A Study of the Effect of Amino Acid Structure on the Stabilities of the Complexes Formed with Metals of Group II of the Periodic Classification

By D. J. PERKINS

Department of Chemical Pathology, St George's Hospital Medical School, London, S.W. 1

(Received 18 May 1953)

In a previous structural study of stability constants of α -amino acid metal complexes (Perkins, 1952) the work was restricted to amino acids closely related to glycine. In this paper the work is extended to a wide range of α -amino acids to discover the effect of different functional groups. Most of the amino acids are closely related to α -alanine and norvaline. Albert (1952) has published stability constants for the complexes of a number of these amino acids with a selection of metal ions. In the present work only the metals beryllium, zinc, cadmium and mercury of group II of the periodic classification were used.

EXPERIMENTAL

Materials. The following A.R. salts were used in 0.01 M solution: $\text{BeSO}_4 \cdot 4\text{H}_2\text{O}$; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$; $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$. $\text{Hg}(\text{NO}_3)_2$ was made up in 0.025 M- HNO_3 and excess acid back-titrated in the presence of the amino acid investigated.

The amino acids were obtained from British Drug Houses Ltd. and Roche Products Ltd., except for δ -aminovaleric acid, for a sample of which I am indebted to Dr T. S. G. Jones and Dr S. Wilkinson of the Wellcome Research Laboratories, Beckenham, Kent. All the amino acids were dried *in vacuo* at 100° for at least 48 hr., with the exception of cysteine hydrochloride, asparagine and glutamine which were dried in a vacuum over silica gel for 48 hr. The purity of each amino acid was checked by paper chromatography.

Titrations. The ionization constants of each amino acid were determined by the potentiometric method of Albert (1950) in 0.01 M aqueous solution, except for tyrosine and diiodotyrosine which were determined in 0.002 M solution.

Titration in the presence of metallic ions were usually carried out in 0.01 M amino acid and 0.005 M metal solution. The exceptions were tyrosine and diiodotyrosine, which were used in 0.002 M solution, and cysteine and histidine, which were used in 0.0025 M solution.

Calculations. The two systems to be considered in calculating the stability of each complex are:

(a) $\text{M}^{2+} + \text{amino acid} = (\text{complex I})^+$, with dissociation constant $= K_1$.

(b) $(\text{complex I})^+ + \text{amino acid} = \text{complex II}$, with dissociation constant $= K_2$, where M^{2+} is a bivalent metal. The overall stability of the final complex, $\log K_s$, is given by

$$\log K_s = \log K_1 + \log K_2.$$

The calculation of $\log K_s$ takes account of the proportion of amino acid in the form available for chelation at a particular pH. This form of the amino acid is that in which the carboxyl group is ionized and the amino group un-ionized.

All values for $\log K_s$ were calculated from the point at which one molecule of the amino acid was bound to the metal ion. At this point the following equation was used:

$$\log K_s = -2 \log [\text{Sc}],$$

where $[\text{Sc}]$ is the concentration of free amino acid available for chelation at this pH (Albert, 1950). The more critical method of calculating values for either $\log K_1$ or $\log K_2$ for each point of the titration curve was also used. The stability constants of the complexes of histidine were calculated by the method of Albert (1952), which takes into account the additional ionizing group.

RESULTS AND DISCUSSION

Table 1 shows the $\log K_s$ values obtained in this study. The results are grouped according to the chemical structure of the amino acids, and the discussion deals in turn with the effect of each new grouping introduced into the structure of the parent amino acids.

The effect of a β -hydroxyl group. The introduction of a β -hydroxyl group into the α -alanine structure has the effect of slightly decreasing the stability of the metal complexes (Table 1), but alteration of this hydroxy acid by the addition of either a methyl or phenyl group has no further effect. The β -hydroxyl group does not ionize, and it is not surprising therefore that it has no appreciable effect on the metal complexes.

The effect of aromatic substituents. This effect is shown diagrammatically in Fig. 1. The introduction of an aromatic ring into the α -alanine structure produces a decrease in complex stability. Further substitution in the ring with a hydroxyl group in the para position produces an even more dramatic decrease in stability. Although another ionizing group has been introduced, this does not interfere with complex formation, in agreement with Albert's (1952) findings. The introduction of a second hydroxyl group in the aromatic ring, as in 3:4-dihydroxyphenylalanine, produces a considerable increase in complex stabilities. A similar effect is observed with diiodotyrosine, in which two large electronegative atoms are attached to the benzene ring. In this compound the hydroxyl group has a pK of 5.32 but does not take part in chelation.

