Quantitative Studies of the Avidity of Naturally Occurring Substances for Trace Metals

1. AMINO-ACIDS HAVING ONLY TWO IONIZING GROUPS

By A. ALBERT
Department of Medical Chemistry, The Australian National University*

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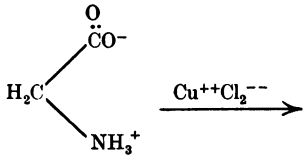
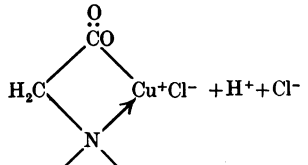
It has long been known that certain naturally occurring substances (namely amino-acids, proteins and porphyrins) form stable complexes with the ions of heavy metals. Although the metals in these complexes no longer react as simple inorganic ions, the complexes are in equilibrium with a (usually exceedingly minute) proportion of inorganic ions from which they were formed. It has recently been found that other biochemically important substances have a similar avidity for metallic ions, namely the pterins, riboflavin and various purines (cf. Albert & Brown, 1949; Albert, 1950). Hence it may be profitable to think of cells and tissues of all kinds as areas in which traces of metallic ions are competed for by the various complex-forming agents present.

Accordingly, it is desirable to know quantitatively, (i) the avidity of each naturally occurring complex-forming agent for the ions of various heavy metals, and (ii) the avidity of each biologically significant heavy metal for various complex-forming agents (including foreign toxic substances).

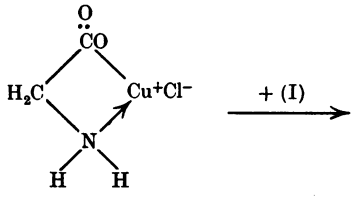


(I)

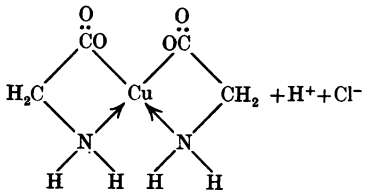


(II)

Up to the commencement of the present work in 1949, very few quantitative investigations of this



(II)



(III)

kind had been made. For example, the only bivalent amino-acids studied were (i) glycine with copper (various authors, see Table 3), with nickel, cobalt, zinc, cadmium and mercury (Flood & Loras, 1945), and with magnesium (Greenwald, 1939); (ii) alanine with copper (Borsook & Thimann, 1932; Keefer, 1946). After the completion of the present work, figures for glycine, alanine, valine and leucine with copper, zinc, cobalt and manganese appeared (Maley & Mellor, 1950).

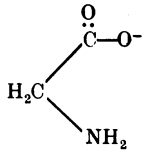
Such comparative neglect is not surprising because the nature of the equilibria concerned in such complex formation was not properly understood before 1941 when Bjerrum's book *Metal Ammine Formation in Aqueous Solution* was published in Denmark. It is now agreed that the equilibrium between a complex-forming agent and an ion is usually thermodynamically reversible: it occurs instantly and without appreciable energy of activation. Hence these equilibria are correctly represented by mass-action equations. Moreover, as Bjerrum was the first to point out, complex formation usually follows a stepwise course. For example the reaction between glycine (I) and cupric chloride gives rise initially to the equilibrium (I) $\rightleftharpoons$ (II).

At a higher pH, another equilibrium occurs, namely (II) $\rightleftharpoons$ (III).

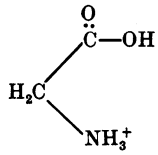
In order to convert these chemical equations to algebraic equations capable of yielding stability constants, the term 'free complex-forming species' will be introduced. This refers to that particular form

* Present Address, 183 Euston Road, London, N.W. 1.

of the complex-forming agent with which the complex is in equilibrium. For example, in the case of glycine, the complex-forming species is the anion (IV) which is present to the extent of only 0·25 % in a solution of glycine at the physiological pH (7·3). The remainder of the glycine is present as the zwitterion (I), a species which is not capable of chelation, but which yields further quantities of (IV) (when the latter is largely removed by chelation) until a new equilibrium is reached. Hence (IV) is an intermediary in the equilibria (I) ⇌ (II) and (II) ⇌ (III) previously given.

