633. *The Stability of Complexes of Ferric Ion and Amino-Acids.*

By D. D. Perrin.

Ferric ion and α-amino-acids form stable 1 : 1 complexes in acid solution. In most cases the stability constants obtained from oxidation–reduction potentials depend linearly on the basic ionisation constants of the amino-acids. Ferric complexes of glutamic acid, aspartic acid, and glycylglycine show increased stability because of additional chelation. Proline, hydroxyproline, and sarcosine complexes are less stable than predicted, possibly because of the difference in the amino-groups taking part in complex formation. Histidine is unusual in that complex formation takes place through the glyoxaline nitrogen atom instead of the α-amino-group. In the stability of its amino-acid complexes, ferric ion exceeds cupric ion and is comparable with mercuric ion.

Albert [1,2] and Perkins [3,4] have measured the stability of α-amino-acid complexes of a number of bivalent cations. Following the observation [5] that ferric ion and glycine form a 1 : 1-complex, we have studied the behaviour of a range of α-amino-acids with ferric ion. Except for cysteine,[6] where linkage appears to be through the sulphur atom, little direct evidence of complex formation between ferric ion and other α-amino-acids had previously been reported. Stability constants have been obtained for 1 : 1 ferric complexes with a range of naturally-occurring α-amino-acids and some related substances.

Experimental

Ferric and ferrous perchlorates were prepared as described previously.[5] The materials investigated were obtained from British Drug Houses Ltd. and Light and Co. Ltd. Arginine was prepared from arginine hydrochloride by ion-exchange on a column of Amberlite resin IRA-400. Ornithine hydrobromide was converted into the nitrate by double decomposition with silver nitrate. Purity was checked by paper chromatography with three solvent systems (*tert.*-butyl alcohol–formic acid–ethyl methyl ketone–water; collidine–lutidine–water; *n*-propyl alcohol–ammonia–water) followed by examination in ultraviolet light and staining with ninhydrin. Traces of other amino-acids (indicated in parentheses) were present in alanine (glycine), glutamic acid (leucine, valine), leucine (valine), methionine (serine or threonine), and proline (hydroxyproline) but did not appreciably affect estimates of the stability constants of the ferric complexes.

Except with histidine, which forms a slightly soluble perchlorate, all measurements were made in sodium perchlorate solutions of unit ionic strength at 20°. The ionisation constants of the amino-acids were obtained by potentiometric titration, with samples dried at 110°. Correction was made for hydrolysis. Where two ionisation constants of an amino-acid were close together they were evaluated by the method described by Britton.[7] All constants, which were expressed in terms of hydrogen activity as measured by glass electrode and of concentrations of amino-acid species, were consistent with published values. Oxidation–reduction potential and spectrophotometric measurements used in obtaining stability constants of the ferric complexes were made as described earlier.[5] Hedström's [8] values of the formation constants of the 1 : 1, 1 : 2, and 2 : 2 ferric–hydroxyl complexes were extrapolated to 20° and unit ionic strength from the results of Siddall and Vosburgh [9] and Mulay and Selwood [10] to give 7×10^{-4}, 4×10^{-7}, and 1×10^{-3}, respectively. These values were used in calculating the small corrections for hydrolysis of ferric ion.

[1] Albert, *Biochem. J.*, 1950, **47**, 531.
[2] *Idem*, *ibid.*, 1952, **50**, 690.
[3] Perkins, *ibid.*, 1952, **51**, 487.
[4] *Idem*, *ibid.*, 1953, **55**, 649.
[5] Perrin, preceding paper.
[6] Cannon and Richardson, *Biochem. J.*, 1929, **23**, 1242; Tanaka, Kolthoff, and Stricks, *J. Amer. Chem. Soc.*, 1955, **77**, 1996, 2004.
[7] Britton, "Hydrogen Ions," Chapman and Hall, London, 4th edn., 1955, vol. 1, p. 217.
[8] Hedström, *Arkiv Kemi*, 1953, **5**, 457; 1953, **6**, 1.
[9] Siddall and Vosburgh, *J. Amer. Chem. Soc.*, 1951, **73**, 4270.
[10] Mulay and Selwood, *ibid.*, 1955, **77**, 2693.

