TABLE VII

CALCULATION OF THE CONCENTRATIONS OF $FeOH(RS)_2^{2-}$ AND $Fe(RS)_3^{3-}$ AND OF $K_{FeOH(RS)_2^{2-}}$ AND $K_{Fe(RS)_3^{3-}}$ IN AMMONIACAL CYSTEINATE SOLUTIONS OF TABLE V

All figures are listed in the sequence corresponding to the exp. No. in Table V.

Concn. of $FeOH(RS)_2^{2-}$ $\times 10^3$ *M*	Concn. of $Fe(RS)_3^{3-}$ $\times 10^3$, *M*	$K_{FeOH(RS)_2^{2-}}$ $\times 10^{33}$	$K_{Fe(RS)_3^{3-}}$ $\times 10^{32}$
7.18	9.28	1.0	1.6
4.96	7.26	1.7	2.5
3.59	4.91	2.2	3.3
5.17	6.93	1.3	2.0
4.94	6.66	1.4	2.1
4.78	5.99	1.5	2.3
1.46	1.29	2.5	3.7
1.42	0.88	1.5	2.3
1.22	0.80	2.1	3.2
0.40	0.16	2.1	3.1
0.40	0.15	2.1	3.4
1.43	1.27	2.4	3.6
1.09	0.78	2.2	3.3
0.87	0.56	2.2	3.4
0.65	0.37	2.2	3.3
0.215	0.071	2.6	3.9
	Mean	1.9	2.9

photometrically in ammoniacal Versenate solutions.

In the calculation of the latter values the dissociation constants of ferric Versenate complexes measured at 20° and 0.1 ionic strength were used instead of those at 25° and ionic strength 0.14–0.16 in the present experiments. This may be partly responsible for the fact that one set of values is about ten times greater than the other set. The main source of the difference in the two sets of values probably is the uncertainty in the solubility product of ferric hydroxide which is involved in the calculation in Table VII. The value of the solubility product depends upon the method of preparation and the age of the precipitate—using a value of 1×10^{-38} for $S_{Fe(OH)_3(s)}$ reported by Jellinek and Gordon[19] yields 3×10^{-34} for the value of $K_{FeOH(RS)_2^{2-}}$ and 5×10^{-33} for that of $K_{Fe(RS)_3^{3-}}$. These are in better agreement with the constants calculated from the experiments with Versene than the constants calculated with a solubility product of 6×10^{-38}. As probable values we propose

$$K_{FeOH(RS)_2^{2-}} = 5 \times 10^{-34}$$
$$K_{Fe(RS)_3^{3-}} = 8 \times 10^{-33}$$

Schubert[5] postulated a dimer formula $\{FeOH(RS)_2\}_2^{4-}$ for the ferric–cysteinate complex. The diffusion coefficient of the ferric–cysteinate complex in solutions of ionic strength 0.12–0.31 containing no gelatin was extrapolated to be 5.7×10^{-6} cm.^{2} sec.$^{-1}$, which is of the same order as that of cystine,[20] and also of that of iron(III)–Versenate complex, $FeY(OH)^{=}$. (5.4×10^{-6} cm.^{2} sec.$^{-1}$).[21] This strongly suggests that the complex is a monomer.

In studies of the ferric–thioglycolate complex Leussing and Kolthoff[7] concluded that the predominant species was $FeOH(TS)_2$ similar to one of the ferric–cysteinate complexes postulated in the present study. Recalculating the dissociation constant of the thioglycolate complex[7] yields a value of 9.4×10^{-33}. This value was obtained in ammoniacal buffers and is based on a solubility product of ferric hydroxide of 6×10^{-38}. Comparison with 1.9×10^{-33} of the corresponding ferric–cysteinate complex, $FeOH(RS)_2^{2-}$, indicates that the stability of both complexes is of the same order of magnitude.

Acknowledgment.—This investigation was supported by a research grant (C-721,C_5) from the National Cancer Institute, U. S. Public Health Service.

[19] K. Jellinek and H. Gordon, *Z. physik. Chem.*, **112**, 236 (1924).

[20] I. M. Kolthoff and C. Barnum, THIS JOURNAL, **63**, 520 (1941).

