- Cohn, E. J. *et al.* (1950). *J. Amer. chem. Soc.* **72**, 465.
 Copenhaver, J. H. Stafford, R. O. & McShan, W. H. (1950). *Arch. Biochem.* **26**, 260.
 Davison, A. N. (1953). *Biochem. J.* **54**, 583.
 Delory, G. E. & King, E. J. (1943). *Biochem. J.* **37**, 547.
 Denz, F. A. (1953). *Brit. J. exp. Path.* **34**, 329.
 Desnuelle, P. (1951). *Bull. Soc. Chim. biol., Paris*, **33**, 909.
 Fodor, P. J. (1950). *Arch. Biochem.* **26**, 331.
 Folley, S. J. & Watson, S. C. (1948). *Biochem. J.* **42**, 204.
 Frazer, A. C. (1948). *Arch. Sci. physiol.* **2**, 15.
 Ganguly, J. & Deuel, H. J. (1953). *Nature, Lond.*, **172**, 120.
 Gomori, G. (1945). *Proc. Soc. exp. Biol., N.Y.*, **58**, 362.
 Gomori, G. (1949). *Proc. Soc. exp. Biol., N.Y.*, **72**, 697.
 Gottlieb, S. & Marsh, P. B. (1946). *Analyt. Chem.* **18**, 16.
 Huggins, C. & Lapides, J. (1947). *J. biol. chem.* **170**, 467.
 King, E. J. (1951). *Micro-analysis in Medical Biochemistry*. London: Churchill.
 King, E. J. & Delory, G. E. (1939). *Biochem. J.* **33**, 1185.
 Klotz, I. M. (1946). *J. Amer. chem. Soc.* **68**, 2299.
 Krause, R. F. & Alberghini, C. (1950). *Arch. Biochem.* **25**, 396.
 McGugan, W. A. & Laughland, D. H. (1952). *Arch. Biochem. & Biophys.* **35**, 428.
 Mendel, B., Myers, D. K., Uyltort, I. E., Ruys, A. C. & Bruyn, W. M. de (1953). *Brit. J. Pharmacol.* **8**, 217.
 Mounter, L. A. & Whittaker, V. P. (1953). *Biochem. J.* **54**, 551.
 Myers, D. K. & Mendel, B. (1953). *Biochem. J.* **53**, 16.
 Nachlas, M. M. & Seligman, A. M. (1949). *J. nat. Cancer Inst.* **9**, 415.
 Ord, M. G. & Thompson, R. H. S. (1950). *Biochem. J.* **46**, 346.
 Reiser, R., Bryson, M. J., Carr, M. J. & Kuiken, K. A. (1952). *J. biol. Chem.* **194**, 131.
 Reiser, R. & Williams, M. C. (1953). *J. biol. Chem.* **202**, 815.
 Richter, D. & Croft, P. G. (1942). *Biochem. J.* **36**, 746.
 Schönheyder, F. & Volqvartz, K. (1945a). *Acta Physiol. scand.* **10**, 62.
 Schönheyder, F. & Volqvartz, K. (1945b). *Acta physiol. scand.* **9**, 57.
 Sylvester, N. D., Ainsworth, A. N. & Hughes, E. B. (1945). *Analyst*, **70**, 295.
 Verzár, F. (1948). *Arch. Sci. physiol.* **2**, 43.
 Vincent, D. & Lagreu, R. (1949). *Bull. Soc. Chim. biol., Paris*, **31**, 216.
 Whittaker, V. P. (1951). *Physiol. Rev.* **31**, 312.
 Willstätter, R. & Waldschmidt-Leitz, E. (1923). *Hoppe-Seyl. Z.* **125**, 132.
 Wilson, I. B., Levine, S. & Freiburger, I. (1952). *J. biol. Chem.* **194**, 613.

A Study of Some Simple Peptide Complexes with Zinc and Cadmium Ions in Aqueous Solution

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Previous studies (Perkins, 1952, 1953a) of metal complexes in aqueous solution were restricted to amino acids. It has already been shown (Monk, 1951a-c; Perkins, 1952) that for the simplest peptide, glycylglycine, complexes are formed which have smaller stability constants than those of glycine. Dobbie, Kermack & Lees (1951) studied the copper complexes of glycylglycine and diglycylglycine and found that the effect of the second peptide bond was to further reduce the stability constant. In this paper a range of simple peptide complexes with zinc and cadmium ions are examined. Some of the results have been the subject of a preliminary communication (Perkins, 1953b).