As discussed [5] for ferric complex formation with glycine, it is reasonable to assume that with most α-amino-acids the ferric complex results from chelation of the metal ion by the amino-acid anion in which the amino-group is not ionised. Results here reported confirm the general correctness of this assumption. Unless otherwise indicated, all stability constants are calculated for this direct reaction.

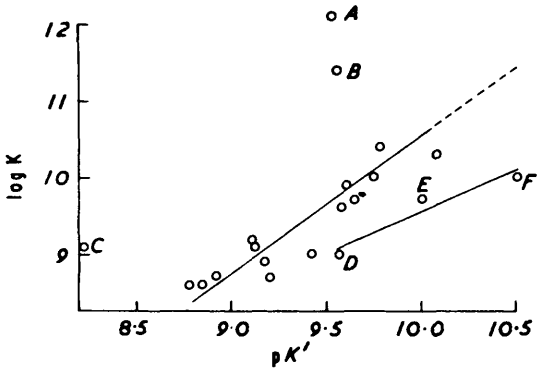
Results and Discussion

Values of log K for the 1 : 1 ferric–amino-acid complexes, obtained from oxidation–reduction potentials, are listed in the Table, together with relevant experimental details.

Stability constants of 1 : 1 *ferric–amino-acid complexes at* 20° *and unit ionic strength.*

	Ionisation constants			log K	
Amino-acid (M)	α-CO_2H	α-NH_2	Other	± S.E. (no. of obs.)	pH
Glycine (for comparison)	2·41	9·76	—	10·0	—
Alanine (1·00)	2·49	9·79	—	10·4 ± 0·03 (17)	0·77—3·35
α-Amino-*n*-butyric acid (0·300) ...	2·31	9·66	—	9·7 ± 0·01 (11)	1·54—3·17
α-Amino*iso*butyric acid (0·500) ...	2·48	10·09	—	10·3 ± 0·03 (9)	1·79—3·06
Valine (0·300)	2·38	9·59	—	9·6 ± 0·02 (14)	1·26—3·21
Leucine (0·200)	2·37	9·62	—	9·9 ± 0·02 (10)	1·45—2·98
β-Phenylalanine (0·150)	2·21	9·18	—	8·9 ± 0·03 (9)	1·47—2·66
Serine (0·300)	2·26	9·12	—	9·2 ± 0·04 (11)	1·32—2·73
Threonine (0·100)	2·24	8·86	—	8·6 ± 0·03 (9)	1·38—2·56
Aspartic acid (0·050)	2·00	9·56	3·78	11·4 ± 0·03 (10)	1·64—2·27
Glutamic acid (0·050)	2·39	9·54	4·21	12·1 ± 0·02 (8)	1·56—2·33
Asparagine (0·150)	2·09	8·79	—	8·6 ± 0·01 (8)	1·52—2·40
Arginine (0·110)	2·19	9·21	(12·5 [a])	8·7 ± 0·03 (9)	1·62—2·62
Ornithine (0·210)	2·11	8·93	10·59	8·7 ± 0·05 (7)	1·76—2·85
Proline (0·050)	2·02	10·52	—	10·0 ± 0·03 (9)	1·62—2·55
Hydroxyproline (0·050)	1·93	9·58	—	9·0 ± 0·03 (8)	1·70—2·48
Histidine (0·100)	1·82 [a]	9·20 [a]	6·08 [a]	4·7[b] ± 0·04 (7)	1·80—2·92
Tryptophan (0·050)	2·39	9·43	—	9·0 ± 0·03 (10)	1·82—2·92
Methionine (0·200)	2·26	9·13	—	9·1 ± 0·02 (9)	1·49—2·85
Sarcosine (0·100)	2·18	10·02	—	9·7 ± 0·03 (9)	1·73—2·79
Glycylglycine (0·500)	3·16	8·23	—	9·1 ± 0·05 (7)	1·37—3·03

[a] Ref. 2. [b] See text; $I = 0·1$.



