



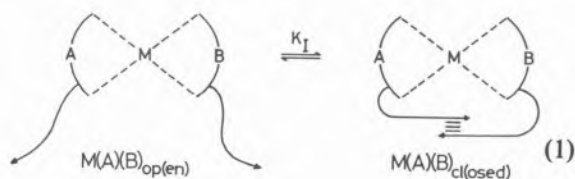
PS5.2 — TH

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INTRAMOLECULAR HYDROPHOBIC AND STACKING INTERACTIONS IN BINARY AND TERNARY AMINO ACID COMPLEXES

Selectivity is one of the outstanding features of biological systems; it is often caused by non-covalent interactions, like aromatic-ring stacking or hydrophobic interactions [1]. After our recent observations [2-4] that both these types of intramolecular ligand-ligand interactions in mixed ligand complexes may be promoted by the addition of ethanol or dioxane to an aqueous solution of these complexes, we screened the stability data available in the literature for evidence of such intramolecular interactions in binary and ternary amino acid complexes.

The considered intramolecular equilibrium is schematically represented in (1).



The position of this equilibrium is independent of the concentration; it is quantified by the dimensionless equilibrium constant K_1 (eq. (2)),

$$K_1 = [M(A)(B)_{cl}] / [M(A)(B)_{op}] \quad (2)$$

and this constant may be calculated from the stability data obtained from potentiometric pH titrations; the percentage of the «closed» isomer follows from K_1 [1,5,6].

There are two careful studies available, which deal with the effect of dioxane on the stability of amino acid complexes: in one [7] the Cu^{2+} /alaninate (Ala^-) system is described and in the other [8] the Cu^{2+} /leucinate (Leu^-) system. For both systems complex stability increases with increasing amounts of dioxane, but the $Cu(Leu)_2$ complex is in the dioxane-water mixtures by 0.2 to 0.7 log units more stable than expected [3]. This additional stability increase may be attributed to intramolecular hydrophobic interactions between the isopropyl residues in $Cu(Leu)_2$; the extent of this interaction (eq. (1): $A=B=Leu$) changes in dependence on the amount of dioxane added to water: $Cu(Leu)_{2/cl}$ reaches 21% in water, 81% in 50% (v/v) dioxane-water, and 64% in 70% (v/v) dioxane-water ($I=0.1$; $25^\circ C$) [3]. This shows that addition of some dioxane *favours* the intramolecular hydrophobic interaction in $Cu(Leu)_2$, while high concentrations of the organic solvent ($>60\%$) destabilize it. This observation of an initial promotion of the interaction contrasts with the common experience made at simple unbridged adducts: they are always destabilized by the addition of dioxane or other organic solvents [4,9]. In several mixed ligand complexes [3,4], however, intramolecular stacking is also promoted by the addition of ethanol or dioxane.

The stability data of other binary and ternary metal ion/amino acid systems are summarized in the Table; they provide further evidence that hydrophobic and aromatic-ring stacking interactions in complexes of amino acids with suitable side chains are quite common.

It must be mentioned in this connection that the increased stability in aqueous solution of the Cu^{2+} bis-complexes of phenylalanine, tyrosine and similar amino acids has been repeatedly attributed to Cu^{2+} /aromatic-ring interactions [10,11]. However, the often used argument based on the *solid-state* structures is not conclusive, because even in the solid state the interaction is weak, if it exists at all; this follows from two different crystal structure analyses of the bis(L-phenylalaninato) copper(II) complex: in one case [12] the phenyl ring is located below Cu^{2+} , while in the other it is not [13]. In addition, in a recent study [14] of Pd^{2+} complexes it is shown that the decreasing interaction energies in the complexes follow the order phenyl-aromatic > phenyl-propyl (or

Table

Evidence from Stability Data for Intramolecular Hydrophobic and Aromatic-ring Stacking Interactions in Some Binary ($A=B$) and Ternary Amino Acid Complexes in Aqueous Solution (25°C; $I=0.05-0.1$)^{a)}

Complex	% M(A)(B) _{cl} for			
	Co ²⁺	Ni ²⁺	Cu ²⁺	Zn ²⁺
<i>binary:</i>				
M(Nva) ₂	13	~2	17	
M(Phe) ₂	46	38	61	53
M(Tyr) ₂	66	38	67	67
M(Trp) ₂			87	
<i>ternary:</i>				
M(Phe)(Nva)	21	~5	11	
M(Tyr)(Nva)	22	9	24	
M(Phe)(Tyr)	50	25	36	

a) These results are abstracted from the data given in Tables VI and VII of references [3] and [5], respectively; where available, the average of several calculations is listed. Abbreviations: Nva, norvalinate; Phe, phenylalaninate; Trp, tryptophanate; Tyr, tyrosinate.