EXPERIMENTAL

Materials. The following AnalaR (British Drug Houses Ltd., Poole) salts were used in 0.01M aqueous solution: $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$. All the peptides were vacuum-dried over P_2O_5 at 100° for 48 hr., weighed and made up in 0.02M solution. The peptides and complexes of glycyl-L-tryptophan and glycyl-L-tyrosine were relatively insoluble, and were therefore used in 0.004M and 0.002M solutions respectively. All the peptides were obtained from Roche Products Ltd. unless otherwise stated. The sample of

triglycylglycine was kindly given by Dr Hanby, Courtaulds Ltd., Maidenhead. The peptides, glycyl-DL-serine (Fischer & Roesner, 1910), glycyl-DL-phenylalanine (Fischer & Schoeller, 1907), DL-alanyl-DL-alanine (Fischer & Raske, 1906) and glycyl-DL-isoleucine (Abderhalden, Hirsch & Schuler, 1909) were synthesized by the methods cited.

The purity of the peptides was checked by two-dimensional filter paper chromatography and nitrogen content using the micro-Kjeldahl procedure.

Potentiometric titration. A Cambridge Instrument Co. Ltd. bench type pH meter was used with a glass electrode and saturated calomel half-cell. The titrations were carried out as outlined by Albert (1950) with minor modifications. The dissociation constant for the α -amino group was determined at 25° under the conditions subsequently used for the titrations in the presence of the metal. These titrations were made on solutions in which the ratio of acid to metal was 2:1. For most titrations equal volumes of stock metal and amino acid solutions were mixed and then stirred with O_2 -free N_2 for 10 min. At the mid-point of the titration the volume was 50 ml. All measurements were made at $25.0 \pm 0.1^\circ$ in a thermostatically controlled water bath.

Calculations. The system to be considered in calculating the stability of each complex is

The calculation of $\log K_1$ takes account of the proportion of the peptide in the form available for chelation at a particular pH. This form of the peptide is that in which the carboxyl group is ionized and the amino group un-ionized. The method of calculating values for $\log K_1$ for each point on the titration curve was that of Albert (1950).

RESULTS

The stability constants of the peptide complexes are shown in Table 1.

Table 1. *The stability constants at 25° for the zinc and cadmium complexes of a range of peptides*

Peptide	pK	Stability constant	
		Zn $\log K_1$	Cd $\log K_1$
Glycine	9.73	5.0	4.4
Glycylglycine	8.21	3.6	3.2
Diglycylglycine	8.02	2.6	2.0
Triglycylglycine	7.91	2.9	2.8
DL-Alanine	9.90	4.9	4.2
DL-Alanyl-DL-alanine	8.51	*	3.0
DL-Alanylglycine	8.17	3.00	2.6
Glycyl-DL-alanine	8.17	4.1	3.6
Glycyl-L-leucine	8.13	3.6	3.1
L-Leucylglycine	7.96	3.1	1.9
L-Leucylglycylglycine	7.66	1.8	1.5
Glycyl-DL-serine	8.34	3.7	3.3
Glycyl-DL-phenylalanine	8.28	3.8	3.3
Glycyl-L-tyrosine	8.22	2.6	2.2
Glycyl-L-tryptophan	8.04	3.3	2.7
Glycyl-DL-isoleucine	7.96	3.8	3.4

* Insoluble complex formed.

Fig. 1. A plot of the concentration $[Sc]$ of histidine available for chelation against the proportion ($\bar{n}$) of the zinc complex formed. The line represents the theoretical result.

DISCUSSION

In the calculation of $\log K_1$ values not all points on the titration curve are suitable. Albert (1950) restricted his calculations to that part of the curve where between 10 and 70 % of the 1:1 metal amino acid (or peptide) complex was formed. Below 10 % it is difficult to obtain reliable values as small errors in the measurement of the volume of added KOH make a considerable difference to the pH observed. Above 70 % formation of the 1:1 complex there is an increasing tendency to form the 1:2 complex. This produces a drift in $\log K_1$ values as shown in Fig. 1, which is a plot of $\bar{n}$ (the proportion formation of the 1:1 complex) against $[Sc]$ (the concentration of peptide available for chelation) for zinc and histidine. The curve remains linear to approximately 50 % formation of the 1:1 complex. The deviation beyond this point arises from neglecting the formation of the 1:2 complex in the calculations (Albert, 1950). The value for $\log K_1$ calculated from the position of 70 % complex formation does not, however, vary by more than 0.23 unit from the mean value obtained from the 10–50 % range. The example shown above is an extreme case as, unlike the peptide experiments, two ionizing groups have to be titrated because the zinc ions react with both of them. This produces a dilution effect, which has not been corrected for, and a further source of error in that two pK values are used in the calculations. The individual peptide $\log K_1$ values averaged were within the range ± 0.15 unit.

