THE HEAT OF REACTION AND THE POTENTIAL FOR THE OXIDATION OF CYSTEINE TO CYSTINE

By

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CHAPTER I

INTRODUCTION

The potential for the cysteine-cystine half-reaction is of biological importance. Although several attempts have been made to determine it experimentally, the subject is still in a state of controversy (1).

The purpose of this thesis is to determine by direct calorimetric measurements ΔH^o for the process:

$$\text{cysteine(aq)} \longrightarrow \tfrac{1}{2}\,\text{cystine(aq)} + H^+ + e$$

This can be combined with heats of solution and existing entropy data to calculate the free energy change, and thus the potential.

Enthalpy measurements were made on two systems, cysteine-ferricyanide and cysteine-iodine. The former experiments are described in Chapter III, the latter in Chapter IV. The results obtained were not consistent within the probable experimental error. Since these results depend on the literature values for the ferricyanide and iodine half-reactions, it was then decided to determine ΔH for the ferricyanide-iodine system. The result of this determination is not in agreement with the literature values, but is consistent with the other results of the present work. These enthalpy measurements were made in a nonisothermal, constant temperature environment calorimeter; this apparatus was checked by measuring the heat of solution of tris(hydroxymethyl)aminomethane.

Chapter V presents the method and procedure used in calculating the cysteine-cystine potential; this potential is discussed and compared with the values obtained by various other methods.

Chapter II reviews the pertinent literature.

Chapters III, IV, and V are written in a form suitable for publication in a journal; for this reason, some details have been omitted from these chapters, that are described in the Appendix.

CHAPTER II

SELECTED LITERATURE REVIEW

This chapter is divided into six sections. The first section reviews the thermodynamic principles and conventions used in the remaining chapters. The other five sections are arranged in the same sequence as the subjects of the following chapters. The second section deals with the oxidation of cysteine by ferricyanide and the third section with the standard enthalpy change for the ferricyanide-ferrocyanide half-reaction. In the fourth and fifth sections the oxidation of cysteine by iodine and and the standard enthalpy change for the triiodide-iodide half-reaction are covered, respectively. The potential for the oxidation of cysteine to cystine is considered in the sixth section.

Review of Applicable Thermodynamic Principles and Conventions

Many excellent textbooks of thermodynamics exist that could be used for reference; in this work, Rossini's "Chemical Thermodynamics" (2) was repeatedly consulted. The conventions used for the standard states and the symbols for the thermodynamic functions are those most commonly accepted (2). Rossini's principles of thermochemical investigations were used as an outline for the present investigation.

Half-reactions have been treated thermodynamically in the same way as complete reactions. This is permissible if the thermodynamic properties of aqueous ions are all based on the same conventions. The ones used are those suggested by Noyes (3):

1. Aqueous hydrogen ion in its standard state has zero values for the quantities ΔH_f° and ΔF_f°; the value of S° is equal to half the molar entropy of hydrogen gas at one atmosphere fugacity.

2. An electron has zero values for the quantities ΔH_f°, ΔF_f°, and S°. The entropies of ions at 25° tabulated by the National Bureau of Standards are based on the convention that S° for H^+ is zero; then the S° for e is 15.606 cal/mole deg, although this was not specifically stated. If zero values are assumed for both H^+ and e, as has been done by some authors, erroneous results are obtained for ΔS of half-reactions, although the errors eventually cancel when the complete reaction is considered.

The convention used with respect to the sign of oxidation-reduction potentials is the one recommended by the International Union of Pure and Applied Chemistry (4), which is also used by Clark (1) and most commonly accepted among biochemists. This convention assigns a negative potential to the half-reaction that occurs at the electrode at which oxidation takes place (anode) in an emf cell. Consistent with this choice, a negative sign is assigned to standard half-reactions of reducing agents stronger than hydrogen (in their respective standard states). The symbol E_{h7} is used for the potential of the cysteine-cystine system subject to the conditions that the $[\text{cysteine}]^2 = [\text{cystine}]$ and that the pH = 7.

Oxidation of Cysteine by Ferricyanide

The use of potassium ferricyanide as an oxidizing agent for mercaptans and mercapto groups in proteins has been reviewed (5, 6). In this section, only the reaction with cysteine to give the corresponding disulfide, cystine, will be considered.

Three papers, by Mirsky (7), by Waddill and Gorin (8), and by Khomenko and Tsinkalovs'ko (9) report that the reaction of cysteine with ferricyanide in phosphate buffer (neutral medium) quantitatively converts the mercapto groups to disulfides. The procedure has accordingly been employed for the analytical determination of cysteine (10, 11, 12). It has been shown by Gorin and Godwin (13) and Tysarowski and Konecka (14) that ethylenedinitrilotetraacetate greatly inhibits the reaction, which indicates that it may be catalyzed by metal ions.

Although the literature was carefully searched for mention of the determination of the heat of the oxidation of cysteine to cystine by ferricyanide, none was found. This is the subject of Chapter III.

ΔH° for the Ferricyanide-Ferrocyanide Half-Reaction

In 1936, Bichowsky and Rossini (15) considered all of the data available for the heats of formation of ferrocyanide (aq) and ferricyanide (aq) and assigned "best" values of 121.6 and 146.7 kcal/mole, respectively, at 18°. The difference yields a value of -25.1 kcal for ΔH° of the half-reaction:

$$Fe(CN)_6^{-3}\,(aq) + e \longrightarrow Fe(CN)_6^{-4}\,(aq) \qquad (I)$$

The National Bureau of Standards (16), taking the same original references as Bichowsky and Rossini, lists -113.5 kcal/mole and -28.2 kcal/mole for the heats of formation of potassium ferrocyanide (aq) and ferricyanide (aq), respectively, at 25°. Combination of these values with the heat of formation of K^+ (16) yields a value of -25.26 kcal for ΔH° of process (I).

Several years later Stephenson and Morrow (17) measured the heat capacity of $K_3Fe(CN)_6$ (s) and combined this with the potential of the ferrocyanide-ferricyanide couple (18) and other thermodynamic data (16) to obtain the thermodynamic properties of ferrocyanide (aq) and ferricyanide (aq). From their data a value of -25.1 kcal/mole can be calculated for ΔH° of process (I).

Hepler _et al._ (19) reacted liquid bromine with ferrocyanide and obtained a value for ΔH of -4.2 ± 0.8 kcal/mole of bromine. As pointed out by Hepler _et al._ (19) this value has a greater uncertainty than is desirable, because of the slowness of the reaction. Combination of this value with the heat of formation of Br^- (16) gives a ΔH° of -26.8 ± 0.8 kcal for process (I).

Atanasyants (20) has measured the temperature variation of the emf of the ferrocyanide-ferricyanide and iodine-iodide cell at various concentrations from which was obtained ΔS for the oxidation of ferrocyanide with I_2 (aq). Combination of this with Latimer's (21) value for the emf of the cell gives a value of +9.66 kcal for ΔH of the reaction. From this value and the heats of formation of I_2 (aq) (22) and I^- (16) a value of -25.83 kcal is obtained for ΔH° of half-reaction (I).

Guzzetta and Hadley (23) measured the heats of complexation of Fe^{+2} and $KFe(SO_4)_2 \cdot 12H_2O(s)$ with CN^-, -73.7 ± 0.54 and -68.0 ± 0.23 kcal/mole, respectively. These authors did not reduce their values to the standard state. Watt _et al._ (24) estimated the necessary corrections and obtained -76.93 and -70.96 kcal/mole for the heats of complexation of $Fe(CN)_6^{-4}$ and $Fe(CN)_6^{-3}$, respectively. Combination of the values with heats of formation of Fe^{+2}, Fe^{+3}, and CN^- (16) yields 118.17 and 134.24 kcal/mole for the heats of formation of ferrocyanide and ferricyanide, respectively.

Subtraction gives a value of -16.07 kcal for reaction (I), which is much smaller than all the other values; it is therefore felt that this result is not reliable.

Watt et al. (24) have remeasured the heats of complexation of ferrocyanide by direct reaction of the constituent ions and of ferricyanide by an indirect method. The indirect method was devised to eliminate certain problems associated with the direct reaction of Fe^{+3} with CN^-. A value of -25.23 kcal for ΔH° of reaction (I) is calculated from their heats of complexation, -85.77 and -70.14 kcal/mole for $Fe(CN)_6^{-4}$(aq) and $Fe(CN)_6^{-3}$(aq), and the heats of formation of Fe^{+2}, Fe^{+3}, and CN^- (16).

Busey (25) removed the major part of the uncertainty in Stephenson and Morrow's (17) value for the entropy of $K_3Fe(CN)_6$(s). Combining this with the potential of the ferrocyanide-ferricyanide couple (18) and other available values for the thermodynamic properties of $Fe(CN)_6^{-4}$(aq) and $Fe(CN)_6^{-3}$(aq) he calculated the heats of formation, respectively, as 130.2 and 150.6 kcal/mole. However, he assumes the electron in a half-reaction to have an entropy of zero, while the other entropy values were based on the convention that the entropy of the electron is equal to half the molar entropy of hydrogen gas (3). Correction of his calculations gives the values of 111.6 and 136.6 kcal/mole for the heats of formation of ferrocyanide and ferricyanide. The difference gives a value of -25.0 kcal for ΔH° of process (I).

It is clear from this literature survey that most values for ΔH° of half-reaction (I) are in the range -25.0 to -26.0 kcal. It is the author's feeling that the value of -25.23 kcal obtained from the data of Watt et al. is the best value. This value is the one used in Chapters III and IV.

Oxidation of Cysteine by Iodine

One of the earliest users of iodine was K. Mörner (26), who reported in 1902 that cysteine could be estimated quantitatively by titration with iodine in 10% hydrochloric acid solution. Starch was used as an indicator. Several years later Okuda (27) determined cystine in a protein hydrolyzate by reducing it with zinc and titrating the resulting cysteine with iodate-iodine under conditions carefully controlled with respect to acid concentration, temperature, and volume. Virtue and Lewis (28) modified Okuda's method by using iodine in potassium iodide as the titrant; the excess iodine was back titrated with sodium thiosulfate. Lucas and King (29) also used iodine as the oxidizing agent in the analysis of cysteine.

In most of the above cases it was assumed that the oxidation product was the disulfide; however, some workers, e.g. Shinohara (30), showed that the amount of iodine consumed depends upon the acid concentration, cysteine concentration, temperature, the time taken for titration etc. In 1935, Lavine (31) showed that the stoichiometry was well defined, i.e., quantitative oxidation of cysteine to cystine took place, in 1 <u>M</u> HCl and 1 <u>M</u> KI at room temperature. These are the conditions under which the reaction was studied in Chapter IV.

The heat of the oxidation of cysteine with iodine has not been measured previously; Chapter IV describes this measurement.

ΔH° for the Triiodide-Iodide Half-Reaction

In the present study a value of ΔH° is needed for the half-reaction:

$$\tfrac{1}{2}I_3^-(aq) + e \longrightarrow \tfrac{3}{2}I^-(aq) \quad .$$

This may be obtained by subtracting $\frac{1}{2}\Delta H_f^\circ$ of I_3^- from $\frac{3}{2}\Delta H_f^\circ$ of I^-.

The National Bureau of Standards (16) lists for the heat of formation of I^-(aq) a value of -13.37 kcal/mole, which is generally accepted. However, for the heat of formation of I_3^-(aq) values have been reported that range from -11.7 to -12.9 kcal/mole (32); the National Bureau of Standards gives the best value as -12.4 kcal/mole.

