632. *Complex Formation between Ferric Ion and Glycine.*

By D. D. Perrin.

In acid solution ferric ion and glycine form a stable 1 : 1 complex. A consistent value of 10^{10} for the stability constant of the complex was obtained from measurements of oxidation–reduction potential and spectrophotometry. The ferric–glycine complex is more stable than the corresponding cupric complex.

Although ferrous ion, like most bivalent cations, forms chelate compounds with α-amino-acids [1,2,3] little evidence of complex formation between ferric ion and amino-acids has been reported. An unstable violet complex is formed by ferric ion in cysteine solutions; [4] over the range pH 10—11, the complexes $FeOH(RS)_2^{2-}$ and $Fe(RS)_3^{3-}$, where RS is the cysteine di-anion, are present.[5] It is likely that these complexes involve linkages through the sulphur atoms. For the remaining amino-acids the most positive finding seems to be that with phenol as solvent ferric ion is moved along a paper chromatogram by histidine and to a much smaller extent by a number of other amino-acids.[6] On the other hand, measurement of pH changes during acid–base titration of amino-acid solutions containing ferric ion did not suggest any interaction, except possibly for aspartic acid.[2,3] However, this procedure was somewhat uncertain because under the necessary experimental conditions hydrolysis of ferric ion was considerable and led, at an early stage in the titration, to the separation of a basic sulphate. Hydrated ferric ion is appreciably hydrolysed even in solutions of pH 2, hydrolysis increasing very sharply as the solution is made less acid, and the solubility of ferric hydroxide is so low (solubility product less than 10^{-35} at 20° c [7]) that unless strongly complex-forming reagents are present, precipitation occurs from neutral or weakly acid solutions even for micromolar concentrations of ferric ion.

Formation of metal complexes with amino-acids should involve a large favourable entropy change because of the accompanying reduction of ionic charge.[8] As this effect is greater the smaller the ion and the higher its charge,[9] ferric ion should form more stable complexes with anions than does ferrous ion. Differences in electronegativity point to the same conclusion. The behaviour of ferric ion with glycine, the simplest α-amino-acid, has therefore been re-examined by measurements of oxidation–reduction potentials and spectrophotometry.

Experimental

All reagents were of analytical grade. Ferric hydroxide, prepared by precipitation from hot ferric sulphate solution with ammonia, was washed thoroughly at the centrifuge and dissolved in excess of perchloric acid. The ferric ion concentration was checked by reduction with zinc amalgam and titration with standard potassium dichromate. Perchloric acid in the ferric perchlorate solution was determined by back-titration of excess of hot sodium hydroxide solution, allowance being made for the complete hydrolysis of the ferric perchlorate. Ferrous perchlorate, prepared by double decomposition between barium perchlorate and ferrous sulphate, was standardised against potassium dichromate.

To minimise formation of ferric hydroxyl complexes and colloidal ferric hydroxide all measurements were made at pH 0·5—4·0. High concentrations of glycine were necessary because of the very small fraction present as anion under these conditions. As glycine acts as a buffer over this pH range, high concentrations of acid were also required. Ferric ion forms

[1] Flood and Lorås, *Tidsskr. Kjemi Bergvesen Met.*, 1945, **5**, 83; Maley and Mellor, *Nature*, 1950, **165**, 453.
[2] Albert, *Biochem. J.*, 1950, **47**, 531.
[3] *Idem*, *ibid.*, 1952, **50**, 690.
[4] Cannan and Richardson, *ibid.*, 1929, **23**, 1242.
[5] Tanaka, Kolthoff, and Stricks, *J. Amer. Chem. Soc.*, 1955, **77**, 1996, 2004.
[6] Gabrio and Tishkoff, *Science*, 1950, **112**, 358.
[7] Evans and Pryor, *J.*, 1949, S157.
[8] Williams, *J.*, 1952, 3770.
[9] Powell and Latimer, *J. Chem. Phys.*, 1951, **19**, 1139.