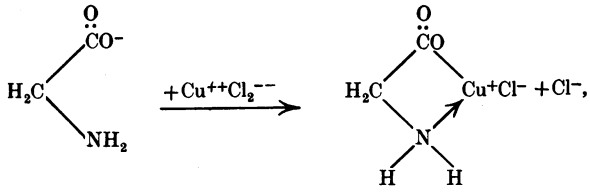


(IV)



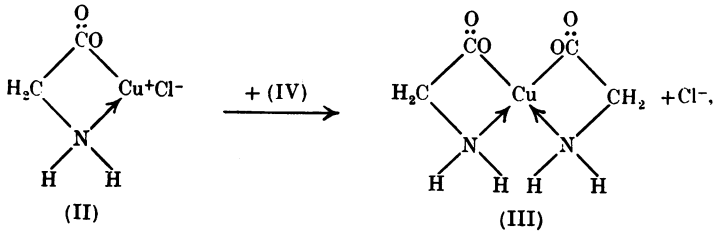
(V)

Accordingly, the equilibria which are relevant to the calculation of stability constants are as follows:



(IV) (II)

for which equation (i) is the appropriate algebraic expression, and



(II) (III)

to which equation (ii) refers.

$$K' = \frac{[\text{complex (II)}]}{[\text{free metallic ions}]\,[\text{free complex-forming species}]} \quad \text{(i)}$$

$$K'' = \frac{[\text{complex (III)}]}{[\text{complex (II)}]\,[\text{free complex-forming species}]} \quad \text{(ii)}$$

By combining these two chemical equations or, which is the algebraic equivalent, multiplying (i) and (ii), an overall stability constant (K_s) for the entire reaction is obtained, hence

$$K_s = \frac{[\text{complex (III)}]}{[\text{free metallic ions}]\,[\text{free complex-forming species}]^2}$$

or, more succinctly,

$$K_s = K' . K''. \quad \text{(iii)}$$

In proportion as complex formation occurs, hydrogen ions are liberated as shown in the original chemical equations. The hydrogen-ion concentration produced in this way provides a means of measuring the avidity of a complex-forming agent for a given metal (Bjerrum, 1941).

The work to be described in this paper comprises the determination of K_s for the reaction between amino-acids having only two ionizing groups and the following ions, Cu^{++}, Ni^{++}, Zn^{++}, Co^{++}, Cd^{++}, Fe^{++}, Mn^{++}, Mg^{++} and Fe^{+++}. The customarily complicated equations associated with this type of work have been simplified wherever possible.

METHODS

(All determinations were carried out at 20°.)

Materials. The metallic ions were used in the form of the following salts of A.R. purity: $CuCl_2$, $Ni(NO_3)_2$, $ZnCl_2$ (prepared by double decomposition of $ZnSO_4$ and $BaCl_2$ and then standardized gravimetrically), $Co(NO_3)_2$, $CdSO_4$, $FeSO_4$, $MnSO_4$, $MgSO_4$, and ferric ammonium sulphate. In addition, some preliminary studies were carried out with $Cu(NO_3)_2$, $CuSO_4$ and $ZnSO_4$. Stock solutions (0·05 M) were used except for the Fe^{++}, Fe^{+++} and Mn^{++} salts. The 0·05 M solution of ammonium molybdate contained 0·883 g. of A.R. $(NH_4)_6Mo_7O_{24}.4H_2O$ in 100 ml. and was brought to pH 5 with 0·5 equiv. of KOH before use.

The glycine (A.R.), α-alanine, valine (microbiologically tested), phenylalanine, serine (microbiologically tested), methionine, asparagine, tryptophan and taurine were obtained from British Drug Houses Ltd. The norleucine was from Ashe Laboratories Ltd., the β-alanine from L. Light and Co. Ltd. and the proline from British Chemicals and Biologicals Ltd. These acids were all dried for 1 hr. at 120° before weighing. The purity of the amino-acids was checked: (i) by paper chromatography in butanol-acetic acid, the papers being examined for adventitious spots in ultraviolet light of wavelength 254 mμ. and also (by daylight) after spraying with ninhydrin; (ii) by potentiometric titration with 0·1 N-KOH and examination of the entire curve for deviation from

the calculated shape. The α-alanine, β-alanine and valine failed to pass these tests and were recrystallized from water until satisfactory.

Titrations. Each amino-acid (in 0·01 M-aqueous solution wherever possible) was titrated with 0·1 N-KOH (carbonate-free), first in the absence of metals and then in the presence of 1 equiv. of the appropriate metal salt (0·005 M for bivalent and 0·0033 M for tervalent ions). Thus the molar ratio of complex-forming agent to metal was 2:1 for all bivalent ions. The total volume was 50 ml., whether metals were present or not. The amount of alkali used for each titration was 5 ml. (i.e. the equivalent of the amount of complex-forming agent taken) and this was added in ten equal portions. The pH was recorded after each addition by means of a Cambridge Instrument Company's glass-electrode potentiometer set with a sintered-glass calomel half-electrode. N_2 was used for stirring and to maintain an inert atmosphere above the solutions. Boiled-out water was used for the Co^{++}, Fe^{++} and Mn^{++} titrations.

