

50. *The Stability of Iron Complexes. Part III.¹ A Comparison of 1 : 1 Ferric and Ferrous Amino-acid Complexes.*

By D. D. PERRIN.

The stability constants of twenty 1 : 1 ferrous complexes with α -amino-acids were obtained in water at 20° and $I = 1$, in a medium consisting essentially of potassium chloride, by potentiometric titration. The constants vary with the proton, acid, dissociation constants of the amino-acids, but to a much smaller extent than for the corresponding ferric complexes. Molar electrode potentials for the $\text{Fe}^{3+} : \text{Fe}^{2+}$ amino-acid complexes, calculated from these results, are linearly related to the logarithms of the dissociation constants of the amino-acids. The slope of the line is probably somewhat greater than similar slopes for the iron 1 : 1 polyaza-1-naphthol and 5-substituted 1 : 10-phenanthroline complexes.

LITTLE quantitative information exists on the relative stabilities in aqueous solutions of ferric and ferrous complexes with a series of similar ligands. Albert and Hampton² obtained stability constants of 1 : 1 ferric and ferrous complexes with seven polyaza-1-naphthols ("aza-oxines") and Smith and Richter³ measured the oxidation-reduction

¹ Parts I and II, *J.*, 1958, 3120, 3125.

² Albert and Hampton, *J.*, 1954, 505.

³ Smith and Richter, *Ind. Eng. Chem. Anal.*, 1944, **16**, 580.

potentials of iron chelates of four substituted 1 : 10-phenanthrolines. Although stability constants for both ferric and ferrous iron with a further eighteen organic ligands have been tabulated,⁴ the ligands were of so many different types, and experimental conditions varied so widely, that few quantitative comparisons are possible. Qualitatively, as is to be expected from entropy effects⁵ and electrostatic considerations, ferric complexes are more stable than ferrous complexes when the ligand is an anion; the relative stability appears to increase with the charge on the ligand. In the few cases so far studied where the ligand is a neutral molecule the ferrous complexes are the more stable.

The dissociation constant, K' , of the ligand is also important. In many cases, including the 1 : 1 ferric-amino-acid complexes,¹ the stability constants of a cation with a series of similar ligands follow approximately the relation $\log K = \alpha pK' + c$, where α and c are constants. It has been suggested that this relation is somewhat fortuitous and is obeyed so widely only because most ligands are at the same time both π - and σ -electron donors (or acceptors).⁷ Although the value of α appears to increase with the electronegativity of the cation, from about 0.3 for Ag^+ in complexes with secondary amines⁸ to 1.7 for Fe^{3+} in amino-acid complexes,¹ factors affecting α have not been studied in detail. It has also been suggested that from thermodynamic reasoning α should be unity;⁹ on the other hand it has been stated that there is no reason to expect this value.⁵ From a simple electrostatic model α should increase with increasing cationic charge and decreasing distance of separation of the cation and ligand.⁷ The effect of the dissociation constants of similar ligands on the relative stabilities of ferric and ferrous complexes, and hence on their standard oxidation-reduction potentials, has been very little studied, except that it has been observed that the potentials of iron complexes of 5-substituted 1 : 10-phenanthrolines varied with pK' of the corresponding *para*-substituted benzoic acids¹⁰ and of the ligands themselves.¹¹ Such a study has recently been commenced in mixed aqueous organic solvents.¹²

The results now reported for 1 : 1 ferrous complexes with α -amino-acids are part of an investigation of the effects of the charge and the dissociation constant of a ligand on the stability in aqueous solution of its ferric and ferrous complexes. Amino-acids are convenient complex-forming species for this purpose because the stability constants of the corresponding ferric complexes are known. However, although stability constants of many 1 : 2 ferrous-amino-acid complexes have been recorded,⁴ values for the 1 : 1 ferrous complexes have been published only for glycine,¹³ glutamic acid,¹³ lysine,¹⁴ cysteine,¹⁴ arginine,¹⁴ and ornithine.¹⁴

EXPERIMENTAL

The amino-acids were of the same quality as in Part II. All other reagents were of analytical grade. Ferrous perchlorate solutions were prepared by double decomposition of carefully neutralised barium perchlorate and ferrous sulphate solutions. Ferric ion was removed by passage through a column of the ferrous salt of a cation-exchange resin (Amberlite IR-120). The eluates, which gave only weak tests for ferric ion on addition of sodium thiocyanate, were stored under nitrogen. Ferrous concentrations were determined by titration

⁴ Bjerrum, Schwarzenbach, and Sillén, "Stability Constants. Part 1: Organic Ligands," The Chemical Society, London, Spec. Publ. No. 6, 1957.