[21] I. M. Kolthoff and C. Auerbach, *ibid.*, **74**, 1452 (1952).

MINNEAPOLIS, MINNESOTA

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE UNIVERSITY OF MINNESOTA]

Oxidation of Ferrous–Cysteinate Complex by Cystine. Oxidation Potential of the Cystine–Cysteine System

BY NOBUYUKI TANAKA,[1] I. M. KOLTHOFF AND W. STRICKS

RECEIVED NOVEMBER 4, 1954

Upon addition of ferrous iron to an air-free ammoniacal solution containing cystine and cysteine some ferrous iron is oxidized by cystine to a violet ferric cysteinate complex. From spectrophotometric determinations of the ferric complex the equilibrium constants for the reactions $2Fe(II)(RS)_2^{2-} + {}^{-}RSSR^{-} \rightleftarrows 2Fe(III)(RS)_3^{3-}$ and $2Fe^{++} + {}^{\pm}RSSR^{\pm} + 2H^{+} \rightleftarrows 2Fe^{+++} + 2RSH^{\pm}$ were calculated to be equal to 2.5×10^{-3} and 5.3×10^{-24}, respectively, at 25°. From the latter value the oxidation potential of the cystine–cysteine system was calculated to be +0.08 volt *vs.* N.H.E. at 25°.

In a study of ferrous– and ferric–cysteine complexes[2] we observed the development of a violet color upon addition of an air-free ferrous iron solution to an air-free ammoniacal mixture of cystine having a large concentration in cysteine. On standing the color intensity increased to a constant value. Other conditions being the same the color became more intense with increasing cystine concentration. Spectrophotometrically the color was found to be similar to that of ferric cysteinate which is formed on the addition of ferric iron to a cysteine solution.[2]

These observations indicate that under proper experimental conditions cystine can oxidize ferrous cysteinate to the ferric complex. Our observations are at variance with those of Michaelis and Barron[3] who found no indication for the oxidation

---

[1] On leave of absence from Tokyo University, Japan.

[2] N. Tanaka, I. M. Kolthoff and W. Stricks, THIS JOURNAL, **77**, 1996 (1955).

[3] L. Michaelis and E. S. G. Barron, *J. Biol. Chem.*, **83**, 191 (1929).

of ferrous cysteinate by cystine while cobalto cysteinate could be oxidized under their experimental conditions.

In the present study the equilibrium constants of the reactions

$$2Fe(II)(RS)_2^{2-} + {}^-RSSR^- \rightleftharpoons 2Fe(III)(RS)_3^{3-} \quad (1)$$

and

$$2Fe^{++} + {}^{\pm}RSSR^{\pm} + 2H^+ \rightleftharpoons 2Fe^{+++} + 2RSH^{\pm} \quad (2)$$

were calculated from concentrations of the ferric complex measured spectrophotometrically in air-free ammoniacal solutions containing varying concentrations of the ferrous complex, cystine and uncombined cyteine. From the equilibrium constant of reaction (2) the oxidation potential of the cystine–cysteine system

$${}^{\pm}RSSR^{\pm} + 2H^+ + 2e^- \rightleftharpoons 2RSH^{\pm} \quad (3)$$

was calculated. This method provides a chemical measurement of the equilibrium constant of this reaction.

Experimental

The optical density and the pH of the air-free reaction mixtures were determined in a similar way as described previously.[2] The optical density (extinction) was measured with a Beckman spectrophotometer model DU at a slit width of 0.035 mm. at 580 mμ and at 25°. The length of the light path (approx. 1 cm.) was calibrated by means of a standard cell of known length. The values of the optical density are referred to a light path of 1.000 cm.

Stock solutions of sodium hydroxide, ammonia and ammonium nitrate were 0.8 M in NaOH, 4 M in NH_3 and 1 M in NH_4NO_3, respectively. The stock solution of ferrous chloride which was prepared as described previously[2] was 0.5 M in ferrous iron.