Dependence of stability constant of ferric complex on basic ionisation constant of amino-acid.

Anomalous acids:
A, glutaminic acid.
B, aspartic acid.
C, glycylglycine.
D, hydroxyproline.
E, sarcosine.
F, proline.

The correction for hydrolysis of ferric ion was less than $0·05 \times \{([Fe^{3+}]_0 - [Fe^{3+}])/[Fe^{3+}]\}$ for all amino-acids except proline, hydroxyproline, and tryptophan (less than 0·10 ×) and histidine (less than 0·25 ×). The rapid increase in magnitude of this correction with increase in pH and the insolubility of ferric hydroxide set an upper limit to the accessible range of pH. The rapid fall of anion concentration with decrease in pH determined the lower limit at which significant differences in free ferric ion concentration could be measured.

Except for histidine, all amino-acids gave consistent values of log K over the experimental range of pH. As with glycine, no evidence of higher complex formation was found.

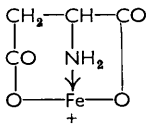
For the combination of a cation with a series of similar ligands Bjerrum [11] found log

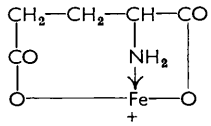
[11] Bjerrum, *Chem. Rev.*, 1950, **46**, 381.

$K = \alpha pK' + c$, where α and c were constants, and K' was the ionisation constant of the ligand. If the factors governing the binding of protons and ferric ions by amino-acid anions are similar, Bjerrum's equation should apply to the present results. Although such a treatment is a considerable over-simplification, the Figure shows that for most of the acids studied a roughly linear relation is, in fact found between log K and the appropriate pK', with $\alpha = 1{\cdot}8$ and $c = -7{\cdot}5$.

Aspartic and glutamic acids do not fit this relation. The much greater than predicted stabilities of their ferric complexes (by 1·8 and 2·5 log units) probably indicate the formation of terdentate complexes involving the additional carboxyl group. The possibility that only the two carboxyl groups or the β- or γ-carboxyl and the amino-group take part in complex formation was not supported by the experimental results. Values of the stability constants calculated on these assumptions varied considerably with pH. Atomic models (Courtaulds) of aspartic and glutamic acids showed that the terdentate structures (I)

(I)





(II)

and (II) should be possible and suggested that the somewhat lower stability of the ferric–aspartic acid complex was due to steric effects. For an ionic radius of 0·60 Å,[12] formation by aspartic acid of a second, six-membered, ring between ferric ion and a β-carboxylic oxygen atom would involve some strain owing to the bond directions. With glutamic acid the decrease in stability of the ferric complex that would be expected from the increase in ring size is apparently more than offset by the removal of this strain. Results for ferric ion differ from those for bivalent cations where, in most cases, the stability of the 1 : 2 glutamic acid complex is appreciably less than that of the 1 : 2 aspartic acid complex and is comparable with the value for glycine.[2,4] The ferric complex of asparagine (the β-amide of aspartic acid) does not show enhanced stability. This result is in line with the known weakness of the amide group in chelation.

The ferric complexes with proline, hydroxyproline, and sarcosine are all less stable than predicted from results for other amino-acids (by 1·4, 0·7, and 0·8 log unit). However, as the alkyl group they contain in place of one of the amino-hydrogen atoms probably gives rise to a steric effect in complex formation, these three acids should be considered as members of a related, but distinct, series of ligands.