⁵ Williams, in "Special Lectures in Biochemistry, 1954—1955," University College, London, p. 43.

⁶ Keller and Parry, in "The Chemistry of the Co-ordination Compounds," ed. Bailar, Chapman and Hall, London, 1956, p. 180.

⁷ Jones, Poole, Tomkinson, and Williams, *J.*, 1958, 2001.

⁸ Bjerrum, *Chem. Rev.*, 1950, **46**, 381.

⁹ Irving and Rosotti, *J.*, 1954, 2910.

¹⁰ Ewens, *Nature*, 1945, **155**, 398.

¹¹ Brandt and Gullstrom, *J. Amer. Chem. Soc.*, 1952, **74**, 3532.

¹² Tomkinson and Williams, *J.*, 1958, 2010.

¹³ Albert, *Biochem. J.*, 1953, **54**, 646.

¹⁴ *Idem, ibid.*, 1952, **50**, 690.

TABLE I. Derivation of stability constants for 1 : 1 ferrous-amino-acid complex.

	[Fe ²⁺] = 0.005M, [HL] = 0.01M, I = 1M (KCl)										
	$\bar{n} =$	0.06	0.08	0.10	0.12	0.16	0.20	0.24	0.30	0.40	0.50
(a) Glycine.											
pH	—	—	6.80	6.95	7.08	7.24	7.38	7.48	7.62	7.80	—
—log [L ⁻]	—	—	4.980	4.835	4.710	4.560	4.430	4.341	4.217	4.065	—
log K ₁	—	—	3.91	3.87	3.83	3.82	3.80	3.80	3.80	3.81	—
(b) Alanine.											
pH	—	—	7.08	7.26	7.40	7.58	7.72	7.80	7.89	8.00	—
—log [L ⁻]	—	—	4.730	4.555	4.420	4.250	4.120	4.051	3.977	3.896	—
log K ₁	—	—	(3.66)	3.59	3.54	3.51	3.48	3.52	3.57	(3.68)	—
(c) α-Amino-n-butyric acid.											
pH	—	—	7.20	7.34	7.44	7.60	7.74	7.83	7.95	8.10	—
—log [L ⁻]	—	—	4.480	4.345	4.250	4.100	3.970	3.891	3.787	3.656	—
log K ₁	—	—	3.41	3.38	3.37	3.36	3.34	3.36	3.38	3.43	—
(d) α-Aminoisobutyric acid.											
pH	7.38	7.61	7.72	7.81	7.88	7.96	—	8.06	8.15	—	—
—log [L ⁻]	4.725	4.500	4.395	4.305	4.250	4.180	—	4.107	4.046	—	—
log K ₁	3.52	3.43	3.42	3.42	3.51	3.56	—	(3.72)	(3.85)	—	—
(e) Valine.											
pH	6.95	7.14	7.30	7.39	7.55	7.68	—	7.87	8.02	—	—
—log [L ⁻]	4.655	4.470	4.315	4.230	4.080	3.960	—	3.797	3.676	—	—
log K ₁	3.46	3.40	3.37	3.35	3.34	3.34	—	3.39	3.46	—	—
(f) Leucine.											
pH	6.96	7.16	7.28	7.37	7.52	7.63	—	7.86	7.96	—	—
—log [L ⁻]	4.675	4.480	4.365	4.280	4.140	4.040	—	3.837	3.766	—	—
log K ₁	3.48	3.41	3.40	3.40	3.40	3.42	—	3.43	(3.56)	—	—
(g) β-Phenylalanine											
pH	—	6.78	6.96	7.08	7.24	7.36	7.48	7.60	7.77	—	—
—log [L ⁻]	—	4.420	4.215	4.130	3.980	3.870	3.761	3.657	3.516	—	—
log K ₁	—	3.35	3.25	3.25	3.24	3.24	3.23	3.25	3.28	—	—
(h) Serine.											
pH	6.44	6.62	6.74	6.86	7.02	7.15	—	7.39	7.56	—	—
—log [L ⁻]	4.695	4.520	4.405	4.290	4.140	4.020	—	3.807	3.666	—	—
log K ₁	3.50	3.45	3.44	3.41	3.40	3.39	—	3.40	3.43	—	—
(i) Threonine.											
pH	6.31	6.50	6.63	6.73	6.88	7.00	—	7.25	7.42	—	—
—log [L ⁻]	4.565	4.375	4.255	4.160	4.020	3.910	—	3.687	3.546	—	—
log K ₁	3.37	3.31	3.29	3.28	3.28	3.28	—	3.28	3.31	—	—
(j) Aspartic acid.											
pH	6.08	6.20	—	6.38	6.54	6.66	—	6.92	7.12	7.30	—
—log [L ⁻]	5.524	5.409	—	5.239	5.089	4.979	—	4.746	4.575	4.413	—
log K ₁	4.32	4.34	—	4.36	4.35	4.35	—	4.33	4.33	4.31	—
(k) Glutamic acid.											
pH	6.78	6.97	—	7.20	7.34	7.47	—	7.70	7.86	—	—
—log [L ⁻]	4.775	4.590	—	4.370	4.240	4.120	—	3.917	3.786	—	—
log K ₁	3.58	3.52	—	3.49	3.50	3.49	—	3.51	3.56	—	—
(l) Asparagine.											
pH	—	6.26	6.40	6.52	6.69	6.84	6.95	7.10	7.30	—	—
—log [L ⁻]	—	4.550	4.415	4.300	4.140	4.000	3.901	3.767	3.596	—	—
log K ₁	—	3.48	3.45	3.42	3.40	3.37	3.37	3.35	3.34	—	—
(m) Arginine.											
pH	6.70	6.90	—	7.15	7.32	7.44	—	7.68	7.82	—	—
—log [L ⁻]	4.525	4.330	—	4.090	3.930	3.820	—	3.607	3.496	—	—
log K ₁	(3.33)	3.26	—	3.21	3.19	3.19	—	3.20	3.27	—	—
(n) Ornithine.											
pH	6.48	6.75	6.88	6.98	7.17	7.29	—	7.53	7.70	—	—
—log [L ⁻]	4.465	4.200	4.075	3.980	3.800	3.690	—	3.477	3.336	—	—
log K ₁	(3.27)	3.13	3.11	3.10	3.10	3.06	—	3.06	3.10	—	—
(o) Proline.											
pH	7.20	7.42	7.57	7.66	7.76	7.82	—	7.96	8.05	—	—
—log [L ⁻]	5.335	5.220	4.975	4.890	4.800	4.750	—	4.637	4.576	—	—
log K ₁	4.14	4.05	4.01	4.01	4.06	4.13	—	(4.25)	(4.38)	—	—