All measurements were carried out in ammonia buffers of pH of about 10. Appropriate volumes of stock solutions of ammonium hydroxide, ammonium nitrate, sodium hydroxide,[4] distilled water and a weighed amount of cystine (Merck reagent product) were placed into an absorption cell and the solution made air-free with nitrogen. To this solution was added a weighed amount of cysteine hydrochloride (Pfanstiehl C.P. product), whereupon the cell was sealed and the passage of nitrogen continued for about 40 minutes in order to remove traces of oxygen. Now a given volume of an air-free stock solution of ferrous chloride was injected into the mixture with the aid of a syringe. After the iron addition the mixture is colorless and turns violet on standing. The cells were kept in a thermostat at 25° and the absorption of the solution was measured at various periods of time until a constant value for the optical density was obtained. In all experiments equilibrium was attained within 20 hours after the injection of the ferrous solution. Experiments with solutions of various initial concentrations of RSH, RSSR and Fe(II) have been carried out. The details of these experiments are given in Table I.

TABLE I

OPTICAL DENSITY OF AMMONIACAL MIXTURES OF CYSTINE, CYSTEINE AND FERROUS IRON

Component added,[a] M					
$[RSH]_t$	$[RSSR]_t$	$[Fe]_t$	pH	Optical density	Concn. of Fe(III) complex × 10^4, M
0.0998	0.00474	0.0201	10.12	0.299	0.98
.0982	.01013	.0201	10.17	.428	1.40
.1030	.0251	.0201	10.08	.656	2.15
.1201	.01010	.0302	10.07	.637	2.09
.1495	.01025	.0201	10.03	.416	1.36

[a] All mixtures were approximately 1 M in ammonia and 0.1 M in ammonium nitrate.

Equilibrium Constant of Reaction (1).—Under our experimental conditions ferric cysteinate was present only as $Fe(RS)_3^{3-}$.[2] The predominant ferrous complex was $Fe(RS)_2^{2-}$ while $FeOHRS^-$ amounted to only 5–10% of the total concentration of ferrous iron. The equilibrium concentration of ferric cysteinate was calculated from the optical density and the molar extinction coefficient of ferric cysteinate ($3.05 \times 10^3\ M^{-1}$ cm.$^{-1}$ at 580 mμ).[2] The concentration of the ferrous cysteinate $Fe(RS)_2^{2-}$ was calculated by making use of the following equations

$$[FeOHRS^-] + [Fe(RS)_2{}^{2-}] + [Fe(RS)_3{}^{3-}] = [Fe]_t \quad (4)$$

$$[FeOHRS^-] + 2[Fe(RS)_2{}^{2-}] + 3[Fe(RS)_3{}^{3-}] + [RSH]_{\text{uncomb.}} = [RSH]_t \quad (5)$$

Introducing the dissociation constants of $FeOHRS^-$ and $Fe(RS)_2^{2-}$ and the acid constants of cysteine (K_C, K_D),[2] eq. 4 and 5 were rearranged

$$[NH_2RS^-COO^-]^2 + [NH_2RS^-COO^-]\left\{\frac{\xi}{\eta} + \frac{2a-b}{k}\right\} + \frac{\xi}{\eta}\,\frac{a-b}{k} = 0 \quad (4')$$

$$[Fe^{++}] = \frac{a}{\xi[NH_2RS^-COO^-] + \eta[NH_2RS^-COO^-]^2} \quad (5')$$

where

$$\xi = \frac{K_w}{K_{FeOHRS^-} \times a_{H^+}} \times \frac{\gamma_{Fe^{++}} \times \gamma_{NH_2RS^-CO^-}}{\gamma_{FeOHRS^-}}$$

$$\eta = \frac{1}{K_{Fe(RS)_2{}^{2-}}} \times \frac{\gamma_{Fe^{++}} \times \gamma^2_{NH_2RS^-COO^-}}{\gamma_{Fe(RS)_2{}^{2-}}}$$

$$a = [Fe]_t - [Fe(RS)_3{}^{3-}]$$

$$b = [RSH]_t - 3[Fe(RS)_3{}^{3-}]$$

$$k = 1 + \frac{a_{H^+}}{K_C} \times \frac{\gamma_{NH_2RS^-COO^-}}{\gamma_{NH_3{}^+RS^-COO^-}} + \frac{a_{H^+}}{K_D} \times \frac{\gamma_{NH_2RS^-COO^-}}{\gamma_{NH_2RSHCOO^-}}$$