Stern and Passchier (32) measured the heat of solution of I_2(s) with excess I^-(aq), where the principal product is I_3^-(aq), from which they calculated a value of -12.72 ± 0.15 kcal/mole for ΔH_f° of I_3^-(aq). Also, Hepler *et al.* (33) measured ΔH for this reaction; a value of -12.0 kcal/mole was obtained for ΔH_f° of I_3^-(aq). These authors also measured the heat of reaction of bromine with excess aqueous iodide. Combining this ΔH with the ΔH_f° of I^-(aq) and Br^-(aq) (16), they calculated a heat of formation of I_3^-(aq) of -11.7 kcal/mole.

Bichowsky and Rossini (15) list the heat of formation of I_3^-(aq) as -12.14 kcal/mole. However, in the second section of their book they calculate a value of -12.6 kcal/mole from the temperature coefficient of the equilibrium constant determined by Jones and Kaplan (34) at 0° and 25°. Several other determinations of the equilibrium constant for the complexing of iodine with iodide ion have been reported, see Davies and Gwynne (35).

In Chapter IV a value of ΔH° for the triiodide-iodide half-reaction is determined from the heat of reaction of ferrocyanide with triiodide ion. Several studies have been made to determine the kinetics and the equilibrium constant of this reaction; Reynolds (36) gives a summary of them. However, the heat of this reaction has not been measured previously.

The Potential for the Cysteine-Cystine Half-Reaction

Several attempts have been made to measure the oxidation-reduction potential of the system:

$$\text{cySH} \longrightarrow \tfrac{1}{2}\text{cySScy} + \text{H}^+ + e$$

The values reported by several groups of investigators vary from -0.14 to -0.39v for E_{h7}. For a comprehensive review refer to Clark (1) or Cecil and McPhee (37).

The first potentiometric study of the cysteine-cystine system was made by Dixon and Quastel (38) using platinum and gold electrodes. They found that the potential was independent of the cystine concentration, i.e. the system is irreversible. Several years later Williams and Drissen (39) titrated cysteine electrometrically with iodine, potassium dichromate, and potassium iodate, and found a different potential with each oxidizing agent. Ghosh <u>et al</u>. (40) claimed to have obtained a reversible system by reduction of the disulfide at a mercury cathode. This, according to them, eliminated oxygen and an oxide film from the surface of the electrode and thus allowed it to act as an inert electrode. Green (41) confirmed Ghosh's work, but maintained that mercury cysteinate was formed and responsible for the reversibility of the system.

Fruton and Clarke (42) used dyes of known potentials. They obtained for the cysteine-cystine potential -0.222v at pH 7; similar potentials were obtained for other sulfhydryl-disulfide systems, which is unexpected. Approximately the same potential was obtained whether they started with the mercaptan or disulfide, which indicated that the system was reversible. Borsook <u>et al</u>. (43) repeated the work of Fruton and Clarke and confirmed them in principle, but not in detail.

A potential of -0.21v, close to the one obtained by Fruton and Clarke, was determined by Cleland (44) by equilibrating the cysteine-cystine system with the DPN^+ - DPNH system in the presence of lipoamide and dihydrolipoic dehydrogenase.

Ryklan and Schmidt (45) found a cysteine-cystine potential of -0.14v by potentiometric titration of cysteine with iodine in 1 M KI, using platinum electrodes. They found that iodide ion catalyzes the cysteine-cystine equilibrium and demonstrated that cystine affects the potential even in absence of iodide ion or other catalyst. Freedman and Corwin (46) repeated some of the work of Ryklan and Schmidt and concluded that their results do not represent valid measurements of the cysteine-cystine potential. Also, they repeated the experiments of Ghosh *et al.* (40) and concluded that mercuric cysteinate was the potential-determining oxidant in their experiments. Kolthoff *et al.* (47) claimed that even though mercuric cysteinate is formed, it is rapidly reduced at the mercury electrode, therefore the overall reaction is the desired reaction.

In 1955 Tanaka *et al.* (48) determined the equilibrium constant for the reaction of ferrous ion with cystine. By combining the equilibrium constant with the potential for the ferric-ferrous ion system they calculated a standard potential of 0.08v, which corresponds to a value of -0.33v for E_{h7}.

Borsook *et al.* (43) obtained a potential for the system of interest from thermal measurements. They calculated a potential of -0.39v from the free energy change of the cysteine-cystine half-reaction. The free energy change was calculated from the familiar thermodynamic relationship:

$$\Delta F = \Delta H - T\Delta S$$

The heat capacity and the heat of combustion measurements of Huffman and

Ellis (49, 50) were used to calculate ΔS and ΔH, respectively. The method is certainly sound, however the determination of the heat of reaction may not be very accurate, since it involves the difference between two very large numbers in which a small percentage error leads to a large absolute error. Sunner (51) again measured the heats of combustion of cysteine and cystine and recalculated the combustion data of Huffman and Ellis (50) on the basis of a different final state. From the two sets of data the respective values of +1.91 and +2.05 kcal can be calculated for the difference in one half the heat of formation of cystine and the heat of formation of cysteine. By combining these with entropy data the values of -.390v and -.384v can be calculated for E_{h7} of the cysteine-cystine system.

In Chapter V a value of E_{h7} is calculated using the value of ΔH obtained in earlier chapters.

CHAPTER III

(As stated in the Introduction, this chapter is written
in a form suitable for publication)

HEAT OF THE OXIDATION OF CYSTEINE TO CYSTINE BY POTASSIUM FERRICYANIDE

Summary

ΔH°_{298} for the reaction:

$$\text{cysteine(aq)} + \text{Fe(CN)}_6^{-3}\text{(aq)} \longrightarrow \tfrac{1}{2}\ \text{cystine(aq)} + \text{Fe(CN)}_6^{-4}\text{(aq)}$$

has been determined to be -22.80 ± 0.08 kcal/mole. Combination of this value with the ΔH° for the Fe(CN)_6^{-3} - Fe(CN)_6^{-4} half-reaction and with the appropriate heats of solution and dilution, yields a value of +2.19 ± 0.08 kcal/mole for ΔH° of the reaction:

$$\text{cysteine(s)} \longrightarrow \tfrac{1}{2}\ \text{cystine(s)} + \tfrac{1}{2}\text{H}_2\text{(g)}.$$

Introduction

The oxidation potential for the system:

$$\text{cysteine(aq)} \longrightarrow \tfrac{1}{2}\ \text{cystine(aq)} + \text{H}^{+} + \text{e}$$

has been determined by several groups of investigators, but the results have not been consistent (1). It is the purpose of the present paper and of the two that follow to determine the heat of the reaction in solution, which can be combined with other existing thermodynamic data to give the desired potential.

The heat of the oxidation of cysteine with $Fe(CN)_6^{-3}$ was measured with a solution calorimeter of the nonisothermal, constant-temperature-environment type. The heat of solution of tris(hydroxymethyl)aminomethane (TRIS) in 0.1 M hydrochloric acid was used to check the calorimeter system. The experimental result of the cysteine-$Fe(CN)_6^{-3}$ reaction was combined with the standard enthalpy change for the $Fe(CN)_6^{-3}$ - $Fe(CN)_6^{-4}$ half-reaction and appropriate heats of solution and dilution data to determine ΔH°_{298} for the reaction:

$$\text{cysteine(s)} \longrightarrow \tfrac{1}{2}\ \text{cystine(s)} + \tfrac{1}{2}H_2\text{(g)}.$$

This result can be compared to previously obtained heats of combustion measurements.

Experimental

Materials

L - Cysteine hydrochloride hemihydrate (M. W. 166.6) was from the California Corporation for Biochemical Research (Grade A); L - cysteine was from Sigma Chemical Company; the SH titers determined by the N-ethylmaleimide procedure (52) were 96.9% and 101.7%, respectively. Potassium ferricyanide (Baker Analyzed Reagent, assay: 100.0%) was used without further purification. Tris(hydroxymethyl)aminomethane (TRIS) from Eastman Chemical Company (purity 99.95+%) was dried to constant weight before use. The 0.1 M hydrochloric acid used in the calibration experiments was obtained by dilution of concentrated hydrochloric acid (Baker Analyzed Reagent). The water used was obtained from the condensation of steam and passed through a column of Rexyn IRG-501 (H-OH) ion-exchange resin. The pH 5.0 buffer was 0.06 M in acetic acid and 0.14 M in sodium acetate.

Apparatus

The calorimeter was of the nonisothermal, constant-temperature-environment type. The environment was a 21.5-liter water bath contained in a polystyrene ice-chest with cover. The bath was stirred and its temperature was maintained at 25.000 ± 0.002° with a proportional controller (Sargent Thermonitor, Model S). The air above the water was insulated by the cover and its temperature was maintained at 25.0 ± 0.1°.

The calorimeter vessel was a 600 ml silvered Dewar flask (Fig. 1,j). It was closed at the top by a cork stopper (h), which was attached to and covered by a brass cap (i). When in operation, the top of the vessel was 4 cm above the water in the bath, and the cap helped to maintain the top of the calorimeter at the bath temperature (53).

The stirrer (m) was made from glass rod. It was actuated by a 600 r.p.m. synchronous motor. The pitch and size of the propeller blades were such that the reactants were rapidly mixed, but the heat of stirring was small.

The ampule holder (o) consisted of a hollow Teflon cylinder with many vertical slots and a screw-on cap (s) fitted with two tantalum spikes (t). The Lucite plunger (p) extended from the exterior into the holder and rested on a tantalum ring (q) which encircled the shoulder of the ampule (r) as shown. A sharp blow to the end of the plunger broke the glass ampule against the spikes.

The heater (k) consisted of a stainless-steel spool (e) on which was wound approximately 16 ft. of size #26 silk-covered Evanohm resistance wire (f). An O-ring (d) was fitted over each end of the spool, and this was force-fitted into a tantalum sleeve (g); its ends were coated with paraffin wax (c) to make a water-tight seal. The heater was