complexes with most inorganic anions, but a complex is not formed between ferric ion and perchlorate ion for perchlorate concentrations [10] up to 3M. Under the present experimental conditions about 4% of the ferric ion not present in complexes would exist [11] as ferric perchlorate ion-pairs. This would not significantly affect the results. Perchloric acid was used throughout, with the addition of sodium perchlorate to maintain a constant ionic strength of 1·0. In computing ionic strength the zwitterion form of glycine was considered as a neutral molecule.[12]

Oxidation–Reduction Potentials.—The potentiometer was a Tinsley type 4046B, easily readable to 0·1 mv, and was used with a Pye " Scalamp " galvanometer. Two bright platinum electrodes in the solution were connected to a large saturated calomel electrode by an ammonium nitrate (1·60M)–sodium nitrate (0·20M)–agar bridge. (Potassium chloride or nitrate bridges

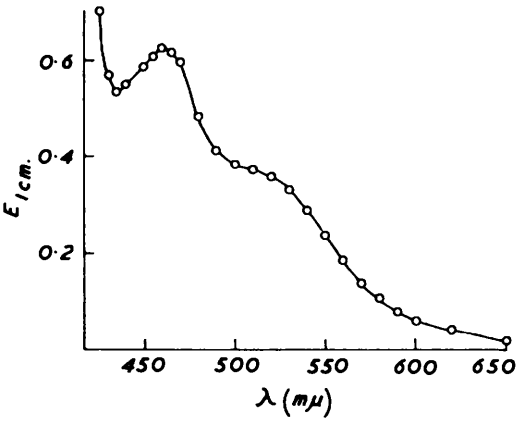

FIG. 1. *Absorption spectrum of ferric–glycine complex.*
Ferric ion, 0·0166M; glycine, 1·20M; pH 2·23.

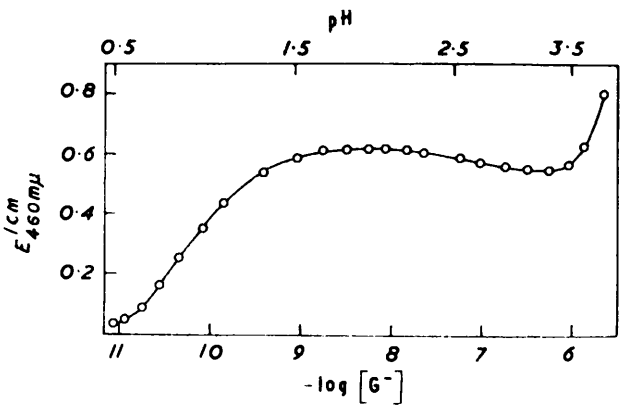

FIG. 2. *Variation of optical density with glycine anion concentration.*
Ferric ion, 0·0166M.

were unsatisfactory owing to precipitation of potassium perchlorate at the liquid junction.) Adjusted to pH 7, this salt mixture gave equal cation and anion conductances but did not precipitate ammonium perchlorate. All pH measurements were made on a Cambridge bench model pH meter with a glass electrode–saturated calomel electrode combination, separated by an ammonium nitrate–sodium nitrate bridge. The pH standard taken was 0·050M-potassium hydrogen phthalate, pH 4·00. Commercial nitrogen, passed through Fieser's solution, was used to stir the solution in the electrode vessel and to maintain an inert atmosphere. All potentiometric measurements were made at 20° ± 0·1°.

From the ionisation constants of glycine and the stability constants of the ferrous–glycine complexes [13] it can be calculated that, even at a glycine concentration of 1M, ferrous complex

[10] Rabinowitch and Stockmayer, *J. Amer. Chem. Soc.*, 1942, **64**, 335.
[11] Sutton, *Nature*, 1952, **169**, 71.
[12] King, *J. Amer. Chem. Soc.*, 1945, **67**, 2178; Scatchard and Kirkwood, *Phys. Z.*, 1932, **33**, 297.
[13] Albert, *Biochem. J.*, 1953, **54**, 646.

