CLXXXI. DISSOCIATION CONSTANTS OF CYSTINE, CYSTEINE, THIOGLYCOLLIC ACID AND α-THIOLACTIC ACID.

By ROBERT KEITH CANNAN
and BERT CYRIL JAMES GABRIEL KNIGHT.

From the Department of Physiology and Biochemistry, University College, London.

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Mathews and Walker [1909] and, in greater detail, Dixon and Tunnicliffe [1923] have demonstrated the great significance of the hydrogen ion activity in relation to the rates of oxidation and of autoxidation of a number of thio-acids. To assist the analysis of these results and of certain observations of our own it became desirable to know the dissociation constants of cysteine, thioglycollic acid and thiolactic acid. It was thought worth while at the same time to determine the corresponding constants of cystine in order that a gap in the electrochemical data on the natural amino-acids might be filled.

With respect to cysteine, thioglycollic acid and thiolactic acid no difficulty was encountered in obtaining reproducible hydrogen electrode titration curves from which the several constants could be deduced. The method was, however, not available in the case of cystine as this substance suffers reduction at the hydrogen electrode surface with consequent depolarisation of the electrode. The quinhydrone electrode suggested itself as an alternative since its potential is positive to that which, on chemical and other grounds, may reasonably be attributed to solutions of cystine. This electrode, indeed, proved satisfactory within its limitations but was rejected because it does not permit the extension of a titration to the low concentrations of hydrogen ions at which stages in the dissociation of cystine occur.

An oxidation-reduction electrode was sought, therefore, which would be available over the whole significant p_H range and whose equilibrium would not be disturbed by the presence of cystine. The methylene blue-methylene white electrode fulfilled these requirements.

Experimental.

The titration vessel used for the hydrogen electrode titrations was similar to that employed by Clark and Cohen [1923] for oxidation-reduction titrations. This carried a pair of palladium-coated, gold-plated platinum electrodes and a supply tube for hydrogen gas. A saturated calomel electrode and a

saturated potassium chloride bridge completed the cell, which was immersed in a kerosene thermostat at 30°. The E.M.F. was determined potentiometrically against a standard Weston cell. At no significant points on the titration curves did the two electrodes differ by more than 0·1–0·3 mv. In the type of cell employed the hydrogen stream is delivered through the solution but not directly over the electrode surfaces. Consequently the initial attainment of equilibrium at the electrode occupies some time after the introduction of the solution to be titrated: 20–30 minutes was found adequate. Subsequent adjustments to additions of titrating agent are rapid, and true equilibrium values can be determined in 2–3 minutes after each addition.

For our own purposes we required to know the p_H of approximately 0·05 molar solutions of the sulphydryl compounds neutralised to varying degrees with 0·5 molar CO_2-free sodium hydroxide. For this reason the electrometric titrations were performed with solutions of similar strength. It was appreciated that the constants derived from observations on solutions of such ionic strength are subject to a significant correction for activity. No such correction has been applied because, in the case of the two ampholytes, its calculation is uncertain, and because most of the data in the literature on the constants of the natural amino-acids are uncorrected values (K') referable to solutions of about 0·1 molar strength. Some indication of the magnitude of the activity correction was obtained by repeating the titrations on 0·01–0·02 molar solutions. A further advantage in working at fairly high concentration is the more extensive data which can be obtained on the constants functioning at the extremes of the p_H scale.

Cystine was prepared by the acid hydrolysis of hair and recrystallised several times. From this, cysteine hydrochloride was obtained by reduction with zinc and hydrochloric acid, precautions being taken against re-oxidation during crystallisation. Thioglycollic acid (B.P. 109–110°/16 mm.) was prepared from monochloroacetic acid and sodium disulphide followed by reduction of the dithiodiglycollic acid with zinc and hydrochloric acid. α-Thiolactic acid (B.P. 107–110°/17 mm.) was prepared by the method of Lovén [1884] from α-chloropropionic acid. These acids were redistilled at low pressure in nitrogen.

The titration curves are assembled in Fig. 1, in which the "corrected equivalents of base" [Simms, 1926] are plotted against the observed p_H values.

Table I, which is later explained, records some of the data from which the curve for cysteine has been constructed and serves to exhibit the order of consistency attained throughout the work.