larger alkyl residue) > ... > Pd²⁺-aromatic >> Pd²⁺-aliphatic ~ 0. This means, the Pd²⁺-aromatic interactions are already weaker than intramolecular hydrophobic or stacking interactions, and a Cu²⁺-aromatic interaction is expected to be even weaker than the Pd²⁺-aromatic one. Indeed, in our studies [2-4] of phenylalkanecarboxylate-Cu²⁺ complexes we could not discover any hint for such an interaction in solution.

The following points which are partly based on the data of the Table argue also *against* a significant Cu²⁺-aromatic interaction in solution but *for* intramolecular ligand-ligand interactions in many amino acid complexes: (i) Always the stability constant for the bis-complex is larger than expected, which is convincingly explained by an intramolecular stack formation. (ii) The increased stability can *not* result from an influence of the second ligand on Cu²⁺, making it more suitable for a Cu²⁺-aromatic interaction (as suggested [10,11]), because, *e.g.*, M(Phe)(Gly) or M(Phe)(Ala) show no increased stability, while M(Phe)(Nva) or M(Phe)(Tyr) do so (Table). (iii) Thermodynamic results also support the postulation of intramolecular ligand-ligand interactions: ΔH_1 for the reaction between Cu²⁺ and Trp⁻ or Ala⁻ are very similar, while ΔH_2 for the addi-

tion of the second Trp⁻ to Cu(Trp)⁺ is about 5 kJ/mol more exothermic than for the reaction Cu(Ala)⁺ + Ala⁻ → Cu(Ala)₂ [15]; this agrees with other studies showing that formation of stacking adducts is not solely entropy driven [16]. (iv) The mentioned Cu²⁺/leucinate results could not be explained without a ligand-ligand interaction. (v) The increased binding tendency of the second ligand is a general feature and occurs with many other metal ions aside from Cu²⁺: for the complexes with Co²⁺, Ni²⁺ and Zn²⁺ the increased stability can hardly be attributed to metal ion-aromatic or metal ion-hydrophobic interactions. Indeed, the data of the Table reveal that the situation is governed by the ligands and *not* by the metal ions. In conclusion: most of the indicated results, if considered for themselves, would not provide very convincing arguments. It is the wealth of data pointing into the same direction which, if combined, provide strong evidence that hydrophobic and aromatic-ring stacking interactions indeed occur in binary and ternary complexes of amino acids with suitable side chains.

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MODELS FOR METAL-PROTEIN

INTERACTION:

COPPER(II) COMPLEXES WITH A CYCLIC PEPTIDE HAVING SIDE-CHAIN IMIDAZOLYL AND CARBOXYL GROUPS

Considerable effort has been devoted to the study of macrocyclic ligands and their metal complexes. Thus, introduction of a metal ion into functionalized cyclic peptides may be an appropriate model for the studies of binding by metalloenzymes. Such cyclic peptides can be designed to incorporate amino acid side chains that are important for the function of various enzymes. For example, the imidazolyl and the carboxyl groups of histidine

and glutamic acid seem to play an important role in the coordination of proteins or naturally occurring peptides to metal ions. The investigation of simple cyclic peptides having side-chain imidazolyl and carboxyl groups as model might be of significant value in elucidating the details of enzyme mechanisms, particularly in aqueous solution.

We chose to synthesize cyclo-(Gly-L-Glu-Gly-L-His-Gly-Gly-L-His-Gly) (hereinafter denoted G5H2Gu). Complexation of G5H2Gu with the transition metal ion Cu(II) in aqueous solution over a wide pH range and with different peptide/metal ratios, has been studied by using carbon-13 and proton NMR, ESR and Visible Spectroscopy.

The results obtained are discussed in terms of different complexes depending on the pH and are compared with a cyclic peptide having different side-chains and a different cavity size.



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THE ROLE OF THE TOSYL GROUP ON THE COORDINATION ABILITY OF N-PROTECTED AMINOACIDS. SOLID STATE BEHAVIOR OF N-TOSYLVALINATE COPPER(II) COMPLEXES

Among N-protected aminoacids, as N-acetyl-, N-benzoyl-, N-benzyloxycarbonyl and N-toluenesulfonyl-aminoacids, only the latter class of deri-