TABLE III

TITRATION OF BLOOD SERUM WITH $Cu^{(II)}$

Medium—0.1 M NH_3 + 0.1 M NH_4NO_3, pH 9.2; R.p.e. at 600 r.p.m., −0.4 volt.

Serum fraction	$Cu^{(II)}$ value, as g. % albumin
Albumin	3.41
α_1-globulin	0.25
α_2-globulin	0
β-globulin	0
γ-globulin	0
Total	3.66
Whole serum, by $Cu^{(II)}$	3.68
Whole serum, tyrosine (cor.)	3.70

value for the whole serum was obtained. Results of copper(II) titrations on both whole serum and the fractions are given in Table III. While the unusually close agreement between values is certainly fortuitous, it is significant that over 93% of the copper titer of the whole serum is accounted for in the albumin fraction. The remaining 6% occurs in the α_1-globulin fraction and can be attributed to overlap of the albumin and α_1-globulin fractions.

The amperometric copper titration now is being applied to the rapid and specific determination of albumin in blood serum. Results will be reported elsewhere.

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Some Metal Complexes of Glycine Peptides[1a,b]

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The formation constants of Cd(II), Ni(II) and Zn(II) complexes of glycylglycine, glycylglycylglycine, tetraglycine and glycine amide have been determined. With each of these three metal cations, the glycine amide and the three peptide complexes are about equally stable. With Mg(II) cation, the glycylglycine and tetraglycine complexes are equally stable. It is shown that the three glycine peptides probably have common coördination sites, which are probably the terminal amino group and the immediately adjacent peptide group.

Introduction

Evans and Monk[2] reported the formation constants of several metal complexes of glycylglycine and glycylglycylglycine, and suggested that the metal-peptide bonds do not involve the terminal amino and charged carboxylate groups. More recently, Li, *et al.*,[3] obtained the formation constants of Co(II) complexes of glycylglycine, glycylglycylglycine and tetraglycine, using the methods of pH and ion exchange, and found that these three glycine peptides are about equally stable. They postulated that the three glycine peptides probably have common coördination sites toward Co(II), and that the sites are probably the terminal amino group and the immediately adjacent peptide group. It is of interest to continue the study of the peptide complexes with other metal ions, and this paper presents the results on the Cd(II), Zn(II), Ni(II) and Mg(II) complexes.

Experimental

Materials.—The glycine peptides and glycine amide were purchased from Nutritional Biochemicals Corp., Cleveland, Ohio. Stock solutions of metal salts were prepared and analyzed by conventional means. All chemicals were of C.P. grade.

Procedure.—Measurements of pH were made with a Beckman Model G pH meter with external electrodes. Only freshly prepared solutions of the peptides were used, and nitrogen gas was bubbled through the solution. The instrument was standardized against Beckman standard buffer solutions, pH 4 and 7.

Polarographic current–voltage curves were made manually with a Fisher Elecdropode. All potentials were measured against a saturated calomel electrode (S.C.E.) and the half-wave potentials were corrected for the IR drop.

Results

(A) Titration of 1:1 (Peptide:Metal Ion) Molar Mixtures.—The formation constants of the 1:1 complexes have been determined by pH titration of solutions containing 0.01 M glycine peptide or glycine amide, 0.01 M metal(II) nitrate and 0.12 M KNO_3. As examples, the titration data for the Ni(II) complex of tetraglycinate, and the Cd(II) complex of glycylglycylglycinate at 25° are given in Table I. The symbols (A^-) and $\bar{n}$ represent the total concentration of free peptide anion in solution and the average number of moles of peptide anion bound per mole of the divalent metal ion, respectively.

Values of k_1 are calculated for data in the region $\bar{n}<0.5$ only, because at higher $\bar{n}$ values, protons from the amide groups in the peptides are titrated. A summary of the formation constants of the 1:1 complexes of Cd(II), Zn(II), Ni(II) is given in Table II. Our values of log k_1 (u = 0.15) of the glycylglycinate and glycylglycylglycinate complexes are in general about 0.3 log unit lower than the corresponding values listed by Evans and Monk[2] at u = 0. Part of the difference probably is due to the difference in ionic strength. No value for the tetraglycinate complex of these metal cations has been reported in the literature.

