Influence of Solvent Composition (Water–Dioxane Mixtures) on the Formation Degree of Intramolecular Aromatic-Ring Stacks in Binary Cu(L-Phenylalaninate)$_2$, Cu(L-Tryptophanate)$_2$, and Related Complexes

Guogang Liang* and Helmut Sigel**

Institute of Inorganic Chemistry, University of Basel, Spitalstraße 51, CH-4056 Basel, Switzerland

Z. Naturforsch. **44b**, 1555–1566 (1989); received August 11, 1989

Complex Stability, Solvent Influence, Copper Amino Acid Complexes, Phenylalanine Complexes, Stacking Interactions, Tryptophan Complexes

The stability constants of the binary $Cu(AA)^+$ and $Cu(AA)_2$ complexes, where AA^- = L-phenylalaninate (Phe^-) or L-tryptophanate (Trp^-), have been determined by potentiometric pH titrations in water, and in 30, 50, 70 and 80% (v/v) dioxane–water mixtures (I = 0.1 M, $NaNO_3$; 25 °C); the corresponding data for the complexes with L-alaninate (Ala^-), L-valinate (Val^-), L-norvalinate (Nva^-), and L-leucinate (Leu^-) are taken from our recent work (G. Liang, R. Tribolet, and H. Sigel, Inorg. Chim. Acta **155**, 273 (1989)). The overall stability of $Cu(AA)^+$ and $Cu(AA)_2$ is governed for all amino acetates (AA^-) by the polarity of the solvent, while the extent of the intramolecular stack formation between the aromatic side chains in $Cu(AA)_2$ is influenced by the hydrophobic solvation properties of the organic solvent molecules (*i.e.*, the ethylene units of dioxane). Based on the stability difference $\Delta\log K^*_{AA} = \log K^{Cu(AA)}_{Cu(AA)_2} - \log K^{Cu}_{Cu(AA)}$, it is shown that $Cu(Phe)_2$ and $Cu(Trp)_2$ are more stable than $Cu(Ala)_2$, and this increased stability is used for evaluating the extent of the stack formation (= closed form) in $Cu(Phe)_2$ and $Cu(Trp)_2$: the percentages of the closed forms vary between about 25 and 80% (based on $Cu(AA)_{2tot}$), and those for $Cu(Val)_2$, $Cu(Leu)_2$ and $Cu(Nva)_2$ between about 10 and 30%. The formation degree of the intramolecular side-chain adduct in $Cu(AA)_2$ decreases (in most solvents), as one might expect, within the series: $Cu(Trp)_2 > Cu(Phe)_2 > Cu(Val)_2 \gtrsim Cu(Leu)_2 \gtrsim Cu(Nva)_2$. The corresponding observations are made with $M(AA)_2$ complexes of Co^{2+}, Ni^{2+}, and Zn^{2+}. The influence of the organic solvent on the intramolecular hydrophobic and stacking adducts differs somewhat: (i) Stack formation in $Cu(Phe)_2$ and $Cu(Trp)_2$ is slightly inhibited by the presence of dioxane, but even in 50% (v/v) dioxane–water the formation degree of the aromatic-ring stacks is still more than 50%. (ii) Addition of some dioxane to an aqueous solution containing $Cu(Val)_2$, $Cu(Leu)_2$ or $Cu(Nva)_2$ favors the formation of the aliphatic side-chain adducts; the largest formation degree being reached close to a content of 70% dioxane. Both observations contrast with the general experience made at unbridged hydrophobic or stacking adducts: these are considerably destabilized already by the addition of relatively small amounts of an organic solvent to an aqueous solution. Such a destabilization of the closed $Cu(AA)_2$ species occurs only at high concentrations of the organic solvent (usually more than 70%). It should be added that the organic solvent most probably influences the structure of the intramolecular ligand-ligand adducts giving rise to a whole series of "closed" species; a resolution is presently not possible and therefore the whole stability increase is attributed to a (single) so-called "closed" species to allow a quantification of the effect. The relevance of amino acid side-chain interactions regarding cooperativity, selectivity, evolutionary aspects, and low polarity regions, as in active-site cavities of proteins, are shortly indicated.

The side chains of amino acids (AA) are important for the selective interactions in bio-systems, influencing structure, specificity, and activity. For example, in globular proteins close interactions between the aromatic side chains of phenylalanine, tyrosine and tryptophan are common [1, 2], and the corresponding residues often also form part of a hydrophobic pocket designed to bind an aromatic substrate [3], like tryptophan [4]. Stacking interactions between the indole or phenyl residues of tryptophan or phenylalanine, respectively, and nucleotide bases are well-known [5, 6], and promotion of these interac-

Abbreviations: AA, amino acid; AA^-, amino acetate = amino acid monoanion; Ala^-, L-alaninate; ATP^{4-}, adenosine 5′-triphosphate; BzOH, benzyl alcohol; Leu^-, L-leucinate; *i*-Leu^-, *iso*-leucinate; M^{2+}, general divalent metal ion; Nle^-, norleucinate; Nva, L-norvalinate; Phe^-, L-phenylalaninate; Phen, 1,10-phenanthroline; Trp^-, L-tryptophanate; Tyr^-, tyrosinate; Val^-, L-valinate.

* Work done at the University of Basel during a leave from the Academy of Beijing Traditional Medicine, Beijing, People's Republic of China.

** Reprint requests to Prof. Dr. Helmut Sigel.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0932–0776/89/1200–1555/$ 01.00/0

tions in aqueous solution for low-molecular-weight associates *via* the formation of mixed ligand metal ion complexes has been demonstrated [7–10].