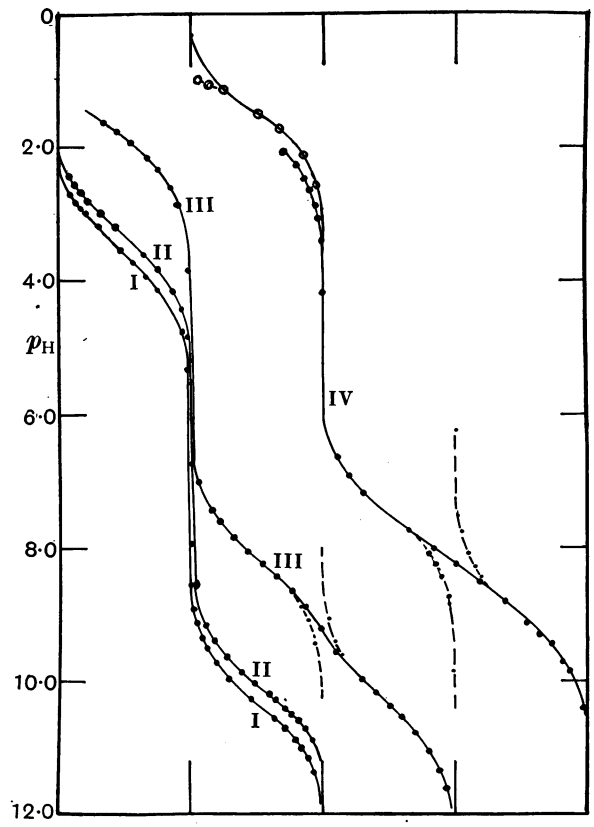
It is apparent, in respect of thioglycollic acid and thiolactic acid, that the two constants differ so greatly in magnitude that the curve may be treated as that of a mixture of two monobasic acids, whose constants are derived from the relation

$$p_{K'} = p_H - \log \frac{\alpha}{1-\alpha}.$$

Table I. *Titration of* 0·1 *M cysteine hydrochloride with M sodium hydroxide.* 30°.

$p_{K_1'}=1{\cdot}86$ $p_{K_2'}=8{\cdot}14$ $p_{K_3'}=10{\cdot}34$

				Corrected equivalents of base	
p_H	a_1	a_2	a_3	Calculated	Found
1·626	0·368	—	—	0·368	0·343
1·675	0·395	—	—	0·395	0·379
1·841	0·489	—	—	0·489	0·485
2·030	0·597	—	—	0·597	0·596
2·151	0·661	—	—	0·661	0·675
2·340	0·769	—	—	0·769	0·755
2·860	0·909	—	—	0·909	0·907
7·000		0·0676	0·0000	1·068	1·081
7·607	One equivalent	0·2266	0·0004	1·227	1·234
8·046	complete	0·4450	0·0023	1·450	1·447
8·422		0·6516	0·0079	1·667	1·660
8·887		0·8236	0·0290	1·882	1·872
9·200		0·8624	0·0625	1·988	1·979
9·545		0·8334	0·1336	2·101	2·106
9·965		0·6962	0·2935	2·283	2·298
10·160		0·5987	0·3955	2·390	2·404
10·520		0·3971	0·6011	2·599	2·610
10·756		0·2771	0·7219	2·721	2·712
11·035		0·1679	0·8320	2·832	2·798
11·595		0·0526	0·9475	2·948	2·934



Corrected equivalents of base.

Fig. 1.

Curve I=α-Thiolactic acid. Curve II=Thioglycollic acid. Curve III=Cysteine.
Curve IV=Cystine {●—●—● *M*/50 M. blue electrode. ○—○—○ *M*/10 Quinhydrone electrode.

The case of cysteine is more complex. To avoid the necessity for discriminating between acidic and basic constants in the analysis it is of assistance to regard the kation of the hydrochloride as an acid dissociating three hydrogen ions. The constants K_1', K_2', K_3' refer to the successive steps without any present implication as to the specific group which determines the value of each constant.

$$R \begin{cases} NH_3^+ \\ COOH \\ SH \end{cases} \rightarrow R \begin{cases} NH_3^+ \\ COO^- \\ SH \end{cases} \rightarrow R \begin{cases} NH_3^+ \\ COO^- \\ S^- \end{cases} \rightarrow R \begin{cases} NH_2 \\ COO^- \\ S^- \end{cases}$$

It is clear from the curve that the value of K_1' is so much greater than those of K_2' and K_3' that it may be derived separately. The influences of K_2' and K_3', however, significantly overlap. This region of the curve was therefore treated as that of a dibasic acid. Graphic approximations to the values of $p_{K_2'}$ and $p_{K_3'}$ were made and the values of α_2 (primary ion) and α_3 (secondary ion) were calculated from the equations

$$\alpha_2 = \frac{1}{1 + \frac{[H^+]}{K_3'} + \frac{K_3'}{[H^+]}}$$

$$\alpha_3 = \frac{1}{1 + \frac{[H^+]}{K_3'} + \frac{[H^+]^2}{K_2'K_3'}},$$

which are derived from the application of the mass law [Michaelis, 1921]. Thence were calculated the equivalents of base $(1 + \alpha_2 + 2\alpha_3)$ required to bring to the observed p_H values a mixture of three acids having the given constants. These values are compared with the corrected equivalents of base derived from the titration readings. Minor modifications of the graphic values of $p_{K'}$ were then made to improve the alignment of the above data[1].