The pK_a value of each amino-acid (for the equilibrium (I) $\rightleftharpoons$ (IV)) was calculated from nine equidistant points on the titration curve. The means of the results are recorded in Table 2: all the results lay within the range $pK_a \pm 0{\cdot}05$. Although these pK_a results could have been derived from the literature, it was thought preferable to determine them under the experimental conditions to be used with the metallic ions.

The results of these titrations were used to solve the above equation.

Calculations. As the concentration of free metallic ions is not measured in this method, equation (i) is rewritten as (iv) (following the practice of Flood & Loras, 1945) and (ii) as (v). The derivations are given in the Appendix to this paper.

$$K' = \bar{n}/(1-\bar{n})\,[\mathrm{Sc}], \qquad \text{(iv)}$$

$$K'' = (\bar{n}-1)/(2-\bar{n})\,[\mathrm{Sc}], \qquad \text{(v)}$$

where [Sc] is the concentration of free chelating species, as defined above, and $\bar{n}$ is the average number of molecules of complex-forming agent bound by one atom of metal.

These equations can now be solved because the experimental data allow the calculation of [Sc] from equation (vi) and of $\bar{n}$ from equation (viii). Simplified forms of (vi) and (viii) will also be discussed. Equation (vi) is derived from an equation given by Flood & Loras (1945):

$$\log[\mathrm{Sc}] = \log\{[\mathrm{HSc^\circ}] - [\mathrm{KOH}] - [\mathrm{H^+}] + [\mathrm{OH^-}]\} - \log\left\{\frac{[\mathrm{H^+}]}{K_a} + \frac{2[\mathrm{H^+}]^2}{K_a K_{a'}}\right\} \qquad \text{(vi)}$$

where [HSc°] is the concentration of complex-forming agent (all species), before the metal was added, [KOH] is the concentration of KOH which would be present if the complex-forming agent and metal were absent (in the present work [KOH] equals 0·01 M at the end of most titrations), and K_a is the ionization constant of the equilibrium (I) $\rightleftharpoons$ (IV) and $K_{a'}$ is that of (I) $\rightleftharpoons$ (V).

Because of the high affinity of Cu for amino-acids, titration with alkali did not uncover the lower values of $\bar{n}$. Hence another portion of the complex-forming agent was titrated with acid in order to obtain these values and +[HCl] was then substituted for −[KOH] in equation (vi).

The $[H^+]$ term vanishes in equation (vi) above pH 4 and the $[OH^-]$ term vanishes below pH 10. When working between pH 4 and 10, a greatly simplified version of (vi) can be used, namely (vii), for all pH values that are more than 1 unit higher than the $pK_{a'}$.

$$\log[\mathrm{Sc}] = (\mathrm{pH} - \mathrm{p}K_a) + \log\{[\mathrm{HSc^\circ}] - [\mathrm{KOH}]\}. \qquad \text{(vii)}$$

As the $pK_{a'}$ of α-amino-acids is about 2, equation (vii) has been widely used in the present work.

Equation (viii) is the Bjerrum equation for $\bar{n}$ which has been further refined by incorporating the function α (Phillips, 1950).

$$\bar{n} = \frac{[\mathrm{HSc^\circ}] - \alpha\,[\mathrm{Sc}]}{[M^\circ]}, \qquad \text{(viii)}$$

where $[M^\circ]$ is the total concentration of metal (free or combined) and α has the value given by equation (x), except when the pH is more than 1·4 units above $pK_{a'}$ (which usually happened in the present work) when the simplified equation (ix) applies.

$$\alpha = \frac{[\mathrm{H^+}]}{K_a} + 1, \qquad \text{(ix)}$$

$$\alpha = \frac{[\mathrm{H^+}]}{K_a} + \frac{[\mathrm{H^+}]^2}{K_a K_{a'}} + 1, \qquad \text{(x)}$$

$\bar{n}$ can be determined by the greatly simplified equation (xi)

$$\bar{n} = \frac{2[\mathrm{KOH}]}{[\mathrm{HSc^\circ}]}, \qquad \text{(xi)}$$

when the pH is at least 1·4 units lower than pK_a and more than 1·4 units above $pK_{a'}$. Actually, a considerable range of results can be treated by this equation, for example, in the case of glycine all results falling between pH 3·6 and 8·5, i.e. about three-quarters of all the results obtained.

RESULTS

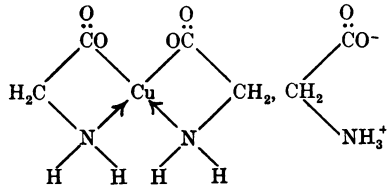
By titrating each amino-acid in the presence of the various metallic ions, pH values were obtained for each addition of alkali: from these readings values for [Sc] and $[\bar{n}]$ were calculated from equations (vi) and (viii). To obtain the lower values of $\bar{n}$ for copper, an additional titration had to be performed with 0·1 N-hydrochloric acid, but the avidity of the other metals was not high enough to necessitate this. Variation in the nature of the anion made no difference to the results, e.g. zinc chloride could be replaced by zinc sulphate and copper chloride by copper nitrate or sulphate, but no pH reading lower than 3·9 was used for sulphate on account of the interference expected (and found) from the second ionization constant of sulphuric acid ($pK = 1{\cdot}9$).