However, "homo-interactions" between the same type of aromatic-ring residues are also common [11]. For example, the interaction between phenylalanine rings in proteins is well documented [1, 12], and the formation of intramolecular stacks in aqueous solutions of binary amino acid complexes, like M(phenylalaninate)$_2$, M(tyrosinate)$_2$ and M(tryptophanate)$_2$ with M^{2+} = Co^{2+}, Ni^{2+}, Cu^{2+} or Zn^{2+}, is also known [13, 14]. In connection with these binary complexes one should note that, analogously to the observations made with mixed ligand or ternary complexes [7–9, 14, 15], the formation of a metal ion-bridge between the aromatic-ring residues considerably favors the stability of the stacks [13]. This property is important for the following reasonings and the design of the experiments.

There is now good evidence that in the active-site cavity of an enzyme or protein the "equivalent solution" or "effective" dielectric constant is lower than in water [16, 17], and this raises the question: How are the stacking properties of aromatic side chains of amino acids influenced under such conditions? It should be recalled that addition of organic solvents like ethanol or dioxane to an aqueous solution not only reduces the polarity of the solvent but also inhibits hydrophobic and stacking interactions in unbridged adducts, *i.e.* lowers their stability [15, 18]. Is the latter a general feature? Does it also hold for bridged adducts?

It seemed to us that simple amino acetate (AA^-) complexes are ideal to tackle these problems, as the stability of such complexes can exactly be determined by potentiometric pH titrations. Hence, we studied the stability of the Cu(AA)$_2$ complexes, where AA^- = L-phenylalaninate or L-tryptophanate (Fig. 1), and evaluated the influence of increasing amounts of dioxane on their stability. It turns out that the formation degree of the intramolecular stacks is still significant even in 70% (v/v) dioxane–water mixtures.

The evaluation of the results to be presented is based on the stability of the Cu(L-alaninate)$_2$ complex [19]. The stabilities of M(glycinate)$_2$ complexes are known [20, 21] to be often exceptional and therefore Cu(glycinate)$_2$ was not considered as being a suitable basis. However, the methyl side chain of alaninate is so short that no hydrophobic ligand-

L-AMINO ACIDS

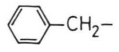
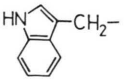
$R-CH(NH_3^+)COO^-$	AA	R^-
Phenylalanine	Phe	
Tryptophan	Trp	
Alanine	Ala	CH_3-
Valine	Val	$(CH_3)_2CH-$
Norvaline	Nva	$CH_3-CH_2-CH_2-$
Leucine	Leu	$(CH_3)_2CH-CH_2-$

Fig. 1. L-Amino acids (AA) considered in this study, together with the structure of their side-chains.

ligand interaction is possible in Cu(Ala)$_2$. This is different with amino acids having a larger aliphatic side chain, like L-valine, L-norvaline or L-leucine (Fig. 1); as shown recently [19], in the corresponding Cu(AA)$_2$ complexes an interligand interaction is occurring to some extent and therefore these previous results are shortly compared with the present ones.

There is one further aspect to be kept in mind: Amino acetates can bind to the equatorial part of the Cu^{2+} coordination sphere in two different ways [22] as shown in Fig. 2, *i.e.* a *cis* and a *trans* isomer may be formed: If both amino acids are of the same chirality both side chains will be on the same side of the complex in the *trans* isomer, and on opposite sides in the *cis* complex. It is obvious, that an intramolecular ligand-ligand interaction can occur only in the *trans* arrangement of a Cu(AA)$_2$ complex. However, solution equilibration rates are rapid for Cu^{2+}, *trans* arrangements are usually preferred [23–26], and the energy barrier for the *cis*/*trans* isomerization in glycinate-like structures is low even in the solid state [27, 28], and consequently most probably even lower in solution [29, 30]. Finally, it should be emphasized

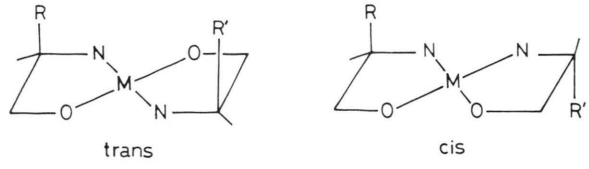


Fig. 2. Possible arrangements of two amino acid anions in the equatorial part [22] of the coordination sphere of Cu^{2+}.

that all complexes to be compared (Fig. 1) have the same 2N/2O donor set coordinated equatorially to Cu^{2+}, and therefore any increased stability, compared with $Cu(Ala)_2$, is to be attributed to intramolecular ligand-ligand interactions.

Experimental

L-Phenylalanine (puriss.) was obtained from Fluka AG, Buchs (Switzerland) and L-tryptophan (biochemical grade) from Merck AG, Darmstadt (F.R.G.). All the other materials were the same as used recently [19].