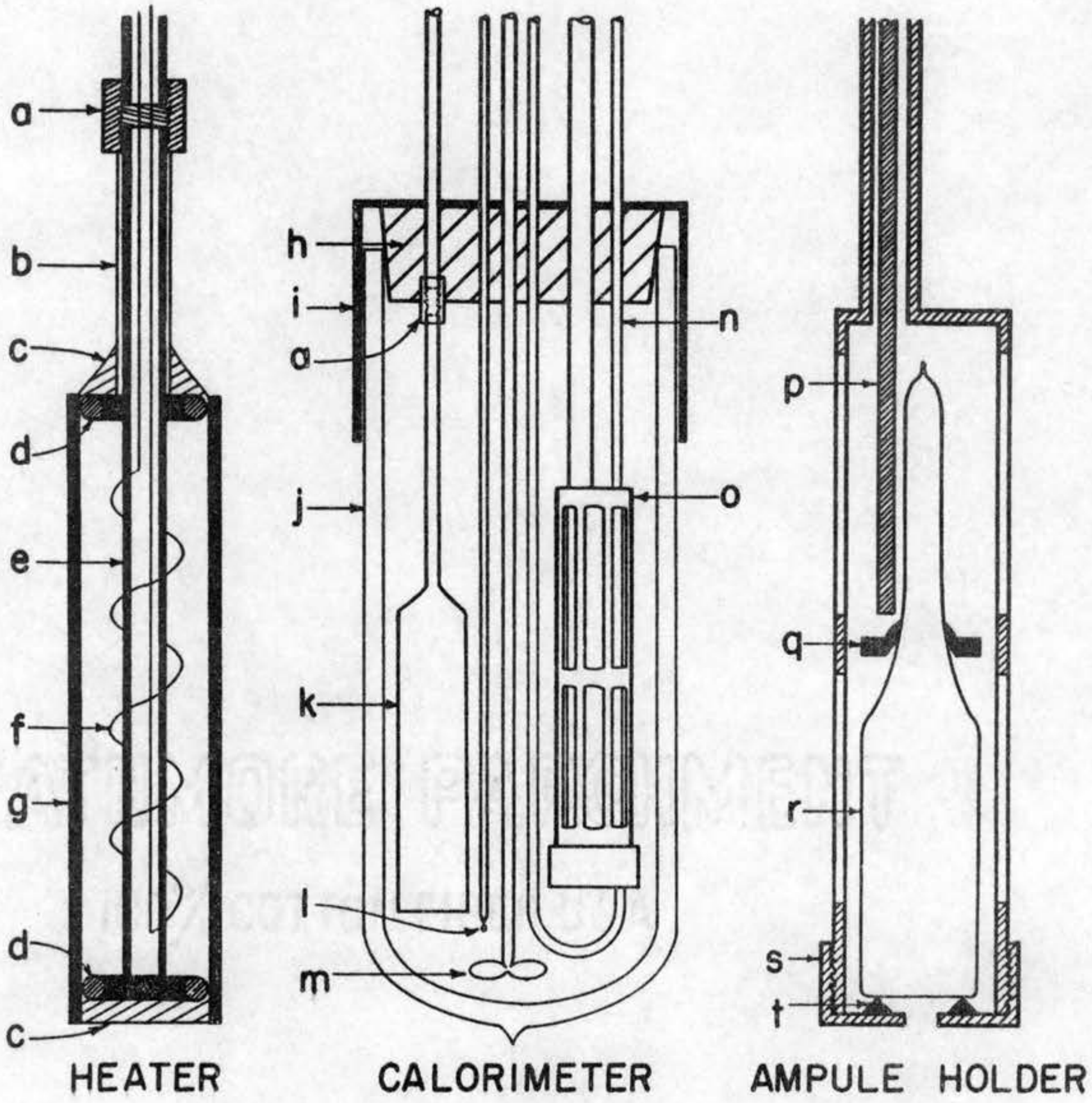


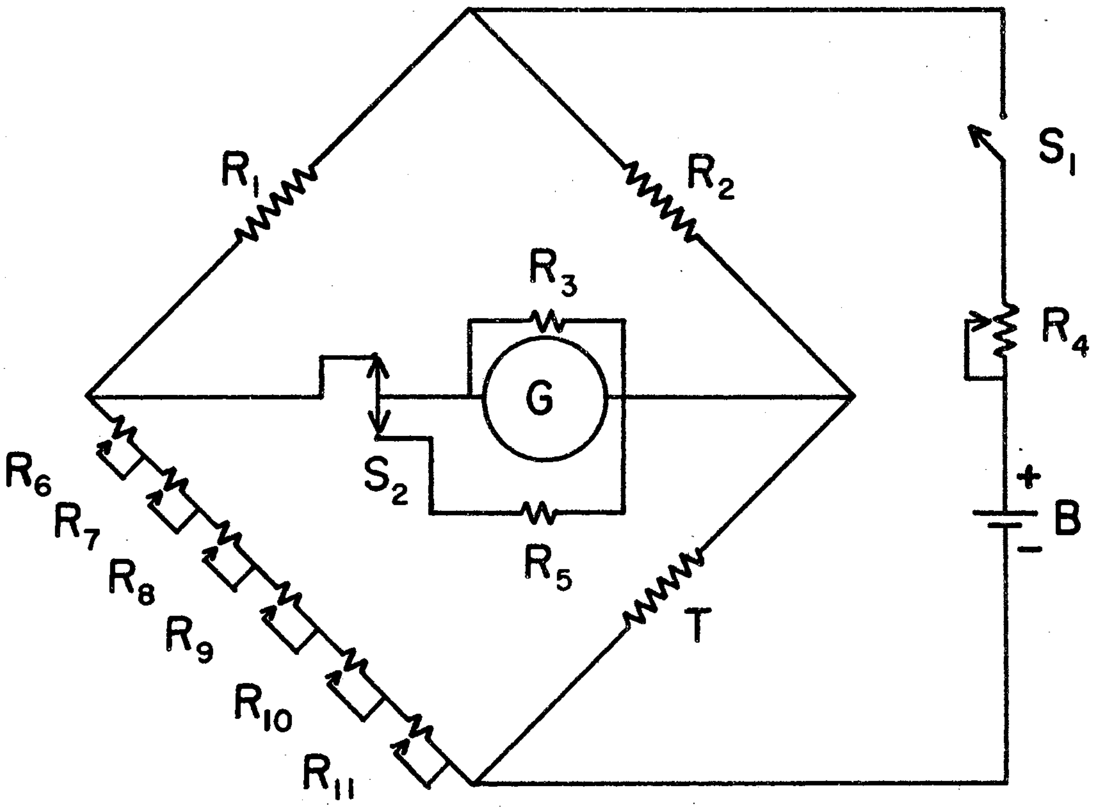
Figure 1. Cross Section of Calorimeter and Components. See Text for Description of Lettered Parts

suspended from the cap by two pieces of stainless steel tubing connected by a Lucite insulator (a); this arrangement minimized the heat exchange due to conduction. A glass sleeve (b) encased the lower tube. The resistances of the heater and the heater including lead-in wires were measured with a Leeds and Northrup Wheatstone bridge that had been calibrated with a NBS certified resistor; they were 49.660 and 50.348 ohms, respectively.

A glass-enclosed thermistor (1) (S-81620, E. H. Sargent Co.), approximately 2000 ohms at 25°, was used to measure the temperature. It formed one arm of a Wheatstone bridge circuit (Fig. 2). Two others arms were type 500 General Radio resistors (2000 ohms ± 0.05%). The fourth arm consisted of six General Radio decade resistors (R_8 - R_{11}) adjustable to 0.01 ohm. The galvanometer was a Leeds and Northrup model 2430-C with a sensitivity of 0.0031 μa/mm.

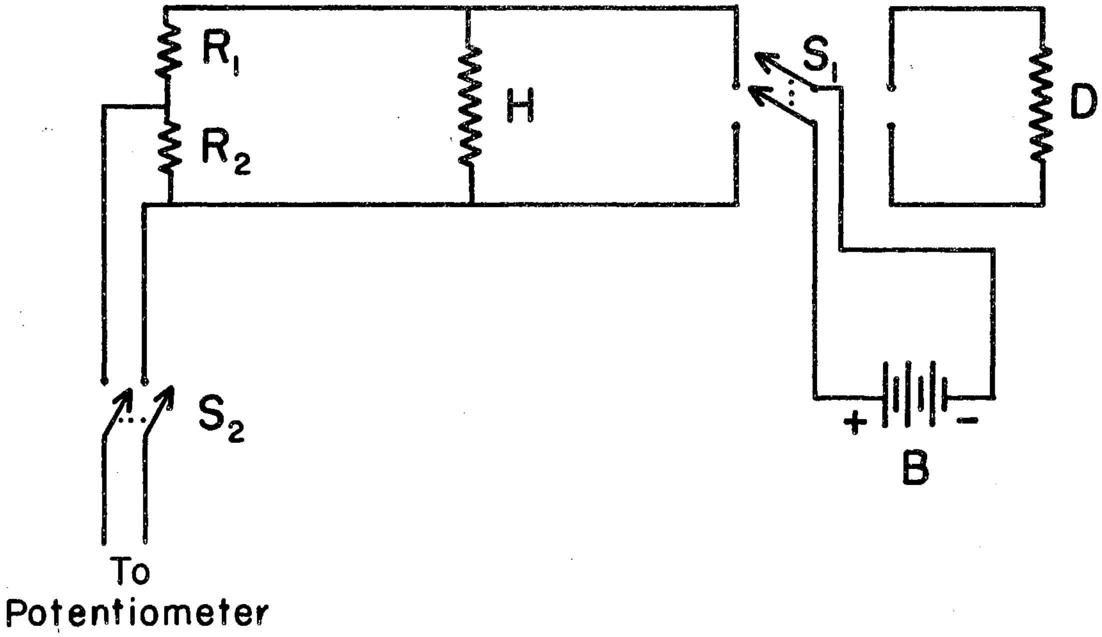
The energizing voltage for the bridge was supplied by a 1.5v battery, which was connected through a decade resistance box. This resistance was adjusted to give the optimum bridge sensitivity; its value was 4000 ohms, which gave a thermistor current of 0.125 ma and a sensitivity of about 1.2×10^{-3} degree/mm. The resulting resistance change was measured to 0.01 ohm, which is equivalent to 0.000125° C.

The electrical circuit for determining the energy equivalent was of conventional design (53) (Fig. 3). A 6v storage battery was the power supply. The heater and a dummy heater having the same resistance were connected to the battery through a three-position knife switch, so that the battery voltage could be applied to either one. Two General Radio type 500 resistors, R_1 (20 K ± 0.05%) and R_2 (1 K ± 0.05%), in series were mounted in parallel with the heater. The potential drop across R_1 +



R_1	2,000 ohms	S_1	Power switch
R_2	2,000 ohms	S_2	Galvanometer switch
R_3	470 ohms		
R_4	Sensitivity adjustment		
R_5	56 ohms	T	Thermistor
R_6	1,000 ohms/step		
R_7	100 ohms/step		
R_8	10 ohms/step	B	1.5v D.C.
R_9	1 ohms/step		
R_{10}	0.1 ohms/step		
R_{11}	0.01 ohms/step	G	Galvanometer

Figure 2. Schematic Diagram of Bridge Circuit



R_1	20,000 ohms
R_2	1,000 ohms
H	Calorimeter heater
D	Dummy heater
S_1	Power switch
S_2	Potentiometer switch
B	6v D.C.

Figure 3. Schematic Diagram of Electrical Calibration Circuit

R_2, which is equal to the potential drop across the heater, was obtained by multiplying the measured drop across R_2 by the factor 21(49.660/50.438). The ratio (49.660/50.438) is the resistance of the heater to that of the heater plus lead-in wires. The potential was measured with a Leeds and Northrup no.7552 potentiometer using an unsaturated Eppley standard cell no. 7309, which has a negligible temperature coefficient, and a 2v Williard constant voltage storage battery no. 2799-A. The potentiometer and standard cell were checked against another standard cell of known voltage. The duration of the electrical energy input was measured with an electric timer (Precision Scientific) to 0.1 sec.

Calibration Procedure

Approximately 2.5 g of solid TRIS was placed in a thin-bottomed 5 ml glass ampule with the aid of a small funnel, and its weight was determined by the difference in the weight of the empty and full ampule. The ampule was then carefully sealed in a flame. It was inserted into the holder through the bottom and the bottom cap was screwed into place. To the calorimeter vessel was added 500 ml of 0.1 M HCl. Then the cap was placed over the mouth of the Dewar flask and the stirrer started. The temperature was measured and, if necessary, adjusted to approximately 24.5° by circulating water through the cooling loop (n); the water was then removed from the tube. The entire system was allowed to equilibrate for at least 30 min; then resistance readings were taken every 5 min for an "initial" period of at least 30 min. At 25° the ampule was smashed against the tantalum spikes. Resistance readings were taken every minute for approximately 20 min and then every 5 min for a "final" period of at least 30 min. A typical plot of time vs. resistance is shown in Fig. 4.