Table 1 gives a typical example of this stage of the process together with values for K' and K'' (as logs) calculated by equations (iv) and (v), respectively. This table also gives examples of values for K_s obtained by combining K' and K'' (according to equation (iii)) and of K_s calculated by equation (xii) (see below). The latter is rather the more accurate of the two, but the differences were usually found to be small. The application of (iv) or (v) to any given pH reading led to either K' or K'' (application of the inappropriate equation gave a negative value which was discarded).

Table 1. *Potentiometric titration of* L-*asparagine* (0·01 M; $pK_a = 8·85$) *and cadmium sulphate* (0·005 M); *all in* 50 *ml.*

0·1 N-KOH (ml.)	pH	log [Sc]	$\bar{n}$	log K'	log K''	
0	4·81	—	—	—	—	—
0·25	6·12	$\bar{5}·25$	0·10	3·80	—	—
0·5	6·50	$\bar{5}·60$	0·20	3·80	—	—
1·0	6·85	$\bar{5}·90$	0·40	3·92	—	—
1·5	7·20	$\bar{4}·20$	0·57	3·93	—	—
2·0	7·45	$\bar{4}·38$	0·74	—	—	—
2·5	7·70	$\bar{4}·55$	0·93	—	—	Value of log [Sc] when $\bar{n} = 1·00$, found graphically, is 4·61, whence log K_s = 6·78 (by equation xii)
3·0	7·95	$\bar{4}·70$	1·11	—	—	
3·5	8·21	$\bar{4}·84$	1·26	—	2·70	—
4·0	8·50	$\bar{4}·95$	1·42	—	2·91	—
4·5	8·93	$\bar{3}·08$	1·56	—	3·03	—
5·0	9·32	—	—	—	—	—
			Mean	3·87	2·90	whence log $K_s = 6·77$ (by equation iii)

The values obtained for log K' and K'' (from (iv) and (v)) were converted to antilogarithms, averaged and reconverted to logarithms. Not all values were selected for this process, as they are not all of equal accuracy. For example, when $\bar{n}$ lies between 0·7 and 1·3, some molecules of (II) are beginning to take on another molecule of glycine before all the molecules of (I) have reacted with the metallic ion. Again, when $\bar{n}$ is only a small fraction of $(1 - \bar{n})$, knife-edge conditions prevail, so that a small inaccuracy in calculating $\bar{n}$ makes a large error in K'. Finally, when $\bar{n}$ is approaching 2, the conditions are often such (even in 0·01 M-solutions and in the absence of excess glycine) that some of the molecules of the complex (III) form a weak association (e.g. VI) with an extra molecule of glycine (cf. Flood & Loras, 1945). Hence the most reliable values of [Sc] for calculating K' or K'' are dependably found from $\bar{n} = 0·10$ to 0·70 and from 1·30 to 1·70.



(VI)

However the most reliable values of all for K_s are obtainable from equation (xii) which is valid only when $\bar{n} = 1$, in stepwise reactions such as are being considered here (Bjerrum, 1941). The use of this equation (which is derived in the Appendix) avoids all the possible sources of error mentioned above.

$$K_s = 1/[Sc]^2, \qquad \text{(xii)}$$

i.e. $\log K_s = -2 \log [Sc]$.

The correct value of [Sc] corresponding to $\bar{n} = 1$ was not always available from a direct potentiometric reading, but was found by plotting values of $\bar{n}$ against $-\log [Sc]$, as in Fig. 1. Curves obtained in this way have been termed 'formation curves' by Bjerrum (1941), and are valuable because they provide evidence as to whether the reaction under consideration is stepwise or not. Fig. 1 gives the formation curves for the complexes of glycine with nickel, cobaltous and ferrous ions. From such curves it was found that all the α-amino-acids reported here gave

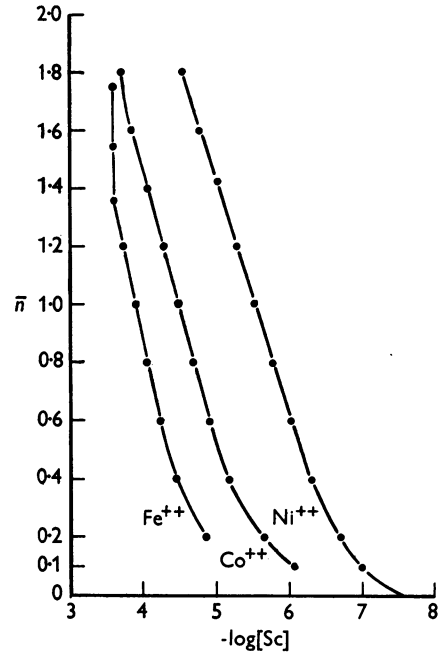


Fig. 1. Formation curves of the glycine-ferrous, glycine-cobaltous and glycine-nickel complexes. The average number of molecules ($\bar{n}$) of glycine united to one atom of metal has been plotted against the negative logarithm of the concentration of the chelating species of glycine [Sc].