For most of the remaining ferric–amino-acid complexes, the greatest single factor governing their stability appears to be the basic ionisation constant of the amino-acid. The low values of this constant for serine, threonine, and asparagine may be due either to inductive effects or to the formation of hydrogen bonds between the carboxylic oxygen and the β-hydroxyl or the amide group. The depression of pK' produced by introducing the hydroxyl or amide group is similar to that found in comparing 2-aminoethanol with ethylamine.[13]

The terminal basic groups in arginine and ornithine do not appear to take part in ferric complex formation. On the other hand, when the stability constant of the ferric–histidine complex was calculated for complex formation involving the α-amino- and the carboxyl group no constancy was found, values of log K changing by one unit for a change of one pH unit. This was also true for calculations where complex formation was assumed to take place through the glyoxaline-nitrogen atom and the α-amino-group, by analogy with complexes with some bivalent cations.[2] A steady value of the stability constant was obtained, however, if ferric ion was assumed to be bound between the glyoxaline-nitrogen

[12] Pauling, "The Nature of the Chemical Bond," Cornell Univ. Press, Ithaca, New York, 1939, p. 330.

[13] Bruehlam and Verhoek, *J. Amer. Chem. Soc.*, 1948, **70**, 1401.

atom and the carboxyl group, the α-amino-group remaining ionised. The weakness of the 7-membered ring formed in this way is shown by the low value of the stability constant.

Attempts to measure the stability constant of a ferric–cysteine complex in acid solution were unsuccessful; reduction of ferric ion by cysteine was very rapid. No difficulties were encountered with methionine.

Results for glycylglycine showed the ferric complex to be formed by linkage through the amino- and the carboxyl group as in simple amino-acids. So large a ring structure would not be very stable but chelation also probably occurs through the amide group, giving rise to a terdentate structure containing two 5-membered rings.[14] Hence, although the amide group is only a weak donor, and the stability constant of the complex is less than for ferric–glycine, the complex is 1·8 log units more stable than would be expected for an amino-acid of the same pK'.

All ferric–amino-acid complexes were amber-coloured. The absorption spectra of the aspartic and glutamic acid complexes resembled closely the ferric–glycine spectrum. Most of the other amino-acid complexes showed little or no evidence of the maximum near 460 mμ but only a general absorption increasing steadily towards the blue end of the spectrum. As an approximate check on the value found from oxidation–reduction potentials for the stability constant of the ferric–glutamic acid complex, estimates were obtained by measuring the dependence of optical density on pH. The approximate constancy, around pH 3, of the optical density of a solution containing ferric ion (0·0083M) and glutamic acid (0·0500M) was assumed to be due to complete complex formation. The measured optical densities, over the range pH 1·36—1·99 at 25° and $I = 1$, gave log $K = 12{\cdot}4$ (range 12·1—12·7). The calculation was very sensitive to errors in pH or pK'; the maximum deviation in log K could be caused by an error of 0·1 pH unit. Similar measurements between pH 1·27—1·87 gave for the ferric–aspartic acid complex a value of log $K = 11{\cdot}5$ (range 11·3—11·6). For both acids these results confirm the more accurate values obtained from oxidation–reduction potentials.

The approximate relation,[1] log $K_1 = \frac{1}{2}$ (log K_S − 1), where K_S is the stability constant of the 1 : 2-complex, enables published data on amino-acid complexes with bivalent cations to be compared with the present results. From these results it appears that in relation to Mellor and Maley's[15] series of bivalent metals, ferric ion should be placed well above cupric ion[1,2] and near mercuric ion.[3,4]

By analogy with bivalent cations, 1 : 2 and 1 : 3 ferric complexes might be expected. However, under our conditions no evidence of complexes higher than 1 : 1 was obtained.

The ionisation constants used in this work were determined by Mr. F. Robinson, whom the author thanks.

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The Australian National University, Canberra. [*Received, March 24th*, 1958.]

[14] Martell and Calvin, "Chemistry of the Metal Chelate Compounds," Prentice-Hall, New York, 1952, p. 139.
[15] Mellor and Maley, *Nature*, 1948, **161**, 436.