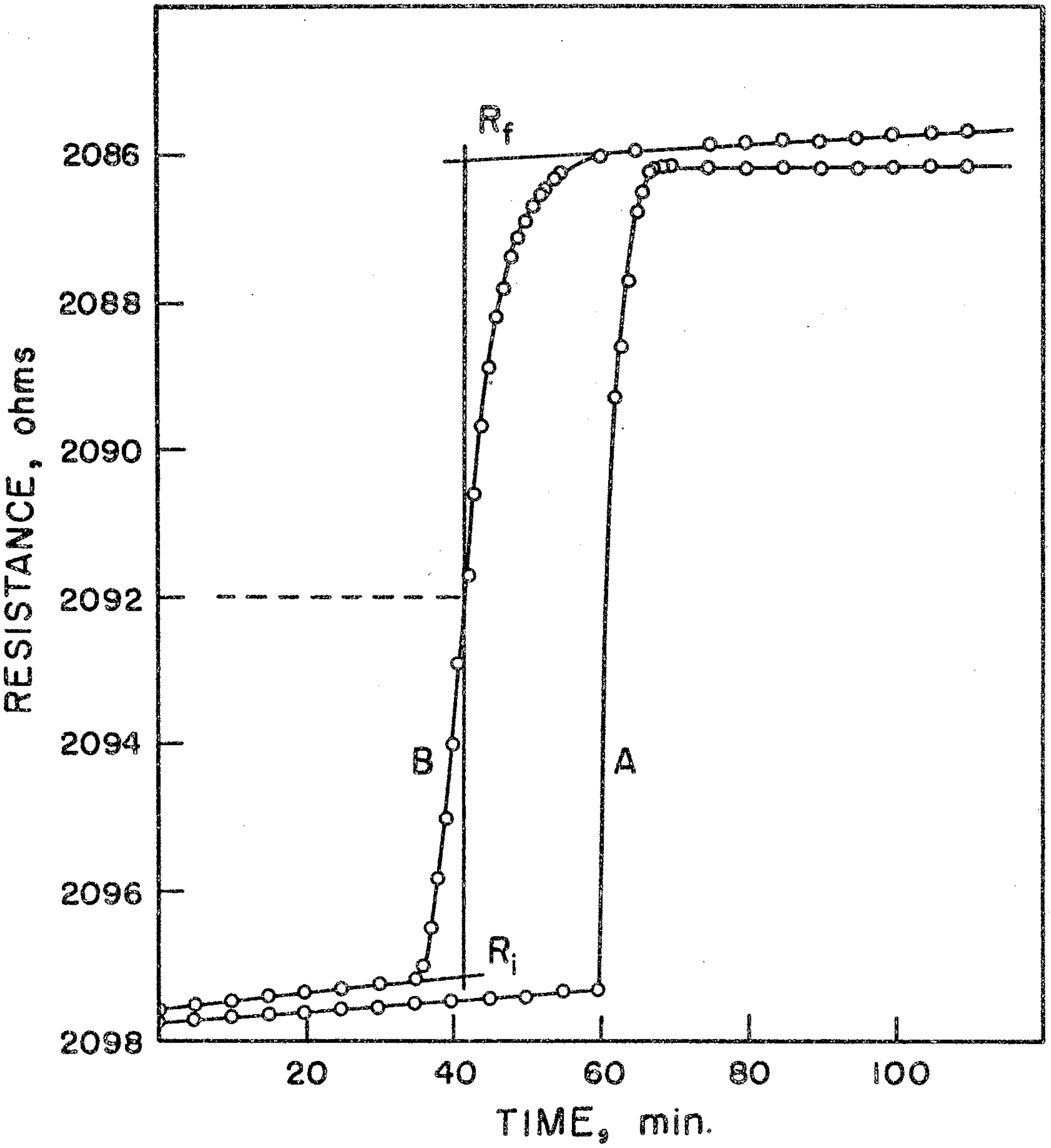


Figure 4. Time vs. Resistance Plot: (A) TRIS reaction, (B) Electrical Calibration Curve (R_i and R_f are Obtained by Extrapolation as Shown)

After the "final" period the system was again brought to approximately 24.5°, permitted to come to thermal equilibrium as before, and then heated electrically over the same temperature range that had been given by the chemical reaction. Thus, the assigned temperature of the calorimetric process was the initial temperature of the reaction period, i.e. 25° (54). The battery of the electrical calibration circuit was discharged through the dummy heater for 1 hr or more before switching to the calorimeter heater.

Reaction Procedure

The reactant, potassium ferricyanide in buffer, was measured into an ampule with a 5 ml burette. The other reactant, cysteine, was dissolved in buffer and 500 ml of the solution was placed in the calorimeter vessel. Cysteine was used in slight excess of the stoichiometric amount. The duration of the "reaction" period was shorter than that of the TRIS reaction; the rest of the procedure was the same.

The heat of dilution of potassium ferricyanide from the ampule concentration to the vessel concentration was determined by repeating the experiment just described with cysteine not present.

The heat of solution of cysteine was determined by placing cysteine (free base) in an ampule and breaking this in acetate buffer of pH 5, which is the isoelectric point of the amino acid.

The heat of ampule breakage was determined by breaking an ampule containing water into the calorimeter vessel containing water.

Calculations

The following equation was used to calculate the enthalpy change:

$$-\Delta H = [(\text{electrical energy})/n]\ [\Delta T_r/\Delta T_e] \qquad (1)$$

where n is the number of moles of the reactant that is taken in limiting amounts. The fraction $\Delta T_r / \Delta T_e$, which was always close to one, was the ratio of the reaction temperature change to the electrical calibration temperature change. The value of the electrical energy in calories was calculated from the equation:

$$\text{electrical energy(cal)} = V^2\text{(volts) } t\text{(sec)}/4.1840\ R\text{(ohms)} \qquad (2)$$

where V was the potential drop measured across the heater and R its resistance.

The quantity $\Delta T_r / \Delta T_e$ was determined from the expression:

$$\Delta T_r / \Delta T_e = \log(R_i / R_f)/\log(R_i' / R_f') \qquad (3)$$

R_i and R_f were the initial and final resistances of the "reaction" period, whereas the primed values were the corresponding values for the electrical calibration. Equation (3) can be derived from:

$$\log R = A + B / (T + C) \qquad (4)$$

which is the most accurate relationship between the resistance and temperature of a thermistor (3); A, B, and C are the thermistor constants.

The method used to obtain R_i and R_f is discussed by Sturtevant (55). They were found by extrapolating the "initial" and "final" periods to the time at which the temperature of the calorimeter was equal to the average of the values at the start and finish of the "reaction" period. Fig. 4 shows this method applied to a typical calibration experiment. Since the reaction time for the ferricyanide-cysteine reaction was much shorter, R_i was taken as the resistance at the time the ampule was broken and R_f was obtained by extrapolating the "final" period back to this time.

Results

All results are expressed in terms of the "defined" thermochemical calorie, 4.1840 absolute joules and refer to processes at 25°. The uncertainty intervals are given as twice the standard deviation of the mean, $2[\Sigma d^2/n\ (n-1)]^{\frac{1}{2}}$.

Fig. 4 shows a representative resistance vs. time curve for the solution of TRIS in 0.1 $\underline{M}$ hydrochloric acid (Process I). Table I tabulates the data for three experiments; n is the number of moles of TRIS and ΔR is the corrected resistance change due to the reaction. The average of seven measurements was found to be -7135 ± 6 cal/mole.

From three experiments it was found that the heat of ampule breakage was essentially zero, therefore no correction was made for it.

In the reaction between ferricyanide and cysteine, the initial concentration of potassium ferricyanide, i.e. of the reactant in the ampule, and of cysteine, and the final concentrations of the products were as specified below (Process II):

$$\underset{(0.6\ \underline{M})}{Fe(CN)_6^{-3}} + \underset{(0.008\ \underline{M})}{cySH} \longrightarrow \underset{(0.006\ \underline{M})}{Fe(CN)_6^{-4}} + \underset{(0.004\ \underline{M})}{\tfrac{1}{2}cySScy} + H^+ \qquad \text{(II)}$$

The completeness of the reaction was shown by the absence of $Fe(CN)_6^{-3}$ in the products. Table I gives -21.18 ± .08 kcal/mole as the average of 4 experiments; n is the number of moles of ferricyanide, the substance which is in limiting amounts, and ΔR is the same as before.

The final concentration of cystine, $4 \times 10^{-3}\ \underline{M}$, exceeded its solubility at pH 5, which is about 4.8×10^{-4} moles/liter (56). However, the precipitation of cystine was slow. No observable precipitation occurred in the first fifteen minutes of the "final" period and several hours were required for complete precipitation.

Approximately fifteen minutes after the ferricyanide-cysteine reaction had taken place, it was observed that the rate of resistance change gradually increased, i.e., the slope of the "final" period changed as shown in Fig. 5. This phenomenon was not observed during the "final"

TABLE I

HEATS OF THE CALORIMETRIC PROCESSES (I - IV)

Process	$n \times 10^3$	ΔR	ΔH (kcal/mole)
I	19.6899	20.88	- 7.142
	20.8435	22.09	- 7.130
	19.9926	21.21	- 7.139
II	3.04625	9.65	-21.27
	3.05633	9.67	-21.15
	3.10905	9.71	-20.95
	3.08269	9.73	-21.34
			-21.18 ± 0.08
III	3.0430	0.72	+ 1.62
	3.0414	0.72	+ 1.62
	3.0440	0.72	+ 1.62
			+ 1.62 ± 0.00
IV	9.4520	3.80	+ 2.68
	7.0442	2.84	+ 2.69
	9.0904	3.66	+ 2.70
			+ 2.69 ± 0.00