Table 2. *Stability constants of the complexes of amino-acids and metallic cations*

(Values of log K_s calculated from the values of log [Sc] at $\bar{n} = 1{\cdot}00$, by equation xii.)

Amino-acid	pK_a	$pK_{a'}$	Cu^{++}	Ni^{++}	Zn^{++}	Co^{++}	Cd^{++}	Fe^{++}	Mn^{++}	Mg^{++}	Fe^{+++}
Glycine	9·86	2·22	15·4	11·0	9·3	8·9	8·1	7·8	5·5	4 (approx.)	Nil
L-Proline	10·68	1·93	16·8	11·3	10·2	9·3	8·7	8·3	5·5 (approx.)	<4	Nil
DL-Tryptophan*	9·55	2·20	15·9	10·2	9·3	8·5	8·1	7·6	5 (approx.)	<4	Nil
L-Asparagine	8·85	2·14	14·9	10·6	8·7	8·4	6·8	6·5	4·5 (approx.)	4 (approx.)	Nil
DL-Norleucine†	9·96	2·25	15·5	11·1	10·4	9·4	8·7	8·6	5 (approx.)	<4	Nil
DL-Alanine	9·97	2·22	15·1	—	—	8·4	—	7·3	—	—	—
DL-Valine	9·72	2·20	15·1	—	—	8·6	—	6·8	—	—	—
DL-Phenylalanine	9·31	2·04	14·9	—	—	7·9	—	6·3	—	—	—
DL-Serine	9·24	2·20	14·6	—	—	8·0	—	7·0	—	—	—
DL-Methionine	9·34	2·20	14·7	—	—	7·9	—	6·7	—	—	—
β-Alanine	10·36	3·60	12·9	—	—	7‡ (approx.)	—	4‡ (approx.)	—	—	—
Taurine	9·08	1·5 (approx.)	8‡ (approx.)	—	—	4‡ (approx.)	—	Nil	—	—	—

* Because of its sparing solubility, tryptophan was titrated at 0·005 M.

† Because of the sparing solubility of its complexes, titrations of norleucine were carried out at 0·0013 M.

‡ These values were obtained from log K' (at low values of $\bar{n}$) by adding (log $K' - 1$), a method which works well with α-amino-acids but which may not be valid here. At higher values of $\bar{n}$, there was precipitation.

stepwise addition with all the divalent metals studied.

Table 2 contains all the values of log K_s calculated from (xii), reported to one place of decimals. The common practice of reporting to two places is to be

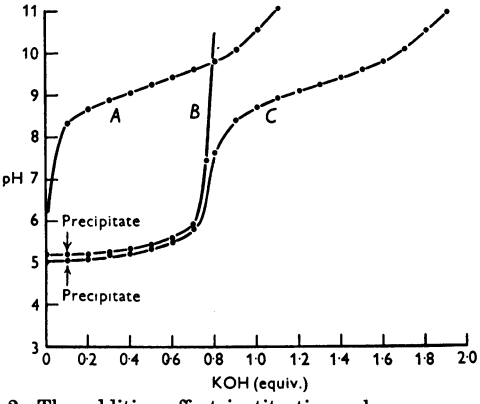


Fig. 2. The additive effect in titration, when no complex formation takes place. *A*, 0·01 M-boric acid; *B*, 0·005 M-cupric chloride; *C*, 0·01 M-boric acid + 0·005 M-cupric chloride.

deprecated as the figures depend on differences between pairs of readings from a pH apparatus where the second place of decimals has the usual uncertainty. Values obtained from (iii) were found to be close or identical in all cases where the comparison could be made. (This comparison was not usually possible for Mn^{++} and Mg^{++} because high values of $\bar{n}$ did not appear before one equivalent of alkali had been added. Again, the sparing solubility of the 2:1 complexes of proline with zinc and cadmium made few values of $\bar{n}$ available above 1·0.)