formation is negligible in solutions more acid than pH 4. Under such conditions the oxidation–reduction potentials of solutions of known ferrous-ion concentration can be used to study complex formation involving ferric ion.[14] At 20° the potential of the cell

$$\mathrm{Pt}\ \Big|\ \begin{matrix}\mathrm{Fe^{3+}} & \mathrm{ClO_4^-}\\ \mathrm{Fe^{2+}} & \mathrm{H^+}\\ \multicolumn{2}{c}{\text{glycine}}\end{matrix}\ \Big|\ \text{sat. KCl}\ \Big|\ \text{calomel}$$

is $E = E_0 + 0{\cdot}0581 \log (a_{Fe^{3+}}/a_{Fe^{2+}}) + E_j$, where E_0 is a constant and E_j is the liquid-junction potential. If the approximations are made that at constant ionic strength the liquid-junction potential and the activity coefficients of ferric and ferrous ions do not change appreciably when glycine is added to the solution or the pH of the solution is altered it follows that

$$(E_0' - E)/0{\cdot}0581 = \log ([Fe^{3+}]_0/[Fe^{3+}]) \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where E_0' is the potential of the experimental ferric–ferrous ratio under conditions where no complex formation occurs. If ferric ion forms 1 : 1, 1 : 2, and higher complexes with overall stability constants K_1, K_1K_2, . . ., the mass-action equation can be written

$$[Fe^{3+}]_0/[Fe^{3+}] = 1 + K_1[G^-] + K_1K_2[G^-]^2 + \ldots \quad . \quad . \quad . \quad . \quad . \quad (2)$$

where G^- is the glycine anion. To allow for hydrolysis of ferric ion additional terms must be added to the right-hand side of eqn. (2). Estimates of contributions due to the species $FeOH^{2+}$, $Fe(OH)_2^+$, and $Fe_2(OH)_2^{4+}$ at 20° and $I = 1$, based on published data,[15,16,17] are $1 \times 10^{-3}/[H^+] + 4 \times 10^{-7}/[H^+]^2 + 2 \times 10^{-3}[Fe^{3+}]/[H^+]^2$. By plotting $[Fe^{3+}]$, obtained from eqn. (1) and corrected for hydrolysis, against $[G^-]$ and endeavouring to fit the experimental data by an equation of the form, $y = 1 + ax + bx^2 + \ldots$, evidence of complex formation can be sought and approximate values of the stability constants obtained.

Spectrophotometry.—All measurements were made at 25° ± 1° on a Hilger Uvispek H700/305 spectrophotometer.

Concentrated glycine solutions buffered with perchloric acid and containing ferric ion are amber-coloured, the intensity increasing to a maximum with increase of pH. The absorption spectrum, shown in Fig. 1, is not due to the ion $FeOH^{2+}$.[10]

If the assumption is made that at low glycine anion concentrations only one complex, $FeG_n^{(3-n)+}$ is formed, the variation of optical density with change of pH enables the glycine : ferric ion ratio of the complex, and the stability constant, to be determined. The results shown in Fig. 2 were obtained by progressive addition of 5M-perchloric acid to a solution initially of pH 3·85 and 2M in glycine. The optical densities are for a total ferric ion concentration of $1{\cdot}66 \times 10^{-2}$M. Because the complex is the only light-absorbing species present below about pH 2, the flat portion of the curve in Fig. 2 indicates almost complete conversion of ferric ion to ferric–glycine complex. Over this flat portion the molecular extinction coefficient of the complex is given by:

$$E_{1\,\mathrm{cm.}} = \varepsilon[FeG_n^{(3-n)+}] \simeq \varepsilon[Fe^{3+}]_0$$

Hence values of $[FeG_n^{(3-n)+}]$ and $[Fe^{3+}]$ can be calculated at lower glycine anion concentrations. But, at constant ionic strength, $K_n = [FeG_n^{(3-n)+}]/[Fe^{3+}][G^-]^n$. Therefore,

$$\log ([FeG_n^{(3-n)+}]/[Fe^{3+}]) = \log K_n + n \log [G^-] \quad . \quad . \quad . \quad . \quad (3)$$

The plot of the left-hand side of eqn. (3) against $\log [G^-]$ gives n and $\log K_n$.