An attempt was made to determine K_1 values of the cobalt glycylglycine complexes by measurement of colour intensity. This experiment failed because the intense colour measured was not a property of the simple complex but one of an oxygen-containing derivative (Gilbert, Otey & Price, 1951).

In Table 1 the constants for the complexes of composition 1 metal:2 peptide are not recorded as these complexes were mostly insoluble.

A comparison of the stability constants of the peptides and their constituent amino acids shows that there is a considerable decrease on peptide formation. This is not only shown by the homologous glycine and α -alanine series but also by the peptides in which two different amino acids are united.

The length of peptide chain. The progressive lengthening of the peptide chain from glycine to diglycylglycine and α -alanine to alanylalanine shows for the 1:1 complexes that the largest decrease in stability constants occurs when the first and second peptide bonds are introduced. Dobbie *et al.* (1951) found the same effect for the copper complexes of glycylglycine and diglycylglycine. The series leucylglycine and leucylglycylglycine shows

the same effect. In one tetrapeptide examined, the introduction of the third peptide bond produced a small increase in stability constants.

The effect of introducing other amino acid residues, which are aliphatic homologues of glycine, is shown to be small by the study of alanylglycine, glycylalanine, leucylglycine and glycylleucine. When, however, the residues were reversed in order in each peptide a considerable alteration in stability constants was observed.

Peptides of glycine. A comparison between the stability constants of glycylglycine and a range of glycyl peptides is shown in Table 1. In each example the effect of the peptide bond is found to be that of decreasing the stability constants below those of the parent amino acids. The same order was followed, within experimental error, for the stability constants for these complexes, as was found for the amino acids forming the second residue (Perkins, 1952, 1953*a*). This means that it may be possible to predict roughly the order of stability constants for a simple peptide on the basis of its composition.

Smith (1953) discussed the mode of action of manganese as an activator of dipeptidase and suggested that a coordination complex was formed between the metal and the substrate. He found that zinc, cadmium and silver ions were inhibitors for the enzyme. Each of these metals forms complexes with glycylglycine with larger $\log K_1$ values than that for manganese (Monk, 1951*a-c*) and would therefore be expected to act as inhibitors if a substrate-metal complex is formed. It is in biological systems of this nature that a knowledge of the relative stability constants may become of importance.

SUMMARY

1. Zinc and cadmium complexes of peptides have lower stability constants than the corresponding complexes of free amino acids.

2. Examination of the homologous peptide series glycine to triglycylglycine shows that complex stabilities decrease further with the introduction of the first and second peptide bonds but increase slightly with the third.

3. The order in which the amino acid residues of the peptide occur is shown to be important.

4. The glycyl peptides of a number of amino acids have complex stability constants which can be roughly predicted from their amino acid content.

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REFERENCES

- Abderhalden, E., Hirsch, P. & Schuler, J. (1909). *Ber. dtsh. chem. Ges.* **42**, 3394.
 Albert, A. (1950). *Biochem. J.* **47**, 531.
 Dobbie, H., Kermack, W. O. & Lees, H. (1951). *Biochem. J.* **50**, v.
 Fischer, E. & Raske, K. (1906). *Ber. dtsh. chem. Ges.* **39**, 3989.
 Fischer, E. & Roesner, H. (1910). *Liebigs Ann.* **375**, 199.
 Fischer, E. & Schoeller, W. (1907). *Liebigs Ann.* **357**, 1.
 Gilbert, J. B., Otey, M. C. & Price, V. E. (1951). *J. biol. Chem.* **190**, 377.
 Monk, C. B. (1951*a*). *Trans. Faraday Soc.* **47**, 285.
 Monk, C. B. (1951*b*). *Trans. Faraday Soc.* **47**, 292.
 Monk, C. B. (1951*c*). *Trans. Faraday Soc.* **47**, 297.
 Perkins, D. J. (1952). *Biochem. J.* **51**, 487.
 Perkins, D. J. (1953*a*). *Biochem. J.* **55**, 649.
 Perkins, D. J. (1953*b*). *Biochem. J.* **55**, xxviii.
 Smith, E. L. (1953). *The Chemical Structure of Proteins*, Ciba Symposium, p. 128. London: Churchill.