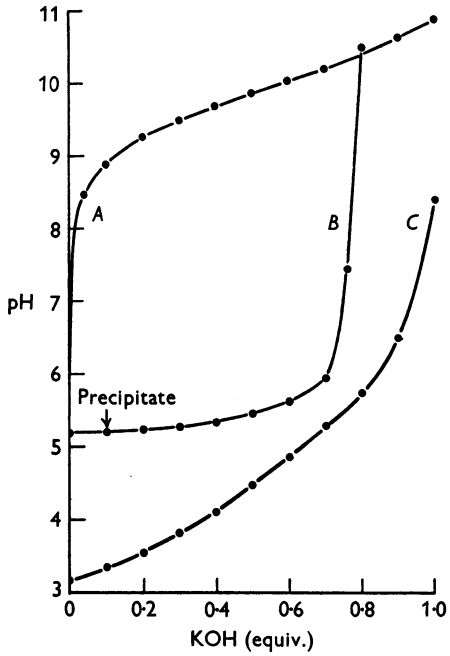


Fig. 3. The non-additive effect in titration, when complex formation takes place. *A*, 0·01 M-glycine (1 equiv.); *B*, 0·005 M-cupric chloride (1 equiv.); *C*, 0·01 M-glycine + 0·005 M-curpric chloride.

In some cases, Mg^{++} gave no evidence of complex formation: the titration curves exactly duplicated

those obtained in the absence of Mg^{++}. However, it is not easy to recognize a value of K' lying between 0 and 1·8 by the present method so that a complex of low stability could be overlooked.

In no case did Fe^{+++} form a complex, the titration curves always coinciding with those obtained in the absence of the amino-acid. The precipitate obtained in the early stages of the titration of glycine and ferric ammonium sulphate was entirely inorganic and agreed with the properties of a basic ferric sulphate $(Fe_2O_3)_2SO_3$; identical material was obtained after the addition of 1·67 ml. of 0·1 N-alkali, i.e. one-third way through the titration where $\bar{n}$ would normally have the value of 1.

Fleck & Ward (1933) observed the combination of oxine and the molybdenyl cation (MoO_2^{++}) from ammonium molybdate. In the present work, an attempt to follow any similar combination by glycine was made difficult by interference with $\log K''$ by the pK_a of ammonia. However a steady value for $\log K'$ (3·7) was obtained. Hence $\log K_s$ is approx. 6·4 if it is assumed that here $\log K_s$ is approximately equal to $(2 \log K' - 1)$ as is the case with the combinations listed in Table 2.

DISCUSSION

When a mixture of two substances which do not mutually form a complex is titrated with alkali, the curve obtained reproduces, in turn, the component curves, as is shown in Fig. 2 (boric acid and cupric chloride). When, however, the two substances can form a complex by liberation of hydrogen ions (as is the case with the α-amino-acids), an entirely different picture is seen. The new curve no longer traverses, in turn, the component curves, but strikes a path that is independent of that of the complex-forming agent and is almost always independent of that of the metal. Most important of all, the usual precipitates of metallic hydroxides (or basic salts) no longer take place upon the first addition of alkali. This is shown plainly in Fig. 3 (glycine and cupric chloride). The extent of the displacement (curve *A* to curve *C*) forms a measure of the avidity of a particular complexing agent for a given metal, and is dealt with algebraically in equations (i)–(xii).

The results obtained in this way (Table 2) show that the differences in the avidity of the (divalent) α-amino-acids for any particular metallic ion are not strikingly large (e.g. the affinity for cobalt varies over only a 30-fold range). Proline has the greatest avidity, whereas the following have the least: asparagine, serine, methionine and phenylalanine.

Apparently, all the bivalent amino-acids exert the same order of preference for the various metallic ions, e.g. Cu^{++} is always preferred to Ni^{++}, then follow (in order) Zn^{++}, Co^{++}, Cd^{++}, Fe^{++}, Mn^{++} and Mg^{++}. This is shown to be true for glycine, norleucine, asparagine, proline and tryptophan, and the given sampling (Cu^{++}, Co^{++}, Fe^{++}) indicates that it is, most likely, true also for alanine, valine, phenylalanine, serine and methionine. No measurements were made on norvaline, leucine, isoleucine, glutamine and threonine as they have close analogues in Table 2 and it seemed highly likely that similar results would be obtained. No complexes are formed with Fe^{+++}.

The order of metals found is in agreement with Mellor & Maley's (1948) series of divalent metals, namely:

$$Hg > Cu > Ni > Pb > \begin{cases} Co \\ Zn > Cd > Fe > Mn > Mg. \end{cases}$$

This order has been demonstrated for the following complex-forming agents, ammonia, ethylenediamine, propylenediamine, salicylaldehyde, acetylacetone, 8-hydroxyquinoline (Mellor & Maley, 1948; Maley & Mellor, 1949), salicylaldehyde-5-sulphonic acid (Calvin & Melchior, 1948) and 2:2′:2″-triaminotriethylamine (Ackermann, Prue & Schwarzenbach, 1949). Moreover, Flood & Loras (1945) have demonstrated an order for glycine consistent with that found in the present work, namely:

$$Hg > Cu > Ni > Zn > Co > Cd.$$

It will be shown in Part 2, that the pteridines depart considerably from this order.