(B) Titration of 1–10 (Peptide–Metal Ion) Molar Mixtures.—The formation constants of the

(1) (a) This work has been supported by a grant from the National Science Foundation, Grant No. G1926; (b) taken from the M.S. thesis of M. C. M. Chen.

(2) W. P. Evans and C. B. Monk, *Trans. Faraday Soc.*, **51**, 1244 (1955).

(3) N. C. Li, E. Doody and J. M. White, THIS JOURNAL, **79**, 5859 (1957).

TABLE I

TITRATION OF METAL(II) NITRATE–PEPTIDE MIXTURES

(a) 25 ml. solution containing 0.0100 *M* $Ni(NO_3)_2$; 0.0100 *M* tetraglycine; 0.120 *M* KNO_3; plus *v* ml. of 0.100 *M* NaOH

v, ml.	*p*H	−log (A⁻)	*n*	log k_1
0.00	4.65			
.25	5.45	4.55	0.09	3.57
.50	5.88	4.18	.19	3.56
.75	6.17	3.95	.29	3.57
1.00	6.40	3.79	.38	3.58
1.25	6.65	3.62	.47	3.57

(b) 50 ml. solution containing 0.0100 *M* $Cd(NO_3)_2$; 0.0100 *M* glycylglycylglycine; 0.120 *M* KNO_3; plus *v* ml. of 0.1000 *M* NaOH

0.50	6.31	3.76	0.08	2.71
1.00	6.71	3.42	.16	2.70
1.50	7.00	3.19	.23	2.66
2.00	7.20	3.06	.31	2.72
2.50	7.42	2.92	.37	2.70
3.00	7.63	2.81	.44	2.71
3.50	7.88	2.69	.48	2.65

TABLE II

FORMATION CONSTANTS OF GLYCINE PEPTIDE AND GLYCINE AMIDE COMPLEXES, CALCULATED FROM 1:1 MIXTURES, $u = 0.15$, 25°

Peptide	$pK_{NH_3^+}$	log k_1 Cd(II)	Zn(II)	Ni(II)
Glycylglycine	8.12	2.95	3.43	4.18
Glycylglycylglycine	8.02	2.70	3.18	3.72
Tetraglycine	7.95	2.65	3.14	3.57
Glycine amide	8.06		3.28	4.18[3]

1:1 complexes have also been determined by *p*H titration of solutions containing 0.005 *M* glycine peptide and 0.050 *M* metal (II) nitrate. As examples, the titration data for the Cd(II) and Zn-(II) complexes of glycylglycinate, and the Ni(II) complex of tetraglycinate at 25° are given in Table III. The symbol *g* represents the fraction of the total peptide concentration *T* which exists in the free dipolar form ($HA^{\pm}$). The equilibrium constant K^1 is as defined by equation 3. A summary of the formation constants of the 1:1 complexes of Cd(II), Zn(II), Ni(II) and Mg(II) is given in Table IV.

The method of calculation of *g*, K^1 and k_1 is as follows: in the presence of 10-fold excess metal ion, such that only the 1:1 complex exists predominantly, and in the *p*H region where (A^-) may be neglected, these equations readily are obtained

$$T = (HA^{\pm}) + (MA^+) \quad (1a)$$

$$T_M = (M^{+2}) + (MA^+) \quad (1b)$$

$$g = \frac{(HA^{\pm})}{T} = \frac{T - (NaOH) - (H^+)}{T} \quad (2)$$

$$M^{+2} + HA^{\pm} = MA^+ + H^+; \; K^1 = \frac{(MA^+)(H^+)}{(HA^{\pm})} \quad (3)$$

$$K^1 = \frac{(1 - g)(H^+)}{g} \quad (4)$$

$$k_1 = \frac{K^1}{K_2 (M^{+2})} \quad (5)$$

The values of *g*, K^1 and k_1 are calculated therefore by means of equations 2, 4 and 5, respectively.