The potentiometric pH titrations and the evaluations of the experimental data were carried out exactly as described [19]. The only point to be emphasized again is the following: $K^{Cu}_{Cu(AA)}$ and $K^{Cu(AA)}_{Cu(AA)_2}$ were determined from solutions containing the ratios of $[Cu^{2+}]$:[AA] = 1:2, 1:2.5, and 1:3. In total always 9 independent titrations were made and evaluated by taking into account the species H^+, $H_2(AA)^+$, H(AA), AA^-, Cu^{2+}, $Cu(AA)^+$, and $Cu(AA)_2$. In addition, 6 titrations were carried out of solutions containing the ratios of $[Cu^{2+}]$:[AA] = 1:1 and 2:1; these were evaluated by considering also the mentioned species, but by using $K^{Cu(AA)}_{Cu(AA)_2}$ from the previous set of experiments. The resulting values for $K^{Cu}_{Cu(AA)}$ from the two experimental sets were always in excellent agreement; the final results given in the tables are the averages from all determinations. This double-determination of $K^{Cu}_{Cu(AA)}$ was made to obtain the difference, $\Delta\log K^{*}_{Cu} = \log K^{Cu(AA)}_{Cu(AA)_2} - \log K^{Cu}_{Cu(AA)}$, as precise as possible; this difference is crucial for the calculation of the formation degree of the species with an intramolecular ligand-ligand interaction (see Sections 1 and 3).

Results and Discussion

1. Definition of the equilibrium constants and stabilities of the binary $Cu(AA)^+$ and $Cu(AA)_2$ complexes

The equilibrium constants were determined by potentiometric pH titrations (I = 0.1 M, $NaNO_3$; 25 °C). The acidity constants of the protonated amino acids, $H_2(AA)^+$ (eq. (1) and (2)), and the stability constants of their binary $Cu(AA)^+$ (eq. (3)) and $Cu(AA)_2$ (eq. (4)) complexes are defined by the following equilibria:

$$H_2(AA)^+ \rightleftharpoons H(AA)^{\pm} + H^+ \quad (1a)$$

$$K^{H}_{H_2(AA)} = [H(AA)^{\pm}][H^+]/[H_2(AA)^+] \quad (1b)$$

$$H(AA)^{\pm} \rightleftharpoons AA^- + H^+ \quad (2a)$$

$$K^{H}_{H(AA)} = [AA^-][H^+]/[H(AA)] \quad (2b)$$

$$Cu^{2+} + AA^- \rightleftharpoons Cu(AA)^+ \quad (3a)$$

$$K^{Cu}_{Cu(AA)} = [Cu(AA)^+]/[Cu^{2+}][AA^-] \quad (3b)$$

$$Cu(AA)^+ + AA^- \rightleftharpoons Cu(AA)_2 \quad (4a)$$

$$K^{Cu(AA)}_{Cu(AA)_2} = [Cu(AA)_2]/[Cu(AA)^+][AA^-] \quad (4b)$$

The relative stability of the two binary complexes towards each other may be quantified by considering equilibrium (5a):

$$Cu(AA)^+ + Cu(AA)^+ \rightleftharpoons Cu(AA)_2 + Cu^{2+} \quad (5a)$$

$$10^{\Delta\log K_{AA}} = \frac{[Cu(AA)_2][Cu^{2+}]}{[Cu(AA)^+]^2} \quad (5b)$$

The corresponding equilibrium constant, as defined in eq. (5b), may be calculated according to eq. (6):

$$\Delta\log K^{*}_{AA} = \log K^{Cu(AA)}_{Cu(AA)_2} - \log K^{Cu}_{Cu(AA)} \quad (6)$$

It is evident that equilibrium (5a) is expected to be on its left side due to the general rule [31] that $K^{M}_{M(L)} > K^{M}_{M(L)_2}$. Indeed, statistical considerations [31] for $\Delta\log K^{*}_{M}$, assuming an octahedral (oh) coordination sphere for M^{2+} and the coordination of two identical bidentate ligands, lead to $\Delta\log K_{oh} = \log(5{:}2/12{:}1) = -0.68$. For the tetragonal or Jahn-Teller distorted octahedral coordination sphere of Cu^{2+} a statistical value is more difficult to assess; one estimates [31] $\Delta\log K^{*}_{Cu/statist} \simeq \log(1{:}2/8{:}1) = -1.20$.

The equilibrium constants (eq. (1–4)) measured for the systems with L-alanine [19], L-phenylalanine and L-tryptophan are summarized in Table I for water and 30%, 50%, 70% and 80% (v/v) dioxane–water mixtures as solvents. The constants valid for these solvents are given together with the mol fractions of dioxane in the solvents and the corresponding dielectric constants [32].

It is interesting to note already here that the values for the stability differences $\Delta\log K^{*}_{AA}$ (eq. (5, 6)) for the complexes with phenylalaninate and tryptophanate are less negative than the corresponding values for alaninate (Table I). This situation is evidently reflected in Fig. 3, where $\Delta\log K^{*}_{AA}$ is plotted in dependence on the amount of dioxane added to water: only at high concentrations of dioxane the properties of the Cu^{2+} systems with Phe^- and Trp^- are becoming more and more Ala^--like. Hence, already this simple comparison demonstrates the occurrence of a significant intramolecular ligand-ligand interaction in the $Cu(AA)_2$ complexes of Phe^- and Trp^- in most solvent mixtures. A more quantitative evaluation of these data is given in Section 3.

Clearly, a relatively large, *i.e.* less negative value for $\Delta\log K^{*}_{AA}$ may originate either from a low stabili-

Table I. Negative logarithms of the acidity constants of L-alanine, L-phenylalanine and L-tryptophan (eq. (1, 2)) and logarithms of the stability constants of the corresponding binary $Cu(AA)^+$ (eq. (3)) and $Cu(AA)_2$ (eq. (4)) complexes, together with the stability differences $\Delta\log K^*_{AA}$ (eq. (5)), in dependence on the amount of dioxane added to water at I = 0.1 M ($NaNO_3$) and 25 °C[a].