The lack of affinity for ferric ions shown by the α-amino-acids forms a striking contrast with the behaviour of 8-hydroxyquinoline and 8-hydroxyquinoline-5-sulphonic acid which combine with Fe^{+++} as avidly as they do with Cu^{++}.

The $\log K_s$ values of Table 2 are compared in Table 3 with all such values as have appeared in the literature. In general, the agreement is good, in view of the fact that each of the sources given in Table 3 used a different method of calculating the results and, in some cases, a different experimental method from those used in the present work.

Some values for β-alanine and taurine are included in Table 2. Of these, only the complex of β-alanine with copper could be measured accurately. Although moderately stable, the copper was fifty times less strongly held than in the least avid of the α-amino-acids. The corresponding figure for the addition of the first two nitrogens in the complex between copper and ammonia, $Cu^{++}(NH_3)_4$, is 8·16 (Bjerrum, 1941).

If at some pH value all the amino-acids in Table 2 were equally well ionized, their success in competing for traces of metallic ions at that pH would be in proportion to their stability constants alone. However, at the physiologically interesting pH of 7·3, the amount of anion (e.g. IV) present varies from 0·04 % in the case of proline to 2·45 % in the case of asparagine, i.e. over a 60-fold range. It is evident from equation (ii) that a sufficient excess of anion (the chelating species) could enable a given substance to

Table 3. *Comparison of results for log K_s (from Table 2) with those in the literature*

Amino-acid		Cu^{++}	Ni^{++}	Zn^{++}	Co^{++}	Cd^{++}	Mn^{++}	Mg^{++}
Glycine	(1)	15·4	11·0	9·3	8·9	8·1	5·5	4 (approx.)
	(2)	15·1	—	—	—	—	—	—
	(3)	15·1	—	—	—	—	—	—
	(4)	15·2	10·6	8·9	8·4	7·1	—	—
	(5)	15·4	—	9·7	8·9	—	6·6	—
	(6)	15	—	—	—	—	—	—
	(7)	—	—	—	—	—	—	4 (approx.)
Alanine	(1)	15·1	—	—	8·4	—	—	—
	(2)	15·0	—	—	—	—	—	—
	(5)	14·8	—	—	8·8	—	—	—
Valine	(1)	15·1	—	—	8·6	—	—	—
	(5)	14·5	—	—	8·2	—	—	—

(1) Present work.
(2) Keefer (1946), by polarography (pH 9·8–11·8).
(3) Laitinen, Onstott, Bailar & Swann (1949), by polarography (pH 6·3–7·9).
(4) Flood & Loras (1945), potentiometry with glass electrode.
(5) Maley & Mellor (1950), potentiometry with glass electrode.
(6) Riley & Gallafent (1931), potentiometry with copper electrode (cf. Borsook & Thimann, 1932).
(7) Greenwald (1939).

compete successfully with a substance of somewhat higher K_s and higher basic pK_a. This actually occurs at pH 7·3 in the present series and asparagine can compete successfully with glycine and glycine with proline for all the metals used. This effect is greatest where the less avidly bound metals are concerned, e.g. asparagine is five times as successful as proline in securing Fe^{++}, and seven times as successful in securing Mn^{++}.

This situation (namely that pK_a as well as K_s determine the outcome of competition for a metallic ion) will be enlarged upon in Part 2. It is already evident that the function $\bar{n}$, which represents the fraction (of the theoretically formable amount of complex) actually formed at a given pH, can be of greater interest to biochemists than the stability constant which is derived from it.

SUMMARY

1. The avidities of the bivalent α-amino-acids for the ions of heavy metals have been measured and recorded in the form of stability constants.

2. The determinations were made potentiometrically, using a glass electrode, and consist fundamentally of titrating the complex-forming agent (e.g. glycine) in the absence and in the presence of the various metallic ions. The relevant calculations are outlined and some short cuts indicated.

3. For all amino-acids, the stability constants were found to follow the same order, namely

$$Cu^{++} > Ni^{++} > Zn^{++} > Co^{++} > Cd^{++} > Fe^{++} > Mn^{++} > Mg^{++}.$$

4. For any one metal, the stability constants for the various α-amino-acids varied over only a 100-fold range.

5. It is pointed out that the success of the various amino-acids in competing with one another for a trace of a metallic ion will be proportional not only to their stability constants but also to their basic ionization constants.