Table 1. *Stability constants for complexes of a number of amino acids, grouped according to variation in structure, with some metals of group II of the periodic classification*

Amino acid	Temp. (°)	Ionization constants of		log K_s			
		NH ₃ group	Other groups	Be	Zn	Cd	Hg
DL- α -Alanine	21.0	9.90	—	13.1	9.1	7.6	18.8
DL-Serine	20.0	9.34	—	12.1	8.6	7.4	17.5
DL-Threonine	20.0	9.12	—	11.9	8.6	7.2	17.5
DL- β -Phenylserine	17.0	8.86	—	11.1	8.5	7.0	17.3
DL- β -Phenylalanine	20.0	9.33	—	11.9	8.4	7.2	18.7
DL-Tyrosine	20.0	9.23	10.43	11.1	7.9	6.4	17.1
DL-3:4-Dihydroxyphenylalanine	20.0	9.03	—	11.6	8.7	7.9	—
DL-3:5-Diiodotyrosine	20.0	9.48	5.32	—	8.0	6.9	—
DL-Ornithine, HBr	20.0	8.75	10.73	11.7	7.6	6.1	—
L-Arginine	19.0	9.11	—	12.4	8.0	6.7	17.4
L-Citrulline	20.0	9.69	—	13.0	8.7	7.3	18.8
DL-Lysine	20.0	9.03	—	11.4	7.3	5.8	—
δ -Aminovaleric acid	21.5	10.94	—	—	Nil	—	Nil
ϵ -Aminocaproic acid	21.0	10.96	—	Nil	Nil	Nil	Nil
DL-Aspartic acid	15.0	10.06	—	13.4	10.4	8.8	—
DL-Asparagine	15.0	9.13	—	11.7	8.5	7.1	*
DL-Glutamic acid	15.0	10.06	—	13.0	8.8	7.9	—
L-Glutamine	15.0	9.34	—	12.4	8.4	7.4	*
DL-Tryptophan	20.0	9.57	—	11.6	8.2	7.0	*
DL-Histidine	20.0	9.40	6.14	—	12.0	11.1	*
Histamine, 2HCl	20.0	9.89	6.08	—	9.6	8.0	—
DL-Cysteine	20.0	8.53	10.53	—	17.1	*	20.5
DL-Methionine	18.0	9.31	—	12.0	8.3	7.1	—
DL- α -Amino- <i>n</i> -butyric acid	15.0	9.62	—	12.9	7.2	6.8	18.5
DL-Norvaline	19.0	9.87	—	12.6	8.1	6.6	17.7
DL-Norleucine	18.0	9.92	—	12.8	8.7	7.3	17.8

* Insoluble complex formed.

Fig. 1. The effect of aromatic substitution in α -alanine on the stability of the zinc and cadmium complexes.

Fig. 2. The effect of basic substituents on the stability of the zinc and cadmium complexes of norvaline.

Basic derivatives of the norvaline structure. The addition of a δ -amino group to norvaline produces a decrease in complex stabilities (Fig. 2). No evidence was obtained of this group taking part in the chelation. Albert (1952) has reported that with copper there was a tendency to form an ethylenediamine type of complex. Mercury might be expected to show this tendency, but was not examined because an insoluble complex is formed. Complexes of arginine, in which the norvaline has a terminal guanido group, showed no variation in stability from those of norvaline, but the introduction of a carbamido group, as in citrulline, increased complex stabilities. In neither of these compounds was there evidence of complex formation with any group other than the α -amino group.

The value of 7.6 for the stability constant ($\log K_s$) of the ornithine-zinc complex is in disagreement with that of 6.9 found by Albert (1952). The hydrochloride and hydrobromide of this amino acid were studied in order to verify our value. No impurities were detected by two-dimensional paper-chromatography, although the pK values for the two salts differed. The complex stabilities for the two samples were, however, fortuitously identical. Analysis for nitrogen by the micro-Kjeldahl method showed that the hydrochloride was partially hydrated and the results obtained with this salt were therefore discarded.

The effect of basic terminal groups. The introduction of a terminal amino group into the norvaline structure reduces the complex stabilities. A similar comparison between norleucine and lysine shows that in this pair of compounds, in which the carbon chain is one atom longer, the introduction of a terminal amino group also reduces the complex stabilities. Again with lysine no evidence was found for the ethylenediamine type of complex. Albert (1952) studied the complexes of 1:2-diaminopropionic acid and 1:3-diaminobutyric acid. He was able to show that in ascending this homologous series, chelation through both nitrogen atoms decreased until in the case of ornithine (1:4-diaminovaleric acid) it was of negligible importance.

Although chelation through the terminal amino groups of ornithine and lysine did not occur it was decided to study the derivatives in which the strongly-binding α -amino group was absent. Studies with δ -amino valeric acid and ϵ -amino caproic acid show that no binding occurs. This result confirms that the chain length between the carboxyl and amino groups is important, although there is little evidence for the metal bridging the carboxyl and amino groups in α -amino acids.