The concentration of $Fe(RS)_2^{2-}$ can now be calculated from

$$[Fe(RS)_2{}^{2-}] = \eta[Fe^{++}][NH_2RS^-COO^-]^2.$$

The values of $K_{FeOHRS^-} = 1.7 \times 10^{-13}$, $K_{Fe(RS)_2{}^{2-}} = 1.7 \times 10^{-12}$, $K_C = 3.6 \times 10^{-11}$ and $K_D = 3.1 \times 10^{-11}$ were given in a previous paper.[2] The values of the ionization constants of cystine $pK_3(NH_3{}^+) = 8.00$ and $pK_4(NH_3{}^+) = 10.25$ were taken from Borsook, *et al.*[5] The activity coefficients were estimated in the same way as described previously.[2]

The values of the equilibrium constant of reaction (1)

$$K = \frac{a^2_{Fe(III)(RS)_3{}^{3-}}}{a^2_{Fe(II)(RS)_2{}^{2-}} \times a_{^-RSSR^-}} \quad (6)$$

and the concentrations of the ferrous and ferric

TABLE II

EQUILIBRIUM CONSTANT K IN EQUATION 6

$Fe(II)(RS)_2{}^{2-}$ × 10^2, M	Concn. of $Fe(III)(RS)_3{}^{3-}$ × 10^4, M	${}^-RSSR^-$ × 10^2, M	μ	γ_1[a]	γ_2[a]	γ_3[a]	K × 10^3
1.81	0.98	0.308	0.44	0.57	0.225	0.057	2.7
1.79	1.40	0.685	.44	.57	.225	.057	2.5
1.81	2.15	1.59	.50	.56	.22	.053	2.3
2.71	2.09	0.632	.53	.55	.215	.052	2.6
1.89	1.36	0.625	.57	.545	.21	.050	2.3
						Mean	2.5

[a] γ_1, γ_2, γ_3 refer to activity coefficients of mono-, di- and trivalent ions, respectively.

---

(4) Approximate amount of sodium hydroxide required to neutralize the hydrochloric acid in cysteine hydrochloride and to form the ferrous cysteinate complex.

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

complexes in various equilibrium mixtures are listed in Table II.

Oxidation–Reduction Potential of the Cystine–Cysteine System.—In order to find the oxidation-reduction potential of the cystine–cysteine system, the equilibrium constant of reaction (2)

$$K = \frac{a^2_{\text{Fe}^{+++}} \times a^2_{\text{RSH}^\pm}}{a^2_{\text{Fe}^{++}} \times a^\pm_{\text{RSSR}^\pm} \times a^2_{\text{H}^+}} \qquad (7)$$

was first calculated. The concentration of ferrous ion obtained from equation 5′ was multiplied by the appropriate activity coefficient to yield $a_{\text{Fe}^{++}}$. The activity of ferric ion was calculated from the dissociation constant of ferric cysteinate[2] Fe(RS)_3^{3-} ($K = 8 \times 10^{-33}$) and from the activity of $\text{NH}_2\text{-RS}^-\text{COO}^-$ ion. The concentration of this ion was obtained from equation 4′. The results are summarized in Table III.

TABLE III

CALCULATION OF EQUILIBRIUM CONSTANT K IN EQUATION 7

pH	$a_{\text{Fe}^{++}}$ $\times 10^{10}$	$a_{\text{Fe}^{+++}}$ $\times 10^{31}$	$a_{\text{RSH}^\pm}$ $\times 10^4$	$a^\pm_{\text{RSSR}^\pm}$ $\times 10^5$	K $\times 10^{24}$
10.12	2.79	0.368	3.66	0.707	5.9
10.17	2.64	0.476	3.02	1.24	5.1
10.08	3.04	0.890	4.21	4.34	5.1
10.07	5.20	1.07	4.07	1.76	5.5
10.03	1.30	0.146	8.07	2.03	4.8
				Mean	5.3

The values of ΔF^0 and E^0 for the reaction of eq. 2 were calculated to be

$$\Delta F^0 = 32 \text{ kcal.}$$

$$E^0 = -0.69 \text{ volt } (vs. \text{ N.H.E.}).$$

This value of E^0 with the oxidation potential of the couple

$$\text{Fe}^{++} \rightleftharpoons \text{Fe}^{+++} + e^-, \ (-0.771 \text{ volt } vs. \text{ N.H.E.})^6$$

yields a standard oxidation potential of the reaction

$$\pm\text{RSSR}\pm + 2\text{H}^+ + 2e^- \rightleftharpoons 2\text{RSH}\pm \qquad (3)$$

of +0.08 volt *vs.* N.H.E.