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Appendix

A. Derivation of equations (iv) and (v).

$$\bar{n} = \frac{[II] + 2[III]}{[M] + [II] + [III]},$$

where $[M]$ is the concentration of free metallic ions and II and III are the complexes illustrated in the introduction (p. 531). Then

$$\frac{\bar{n}}{1-\bar{n}} = \frac{[II] + 2[III]}{[M] - [III]} = \frac{[II]}{[M]},$$

when [III] is small, also

$$\frac{\bar{n}-1}{2-\bar{n}} = \frac{[III] - [M]}{2[M] + [II]} = \frac{[III]}{[II]},$$

when $[M]$ is small. Hence (from i)

$$K' = \bar{n}/1 - \bar{n}[Sc], \quad \text{(iv)}$$

provided [III] is small, and

$$K'' = (\bar{n} - 1)/(2 - \bar{n})\,[Sc] \quad \text{(v)}$$

provided $[M]$ is small.

B. Derivation of equation (xii).

When $\bar{n} = 1$, $[II] + 2[III] = [M] + [II] + [III]$; hence

$[M] = [III]$ and, as $K_s = [III]/[M]\,[Sc]^2$,

$$K_s = 1/[Sc]^2. \quad \text{(xii)}$$

The Amino-acid Composition of the Protein Material in Soil

By J. M. BREMNER

Chemistry Department, Rothamsted Experimental Station, Harpenden, Hertfordshire

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Recent work (Kojima, 1947*a*; Bremner, 1949) has shown that a considerable fraction of the organic nitrogen of soil is in the form of protein, about one-third of it being liberated as α-amino nitrogen by acid or alkaline hydrolysis. No method of separating this protein material from other soil constituents has yet been found and information about its amino-acid composition is scattered and incomplete. The following amino-acids have been isolated as hydrolysis products of soil organic matter: leucine (Suzuki, 1906–8; Robinson, 1911; Lathrop, 1917; Kojima, 1947*b*), isoleucine (Robinson, 1911; Kojima, 1947*b*), valine (Kojima, 1947*b*), alanine and proline (Suzuki, 1906–8), arginine and histidine (Schreiner & Shorey, 1910*a*, *b*; Lathrop, 1917; Tokuoka & Dyo, 1937), lysine (Shorey, 1913; Lathrop, 1917), aspartic acid (Suzuki, 1906–8; Kojima, 1947*b*) and tyrosine (Lathrop, 1917). The presence of large amounts of extraneous material in soil hydrolysates makes preparative work so difficult that few workers have attempted more than qualitative isolation of some of the amino-acids present. In consequence the results obtained by isolation are too limited to permit deductions regarding the amino-acid distribution in soil.

The object of the present investigation was to identify the various amino-acids liberated by hydrolysis of soil protein and to determine whether or not the proteins in different soils are similar in their amino-acid composition. The method used for amino-acid analysis, that of partition chromatography on paper, permitted a comparison of the amino-acid distribution in a variety of soils.

Preliminary results of this work have already appeared (Bremner, 1950).

MATERIALS, METHODS AND RESULTS

Soils. The ten soil samples used differed greatly in pH value, organic-matter content and cultural history. The selection included four neutral fen soils (nos. 1–4), an acid fen soil (no. 8), an acid peat (no. 7) and a Russian chernozem (no. 9). The latter was from a bulk stock used by K. K. Gedroiz in his classical investigations on 'base exchange'. The three Rothamsted soils were selected to provide extreme contrasts in cultural history: sample 5 was from a continuous wheat plot (Broadbalk 2*B*) receiving farmyard manure annually, sample 6 was from unmanured land (Hoosfield) fallowed for 3 years after over a 100 years under alternating wheat and fallow, and sample 10 was taken in 1943 from an arable field (Sawyers II) down to grass from 1928 to 1940. The pH values of the samples were determined with the glass electrode, N contents by a micro-Kjeldahl procedure and $CaCO_3$ contents by the Schollenberger (1930) technique (Table 1).

Hydrolysis. The soils were hydrolysed by boiling under reflux for 24 hr. with 6 N-HCl (4 ml./g. of air-dried soil). In the case of sample 3, $CaCO_3$ was removed before hydrolysis by leaching with cold 0·1 N-HCl, which extracts insignificant amounts of organic N from soil. The hydrolysis mixtures were filtered, the residues washed thoroughly with hot water, and the filtrates concentrated several times *in vacuo* to remove HCl. The residues were dissolved in water and the solutions brought to pH 7·0 by addition of NaOH. The precipitates formed on neutralization were removed by filtration, washed with hot water, and the filtrates concentrated to small volume *in vacuo* and desalted by the electrical method of Consden, Gordon & Martin (1947). The brown precipitates formed during desalting were removed by filtration and the filtrates concentrated *in vacuo*. The N contents of the concentrated solutions were determined by a micro-Kjeldahl procedure and sufficient water was added to each to give a total N content of about 2 mg./ml.