Methylene blue-methylene white electrode.

Clark, Cohen and Gibbs [1925] have found that the electrode potential of a given ratio of the oxidised and reduced forms of this system varied not only with different samples of the dye but also with the total concentration present. Fortunately we were able to overcome these difficulties through the gift of a specimen of Sample *F* of the methylene blue for which Clark, Cohen and Gibbs have recorded electrode data. The dye was employed at a similar concentration to that with which they worked (0·0001 *M* total dye). Into a burette, protected from oxygen, was introduced a solution, approximately equimolar, of methylene blue and methylene white. The potential of this mixture at a gold-plated platinum electrode at p_H 3·97 was determined by adding 5 cc. to 35 cc. of 0·057 *M* potassium acid phthalate.

[1] As Simms [1926] has pointed out, these constants are titration constants (G') and are related to the true dissociation constants (K') by the equations

$$K_1' = G_1' + G_2',$$

$$\frac{1}{K_2'} = \frac{1}{G_1'} + \frac{1}{G_2'}.$$

The correction is negligible in the present case.

The potential of the methylene blue-methylene white electrode is related to $[H^+]$ by the following equation [Clark, Cohen and Gibbs, 1925] (which holds acid to p_H 12):

$$E_h = C + 0{\cdot}03006 \log [K_1K_2 [H^+] + K_2 [H^+]^2 + [H^+]^3]$$

where E_h is the potential referred to the normal hydrogen electrode,

$$K_1 = 1{\cdot}4 \times 10^{-6} \text{ and } K_2 = 3{\cdot}0 \times 10^{-5},$$

being dissociation constants of methylene white.

C is a constant given by

$$C = 0{\cdot}533 - 0{\cdot}03006 \log \frac{[\text{methylene white}]}{[\text{methylene blue}]}.$$

For any experimental ratio of the oxidised and reduced forms C can be calculated by determining the potential (E_h) at a known p_H. With this determined the equation may be used to relate observed potentials of the given mixture to the p_H of the solution to which it has been added. The equation is cumbersome, however, and it is convenient that K_1 and K_2 are of such an order that within the range p_H 1–2·5 the relation reduces to $E_h = C - 0{\cdot}09018\ p_H$; and between p_H 7·8–12·00 to

$$E_h = C - 0{\cdot}313 - 0{\cdot}03006\ p_H.$$

In view of the low solubility of cystine the titration was carried out on a 0·02 molar solution containing sufficient standard hydrochloric acid to effect solution. To 35 cc. of this solution were added 5 cc. of the dye mixture and the titration was conducted in purified nitrogen with oxygen-free sodium hydroxide. The excellent agreement between the potentials of two electrodes, their reproducibility, the absence of potential drifts, and the satisfactory analysis to which the data submitted gave assurance that the electrode gave a true measure of the p_H. No precipitation of the cystine was observed at any stage of the titration.

In the case of cystine there are four constants to be determined. By analogy with cysteine and other amino-acids it will be expected that one pair will be active in the acid range and one on the alkaline side of neutrality. The curve shows this to be so and permits each pair of constants to be derived by treating their section of the curve as that of a dibasic acid, as was done in the case of cysteine. As a result it appears that K_1' is too great to be measured. K_2', K_3' and K_4' have been deduced. K_2' was determined also by titration of a 0·1 molar solution of cystine hydrochloride at the quinhydrone electrode. Precipitation of cystine did not occur, even at this concentration, until the titration of the first two equivalents was almost complete.

The third and fourth titration constants lie so close together that they differ slightly from the true constants. Applying the correction of Simms [1926] (footnote, p. 1387) we get for the uncorrected apparent dissociation constants the values in Table II.

Table III is an abbreviation of the data for K_3' and K_4' constructed in the same way as Table I. It serves to show the order of consistency of the methylene blue electrode readings.