% (v/v) Dioxane	mol fract.	ε[b]	$pK^H_{H_2(AA)}$	$pK^H_{H(AA)}$	$\log K^{Cu}_{Cu(AA)}$	$\log K^{Cu(AA)}_{Cu(AA)_2}$	$\Delta\log K^*_{AA}$
L-Alanine[c]							
0	0	78.5	2.40 ± 0.01	9.84 ± 0.01	8.22 ± 0.02	6.84 ± 0.02	−1.38 ± 0.03
30	0.083	52.7	2.79 ± 0.01	9.95 ± 0.02	8.94 ± 0.02	7.48 ± 0.04	−1.46 ± 0.04
50	0.175	35.2	3.18 ± 0.02	10.03 ± 0.01	9.51 ± 0.02	8.00 ± 0.03	−1.51 ± 0.04
70	0.331	18.6	3.61 ± 0.02	10.12 ± 0.01	10.16 ± 0.02	8.65 ± 0.02	−1.51 ± 0.03
80	0.459	11.6	3.98 ± 0.01	10.07 ± 0.01	10.56 ± 0.02	8.95 ± 0.02	−1.61 ± 0.03
L-Phenylalanine							
0	0	78.5	2.34 ± 0.03	9.20 ± 0.01	7.90 ± 0.02	6.95 ± 0.02	−0.95 ± 0.03
30	0.083	52.7	2.70 ± 0.01	9.31 ± 0.01	8.66 ± 0.02	7.53 ± 0.02	−1.13 ± 0.03
50	0.175	35.2	3.05 ± 0.02	9.40 ± 0.02	9.24 ± 0.02	8.05 ± 0.02	−1.19 ± 0.03
70	0.331	18.6	3.46 ± 0.02	9.41 ± 0.01	9.86 ± 0.02	8.56 ± 0.03	−1.30 ± 0.04
80	0.459	11.6	3.86 ± 0.01	9.33 ± 0.01	10.38 ± 0.03	8.89 ± 0.02	−1.49 ± 0.04
L-Tryptophan							
0	0	78.5	2.42 ± 0.02	9.46 ± 0.02	8.19 ± 0.01	7.49 ± 0.03	−0.70 ± 0.03
30	0.083	52.7	2.85 ± 0.02	9.59 ± 0.02	8.89 ± 0.02	8.01 ± 0.02	−0.88 ± 0.03
50	0.175	35.2	3.27 ± 0.02	9.70 ± 0.02	9.54 ± 0.02	8.48 ± 0.03	−1.06 ± 0.04
70	0.331	18.6	3.65 ± 0.02	9.76 ± 0.01	10.20 ± 0.01	9.02 ± 0.03	−1.18 ± 0.03
80	0.459	11.6	4.01 ± 0.02	9.70 ± 0.01	10.68 ± 0.02	9.21 ± 0.02	−1.47 ± 0.03

[a] The errors given are *three times* the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. The values of the error limits for $\Delta\log K^*_{AA}$ were calculated according to the error propagation after Gauss; [b] the dielectric constants for the dioxane/water mixtures are from [32]; [c] these values are taken from Table I of [19].

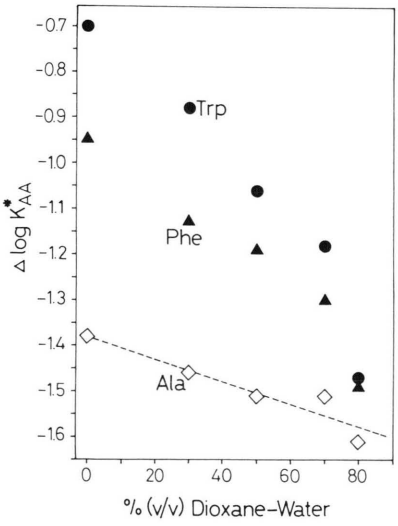


Fig. 3. Dependence for $\Delta\log K^*_{AA}$ (eq. (5, 6)) of the Cu^{2+} systems with L-alaninate (◇), L-phenylalaninate (▲), and L-tryptophanate (●) on the amount of dioxane added to water. The plotted data are from Table I.

ty of $Cu(AA)^+$ or from a high stability of $Cu(AA)_2$ (eq. (6)). In case the reason is a stability-enhancing intramolecular ligand-ligand interaction, the effect should be reflected in $\log K^{Cu(AA)}_{Cu(AA)_2}$ of $Cu(AA)_2$, because the corresponding interaction is possible only in $Cu(AA)_2$ and *not* in $Cu(AA)^+$. To check the validity of this assumption plots of $\log K^{Cu}_{Cu(AA)}$ or $\log K^{Cu(AA)}_{Cu(AA)_2}$ *versus* $pK^H_{H_2(AA)} + pK^H_{H(AA)}$ were constructed for the six amino acids of Fig. 1; for a series of structurally related ligands a straight line should result [33]. As long as an amino acetate (AA^-) coordinates in a glycinate-like way, the stability of the corresponding complex should depend only on the basicity of the amino and carboxylate groups; hence, for a series of $Cu(AA)_2$ complexes with different intensities of ligand-ligand interactions irregular properties should occur.

An example of the mentioned plots is shown in Fig. 4, where the data for water as solvent are given. The afore mentioned expectations are obviously con-

firmed and this holds for all five solvents: (i) There is no correlation for the $Cu(AA)_2$ complexes observed. (ii) The data for the $Cu(AA)^+$ complexes fit with Phe^-, Val^-, Leu^-, Nva^-, and Ala^- on a straight line; only the point due to $Cu(Trp)^+$ is between 0.1 to 0.17 log units above this line depending on the solvent. This means, the $Cu(Trp)^+$ complex is slightly more stable than expected, and therefore $\Delta\log K^*_{Trp}$ is apparently slightly too negative; however, this is without effect for the evaluations in Section 3*.