Results

Ionisation constants for glycine at 20° in a solution of unit ionic strength were determined by potentiometric titration to be 2·43 and 9·76. By using the small temperature corrections given by Owen,[18] values at 25° were obtained as 2·41 and 9·73.

[14] Ågren, *Acta Chem. Scand.*, 1954, **8**, 266; Perrin, *J. Amer. Chem. Soc.*, in the press.
[15] Siddall and Vosburgh, *J. Amer. Chem. Soc.*, 1951, **73**, 4270.
[16] Hedström, *Arkiv Kemi*, 1953, **5**, 457; **6**, 1.
[17] Mulay and Selwood, *J. Amer. Chem. Soc.*, 1955, **77**, 2693.
[18] Owen, *ibid.*, 1934, **56**, 24.

Data from oxidation–reduction potentials are presented in Fig. 3, plotted for compactness on logarithmic scales. The linearity of the results, for a 30,000-fold variation in glycine anion concentration, indicates the formation of a 1 : 1 ferric–glycine complex and the absence of higher complexes. For the lower set of values in Fig. 3 perchloric acid was added progressively to a solution containing ferric ion and glycine, the limiting value of E, obtained at pH 0·6, being taken as E_0'. Subsequent calculation showed that the error from neglect of complex formation

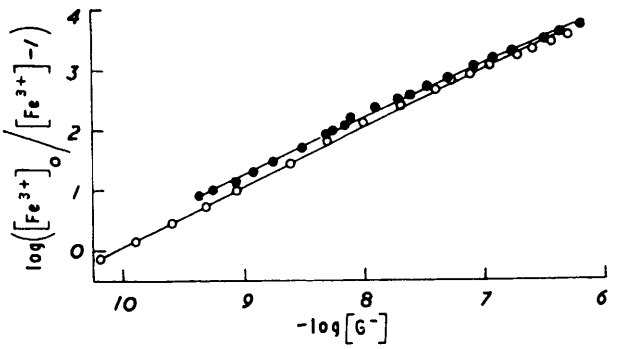

FIG. 3. *Glycine : ferric ion ratio and association constant of the complex, from oxidation–reduction potentials.*

Solid circles: ferric ion, 0·0017M; ferrous ion, 0·0014M; glycine, 0·8—1·0M; pH increased from 1·39 to 4·0.

Open circles: ferric ion, 0·0024M; ferrous iron, 0·0033M; glycine, 0·56—0·67M; pH decreased from 4·1 to 1·0.

at this pH was less than 1 mv. The upper set in Fig. 3 were obtained by measuring E_0' in an acid solution before the addition of glycine in perchloric acid; the pH of the solution was then increased by adding sodium hydroxide. The two values of E_0 so obtained were 0·4861 and 0·4956 v, respectively. If this small difference is due to the effect of glycine on the ferric and ferrous activity coefficients, as distinct from complex formation, the former value of E_0 should be used with both sets of results. This brings both lines in Fig. 3 very close together, with a maximum deviation, at the lowest values, of only 0·1 logarithmic unit, and leads to a stability constant for the complex of log $K_1 = 10{\cdot}0$. Under the experimental conditions correction for hydrolysis of ferric ion was negligible.

Results obtained from spectrophotometry and plotted in Fig. 4 confirm the formation of a 1 : 1 ferric–glycine complex and lead to a value of log $K_1 = 10{\cdot}2$ at 25°.