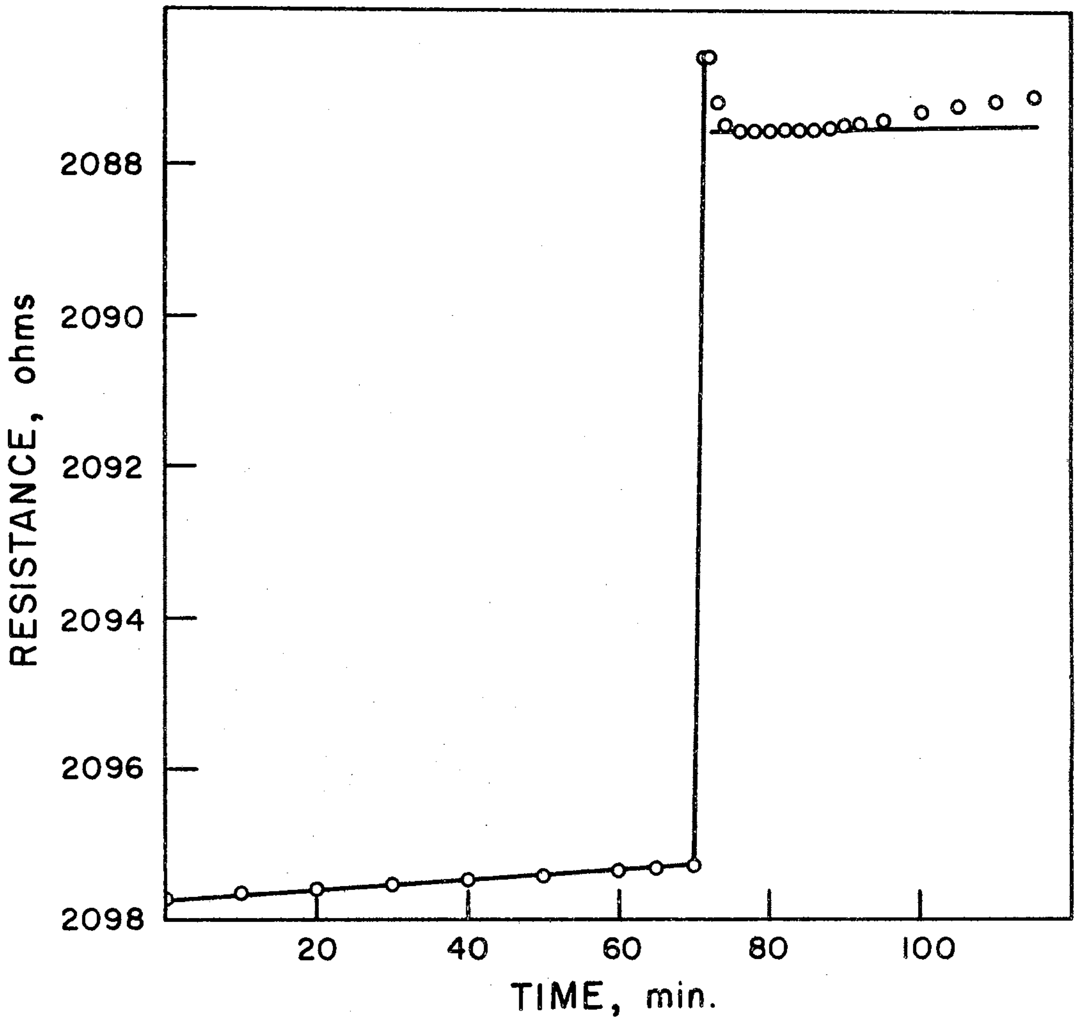


Figure 5. Time vs. Resistance Plot for Reaction of Cysteine With $Fe(CN)_6^{-3}$

period of the TRIS reaction (Fig. 4). This exothermic process was believed to be the precipitation of cystine. In obtaining the corrected resistance change due to reaction, it was assumed that during the first fifteen minutes, a period of constant slope as shown in Fig. 5, the heat produced by the cystine precipitation was negligible.

Process III was:

$$\underset{(0.6\ \underline{M})}{Fe(CN)_6^{-3}} \longrightarrow \underset{(0.006\ \underline{M})}{Fe(CN)_6^{-3}} \qquad \text{(III)}$$

i.e., the heat of dilution of $Fe(CN)_6^{-3}$ from the ampule concentration to the vessel concentration. Table I gives the results of 3 experiments, which average 1.62 ± 0.00 kcal/mole.

Process IV, the heat of solution of cysteine (free base):

$$\text{cySH(s)} + \text{buffer pH 5} \longrightarrow \text{cySH}(0.014\ \underline{M} - 0.018\ \underline{M}) \qquad \text{(IV)}$$

was found to be independent of the final concentration over the range 0.014 M to 0.018 M. Table I gives the results of 3 experiments, which average +2.69 ± 0.00 kcal/mole.

Discussion

Careful studies of the reaction of TRIS with 0.1 M hydrochloric acid by Gunn (57) and by Irving and Wadsö (58) have shown it to be a very satisfactory process for checking and comparing solution calorimeters. These workers report values of -7107 ± 0.9 and -7111 ± 3 (59) cal/mole, respectively.

The result found in this work (7135 ±6 cal/mole), is 0.3% high. This discrepancy is significant, since the precision was better than 0.1%. However, one cannot be certain at this point whether the discrepancy is due to systematic errors in the calibration or to differences in the TRIS samples. The sample used in this work measured in a different calorimeter (60) also gave results about 0.3% high; for this reason the electrical calibration was taken as the basis for the calculations.

By substracting process III from process II the following reaction:

$$\underset{(0.006\ \underline{M})}{Fe(CN)_6^{-3}} + \underset{(0.008\ \underline{M})}{cySH} \longrightarrow \underset{(0.006\ \underline{M})}{Fe(CN)_6^{-4}} + \underset{(0.004\ \underline{M})}{\tfrac{1}{2}cySScy} + H^+ \qquad \text{(V)}$$

was obtained with an enthalpy change of -22.80 ±.08 kcal/mole.

The enthalpy change for the cysteine-cystine half-reaction was obtained from equation V by appropriately combining it with available heats of dilution and the standard enthalpy change for the $Fe(CN)_6^{-4}$ - $Fe(CN)_6^{-3}$ half-cell. The method is illustrated in the following scheme.

$$\underset{(0.006\ \underline{M})}{Fe(CN)_6^{-3}} + \underset{(0.008\ \underline{M})}{cySH} \longrightarrow \underset{(0.006\ \underline{M})}{Fe(CN)_6^{-4}} + \underset{(0.004\ \underline{M})}{\tfrac{1}{2}cySScy} + H^+ \qquad \Delta H = -22.80 \pm 0.08$$

$$\underset{(0\ \underline{M})}{Fe(CN)_6^{-3}} \longrightarrow \underset{(0.006\ \underline{M})}{Fe(CN)_6^{-3}} \qquad \Delta H = +0.15\ (61)$$

$$\underset{(0.006\ \underline{M})}{Fe(CN)_6^{-4}} \longrightarrow \underset{(0\underline{M})}{Fe(CN)_6^{-4}} \qquad \Delta H = -0.32\ (61)$$

$$\underset{(0\ M)}{Fe(CN)_6^{-4}} \longrightarrow \underset{(0\ M)}{Fe(CN)_6^{-3}} + e \qquad \Delta H^\circ = +25.23\ (24)$$

$$\underset{(0.008\ \underline{M})}{cySH} \longrightarrow \underset{(0.004\ \underline{M})}{\tfrac{1}{2}cySScy} + e + H^+ \qquad \Delta H^\circ = +2.26 \pm 0.08$$

To convert this value to standard conditions the heats of dilution of cysteine and cystine are needed, and they have not been determined. However, heats of dilution data are available for several other amino acids (62) in dilute solution; the values are very small and close to one another. Thus, it may be assumed that the heats of dilution of cysteine and cystine would probably be less than 10 cal, and since only the difference would be important it was assumed to be zero. Therefore, the above value of +2.26 ± 0.08 kcal is taken as the standard state value, ΔH°, of the cysteine-cystine half-reaction.

By combining the above ΔH^{o} value with the heat of solution of cysteine, +2.69 kcal/mole (reported here) and one-half the heat of solution of cystine, +2.76 kcal/mole (63), a value of +2.19 ± 0.08 kcal is obtained for process VI.

$$\text{cySH(s)} \longrightarrow \tfrac{1}{2}\text{cySScy(s)} + \tfrac{1}{2}\text{H}_2\text{(g)} \qquad \text{(VI)}$$

From the heats of combustion (50), Borsook and coworkers (48) obtained the value of +1.92 kcal for process VI. This value is subject to a considerable uncertainty because it is obtained by substracting two large numbers, the heats of formation. Borsook <u>et al</u>. mention that there is a possible error associated with the combustion data used, however do not give an estimate. Sunner (51) repeated the measurements of the heats of combustion of cysteine and cystine, and recalculated the data of Huffman and Ellis, based on a final state of free sulfur, to a final state of H_2SO_4 (10^{-4} <u>M</u>); his result for process VI was +1.91 kcal. Application of the correction to the data of Huffman and Ellis would give, according to Sunner, +2.05 kcal. Although these values are in good agreement, there is a large uncertainty attached to ΔH of process I, because it is the difference between two large numbers.

The precision of the value obtained in this work is subject to much less uncertainty. The results obtained from the TRIS reaction indicate the precision of the calorimeter to be about 0.1%, and the accuracy at worst 0.3%. The value obtained for the heat of solution of cysteine is quite reasonable and contains little uncertainty; the heat of solution of cystine (63), calculated from the dependence of solubility on temperature, is probably less precise.

In light of the above the value of ΔH^{o} for reaction VI is taken to be +2.19 ± 0.08 kcal, although it depends on the value used for the standard enthalpy change for the $Fe(CN)_6^{-3}$ - $Fe(CN)_6^{-4}$ half-reaction. The value used is generally accepted (64, 65), however other values are available, see summary in Chapter II.

The succeeding paper reports a ΔH° for the above reaction determined from the oxidation of cysteine with I_3^-; this serves as a check on the method and thermodynamic data used here.

Acknowledgment

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CHAPTER IV

(As stated in the Introduction, this chapter is written
in a form suitable for publication)

THE HEATS OF OXIDATION OF CYSTEINE TO CYSTINE AND OF FERROCYANIDE TO FERRICYANIDE BY IODINE

Summary

For the reaction:

$$\tfrac{1}{2}I_3^-(aq) + cySH_2^+(aq) \longrightarrow \tfrac{3}{2}I^-(aq) + \tfrac{1}{2}H_2cySScy^{+2}(aq) + H^+$$

a value of -9.63 ± 0.05 kcal has been obtained for ΔH_{298} in 1 $\underline{M}$ HI. For the reaction:

$$\tfrac{1}{2}I_3^-(aq) + Fe(CN)_6^{-4}(aq) \longrightarrow \tfrac{3}{2}I^-(aq) + Fe(CN)_6^{-3}(aq)$$

a value of +13.02 ± 0.04 kcal has been obtained for ΔH_{298} in acetate buffer, pH 5. Assuming a ΔH° of -25.23 kcal for the $Fe(CN)_6^{-3}$ - $Fe(CN)_6^{-4}$ half-reaction gives a value of -11.69 ± 0.04 kcal for the half-reaction:

$$\tfrac{1}{2}I_3^-(aq) + e \longrightarrow \tfrac{3}{2}I^-(aq)$$

and a ΔH° of +2.33 ± 0.13 kcal for the reaction:

$$\text{cysteine(s)} \longrightarrow \tfrac{1}{2}\ \text{cystine(s)} + \tfrac{1}{2}H_2(g).$$

The heats of solution of cysteine and cystine in 1 $\underline{M}$ HCl were measured and found to be +1.65 ± 0.07 and +3.47 ± 0.15 kcal/mole, respectively.

Introduction

In the previous paper a value of ΔH° for the half-reaction:

$$\text{cysteine(aq)} \longrightarrow \tfrac{1}{2}\ \text{cystine(aq)} + H^+ + e$$

was obtained from the heat of reaction with ferricyanide ion. It seemed desirable to confirm the value in question by reacting with another oxidant, and iodine was chosen for this purpose. According to Lavine (31) this reagent quantitatively converts cysteine to cystine, but only in 1 M hydroiodic acid medium. In this medium iodine is present as I_3^-, cysteine and cystine as the derivative cations. In order to obtain the value of ΔH for the cysteine-cystine half-reaction, it is necessary to have the heats for the ionizations in question; these have been determined.

The recommended value of ΔH° for the triiodide-iodide half-reaction is -13.86 kcal (16); the value for the cysteine-cystine half-reaction based on this value was not however, in good agreement with that obtained in the preceding paper. It was then decided to measure the heat of the reaction between iodine and ferrocyanide. The result is not in agreement with the accepted values; if the values are made consistent then agreement is obtained for the value of the cysteine-cystine half-reaction.

Experimental

Materials

Iodine (assay: 99.8%) and potassium iodide were both Baker Analyzed Reagent. The potassium ferrocyanide was Merck Reagent grade. The L - cysteine samples were those described in the previous paper. L - Cystine was from Schwarz Laboratories. The 1 M HI solutions were made from potassium iodide and hydrochloric acid using water source described in the previous paper. Because of their susceptibility to air oxidation, the potassium ferrocyanide and cysteine solutions were made just prior to use and were used in excess of the stoichiometric amount. The pH 5 buffer was the same as that mentioned in the previous paper. The pH 7 buffer was 0.0267 M in NaH_2PO_4 and 0.04 M in Na_2HPO_4.