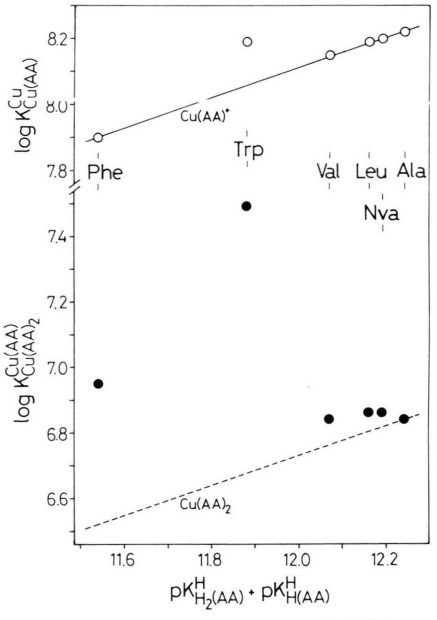


Fig. 4. Relationship between $\log K^{Cu}_{Cu(AA)}$ or $\log K^{Cu(AA)}_{Cu(AA)_2}$ and $pK^H_{H_2(AA)} + pK^H_{H(AA)}$ for the binary $Cu(AA)^+$ (○) and $Cu(AA)_2$ (●) complexes in aqueous solution. The data for Ala, Phe and Trp are from Table I, those for Val, Nva und Leu are from [19] (I = 0.1 M, $NaNO_3$; 25 °C). The points of the $Cu(AA)^+$ complexes for Phe, Val, Leu, Nva and Ala fit on a straight line (full line; regression) with the slope, $m = 0.462 \pm 0.007$ (1σ), and the intercept of the y axis, $y_o = 2.575 \pm 0.082$. The broken line for the $Cu(AA)_2$ complexes is tentatively drawn with the same mentioned slope through the point for Ala.

* Whatever the reason for the slight stability increase in $Cu(Trp)^+$ may be, and in whatever way this may be affected in $Cu(Trp)_2$ by the intramolecular ligand-ligand interaction, its disturbance is on the account of this ligand-ligand interaction, *i.e.* on the stability of $Cu(Trp)_2$, and therefore the stability difference $\Delta\log K^*_{Trp}$ is still exactly describing the position of equilibrium (5a), and also $\Delta\Delta\log K^*$ (eq. (10); Section 3) that of equilibrium (13); hence, the equilibrium constant K^*_I (eq. (7–9)) and consequently the percentage of $Cu(Trp)_{2/cl}$ (eq. (12)) can still be calculated (see Section 3).

In connection with the indicated properties of the $Cu(Trp)^+$ complex it may be mentioned that Martin [21] has described a similar increased stability for $Ni(Trp)^+$, which he attributed to a Ni^{2+}/indole-side chain interaction. If there is indeed a Ni^{2+} or Cu^{2+}/aromatic-ring interaction in $Ni(Trp)^+$ or $Cu(Trp)^+$ has to be left open for the present; other explanations appear also as possible and therefore we are presently further studying this problem. However, the mentioned situation regarding the $M(Trp)^+$ mono-complex should not be confused with the situation in $M(AA)_2$ bis-complexes, where AA^- = tryptophanate, phenylalaninate or tyrosinate; in these bis-complexes the increased stability is clearly due to intramolecular ligand-ligand interactions as outlined previously in detail [13] (for further related examples see also [30]).

2. Solvent influence on the stability of Cu^{2+}-amino acetate complexes

In agreement with observations at complexes of other negatively charged ligands [15, 16, 18, 34], the stability of the $Cu(AA)^+$ and $Cu(AA)_2$ complexes increases with decreasing solvent polarity. This is evident from Fig. 5 where the stabilities of the corre-

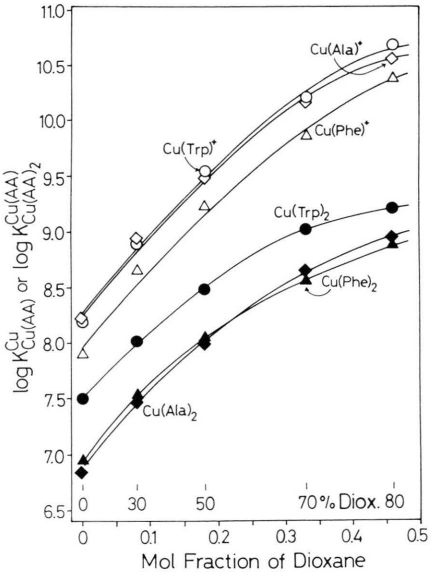


Fig. 5. Plots of $\log K^{Cu}_{Cu(AA)}$ (eq. (3)) or $\log K^{Cu(AA)}_{Cu(AA)_2}$ (eq. (4)) in dependence on the mol fraction of dioxane in water for the binary Cu^{2+} complexes with L-alaninate (◇, ◆), L-phenylalaninate (△, ▲), and L-tryptophanate (○, ●). The plotted data are taken from Table I.

sponding complexes with Ala^-, Phe^-, and Trp^- are plotted in dependence on the mol fraction of dioxane present in water. A more detailed inspection of Fig. 5 reveals further that, *e.g.*, the data for $Cu(Ala)^+$ and $Cu(Trp)^+$ furnish nearly the same curve, while those for the corresponding $Cu(AA)_2$ complexes are on rather different curves; in other words, $Cu(Trp)_2$ is considerably more stable than $Cu(Ala)_2$ and this is again a reflection of the intramolecular stack formation in $Cu(Trp)_2$. Similar arguments could be advanced for the Cu^{2+}/Phe complexes.