(C) Polarographic Studies.—Some 20 polarograms were taken at 25.0° with solutions containing 5×10^{-4} *M* $Cd(NO_3)_2$, varying concentrations of the glycine peptides or of glycine amide hydrochloride, plus NaOH, and sufficient KNO_3 to keep the total ionic strength constant at 0.15. The data are used to calculate the number of moles of ligand *p* coördinated to each cadmium ion and the over-all formation constant, k_f, of the complex by means of the equations[4]

$$\Delta E_{1/2}/\Delta \log (A) = -(RT/nF)p(2.303) \quad (6)$$

$$(E_{1/2})_c - (E_{1/2})_s = -(RT/nF) \ln k_f - p(RT/nF) \ln (A) \quad (7)$$

In these equations, $(E_{1/2})_c$ and $(E_{1/2})_s$ are the half-wave potentials of the Cd(II) in the presence and absence of the ligand, respectively. (A) is the concentration of the free ligand and may be calculated from the total concentration of the peptide or glycine amide, *p*H of the solution and $pK_{NH_3^+}$ of the glycine peptide or glycine amide, the values of which are included in Table II.

In each case the electrode reaction is reversible and $n = 2$ (values of $(E_{3/4} - E_{1/4})$[4b] ranging from 0.029 to 0.033). Plots of $E_{1/4}$ *vs.* log (A) in the concentration range (A) = 0.005 to 0.04 *M* show that $p = 2$ (values of *p* ranging from 1.95 to 2.05) for each of the three glycine peptide and glycine amide complexes. The average values of k_f

TABLE III

TITRATION OF METAL(II) NITRATE–PEPTIDE MIXTURES

25 ml. solution containing 0.0050 *M* peptide; 0.0500 *M* M(II) nitrate; titrated with 0.0987 *M* NaOH under nitrogen, 25°

NaOH, ml.	*p*H	*g*	$K^1 \times 10^{-6}$	log k_1
	(a) Glycylglycine–Cd(II) nitrate			
0.10	5.52	0.92	3.81	2.84
.20	5.84	.84	3.62	2.88
.30	6.04	.78	3.89	2.85
.40	6.22	.69	3.69	2.87
.50	6.38	.61	3.75	2.88
.60	6.52	.53	3.73	2.88
.70	6.66	.45	3.74	2.89
	(b) Glycylglycine–Zn(II) nitrate			
0.10	5.10	0.92	1.45	3.26
.20	5.38	.84	1.26	3.34
.30	5.60	.78	1.41	3.29
.40	5.75	.69	1.25	3.34
.50	5.90	.61	1.24	3.36
.60	6.05	.53	1.27	3.35
.70	6.20	.45	1.30	3.35
	(c) Tetraglycine–Ni(II) nitrate			
0.10	4.68	0.92	55.0	3.51
.20	4.96	.84	47.7	3.59
.30	5.19	.78	54.9	3.53
.40	5.37	.69	52.1	3.55
.50	5.53	.61	53.0	3.56
.60	5.68	.53	54.0	3.55
.70	5.86	.45	59.3	3.53

TABLE IV

FORMATION CONSTANTS OF GLYCINE PEPTIDE COMPLEXES, CALCULATED FROM 1:10 MIXTURES, $u = 0.15$, 25°

	log k_1 Cd	Zn	Ni	Mg
Glycylglycine	2.87	3.32	3.95	1.34
Tetraglycine	2.66	3.01	3.54	1.32

(4) (a) N. C. Li, J. M. White and R. L. Yoest, THIS JOURNAL, **78**, 5218 (1956); (b) N. C. Li and E. Doody, *ibid.*, **74**, 4184 (1952).

($k_f = (CdA_2)/(Cd^{++})(A)^2$ are 5.4, 5.3, 5.2, 5.2 for the Cd(II) complexes of glycylglycine, glycylglycylglycine, tetraglycine and glycine amide, respectively. As an example of the precision obtained in calculating the values of k_f, polarographic results for the Cd(II) complex of glycylglycinate are given in Table V.