Apparatus and Procedure

The calorimeter and auxiliary equipment, as well as the equation and methods used to determine ΔH for a reaction, are described in the previous paper.

The iodine samples were weighed in a closed container to prevent loss due to sublimation. The iodine was dissolved in 5 ml of 1 M KI and the solution was measured into a sample ampule with a 5 ml burette. Only freshly prepared solutions were used and they were exposed to light for the shortest time possible. The ampules were sealed in a flame with care to prevent overheating of the contents. The reaction of iodine with cysteine was carried out in 1 M HI, whereas, iodine was reacted with ferrocyanide in pH 5 acetate buffer and pH 7 phosphate buffer. The "reaction" periods were less than 2 min long, and thus shorter than that of the calibration reaction.

The heat of dilution of I_3^- from the ampule to the vessel concentration was determined in the same medium as the corresponding reaction, either 1 M HI or pH 5 buffer containing 1 M I^-.

The heats of solution of cysteine and cystine were obtained by dissolving the solids in 1 M hydrochloric acid.

Results

All results are expressed in terms of the "defined" thermochemical calorie, 4.1840 absolute joules and refer to processes at 25°. The uncertainty intervals are given as twice the standard deviation of the mean, $2[\Sigma d^2/n(n-1)]^{\frac{1}{2}}$.

According to Lavine (31) the reaction of iodine and cysteine in 1 $\underline{M}$ HI gives cystine quantitatively. In preliminary experiments the stoichiometry was confirmed by reacting an excess of I_3^- with cysteine in 1 $\underline{M}$ HI and back titrating the excess I_3^- with $S_2O_3^=$. The experimental and calculated amounts agreed within less than 2%. The calorimetric process may be represented by the following equation:

$$\underset{(.48\ \underline{M})}{\tfrac{1}{2}I_3^-} + \underset{(0.011\ \underline{M})}{cySH_2^+} \longrightarrow \underset{(1\ \underline{M})}{\tfrac{3}{2}I^-} + \underset{(0.0055\ \underline{M})}{\tfrac{1}{2}H_2cySScy^{+2}} + H^+ \qquad \text{(I)}$$

in which the approximate experimental concentrations are given below each reagent. The completeness of the reaction was shown by the absence of iodine in the products by the starch test.

The heat of dilution of I_3^- from the ampule concentration to the vessel concentration (process II):

$$\underset{(0.48\ \underline{M})}{\tfrac{1}{2}I_3^-} \longrightarrow \underset{(0.0048\ \underline{M})}{\tfrac{1}{2}I_3^-} \qquad \text{(II)}$$

was also determined. The experimental heats for processes (I) and (II) are given in Table II; n is the number of moles of I_3^- and ΔR is the corrected resistance change.

Process III is the reaction:

$$\underset{(0.48\ \underline{M})}{\tfrac{1}{2}I_3^-} + \underset{(0.02\ \underline{M})}{Fe(CN)_6^{-4}} \longrightarrow \underset{(0.02\ \underline{M})}{\tfrac{3}{2}I^-} + \underset{(0.01\ \underline{M})}{Fe(CN)_6^{-3}} \qquad \text{(III)}$$

with the initial concentrations of the reactants and the final concentrations of the products as specified. Ferrocyanide was used in excess and

TABLE II

HEATS OF THE PROCESSES I AND II

Reaction	n X 10^3	ΔR	$-\Delta H(\mathrm{kcal}/\tfrac{1}{2}I_3^-)$
I	4.7057	7.77	10.11
	4.8183	8.00	10.09
	4.7929	7.94	10.15
			10.12 ± 0.02
II	4.8370	.41	0.52
	4.7197	.37	0.47
	4.7503	.36	0.46
	4.7644	.41	0.52
			0.49 ±0.03

the completeness of the reaction was shown by the absence of a starch test. The measurements were made both at pH 5 and pH 7; no difference should be found, provided there would be no appreciable association of the ions with H+. The values were in fact the same within experimental error (Table III), and thus the overall average of +12.89 ± 0.04 kcal was taken as ΔH for reaction III.

The heat of diluting I_3^- from the ampule concentration to the vessel concentration:

$$\underset{(0.48\ \underline{M})}{\tfrac{1}{2}I_3^-} \longrightarrow \underset{(0.0048\ \underline{M})}{\tfrac{1}{2}I_3^-} \qquad \text{(IV)}$$

was determined by breaking an ampule containing I_3^- into buffer containing 1 M I^-. The results are given in Table III. The numbers n and ΔR

TABLE III

HEATS OF THE PROCESSES III AND IV

Reaction	n X 10^3	ΔR	pH	$\Delta H(\mathrm{kcal}/\frac{1}{2}I_3^-)$
III	4.7924	9.33	5.0	12.86
	5.4986	10.61	5.0	12.84
	4.7374	9.07	5.0	12.85
	4.7380	9.14	5.0	12.93
				12.87 ± 0.04
III	4.7787	9.05	7.0	12.95
	4.7787	9.05	7.0	12.88
	4.8118	9.11	7.0	12.89
				12.91 ± 0.04
IV	4.8930	0.09	5.0	- .13
	4.8930	0.09	5.0	- .13

represent the same quantities as before. It might be noted that dilution of I_3^- with buffer only, resulted in a large positive heat effect because the I_3^- was dissociated; this is not the process of interest in this context.

Finally, the heats of the processes were determined:

$$\text{cySH(s)} + \text{H}^+(1\ \underline{\text{M}}) \longrightarrow \text{cySH}_2^+(0.016\ \underline{\text{M}} - 0.018\ \underline{\text{M}}) \qquad \text{(V)}$$

$$\text{cySScy(s)} + 2\text{H}^+(1\ \underline{\text{M}}) \longrightarrow \text{H}_2\text{cySScy}^{+2}(0.009\ \underline{\text{M}} - 0.01\ \underline{\text{M}}) \qquad \text{(VI)}$$

These results are summarized in Table IV.

TABLE IV

HEATS OF SOLUTION FOR CYSTEINE AND CYSTINE IN 1 M HYDROCHLORIC ACID

Solute	n X 10^3	ΔR	ΔH(kcal/mole)
Cysteine	7.83574	2.06	1.64
	8.84449	2.33	1.64
	9.27560	2.51	1.68
			1.65 ± 0.07
Cystine	4.40357	2.39	3.38
	4.85689	2.71	3.47
	4.93895	2.82	3.55
			3.47 ± 0.15

Discussion

The difference between reactions I and II, -9.63 ± 0.05 kcal, is ΔH for the reaction:

$$\underset{(0.0048\ \underline{M})}{\tfrac{1}{2}I_3^-} + \underset{(0.011\ \underline{M})}{cySH_2^+} \longrightarrow \underset{(1\ \underline{M})}{\tfrac{3}{2}I^-} + \underset{(0.0055\ \underline{M})}{\tfrac{1}{2}H_2cySScy^{+2}} + H^+ \qquad \text{(VII)}$$

To obtain ΔH for the cysteine-cystine half-reaction from reaction VII, it is necessary to have ΔH° for the process:

$$\tfrac{1}{2}I_3^- + e \longrightarrow \tfrac{3}{2}I^- \qquad \text{(VIII)}$$

The National Bureau of Standards Circular 500 (16) gives -12.4 kcal/mole as ΔH_f° of I_3^-; however, this value leads to a heat for the cysteine-cystine half-reaction not consistent with the value reported in the previous paper.

The value of ΔH° used for process VIII was calculated from ΔH° for the $Fe(CN)_6^{-3}$ - $Fe(CN)_6^{-4}$ half-reaction, appropriate heats of dilution, and ΔH for the reaction:

$$\underset{(0.0048\ \underline{M})}{\tfrac{1}{2}I_3^-} + \underset{(0.02\ \underline{M})}{Fe(CN)_6^{-4}} \longrightarrow \underset{(0.02\ \underline{M})}{\tfrac{3}{2}I^-} + \underset{(0.01\ \underline{M})}{Fe(CN)_6^{-3}} \qquad \text{(IX)}$$

which is the difference between processes III and IV. The method is illustrated as follows:

$$\begin{array}{ll}
\underset{(0.0048\ \underline{M})}{\tfrac{1}{2}I_3^-} + \underset{(0.02\ \underline{M})}{Fe(CN)_6^{-4}} \longrightarrow \underset{(0.02\ \underline{M})}{\tfrac{3}{2}I^-} + \underset{(0.01\ \underline{M})}{Fe(CN)_6^{-3}} & \Delta H = +13.02 \pm 0.04 \\
\underset{(0.01\ \underline{M})}{Fe(CN)_6^{-3}} \longrightarrow \underset{(\ 0\ \underline{M})}{Fe(CN)_6^{-3}} & \Delta H = -\ 0.40\ (61) \\
\underset{(\ 0\ \underline{M})}{Fe(CN)_6^{-4}} \longrightarrow \underset{(0.02\ \underline{M})}{Fe(CN)_6^{-4}} & \Delta H = +\ 0.94\ (61) \\
\underset{(\ 0\ \underline{M})}{Fe(CN)_6^{-3}} + e \longrightarrow \underset{(\ 0\ \underline{M})}{Fe(CN)_6^{-4}} & \Delta H^\circ = -25.23\ (24) \\
\underset{(0\ \underline{M})}{\tfrac{1}{2}I_3^-} \longrightarrow \underset{(0.0048\ \underline{M})}{\tfrac{1}{2}I_3^-} & \Delta H = +\ 0.03\ (16) \\
\underset{(0.02\ \underline{M})}{\tfrac{3}{2}I^-} \longrightarrow \underset{(0\ \underline{M})}{\tfrac{3}{2}I^-} & \Delta H = -\ 0.05\ (16) \\
\hline
\underset{(\ 0\ \underline{M})}{\tfrac{1}{2}I_3^-} + e \longrightarrow \underset{(0\ \underline{M})}{\tfrac{3}{2}I^-} & \Delta H^\circ = -11.69 \pm 0.04
\end{array}$$

In writing the equations for processes V and VI it was assumed that conversion of cysteine and cystine to the derivative cations would be complete. Calculations based on the published ionization constants (43) indicate that cysteine would be about 93% converted at pH 0, cystine about 91% converted to the double cation; however there is a little uncertainty in these calculations. On the assumption of complete conversion, the heats of the ionization reactions can be calculated from the combination of the above heats of solution at pH 0 with those at pH 5 (previous paper). This gives +1.04 ± 0.07 kcal for cysteine and +2.04 ± 0.15 kcal for cystine. These values are comparable with the heats of ionization of the carboxyl group of the amino acids compiled by Greenstein (66).