As the change in solvent polarity affects not only the stability of the complexes but correspondingly also the basicity of the amino acetates (see Table I), plots of $\log K^{Cu}_{Cu(AA)}$ *versus* $pK^{H}_{H_2(AA)} + pK^{H}_{H(AA)}$ may be constructed as seen in Fig. 6. For the complexes with Ala^-, Phe^-, and Trp^-, as well as with those of Val^-, Nva^-, and Leu^- [19], straight lines are resulting. Similar plots could also be constructed for the $Cu(AA)_2$ complexes, but this is only reasonable for $Cu(Ala)_2$ (see Fig. 6) because this is the only binary 1:2 complex of the amino acetates considered here

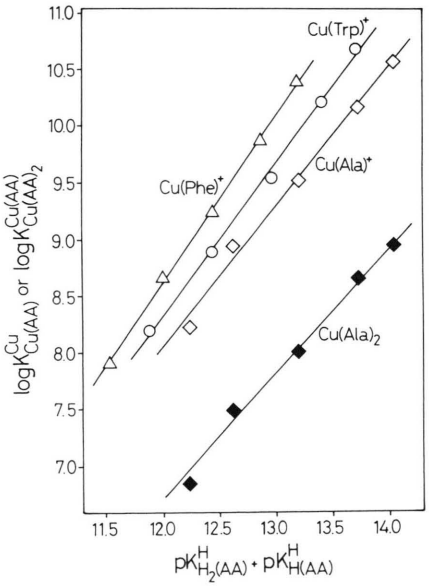


Fig. 6. Relationship between $\log K^{Cu}_{Cu(AA)}$ (eq. (3)) or $\log K^{Cu(AA)}_{Cu(AA)_2}$ (eq. (4)) and $pK^{H}_{H_2(AA)} + pK^{H}_{H(AA)}$ (eq. (1, 2)) for the binary $Cu(AA)^+$ and $Cu(Ala)_2$ complexes with L-alaninate, L-phenylalaninate, and L-tryptophanate. The data are from Table I. The slopes of the straight (regression) lines are $m_{Cu(Phe)} = 1.480 \pm 0.028$ (△), $m_{Cu(Trp)} = 1.350 \pm 0.043$ (○), $m_{Cu(Ala)} = 1.281 \pm 0.024$ ($\pm 1\,\sigma$) (◇), and $m_{Cu(Ala)_2} = 1.172 \pm 0.026$ (◆).

(Fig. 1), in which no intramolecular ligand-ligand interaction occurs. The results of Fig. 6 prove that there is a linear relation between ligand basicity and complex stability resulting from the solvent influence.

3. Solvent influence on the extent of the intramolecular ligand-ligand interaction in $Cu(Phe)_2$, $Cu(Trp)_2$ and related $Cu(AA)_2$ complexes

It may be recalled in this connection that intramolecular hydrophobic and stacking interactions between suitable groups in mixed-ligand complexes have been established by several methods including ^1H NMR shift measurements in organic solvent-water mixtures [13, 18, 34]. Therefore, with the solvent influence on the overall stability of the $Cu(AA)^+$ and $Cu(AA)_2$ complexes in mind, it is interesting to evaluate the extent of the ligand-ligand interaction in $Cu(AA)_2$; this may be done by considering the position of the intramolecular equilibrium (7a) between an *open* (op) and a *closed* (cl) form:

$$Cu(AA)_{2/op} \rightleftharpoons Cu(AA)_{2/cl} \qquad (7a)$$

$$K_I^* = [Cu(AA)_{2/cl}]/[Cu(AA)_{2/op}] \qquad (7b)$$

The dimensionless equilibrium constant K_I is calculated in the usual way [18, 30] with eq. (8) and (9):

$$K_I^* = \frac{10^{\Delta \log K^*_{AA}}}{10^{\Delta \log K^*_{AA/op}}} - 1 \qquad (8)$$

$$K_I^* = 10^{\Delta\Delta \log K^*} - 1 \qquad (9)$$

The crucial parameter by calculating K_I^* from eq. (8) is the following difference, defined as $\Delta\Delta \log K^*$:

$$\Delta\Delta \log K^* = \Delta \log K^*_{AA} - \Delta \log K^*_{AA/op} \qquad (10)$$

Combination of eq. (8) and (10) gives the mentioned eq. (9). Obviously any error in the experimentally determined constants will be the more significant, the smaller the difference is in eq. (10); well-defined error limits of the constants are therefore compulsory.

Use of the experimental results summarized in Table I allows the calculation of K_I^*: the values for $\Delta \log K^*_{AA}$ have been measured, and the stability of the open form is evidently well represented by the stability of $Cu(Ala)_2$, as in this latter case no interaction between the two residues is possible due to the shortness of the methyl side chain; hence, one may define:

$$\Delta \log K^*_{AA/op} = \Delta \log K^*_{Ala} \qquad (11)$$

Table II. Extent of the intramolecular ligand-ligand interaction in the binary Cu(L-phenylalaninate)$_2$ and Cu(L-tryptophanate)$_2$ complexes in dependence on the amount of dioxane added to water: intramolecular and dimensionless equilibrium constant K_I^* (eq. (7–9)) and percentage (eq. (12)) of the closed species $Cu(AA)_{2/cl}$ in different solvents at I = 0.1 M ($NaNO_3$) and 25 °C[a].