TABLE I. (Continued.)

	$\bar{n} =$	0.06	0.08	0.10	0.12	0.16	0.20	0.24	0.30	0.40	0.50
(p) Hydroxyproline.											
pH		6.41	6.60	6.70	6.80	6.96	7.08	—	7.32	7.50	—
$-\log [L^-]$		5.185	5.000	4.905	4.810	4.660	4.550	—	4.337	4.186	—
$\log K_1$		3.99	3.93	3.94	3.93	3.92	3.92	—	3.93	3.95	—
(g) Tryptophan.											
pH		6.77	6.95	7.10	7.20	7.35	7.47	—	7.68	7.84	—
$-\log [L^-]$		4.686	4.511	4.366	4.271	4.131	4.021	—	3.838	3.707	—
$\log K_1$		3.49	3.44	3.40	3.39	3.39	3.40	—	3.44	3.50	—
(r) Methionine.											
pH		6.64	6.84	6.98	7.07	7.24	7.36	—	7.58	7.75	—
$-\log [L^-]$		4.516	4.321	4.186	4.101	3.941	3.831	—	3.638	3.497	—
$\log K_1$		3.32	3.25	3.22	3.22	3.20	3.20	—	3.23	3.27	—
(s) Sarcosine.											
pH		7.18	7.42	7.56	7.68	7.82	7.90	—	8.02	8.08	—
$-\log [L^-]$		4.855	4.620	4.485	4.370	4.240	4.170	—	4.077	4.046	—
$\log K_1$		(3.66)	3.55	3.52	3.49	3.50	3.55	—	(3.69)	(3.86)	—
(t) Glycylglycine.											
pH		6.29	6.52	6.64	6.76	6.94	7.07	—	7.30	7.46	—
$-\log [L^-]$		3.955	3.730	3.615	3.500	3.330	3.210	—	3.007	2.876	—
$\log K_1$		(2.76)	2.66	2.65	2.62	2.59	2.58	—	2.60	2.65	—

Figures in parentheses have been omitted in obtaining average values.