The aforementioned data can be combined to give:

$\frac{1}{2}I_3^- + cySH_2^+ \longrightarrow \frac{3}{2}I^- + \frac{1}{2}H_2\,cySScy^{+2} + H^+$ $\Delta H = -\ 9.63 \pm 0.05$
$(0.0048\ \underline{M})\ (0.011\ \underline{M})(1\ \underline{M})\ (0.0055\ \underline{M})$

$\frac{3}{2}I^- \longrightarrow \frac{3}{2}I^-$ $\Delta H = +\ 0.33$ (16)
$(1\ \underline{M})\ \ (0\ \underline{M})$

$\frac{1}{2}I_3^- \longrightarrow \frac{1}{2}I_3^-$ $\Delta H = +\ 0.03$ (16)
$(0\ \underline{M})\ \ (0.0048\ \underline{M})$

$\frac{3}{2}I^- \longrightarrow \frac{1}{2}I_3^- + e$ $\Delta H^\circ = +11.69 \pm 0.04$
$(0\ \underline{M})\ \ (0\ \underline{M})$

$cySH + H^+ \longrightarrow cySH_2^+$ $\Delta H = +\ 1.65 \pm 0.07$
$(s)\ \ (1\ \underline{M})\ \ (0.016\ \underline{M})$

$\frac{1}{2}H_2\,cySScy^{+2} \longrightarrow \frac{1}{2}cySScy + H^+$ $\Delta H = -\ 1.74 \pm 0.08$
$(0.009\ \underline{M})\ \ (s)\ \ (1\ \underline{M})$

$H^+ + e \longrightarrow \frac{1}{2}H_2\,(g)$ $\Delta H = 0$ (2)

$cySH(s) \longrightarrow \frac{1}{2}cySScy(s) + \frac{1}{2}H_2\,(g)$ $\Delta H^\circ = +\ 2.33 \pm 0.13$

which may be compared to the value of +2.19 ± 0.08 kcal obtained in the preceding paper.

It is felt that a larger uncertainty is attached to the value obtained in the present work. The agreement, although imperfect, adds confidence to the essential correctness of the methods and the thermodynamic data used in the calculations.

CHAPTER V

(As stated in the Introduction, this chapter is written
in a form suitable for publication)

THE CYSTEINE-CYSTINE POTENTIAL

Summary

A value of -0.375 ± 0.007 v has been calculated for E_{h7} of the cysteine-cystine system using a ΔH° of -25.23 kcal for the $Fe(CN)_6^{-3}$ - $Fe(CN)_6^{-4}$ half-reaction. Using a ΔH° of -26.23 kcal for this half-reaction a value of -0.332 ± 0.007v was calculated for E_{h7}. These values are compared and discussed with literature values.

Discussion

The importance of sulfhydryl and disulfide compounds in biological systems has led many investigators to seek the oxidation-reduction potential of the system:

$$\text{cySH(aq)} \rightleftarrows \tfrac{1}{2}\text{cySScy(aq)} + H^{+} + e \qquad \text{(I)}$$

The values reported for this half-reaction vary from -0.14 to -0.39v (1) for E_{h7}, which is the potential calculated for the conditions that $[\text{cySH}]^2 = [\text{cySScy}]$ and pH = 7.

Most of these potentials were obtained by cell measurements or from equilibrium constant determinations using redox systems of known potentials. The cell measurements are in doubt because the cysteine-cystine system is not readily reversible. The results of the equilibrium constant measurements are open to question because it is difficult to match the oxidizing and reducing agents in a manner such that a true equilibrium is established. The experimental methods employed and the problems encountered with the various methods have been reviewed (1, 37, 46, 67).

Borsook, Ellis, and Huffman (43) calculated a potential of - 0.390v from thermal measurements, i.e. third law heat capacity measurements and heat of combustion data. The method is certainly valid in principle, however, the determination of the heat of the cysteine-cystine half-reaction is subject to a large uncertainty, because it involves the difference between two very large numbers.

The present study was undertaken with the expectation that better precision would be attained by measuring the heat of reaction directly. The method used was to measure the heat of the reaction of cysteine with ferricyanide ion and with triiodide ion. Combining these data with ΔH° for the $Fe(CN)_6^{-4}$ - $Fe(CN)_6^{-3}$ and I_3^- - I^- half-reactions, respectively, and with proper heats of dilution, solution, and ionization ΔH° was determined for the cysteine-cystine half-reaction I and for the reaction:

$$cySH(s) \longrightarrow \tfrac{1}{2}cySScy(s) + \tfrac{1}{2}H_2(g) \qquad (II)$$

The details and the values are given in the previous two chapters.

It is felt that the value of ΔH° for reaction II obtained from the ferricyanide-cysteine reaction, +2.19 ± 0.08 kcal, is more reliable than the value, +2.33 ± 0.13 kcal, obtained from the triiodide-cysteine reaction, because the latter value involves more quantities in its calculation. However, the agreement is quite good and the average, +2.26 ± 0.16 kcal, lies within the uncertainty range of both values. Therefore, it is felt that the value of + 2.26 ± 0.16 kcal is the best value for ΔH° of reaction II.

The equations and the quantities, except for ΔH°, used to calculate the potential for the cysteine-cystine system were those given by Borsook and coworkers (43). They have shown that the equation:

$$E_{obs} = \bar{E} - 2.303 \frac{RT}{F} \text{ pH} = \bar{E} - 0.05916 \text{ pH} \qquad (1)$$

is valid in the pH range of 4 to 8. Therefore at pH 7 equation (1) becomes:

$$E_{h_7} = \bar{E} - 0.05916(7) = \bar{E} - .414 \qquad (2)$$

where $\bar{E}$ is given by the relationship:

$$\bar{E} = + \Delta F/23{,}074 \qquad (3)$$

when ΔF refers to the process:

$$\text{cySH}(1\ \underline{M}) \longrightarrow \tfrac{1}{2}\text{cySScy}(1\ \underline{M}) + H^{+} + e \qquad \text{(III)}$$

This value was calculated by appropriate combination of the difference in the free energies of formation of the solid compounds with their free energies of solution to a 1 M solution. Combination of the enthalpy change for reaction II with the entropy data (49) gave the aforementioned difference in the free energies of formation and thus the potential. The method used to calculate E_{h_7} is shown as follows:

For reaction II:

$\Delta H^\circ = +2260 \pm 160$ cal

$\Delta S^\circ = \frac{1}{2}(-286.1)-(-152.3) = +9.3$ cal/deg (49)

$\Delta F^\circ = +2260 \pm 160 - 298.15(9.3) = -513 \pm 160$ cal

$$\begin{array}{lll}
\mathrm{cySH(s)} \longrightarrow \frac{1}{2}\mathrm{cySScy(s)} + \frac{1}{2}\mathrm{H_2(g)} & \Delta F^\circ = -513 \pm 160 & \mathrm{(II)} \\
\frac{1}{2}\mathrm{H_2(g)} \longrightarrow \mathrm{H^+} + e & \Delta F = 0 & \\
\mathrm{cySH}(1\ \underline{M}) \longrightarrow \mathrm{cySH(s)} + \mathrm{H_2O} & \Delta F = -870\ (43) & \\
\underline{\frac{1}{2}\mathrm{cySScy(s)} + \mathrm{H_2O} \longrightarrow \frac{1}{2}\mathrm{cySScy}(1\ \underline{M})} & \underline{\Delta F = +2275\ (43)} & \\
\mathrm{cySH}(1\ \underline{M}) \longrightarrow \frac{1}{2}\mathrm{cySScy}(1\ \underline{M}) + \mathrm{H^+} + e & \Delta F = +892 \pm 160 & \mathrm{(III)}
\end{array}$$

From equation (3):

$\overline{E} = +(892 \pm 160)/23{,}074 = +0.039 \pm 0.007\mathrm{v}$

From equation (2):

$E_{h7} = +0.039 \pm 0.007 - .414 = -0.375 \pm 0.007\mathrm{v}$

The results, +2.19 ± 0.08 and +2.33 ± 0.13 kcal for reaction II, obtained respectively in Chapters III and IV, are dependent upon the value assumed for ΔH° of process IV:

$$\mathrm{Fe(CN)_6^{-3}} + e \longrightarrow \mathrm{Fe(CN)_6^{-4}} \qquad \mathrm{(IV)}$$

The value assumed was -25.23 kcal, but if a more negative value is assumed, ΔH° for the process:

$$\frac{1}{2}\mathrm{I_3^-} + e \longrightarrow \frac{3}{2}\mathrm{I^-} \qquad \mathrm{(V)}$$

calculated in Chapter IV would become more negative, and therefore closer to the value, -13.8 kcal, calculable from the data given by the National Bureau of Standards (16). It was decided to assume for half-reaction IV a value of -26.23 kcal, a quite reasonable value (see Chapter II), and to recalculate on this basis a value for the cysteine-cystine potential.

The value obtained for process V in Chapter IV would become -12.69 kcal. The value of ΔH° for reaction II obtained in Chapter III would be increased to +3.19 ± 0.08 kcal and the one obtained in Chapter IV would become +3.33 ± 0.13 kcal. The average of these two is +3.26 ± 0.16 kcal (for ΔH^o of reaction II); this does not agree nearly as well with the combustion data of Huffman and Ellis (50) and Sunner (51) as the value, +2.26 ± 0.16 kcal, obtained previously. However, the discrepancy does not invalidate the former value, since the uncertainty in the combustion data is large.

Using the same procedure as before a value of -0.332 ± 0.007v is calculated for E_{h7}, which differs from the result of Borsook et al. (43) by 0.058v; these authors estimated the maximum uncertainty in their value as ± 0.046v. The agreement is excellent with the value, -0.33v, calculated by Tanaka et al. (48) from the equilibrium constant of the ferrous-ferric ion and cysteine-cystine system. Also, this potential was obtained by Ghosh et al. (40) by potentiometric measurements. The validity of Ghosh's experimental results were confirmed both by Green (41) and by Freedman and Corwin (46), but the latter questioned the interpretation of the data. Kolthoff et al. (47), on the other hand, supported Ghosh's interpretation and conclusion.

Other investigators have obtained less negative potentials. Fruton and Clarke (42) equilibrated the cysteine-cystine system with various dyes of known potentials, and obtained -0.222v for E_{h7}. Their method has been questioned by Hellerman (68), because side reactions can occur between cysteine and dye. Borsook et al. (43) repeated the experiments of Fruton and Clarke (42) and found their method to be valid, however they were not able to obtain stoichiometric reoxidation of reduced dye by cystine. The value they obtained for E_{h7} was 0.05v more negative than the value calculated by Fruton and Clarke.

By potentiometric titration of cysteine with iodine Ryklan and Schmidt (45) obtained for E_{h7}, -0.14v, a value much more positive than others. During the titration of cysteine with iodine the pH of the solution increased and the authors took no precautions against this, which is one reason their work has been questioned by Freedman and Corwin (46). Under the conditions of changing pH the results are difficult to interpret. Freedman and Corwin, taking precautions to keep the pH constant, tried to repeat Ryklan and Schmidt's work, however were unable to confirm it.

The potential (-0.375 ± 0.007v) obtained in this work using -25.23 kcal as ΔH° of the $Fe(CN)_6^{-3}$ - $Fe(CN)_6^{-4}$ half-reaction is in good agreement with the one calculated by Borsook *et al.* and therefore essentially confirms their thermal data. Using -26.23 kcal as ΔH° of the $Fe(CN)_6^{-3}$ - $Fe(CN)_6^{-4}$ half-reaction a E_{h7} of -0.332 ± 0.007v is calculated, which is in accord with the data of Ghosh *et al.*, Green, and Tanaka *et al.* and differs considerably from the questionable data of Ryklan and Schmidt and Fruton and Clarke. It is the author's view that the correct potential lies between the values -0.375 and -0.332v.

BIBLIOGRAPHY

(1) Clark, W. M., "Oxidation-Reduction Potential of Organic Systems", The Williams and Wilkins Company, Baltimore, 1960, p. 471.

(2) Rossini, F. D., "Chemical Thermodynamics", John Wiley, New York, 1950.

(3) Noyes, R. M., J. Chem. Ed. 40, 2 (1963).