$Cu(AA)_2$	% (v/v) Dioxane	$\Delta\log K^*_{AA}$	$\Delta\log K^*_{AA/op}$	$\Delta\Delta\log K^*$	K_I^*	% $Cu(AA)_{2/cl}$
$Cu(Phe)_2$	0	−0.95 ± 0.03	−1.38 ± 0.03	0.43 ± 0.04	1.69 ± 0.26	63 ± 4
	30	−1.13 ± 0.03	−1.46 ± 0.04	0.33 ± 0.05	1.14 ± 0.25	53 ± 5
	50	−1.19 ± 0.03	−1.51 ± 0.04	0.32 ± 0.05	1.09 ± 0.24	52 ± 6
	70	−1.30 ± 0.04	−1.51 ± 0.03	0.21 ± 0.05	0.62 ± 0.19	38 ± 7
	80	−1.49 ± 0.04	−1.61 ± 0.03	0.12 ± 0.05	0.32 ± 0.15	24 ± 9
$Cu(Trp)_2$	0	−0.70 ± 0.03	−1.38 ± 0.03	0.68 ± 0.04	3.79 ± 0.47	79 ± 2
	30	−0.88 ± 0.03	−1.46 ± 0.04	0.58 ± 0.05	2.80 ± 0.44	74 ± 3
	50	−1.06 ± 0.04	−1.51 ± 0.04	0.45 ± 0.06	1.82 ± 0.37	65 ± 5
	70	−1.18 ± 0.03	−1.51 ± 0.03	0.33 ± 0.04	1.14 ± 0.21	53 ± 5
	80	−1.47 ± 0.03	−1.61 ± 0.03	0.14 ± 0.04	0.38 ± 0.13	28 ± 7

[a] The values for $\Delta\log K^*_{AA}$ (eq. (5)) and their error ranges (*three times* the standard error) are from Table I; $\Delta\log K^*_{AA/op} = \Delta\log K^*_{Ala}$ (eq. (11)). The error limits for $\Delta\Delta\log K^*$ (eq. (10)), K_I^* (eq. (9)), and % $Cu(AA)_{2/cl}$ (eq. (12)) were calculated according to the error propagation after Gauss.

Clearly, knowledge of K_I^* also allows to calculate the percentage of the closed form in equilibrium (7a):

$$\%\ Cu(AA)_{2/cl} = \frac{K_I^*}{1 + K_I^*} \cdot 100 \qquad (12)$$

The results based on eq. (9) through (12) are summarized in Table II.

It may be helpful to emphasize that $\Delta\Delta\log K^*$ (eq. (10)), which is given in the fifth column of Table II, is the difference between the logarithms of two equilibrium constants (eq. (5)) and therefore $10^{\Delta\Delta\log K^*}$ is also quantifying the position of an equilibrium. By making use of the definition (11), this equilibrium may be written as:

$$2\,Cu(AA)^+ + Cu(Ala)_2 \rightleftharpoons Cu(AA)_2 + 2\,Cu(Ala)^+ \qquad (13)$$

It is evident that the coordination spheres of Cu^{2+} on both sides of equilibrium (13) are identical and therefore $10^{\Delta\Delta\log K^*}$ does indeed reflect the extent of the intramolecular ligand-ligand interaction in $Cu(AA)_2$. In those cases where $10^{\Delta\Delta\log K^*} > 1$, equilibrium (13) is displaced towards its right side; with $AA^- = Phe^-$ or Trp^- $\Delta\Delta\log K^*$ varies between 0.12 and 0.68 (Table II), *i.e.* $10^{\Delta\Delta\log K^*}$ is between about 1.3 and 4.8 indicating that the formation of $Cu(Phe)_2$ and $Cu(Trp)_2$ is significantly favored.

To facilitate comparisons, the formation degrees of the closed $Cu(AA)_2$ species with Phe^- and Trp^-, as well as with Val^-, Nva^- and Leu^- (Fig. 1) are plotted in dependence on the mol fraction of dioxane in Fig. 7; the data for the last mentioned three ligands are from [19]. It is evident that the side chains of all five amino acids are suitable for ligand-ligand interactions; the formation degree of the closed species resulting from hydrophobic and stacking interactions is quite significant not only in water but also in dioxane–water mixtures. Comparison of the intramolecular stacking tendency in $Cu(Trp)_2$ and $Cu(Phe)_2$ shows throughout a somewhat larger formation degree of

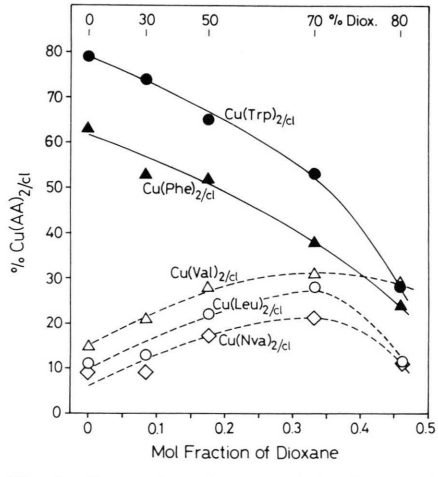


Fig. 7. Formation degree of the intramolecularly closed species (eq. (7, 12)) in the binary $Cu(Phe)_2$ (▲) and $Cu(Trp)_2$ (●) complexes in dependence on the mol fraction of dioxane in water; these plotted data are from Table II. For comparison the corresponding results are shown for $Cu(Val)_{2/cl}$ (△), $Cu(Leu)_{2/cl}$ (○), and $Cu(Nva)_{2/cl}$ (◇); these data are taken from Table II of [19].