The effect of terminal carboxyl and amide groups. The introduction of a β -carboxyl group into α -alanine increases the stabilities of the metal com-

plexes (Fig. 3). The same effect is observed when comparing α -amino-*n*-butyric acid and its related dicarboxylic acid, glutamic acid.

These dicarboxylic acids are interesting because it is theoretically possible that the ring formed by the metal might be through the β - or γ -instead of the α -carboxyl groups. As Albert (1952) has explained, it is not possible to decide by the present method if this does happen. Comparing the glutamic and aspartic acid complexes, it is seen that the effect of moving the terminal carboxyl group further away from the α -groups reduces their enhancing effect on the complex stabilities.

In both series, conversion of the terminal carboxyl group into an amide decreases the complex stability. Although the amides contain a peptide-like amino group no evidence was obtained that this group takes part in chelation. Albert (1950) previously reported that asparagine forms glycine-like complexes.

Fig. 3. A comparison of the stability constants of the cadmium complexes of various amino acids showing the effect of carboxylic acid and amide substituents.

The effect of heterocyclic ring systems on the α -alanine structure. Tryptophan has an α -alanine structure with an indole group substituted at the β -carbon atom. This molecule forms simple glycine-type complexes with metals but of lower stability than those of α -alanine. This is similar to the effect of introducing a β -phenyl group. Histidine, in which the β -carbon atom of α -alanine has a glyoxaline group attached, was found by Albert (1952) to form ethylenediamine-type complexes in which the glyoxaline nitrogen takes part. In the present work the zinc and cadmium complexes were studied and found to have relatively high stability constants. This agrees with the work of Albert with Ni^{2+} , Zn^{2+} and Fe^{3+} .

Sulphur derivatives. Cysteine, in which a sulphhydryl group has been added to the β -carbon atom

of α -alanine, forms the most stable complexes so far examined. The zinc complex had the very high $\log K_s$ value of 17.5. The mercury complex, however, appeared to chelate specifically with the sulphhydryl group. Hughes (1947) showed that in the case of human serum albumin, mercury also combines specifically with the sulphhydryl group. This suggests that mercuric nitrate would be a more specific reagent for sulphhydryl groups than the customary silver nitrate.

In a recent paper (Klotz, Urquhart & Fiess, 1952) on the complexes of sulphhydryl groups of serum proteins, it was observed that the order of stability of the metal complexes was cadmium > zinc. This order could not be compared with that of cysteine as the cadmium complex was insoluble. This is, however, another demonstration of the unusual properties associated with the sulphhydryl group.

Methionine, which is the γ -methylthio derivative of α -amino-*n*-butyric acid, formed complexes of the simple glycine-type. The stabilities of its complexes were almost identical with those of its parent acid. This shows that the sulphhydryl group must be able to ionize to produce any effect. Unfortunately both cystine, the oxidation product of cysteine, and lanthionine, $S(CH_2.CHNH_2.COOH)_2$, were insufficiently soluble to be studied.

This study does not show a single example of the metals of group II forming complexes in order of stability other than mercury > beryllium > zinc > cadmium. Changes in stability constants comparable to the metal effect are only shown by the sulphhydryl and glyoxaline groups.

SUMMARY

1. The stability constants of metal complexes of a wide range of amino acids have been measured by a potentiometric method.
2. The sequence of relative stability for any particular amino acid complexes has been confirmed as mercury > beryllium > zinc > cadmium.
3. The introduction of a β -hydroxyl group into the amino acid structure slightly reduces the stability of the complexes.
4. An aromatic substituent decreases the stability constants. The addition of a para-hydroxyl group further reduces the constant, but this effect is less marked when an ortho-hydroxyl group or two ortho-iodine atoms are also present in the aromatic ring.
5. Terminal amino groups in a simple amino acid reduce, but the carbamido group increases, complex stabilities.
6. No evidence was found for complex formation through groups other than the α -amino group except for histidine and cysteine.
7. Terminal carboxyl groups increase complex stability constants. Amide groups reverse this effect.
8. Histidine has high stability constants and forms complexes through both the glyoxaline and α -amino groups.
9. Cysteine produces complexes with the largest stability constants recorded in the α -amino acid field.

I gladly acknowledge the advice and encouragement of Prof. N. H. Martin during the course of this work and the financial help given by the Research Fund of St George's Hospital and the University of London.

REFERENCES

- Albert, A. (1950). *Biochem. J.* **47**, 531.
Albert, A. (1952). *Biochem. J.* **50**, 690.
Hughes, W. L. jun. (1947). *J. Amer. chem. Soc.* **69**, 1836.
Klotz, I. M., Urquhart, J. M. & Fiess, H. A. (1952). *J. Amer. chem. Soc.* **74**, 5537.
Perkins, D. J. (1952). *Biochem. J.* **51**, 487.