(4) Von Rysselberghe, P., Report of Commission 2, "Proceedings of the 6th Meeting of the International Committee of Electrochemical Thermodynamics and Kinetics" (C.I.T.C.E.) held in Poitiers, 1954, Butterworths Scientific Publications, London, 1955, p. 20-49.

(5) Herriott, R. M., Advan. Protein Chem. 3, 178 (1947).

(6) Chinard, F. P., and Hellerman, L., in Glick, D., Ed., "Methods in Biochemical Analysis", Vol. 1, Interscience, New York, 1954, p. 12.

(7) Mirsky, A. E., J. Gen. Physiol. 24, 709 (1940-41).

(8) Waddill, H. G., and Gorin, G., Anal. Chem. 30, 1069 (1958).

(9) Khomenko O. K., and Tsinkalovs'ka S. M., Ukr. Biokhim. Zh. 34, 888 (1962); Chem. Abstr. 58, 8229 C (1963).

(10) Mason, H. L., J. Biol. Chem. 86, 623 (1930).

(11) Kendall, E. C., Holst, J. E., J. Biol. Chem. 91, 435 (1931).

(12) Mirsky, A. E., J. Gen. Physiol. 24, 725 (1940-41).

(13) Gorin, G., and Godwin, W. E., J. Catalysis 5, 279 (1966).

(14) Tysarowski, W., and Konecka, A., Acta Biochim. Polon. 12, 252 (1965).

(15) Bichowsky, F. R., and Rossini, F. D., "Thermochemistry of the Chemical Substances", Reinhold Publishing Corporation, New York, 1936.

(16) National Bureau of Standards Circular 500, "Selected Values of Chemical Thermodynamic Properties," Washington, D. C., 1952.

(17) Stephenson, C. C., and Morrow, J. C., J. Am. Chem. Soc. 78, 275 (1956).

(18) Kolthoff, I. M., and Tomiscek, W. J., J. Phys. Chem. 39, 945 (1935).

(19) Hepler, L. G., Sweet, J. R., and Jesser, R. A., J. Am. Chem. Soc. 82, 304 (1960).

(20) Atanasyants, A. G., Zh. Fiz. Khim. 37, 2654 (1963).

(21) Latimer, W. M., "Oxidation Potentials", 2nd ed,, Prentice Hall, Inc., New York, 1952.

(22) Mel, H. C., Jolly, W. L., and Latimer, W. M., J. Am. Chem. Soc. 75, 3827 (1953).

(23) Guzzetta, F. H., and Hadley, W. B., Inorg. Chem. 3, 259 (1964).

(24) Watt, G. P., Christensen, J. J., and Izatt, P. M., Inorg. Chem. 4, 220 (1965).

(25) Busey, R. H., J. Phys. Chem. 69, 3179 (1965).

(26) Mörner, K. A. H., Z. Physiol. Chem. 24, 207 (1901).

(27) Okuda, Y., J. Biochem. (Japan) 5, 201 (1925); Chem Abstr. 20, 1094^8 (1926).

(28) Virtue, R. W., and Lewis, H. B., J. Biol. Chem. 104, 415 (1934).

(29) Lucas, C. C., and King. E. J., Biochem. J. 26, 2076 (1932).

(30) Shinohara, K., J. Biol. Chem. 96, 285 (1932).

(31) Lavine, T. F., J. Biol. Chem. 109, 141 (1935).

(32) Stern, J. H., and Passchier, A. U., J. Phys. Chem. 66, 752 (1962).

(33) Wu, Ching-hsien, Birky, M. M., and Hepler, L. G., J. Phys. Chem. 67, 1202 (1963).

(34) Jones, G., and Kaplan, B. B., J. Am. Chem. Soc. 50, 1845 (1928).

(35) Davies, M., and Gwynne, E., J. Am. Chem. Soc. 74, 2748 (1952).

(36) Reynolds, W. L., J. Am. Chem. Soc. 80, 1830 (1958).

(37) Cecil, R., and McPhee, J. R., Advan. Protein Chem. 14, 289 (1959).

(38) Dixon, M., and Quastel, H. H., J. Chem. Soc. 123, 2943 (1923).

(39) Williams, J. W., and Drissen, E. M., J. Biol. Chem. 87, 441 (1930).

(40) Ghosh, J. C., Raychandhuri, S. N., and Ganguli, S. C., J. Indian Chem. Soc. 9, 43 (1932).

(41) Green, D. E., Biochem. J. 27, 678 (1933).

(42) Fruton, J. S., and Clarke, H. T., J. Biol. Chem. 106, 667 (1934).

(43) Borsook, H., Ellis, E. L., and Huffman, H. M., J. Biol. Chem. 117, 281 (1937).

(44) Cleland, W. W., Biochem. 3, 480 (1964).

(45) Ryklan, L. R., and Schmidt, C. L. A., Univ. Calif. Publs. Physiol. 8, 257 (1944).

(46) Freedman, L. D., and Corwin, A. H., J. Biol. Chem. 181, 601 (1949).

(47) Kolthoff, I. M., Stricks, W., and Kapoor, R. C., J. Am. Chem. Soc. 77, 4733 (1955).

(48) Tanaka, N., Kolthoff, I. M., and Stricks, W., J. Am. Chem. Soc. 77, 2004 (1955).

(49) Huffman, H. M., and Ellis, E. L., J. Am. Chem. Soc. 57, 46 (1935).

(50) Huffman, H. M., and Ellis, E. L., J. Am. Chem. Soc. 57, 41 (1935).

(51) Sunner, S., Svensk. Kem. Tidskr. 58, 71 (1946).

(52) Alexander, N. M., Anal. Chem. 30, 1292 (1958).

(53) Skinner, H. H., Sturtevant, J. M., and Sunner, S. in "Experimental Thermochemistry", H. A. Skinner, Ed., Interscience Publishers, Inc., New York, 1962, Chapt. 9.

(54) Rossini, F. D., in "Experimental Thermochemistry", F. D. Rossini, Ed., Interscience Publishers, Inc., New York, 1956, p. 16.

(55) Sturtevant, J. M. in "Physical Methods in Organic Chemistry", Weissberger, Ed., Vol. 1, Part 1, Interscience Publishers, Inc., New York, 1949, p. 744.

(56) Sano, K., Biochem. Z. 168, 14 (1926).

(57) Gunn, S. R., J. Phys. Chem. 69, 2902 (1965).

(58) Irving, R. J., and Wadsö, I., Acta Chem. Scand. 18, 195 (1964).

(59) Wadsö, I., Reported at the 20th Annual Calorimeter Conference, Ames, Iowa 1965.

(60) Freeman, R. D., and Walker, A. D., Private communication.

(61) Lange, E., and Miederer, W., Z. Elektrochem. 60, 34 (1956).

(62) Mason, L. S., Offutt, W. F., and Robinson, A. L., J. Am. Chem. Soc. 71, 1463 (1949).

(63) Greenstein, J. P., and Winitz, M., "Chemistry of the Amino Acids", Vol. 1, John Wiley and Sons, New York, 1961, p. 534.

(64) Rock, P. A., J. Phys. Chem. 70, 576 (1966).

(65) Chadwick, B. M., and Sharpe, O. G., in "Advan. Inorg. Chem. Radiochem.", H. J. Emeléus, and A. G. Sharpe, Eds., Vol. 8, Academic Press, New York, 1966, p. 118.

(66) Greenstein, J. P., and Winitz, M., "Chemistry of the Amino Acids", Vol. 1, John Wiley and Sons, New York, 1961, p. 492.

(67) Boyer, P. D., in "The Enzymes", P. D. Boyer, H. Lardy, and K. Myerbäck, Eds., 2nd ed., Vol. 1, Academic Press, New York, 1959, p. 523.

(68) Hellerman, L., Physiol. Rev. 17, 454 (1937).

(69) Eberson, L., and Wadsö, I., Acta Chem. Scand. 17, 1552 (1963).

(70) Kolthoff, I. M., and Tomsicek, W. J., J. Phys. Chem. 39, 955 (1935).

APPENDIX

Since the preceding chapters were written in a form suitable for publication, some details were omitted for the sake of brevity. These details are presented in this chapter, which is divided into several short sections.

Heat of Solution of TRIS

In Chapter III (Table I) three representative values were given for the heat of solution of TRIS in 0.1 M HCl. The complete set of data is given in Table V where R_i and R_f and the corresponding primed values have the same meaning as in Chapter III.

TABLE V

HEAT OF SOLUTION OF TRIS IN 0.1 M HYDROCHLORIC ACID AT 25°

g of TRIS	$10^3 \times \log R_i/R_f$	$10^3 \times \log R_i'/R_f'$	$-\Delta H$ (cal)
2.38523	4.3476	3.9966	7142
2.52498	4.5965	4.2956	7130
2.42190	4.4134	4.3119	7139
2.62114	4.7791	4.5948	7147
2.29578	4.1928	4.1196	7118
1.25792	2.3048	2.3069	7152
2.56100	4.6481	4.4328	7117
			7135 ± 6

Determination of SH Content of Cysteine

The **L** - cysteine hemihydrate sample was reacted with N-ethylmaleimide (NEM) and with potassium ferricyanide (assay 100.0%). The decreases in absorption of NEM at 300 mμ and in potassium ferricyanide at 418 mμ were used as assay methods for the SH group. The SH content of **L** - cysteine (free base) was determined only by the NEM procedure. The % purity obtained from the SH content determination for the two samples is as follows:

L - cysteine hemihydrate		**L** - cysteine (free base)
NEM	$K_3Fe(CN)_6$	NEM
96.8	99.4	101.4
96.9	100.0	102.0
	98.0	

The Iodine-Triiodide Equilibrium

In the reaction of iodine with cysteine and with potassium ferrocyanide the ampule solution contained about 2.4 X 10^{-3} moles of $I_2(s)$ dissolved in 5 ml of 1 M I^-. The equilibrium constant of the reaction:

$$I_2 + I^- \rightleftarrows I_3^- \qquad (I)$$

is 770 (35). Assuming the activity coefficients to be unity, it may be calculated that more than 99.9% of the I_2 would react to give I_3^- in these conditions. By the same reasoning it was shown that diluting the ampule solution with buffer to a final (iodine and triiodide) concentration of 0.0048 M would cause dissociation, i.e. the reverse of process I. Therefore, to measure ΔH for the dilution process the solution in the ampule was diluted into buffer containing 1 M I^-.

Ionization Enthalpies of the Carboxylic Groups of Cysteine and Cystine

An attempt was made to determine the heats of ionization of cysteine and cystine by the method of Eberson and Wadsö (69), reactions I and II:

$$cySH_2^+ + OH^- \longrightarrow cySH + H_2O \qquad (I)$$

$$H_2cySScy^{+2} + 2OH^- \longrightarrow cySScy + 2H_2O \qquad (II)$$

However, the heat liberated by this method comprises the heat of formation of water which is large; the heat effect of interest, which is quite small, was thus completely swamped. The alternative method described in Chapter IV, i.e. calculation of the heats of ionization from the difference in the heats of solution at two different pH's, is accordingly much to be preferred.

Reaction of $Fe(CN)_6^{-4}$ and I_3^- at pH 0

The reaction between ferrocyanide and triiodide, which occurs readily at pH 5 and pH 7, did not go to completion at pH 0. Dependence of the equilibrium constant on the pH should be expected, since $H_2Fe(CN)_6^{=}$ is a relatively weak acid, e.g., the equilibrium constant for the reaction:

$$HFe(CN)_6^{-3} \rightleftarrows Fe(CN)_6^{-4} + H^+$$

is 5.6 X 10^{-5} at 25° (70).

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