Table II. *Apparent dissociation constants (K') at 30°: not corrected for activity.*

	Approx. molar strength	$p_{K_1'}$	$p_{K_2'}$	$p_{K_3'}$	$p_{K_4'}$	K_1'	K_2'	K_3'	K_4'
Thioglycollic acid	0·1	3·4	10·0	—	—	4×10^{-4}	1×10^{-10}	—	—
	0·01	3·5	10·2	—	—	3×10^{-4}	6×10^{-11}	—	—
Thiolactic acid	0·025	3·6	10·3	—	—	$2{\cdot}5 \times 10^{-4}$	5×10^{-11}	—	—
	0·01	3·7	10·3	—	—	$2{\cdot}0 \times 10^{-4}$	5×10^{-11}	—	—
Cysteine	0·1	1·86	8·14	10·34	—	$1{\cdot}4 \times 10^{-2}$	$7{\cdot}3 \times 10^{-9}$	$4{\cdot}6 \times 10^{-11}$	—
	0·02	1·96	8·18	10·28	—	$1{\cdot}1 \times 10^{-2}$	$6{\cdot}6 \times 10^{-9}$	$5{\cdot}3 \times 10^{-11}$	—
Cystine	0·1	<1·0	1·5	—	—	>1 $\times 10^{-1}$	$3{\cdot}2 \times 10^{-2}$	—	—
	0·02	<1·0	1·7	7·48	9·02	>1 $\times 10^{-1}$	$2{\cdot}0 \times 10^{-2}$	$3{\cdot}3 \times 10^{-8}$	$9{\cdot}6 \times 10^{-10}$

Table III. *Titration of 0·02 M cystine hydrochloride with 0·1 M sodium hydroxide.* 30°.

$p_{K_1'} = < 1{\cdot}00$ $\quad p_{K_2'} = 1{\cdot}72$ $\quad p_{K_3'} = 7{\cdot}48$ $[pG_3' = 7{\cdot}50]$ $\quad p_{K_4'} = 9{\cdot}02$ $[pG_4' = 9{\cdot}00]$

p_H	a_1	a_2	a_3	a_4	Corrected equivalents of base: Calculated	Found
2·26	Assume	0·776	—	—	1·776	1·792
2·47	1 equivalent	0·849	—	—	1·849	1·853
2·63	conpleted	0·889	—	—	1·889	1·887
2·87		0·935	—	—	1·935	1·935
3·07		0·957	—	—	1·957	1·957
6·62			0·1164	0·0005	2·117	2·117
6·90	2 equivalents completed		0·2005	0·0016	2·204	2·206
7·15			0·2611	0·0035	2·268	2·296
7·44			0·4591	0·0127	2·484	2·474
7·72			0·6039	0·0317	2·667	2·652
8·02			0·7109	0·0745	2·870	2·830
8·25			0·7375	0·1342	3·006	3·010
8·52			0·7008	0·2321	3·165	3·188
8·81			0·5899	0·3810	3·352	3·366
9·12			0·4267	0·5628	3·552	3·542
9·30			0·3316	0·6600	3·652	3·630
9·45			0·2609	0·7225	3·706	3·718
9·71			0·1793	0·8360	3·851	3·804
9·85			0·1412	0·8757	3·893	3·854
10·41			0·0375	0·9627	3·963	3·946

Remarks.

The treatment which has been accorded the dissociation of cysteine and cystine has not distinguished acidic and basic constants. It is still a common custom to refer the first dissociation constant of amino-acids to the basic group and the second to the acid group, *i.e.*, an amino-acid is regarded as both a weak acid and a weak base. On this basis the K_1' here reported for cysteine, and the K_1' and K_2' for cystine would be the hydrolysis constants of the NH_2 groups related to K_b by $K_b = \frac{Kw}{K'}$.

Adams [1916] and Bjerrum [1923] on the other hand have adopted the more plausible view that the constant active at an acid reaction is the acidic constant and that functioning at low $[H^+]$ is referable to the NH_2 group. In the case of cystine, therefore, K_1' and K_2' relate to the symmetrical COOH groups and K_3' and K_4' become the hydrolysis constants of the NH_2 groups. The enhanced values of the acid constants and the diminution in strength of

the basic groups in comparison with other amino-acids is to be remarked. In the case of cysteine we may give K_1' to the carboxyl group but there is little to distinguish the influence of the sulphydryl- and amino-groups in the two stages of dissociation in the alkaline range. Comparison with the simple thio-acids which have been titrated would suggest that K_3' of cysteine is determined by the sulphydryl group. On the other hand the – SH group in cysteine is further removed from the COOH than in thioglycollic and in thiolactic acids, and should therefore be a stronger group. Moreover K_3' approximates much more closely to the values of the NH_2 constant of related amino-acids (*e.g.* alanine, serine) than does K_2'. It is probable that a plausible argument could be made for either allocation of K_2' and K_3' in cysteine. A decision seems premature and in any case can only be qualitatively true in view of the fact that in cases where two constants are of similar magnitude both groups involved are concerned in determining the value of each constant.

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