Borsook, *et al.*,[5] determined the heat of combustion of cystine and cysteine, and from their results calculated an oxidation potential of +0.025 volt *vs.* N.H.E., while a value of +0.096 volt was calculated from thermal data of Becker and Roth.[7] These values compare favorably with our value of 0.08 volt and thus substantiate the assumptions made in the calculation of the dissociation constants of ferrous and ferric cysteinates.[2] Table IV lists values reported in the literature of the oxidation potential of the cystine–cysteine system at a pH 7.

TABLE IV

THE OXIDATION POTENTIAL AT 25° AT pH 7.0 FOR: *l*-CYSTINE + $2\text{H}^+ + 2e^- \rightleftharpoons$ 2*l*-CYSTEINE

Method	E_0' at pH 7.0 at 25°, volt *vs.* N.H.E.	Investigators
Potentiometric, cathodically pretreated mercury electrode in cystine–cysteine system	−0.33	Ghosh, Raychandhuri and Ganguli[8,9]
	− .33	Green[10]
Potentiometric, platinum electrode in cystine–cysteine–iodide system	− .15	Ryklan and Schmidt[13]
Thermal data	− .35	Borsook, Ellis and Huffman[5]
Thermal data	− .32	Calcd. from combustion data of Becker and Roth[7]
Equilibrium constant for: Fe(II)complex + cystine $\rightleftharpoons$ Fe(III) complex	− .33	This paper

Potentials measured at the mercury electrode under conditions described by Ghosh, *et al.*[8,9] and by Green[10] are in good agreement with the values obtained from combustion data[5,7] and with our data. This indicates that under proper conditions the mercury electrode can give potentials corrresponding to the equilibrium in equation 3.

Potential measurements at platinum electrodes (potentiometric titrations of cysteine with oxidizing agents)[11–14] apparently do not give correct oxidation potentials of the system. It was stated by Barron[15] that platinum is not a reliable indicator electrode in the cystine–cysteine system. Fruton and Clarke[16] measured the oxidation potential colorimetrically, using dyes of known oxidation–reduction potentials. The large deviations (170 millivolts) of their values from those obtained from thermal data and our value are unexplained. In the method used in this paper the actual oxidation–reduction equilibrium of the system was measured.

Acknowledgment.—This investigation was supported by a research grant (C-721, C_5) from the National Cancer Institute, U. S. Public Health Service.

MINNEAPOLIS, MINNESOTA

---

[6] W. M. Latimer, "Oxidation Potentials," 2nd ed., Prentice-Hall, Inc., New York, N. Y., 1952, p. 223.

[7] G. Becker and W. A. Roth, *Z. physik. Chem.*, **169**, 287 (1934).

[8] J. C. Ghosh, S. N. Raychandhuri and S. C. Ganguli, *J. Indian Chem. Soc.*, **9**, 43 (1932).

[9] J. C. Ghosh and S. C. Ganguli, *Biochem. J.*, **28**, 381 (1934).

[10] D. E. Green, *ibid.*, **27**, 678 (1933).

[11] J. W. Williams and E. M. Drissen, *J. Biol. Chem.*, **87**, 441 (1930).

[12] E. K. Fischer, *ibid.*, **89**, 753 (1930).

[13] L. K. Ryklan and C. L. A. Schmidt, *Univ. Calif. Publ. Physiology*, **8**, 257 (1944).

[14] L. D. Freedman and A. H. Corwin, *J. Biol. Chem.*, **181**, 601 (1949).

[15] E. S. G. Barron, *Advances in Enzymology*, **11**, 201 (1951).

[16] G. S. Fruton and H. T. Clarke, *J. Biol. Chem.*, **106**, 667 (1934).