TABLE I.

	Adenine		AMP		Cytosine		CMP	
	log K_f	k'	log K_f	k'	log K_f	k'	log K_f	k'
No metal	–	4.60	–	2.69	–	0.46	–	0.36
Mg(II)	N.A.	4.1	1.97	2.44	N.A.	0.39	1.75	0.26
Ni(II)	1.47	4.87	2.84	2.57	N.A.	0.49	1.9	0.32
Cu(II)	2.68	9.39	3.18	3.48	2.0	0.46	N.A.	0.45
Ag(I)	N.A.	<20	N.A.	4.26		1.16	N.A.	1.13

With nucleotides, nucleosides and their bases the situation is much more complicated. The metal cations can complex with the phosphate moiety of the nucleotides and/or with the base part of the molecule. This fact is also reflected in the retention behavior of these solutes. The following table shows typical behavior (Table I).

In the table k' is a measure of the partition coefficients and hence the retention times. Mg causes a decrease in the retention of the bases (as well as the monophosphate nucleotides) shown in the table: this metal is known to be bound to the phosphate group only. However, the extent of retention change is roughly the same for all solutes shown. Thus in the concentration range and pH used here, the effect of the Mg ion added to the mobile phase may be related to changes in the ionic strength. Similar arguments can be made when nickel ions are added to the mobile phase although the increase in the retention of adenine should be noted. The use of copper ions gives different results. Purines are known to complex Cu(II) better than pyrimidines. Indeed the retention of adenine and AMP increases significantly while that of cytosine and, to some extent CMP, does not change much. The presence of a heavy metal such as silver in the mobile phase increases the retention of all the solutes drastically. This is clear in view of the strong complexes formed by such metals.

Similar results found with other solutes and metal cations will be discussed. The chromatographic data correlates well with that known about the structures and properties of biologically important complexes. Thus, chromatographic methods can aid scientists in analyzing bioinorganic complexes.

U8

Use of Cyclic Peptides to Mimic the Active Sites of Metalloproteins: ^{13}C and ^{1}H NMR, ESR and Visible Spectra of Copper(II) Complexes with the Cyclo-(Gly–His–Gly–His–Gly–His–Gly) Peptide in Aqueous Solution

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Cyclic peptides have been used to model various aspects of protein conformation and active sites [1]. In such models the usual flexibility of peptide chains is substantially reduced through cyclization. Furthermore, it is generally assumed that specific amino acid residues such as Glu, Asp, His, Lys··· have side chains that can bind to transition-metal ions. We chose to synthesize cyclo-(Gly–His–Gly–His–Gly–His–Gly) (hereinafter denoted (G_4H_3)) because the histidine residue plays an important role in metal ion coordination of metalloproteins and frequently encountered *in vivo*. Complexation of (G_4H_3) with the transition metal ion Cu(II) in aqueous solution over a wide pH range and with different peptide/metal ratios has been studied using carbon-13 and proton NMR, ESR and visible spectroscopy. From analyses of the spectral data, it is concluded that Cu(II) binds at two metal-binding sites depending on the pH. At physiological pH, the binding of Cu(II) at the first site involves the three HisN(3) imidazole groups to give a 1:1 species in a tetracoordinated structure; whereas, at higher pH, the binding of Cu(II) at the second site uses four deprotonated peptide nitrogens.

The present results further confirm the concept of molecular design and the feasibility of designing peptide molecules mimicking the complicated metal-binding sites of biological macromolecules.

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U9

Polarographic Investigations on Stereoselectivity of some Ternary Complexes of Aminoacids with Copper(II)

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The biological significance of histidine residue as a metal binding site in proteins has prompted many workers to investigate copper(II) histidine complexes. It has been found that elucidation of the complex formation between Cu(II) and histidine is not easy since histidine involves a bulky imidazole ring and has three or four coordination sites, and Cu(II) requires a tetragonal coordination [1–3]. The probable electrostatic interactions giving rise to stereoselective aspects of the problem have been mainly investigated using potentiometric, spectral and magnetic measurements [4]. No polarographic studies are reported in the literature. In the present communication the parent and ternary complexes of D- and L-histidinato copper(II) with some L-aminoacids, *viz.* phenylalanine, tryptophan, valine, proline, methionine, leucine, lysine, serine, threonine, alanine, glutamic acid and aspartic acid have been studied in aqueous 0.5 *M* KNO_3 at the dropping mercury electrode under varying experimental conditions with a view to calculate the kinetic parameters. Besides half wave potential ($E_{1/2}$), transfer coefficient (α), the formal rate constant (Kr), the activation energy of the rearrangement of the depolizer (Q_e) and the activation energy of diffusion (Q_D) have been evaluated for each system. Electronic spectra (in solution) of these complexes have also been recorded.

Experimental

Experimental details are described in several earlier communications from this laboratory [5]. The characteristics of dme for one set of measurements are given below:

$h = 40$ cm, $m = 2.458$ mg s^{-1}, $t = 2.0$ s, $m^{2/3}\, t^{1/6} = 1.6234$ $mg^{2/3}\, s^{-1/2}$

Results

Polarographic characteristics for some of these ternary complexes (1:1:1 ratio) are given in Table I. All these complexes are observed to undergo diffusion-controlled single-step two electron irreversible electro-reduction at dme. A perusal of the Table shows that Kr and also i_d increase with increase in temperature in the case of Cu–histidine–alanine/or serine, indicating an easier reduction, which is also supported by the shift of $E_{1/2}$ to comparatively more positive potentials with the rise of temperature. However, in the case of Cu–histidine–aspartic and/or threonine, such trend in $E_{1/2}$ is disturbed. The significance of results will be discussed in detail.

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TABLE I. Polarographic Characteristics of some Mixed-Aminoacid Complexes of Copper(II) at Different Temperatures: h = 40 cm, $[Cu^{2+}]$ = 1 m*M*, KNO_3 = 0.5 *M*, gelatin = 0.005%, pH = 6.5 ± 0.1.

Complexes	Temp. (°C)	$-E_{1/2}$ (V *vs.* SCE)	i_d (μA)		$D \times 10^7$ ($cm^2\ sec^{-1}$)	$Kr \times 10^4$ ($cm\ sec^{-1}$)	Q_D (kcal)	Q_e (kcal)
Cu–histidine–aspartic acid	25	0.189	0.90	0.2766	2.086	6.195		
	30	0.170	0.76	0.3405	1.488	9.743	7.889	7.5733
	35	0.178	1.02	0.2724	2.680	8.768		
Cu–histidine–alanine	25	0.183	0.845	0.3575	1.840	7.979		
	30	0.178	0.920	0.3367	2.181	9.455	5.2690	4.4329
	35	0.178	1.28	0.3347	4.221	12.78		
Cu–histidine–threonine	25	0.183	0.89	0.2570	2.041	6.616		
	30	0.193	1.20	0.2647	3.710	7.413	10.474	2.3312
	35	0.194	1.40	0.2431	5.049	8.230		
Cu–histidine–serine	25	0.203	0.96	0.2483	2.374	4.793		
	30	0.200	1.10	0.2425	3.118	5.779	6.487	3.9574
	35	0.197	1.31	0.2674	4.442	7.519		