TABLE 2. Stability constants of 1 : 1 ferrous-amino-acid complexes, and molar electrode-potentials of ferric-ferrous couples.

(In water at 20° and unit ionic strength of potassium chloride.)

Amino-acid	pK' (Ref. 1) ($\alpha\text{-NH}_2$)	$\log K_1$	E_L° (v)	Amino-acid	pK' (Ref. 1) ($\alpha\text{-NH}_2$)	$\log K_1$	E_L° (v)
Glycine	9.76	3.83	0.380	Glutamic acid ...	9.54	3.52	0.240
Alanine	9.79	3.54	0.340	Asparagine	8.79	3.40	0.440
α -Amino- <i>n</i> -butyric acid...	9.66	3.37	0.370	Arginine	9.21	3.22	0.420
α -Aminoisobutyric acid...	10.09	3.48	0.345	Ornithine	8.93	3.09	0.415
Valine	9.59	3.39	0.380	Proline	10.52	4.07	0.395
Leucine	9.62	3.42	0.362	Hydroxyproline...	9.58	3.94	0.445
β -Phenylalanine	9.18	3.26	0.415	Tryptophan	9.43	3.43	0.415
Serine	9.12	3.43	0.405	Methionine	9.13	3.24	0.400
Threonine	8.86	3.30	0.430	Sarcosine	10.02	3.52	0.380
Aspartic acid	9.56	4.34	0.330	Glycylglycine ...	8.23	2.62	0.360

DISCUSSION

The approximation (ii) provides a rough check on the results if it is assumed that the effect of ionic strength on the stability constants is the same for all amino-acids. From results for glycine (present work and ref. 12) this effect is found to be a decrease of about 0.5 in $\log K_1$ as I increases from 0.01 to 1.0 (KCl). For eight of the ten amino-acids where such a comparison is possible,^{14,17} estimated and observed values of $\log K_1$ do not differ by more than 0.1; only for tryptophan (0.37) is the difference greater than 0.2. On the other hand, previous values¹⁴ for the 1 : 1 ferrous complexes with ornithine and glutamic acid are much higher than were found in the present work (which now places the ferrous-ornithine complex in its correct position in the Irving-Williams²² series).

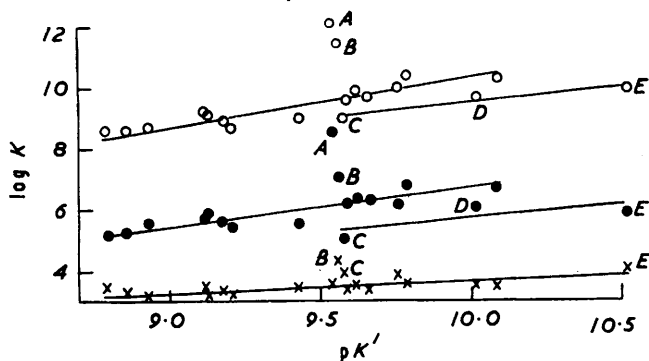
From the Figure the complex-forming ability of an amino-acid with ferrous ion varies much less with the proton, acid, dissociation constant of the amino-acid than was found for ferric ion. In both cases, however, approximately linear relationships are obtained. For the ferrous complexes the slope, α , of the line drawn by the method of least squares is about 0.4. Although subject to considerable uncertainty because of the scatter of the experimental points, values of α of this order of magnitude for the 1 : 1 copper and zinc

²² Irving and Williams, *Nature*, 1948, **162**, 746.

amino-acid complexes are obtained from published^{14,17,23} data if the approximation (ii) is assumed. On the other hand, for the corresponding ferric complexes, α is about 1.7.

The ferrous-aspartic acid complex is more stable than would be predicted from its dissociation constant, probably indicating additional chelation through the β -carboxyl group. The increase in stability is less than for the ferric complex, possibly because of the difference in the charges on the metal ions. The lower charge, and the requirement that the ring formed must be seven-membered, may explain why enhanced stability is not observed for the ferrous-glutamic acid complex. Similarly, and unlike the ferric complex, 1 : 1 ferrous-glycylglycine is less stable than predicted from the dissociation constant of glycylglycine. In contrast to the ferric complexes, the 1 : 1 ferrous-proline and -hydroxyproline complexes are somewhat more stable than expected.