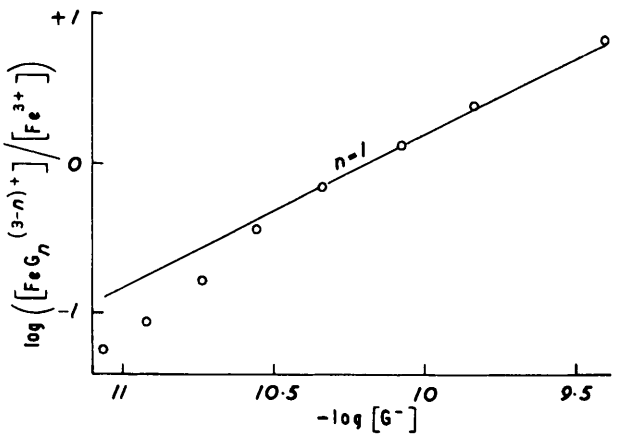

FIG. 4. *Spectrophotometric evaluation of glycine : ferric ion ratio and stability constant of complex.*

Data as for Fig. 2.

In Fig. 2 the slight drop in optical density between pH 2·4 and pH 3·3 is explained quantitatively by formation of the complex, $Fe_2(OH)_2^{4+}$, if the stability constant of the latter lies between the reported [16,17] values of $1{\cdot}2 \times 10^{-3}$ and $7{\cdot}3 \times 10^{-3}$ at 25°. This complex is more significant in these experiments than in the oxidation–reduction potential measurements because its concentration varied with the square of the ferric ion concentration. The rapid increase in optical density above pH 3·5 is believed to be due to the rapidly increasing concentration of colloidal ferric hydroxide, rather than to the formation of a higher ferric–glycine complex.

Discussion

Spectrophotometric and oxidation–reduction potential measurements indicate that ferric ion forms a 1 : 1 complex with glycine. In view of the approximations involved, the two values obtained for the stability constant of the complex are in good agreement. The constancy of the value from oxidation–reduction potentials over a wide range of pH is surprising in view of the change of dielectric constant of glycine solutions with pH. Up to pH 4·5 the dielectric constant increases directly with the glycine zwitterion concentration [19] from a value not very different from that of water, the increase reaching a maximum of approximately 22 units per mole of glycine.[20] These changes should, from considerations such as are used in the Debye–Hückel treatment of ions in solution, exert a considerable effect on the activities, especially of ferric ion.

Similar complex formation has subsequently [21] been shown to occur between ferric ion and a number of other α-amino-acids. It is reasonable to assume that like other metal–amino-acid complexes the ferric complex is a chelate compound formed by binding the metal between a carboxylic oxygen and the amino-nitrogen atom. This assumption is supported by a comparison with the stability constants for metal derivatives of 8-hydroxyquinoline-5-sulphonic acid, where a comparable 5-membered ring is formed:

Values of $\log K_1$ *at* 20°.

Complexing species	Fe^{3+}	Cu^{2+}	Fe^{2+}
Glycine	10·0 [a]	8·5 [b]	4·3 [b]
8-Hydroxyquinoline-5-sulphonic acid	12·4 [c]	12·5 [b]	8·4 [b]

a = present work; *b* = ref. 13; *c* = present work, from oxidation–reduction potentials, I = 0·01—0·08.

A similar trend is also found for metal complexes with tetracycline, the corresponding values being [22] 9·9, 7·8, and 5·3: in this series the metal is presumably bound between two oxygen atoms. In all cases the stability of the ferric complex is close to, or somewhat greater than, that of the cupric complex, while the ferrous complex is 4 to 6 logarithm units less stable than the ferric one.

Because the solubility of ferric hydroxide is so low that, even in the presence of 1M-glycine, precipitation occurs from 0·01M-ferric perchlorate solutions above about pH 4·7, the maximum attainable concentration of glycine anion was very low. This is probably the reason why, although many bivalent cations form 1 : 2 and higher glycine complexes,[1,2] no evidence was obtained of ferric complexes of these types.

Department of Medical Chemistry,
The Australian National University, Canberra. [*Received, March 24th*, 1958.]

[19] Dunning and Shutt, *Trans. Faraday Soc.*, 1938, **34**, 479.
[20] Hedestrand, *Z. phys. Chem.*, 1928, **135**, 36.
[21] Perrin, following paper.
[22] Albert and Rees, *Nature*, 1956, **177**, 433.