TABLE V

POLAROGRAPHIC RESULTS FOR Cd(II) COMPLEX OF GLYCYLGLYCINE, 25°, $u = 0.15$

Total concn., of glycylglycine	*p*H	$-E_{1/2}$	log k_f
0		0.585	
0.02	7.80	.616	5.42
.02	8.20	.630	5.44
.02	8.76	.639	5.40
.04	8.13	.645	5.41
.08	8.16	.663	5.39

Discussion

Ahrland,[5] *et al.*, have shown that the complex formation curve is dependent on concentration of metal ion, if polynuclear complexes exist; while if the complex formation curve is independent of metal ion concentration, the complex is mononuclear. The difference in the metal ion concentration used in sections (A) and (B) is 5-fold, and the agreement in the formation constant values listed in Tables II and IV must mean therefore that the peptide complexes are mononuclear.

The *p*H data of Tables II, IV and our polarographic data show that with each of the three metal cations (Cd(II), Zn(II) and Ni(II)), the glycylglycine, glycylglycylglycine, tetraglycine and glycine amide complexes are about equally stable. With Mg(II), the glycylglycine and tetraglycine complexes are equally stable. Li, Doody and White[3] found that the Co(II) complexes of the three glycine peptides are also about equally stable, and inferred from this and from infrared data that the three glycine peptides probably have common coördination sites, namely, the terminal amino group and the immediately adjacent peptide group. The same conclusion about the coördination sites of the three glycine peptides toward Cd(II), Zn(II), Ni(II) and Mg(II) therefore may be made. With regard to the Zn(II) complexes, it is interesting to note that Li, *et al.*,[3] already have inferred from infrared data that in glycine amide and in glycylglycine, the preferable site of binding in the amide group toward Zn(II) is the carbonyl oxygen.

Acknowledgment.—The authors are indebted to Mr. Richard Brane for carrying out the polarographic experiments on the Cd(II) complexes of glycine amide.

PITTSBURGH, PENNA.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CORNELL COLLEGE]

Studies of Complexes of the Transition Metals. I. Nickel Complexes with Sulfur Containing Ligands

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The formation constants for complexes of nickel(II) with the sulfur containing ligands (thiocarbonate, dithioloxalate and dithiolmalonate) have been determined from spectrophotometric measurements in the visible region of the spectrum. The formulas for these complexes were confirmed to be 1:2 complexes, that is, one nickel ion per two ions of the ligand, by the methods of continuous variation and varying mole ratio. These complexes were studied in aqueous solution of total ionic strength of 0.1. The formation constants (k_1k_2) indicated the order of stability for the complexes to be: dithioloxalate > dithiolmalonate > thiocarbonate. These ligands form complexes with nickel(II) which contain four-membered rings with thiocarbonate, five-membered rings with dithioloxalate and six-membered rings with dithiolmalonate. The order of stability is what one might predict since five- and six-membered rings are known to be more stable. It is also interesting to compare the stability of these complexes with the corresponding oxygen containing complexes. The sulfur-containing ligands form complexes which are more stable than the corresponding oxygen complexes.

Introduction

Nickel(II) forms many coördination compounds with various chelating agents. Three of these reagents are the dithioloxalate, dithiolmalonate and thiocarbonate ions. These ions have been proposed as reagents for the detection of nickel.[1] It has been shown that many of the transition metal ions form colored solutions with dithioloxalate[2] and dithiolmalonate[3] ions, and a colorimetric method for the determination of nickel with dithioloxalic acid dipotassium salt has been studied.[4] X-Ray studies[5] have indicated that dipotassium bisdithioloxalato nickelate(II) exists in a planar configuration.

It was the purpose of this research to study these complexes in dilute solution and to determine the formulas as well as the formation constants. Since each of these complexes is highly colored a spectrophotometric method seemed advantageous.

Experimental Part

Materials.—A 0.0200 *M* standard nickel solution was prepared by dissolving the proper amount of nickel(II) sulfate hexahydrate (Mallinckrodt Analytical reagent) in water at 25°. The concentration of nickel was deter-

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