Dependence of stability constants and oxidation-reduction potentials of iron complexes on dissociation constants of amino-acids.



Anomalous acids:

- A, glutamic acid. C, hydroxyproline. E, proline.
 B, aspartic acid. D, sarcosine.
- $\log K_{Fe^{3+}}$ (ref. 1). ● $\log K_{Fe^{3+}} - \log K_{Fe^{2+}}$. × $\log K_{Fe^{2+}}$.

The difference of 1.3 between $\alpha_{Fe^{3+}}$ and $\alpha_{Fe^{2+}}$ for the 1 : 1 amino-acid complexes gives directly from eqn. (iii) their change of standard oxidation-reduction potential with dissociation constant as -0.075 volt/ pK' unit. The basic skeleton, $-N \cdot C \cdot C \cdot O-$, of the amino-acid chelating system is also found in oxine (8-hydroxyquinoline) and its aza-derivatives. However, probably because of large individual variations in resonance energies of the ligands as different ring carbon atoms are replaced by nitrogen, the stability constants of neither the 1 : 1 ferric nor the 1 : 1 ferrous series comprising 8-hydroxyquinoline,^{2,13} 8-hydroxyquinoline-5-sulphonic acid,^{2,13} and polyaza-1-naphthol² complexes show much correlation with dissociation constants. On the other hand, the difference, $\log K_{Fe^{3+}} - \log K_{Fe^{2+}}$, is roughly linear with pK' and of slope about 0.8, giving $dE_L^\circ/d(pK') \sim -0.045$ v. From recent measurements,¹² the plot of oxidation-reduction potentials of 1 : 3 iron complexes of substituted 8-hydroxyquinolines in 50% dioxan-water against pK' (pK_{OH}) gives a straight line with a slope, $dE_L^\circ/d(pK')$, ~ -0.080 v; except for 5-cyano-8-hydroxyquinoline ($\delta \sim 0.12$ v) the deviation, δ , of points from this line averages 30 mv. However, if potentials are plotted against the sum, $pK_{NH} + pK_{OH}$, which has been suggested as a measure of ligand basicity,⁷ no simple relation is found.¹² If the factors governing the binding of protons and cations are similar, it seems reasonable to expect that the stability constants of metal chelates might depend on the dissociation constant of the more strongly proton-binding of the two chelating groups. Hence, for the substituted 8-hydroxyquinolines where pK_{OH} is 5–8 log units greater than pK_{NH} , pK_{OH} rather than $pK_{NH} + pK_{OH}$ should be used in comparing the observed oxidation-reduction potentials. If this

²³ Perkins, *Biochem. J.*, 1954, **57**, 702; Monk, *Trans. Faraday Soc.*, 1951, **47**, 292, 297.

is done, 5-formyl- and 5-cyano-8-hydroxyquinoline complexes are found to have potentials much nearer the predicted values than if the comparison uses the sums of the pK values. Similarly, if an error of about 10 mv is assumed, the oxidation-reduction potentials of five 1 : 3 iron-substituted phenanthroline complexes (probably in dioxan-water)¹² are linear with pK_{NH} . Results for some 1 : 3 iron complexes of 5-substituted 1 : 10-phenanthrolines in *m*-sulphuric acid^{3,11} show E_L° to vary directly with pK' , within experimental uncertainty, giving $dE_L^\circ/d(pK') = -0.120$ v. If it is assumed that, because the ligands are neutral molecules, the pK' effect is roughly the same for successive complex formation, $dE_L^\circ/d(pK')$ for the corresponding 1 : 1 complexes thus becomes of the order of -0.040 v. For all three groups (amino-acid, 8-hydroxyquinoline, and phenanthroline) of 1 : 1 iron complexes in aqueous solution it appears, therefore, that as the basic strength of the ligand is increased the firmness with which the valency electron is bound to ferrous ion varies in approximately the same manner. Whether this is also true of ligands containing other skeletons, such as $-O\cdot C\cdot C\cdot C\cdot O-$, is at present being examined.

DEPARTMENT OF MEDICAL CHEMISTRY,
THE AUSTRALIAN NATIONAL UNIVERSITY, CANBERRA.

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