$Cu(Trp)_{2/cl}$ (Fig. 7). This agrees with previous observations in aqueous solution [13, 30], and with the expectation that the larger indole moiety of tryptophanate should be somewhat better suitable for the formation of stacks than the smaller phenyl ring of phenylalaninate.

One may also expect that the intensity of an interaction between aromatic rings is more pronounced than between aliphatic residues [13, 30, 35]. This is indeed confirmed by the results shown in Fig. 7, at least for the solutions containing up to 70% dioxane; the formation degree of the intramolecular adducts decreases in the series: $Cu(Trp)_2 > Cu(Phe)_2 > Cu(Val)_2 \gtrsim Cu(Leu)_2 \gtrsim Cu(Nva)_2$. In all cases the intramolecular adducts are destroyed at high concentrations of the organic solvent, which is in agreement with previous experience [18, 34]. To emphasize that the phenomena described here are of general nature, the data of Table III have been compiled [13, 19, 36]. Though these data refer only to aqueous solution it is evident that ligand-ligand interactions also occur to a considerable extent in amino acid complexes of other metal ions with different coordination geometries; the order of the interaction intensities described for the Cu^{2+} complexes is also clearly valid for other $M(AA)_2$ species (*cf.* also Section 4).

4. Structural considerations on the intramolecular ligand-ligand interactions in $M(AA)_2$ complexes

At first we shall consider the situation in aqueous solution, and it should be recalled that the usually preferred [23–26] *trans*-equatorial coordination to Cu^{2+} of two amino acetates with the same chirality gives a complex with both side chains on the same side of the coordination square (*cf.* Fig. 2). With this in mind, one may conclude, based on space-filling molecular models, *e.g.*, for $Cu(Phe)_2$ that the two phenyl moieties are separated at the basis of the plane by about 5.5 Å and inclination of the two phenyl rings towards each other easily allows at the "top" parts of the rings an approach of 3.5 Å (or less), and this is the usual distance in stacking interactions [37–39].

Table III. Estimations for the percentage of the closed species with the intramolecular hydrophobic or aromatic-ring stacking interaction (eq. (7, 12)) for several $M(AA)_2$ complexes in aqueous solution at 25 °C (I = 0.05–0.1 M)[a].

$M(AA)_2$	% $M(AA)_{2/cl}$ for Co^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}
$M(Val)_2$			15 ± 8	
$M(Nva)_2$	13	~2	17/ 9 ± 7	
$M(Leu)_2$			19/11 ± 7	
$M(Nle)_2$	13	~4		
$M(Phe)_2$	46	38	59/63 ± 4	53
$M(Tyr)_2$	66	38	67	67
$M(Trp)_2$		48 ± 8[b]	87/79 ± 2	

[a] The values with an error limit (3σ) are for the Cu^{2+} complexes with Val^-, Nva^- and Leu^- from [19], and for those with Phe^- and Trp^- from Table II of this work; all the other values are the averages (in the case of several values) of the percentages listed in Table VI of [13]; [b] calculated with the constants given in [36] and eq. (7–12).

The described structural arrangement would still provide the space for an apically coordinated water molecule and we suggest that the differences between the $Cu(AA)_2$ and $Ni(AA)_2$ complexes originate here: From the data for $M(AA)_{2/cl}$ in Table III it is evident that the intramolecular ligand-ligand interaction in the Cu^{2+} complexes is more pronounced than in those with Ni^{2+}. The differences between the following stability constants [36], $\log K^{Ni}_{Ni(Ala)} - \log K^{Ni(Ala)}_{Ni(Ala)_2} = 5.50 - 4.66 = 0.84$ and $\log K^{Ni(Ala)}_{Ni(Ala)_2} - \log K^{Ni(Ala)_2}_{Ni(Ala)_3} = 4.66 - 3.29 = 1.37$, indicate that in the $Ni(Ala)_2$ complex the two amino acetates are mainly equatorially arranged with two apical water molecules completing the octahedral coordination sphere. As indicated above, such an apical water at the basis between the two interacting side chains in $Ni(Phe)_{2/cl}$ (or the related species) appears as sterically possible, but a situation without such a water molecule would certainly be preferable. This seems to be achieved with Cu^{2+}, for which penta-coordination is well known (*e.g.*, [24, 40–43]); *i.e.*, a structure with the (more weakly bound) apical water molecule on one side of the equatorial square-plane and the two amino acid side-chains on the other (Fig. 2). The fact, that Cu^{2+} is usually somewhat displaced from the base plane containing the stronger bonds toward the more weakly bound top atom of the pyramid (*e.g.*, [24, 44]) would further favor the side-chain interaction.

Clearly, in complexes of metal ions with tetrahedral (Zn^{2+}, ?) or octahedral (Co^{2+}, Zn^{2+}, ?) coordination spheres and amino acids of the considered type a ligand-ligand interaction is (at least) as easily achieved as in the distorted coordination sphere of Cu^{2+}. Indeed, the formation degrees listed in Table III for the $M(AA)_{2/cl}$ complexes with Co^{2+}, Cu^{2+}, and Zn^{2+} are identical for a given amino acetate within experimental error. Regarding the structure of the

intramolecular stack three points should be added: (i) It is known that stacked aromatic rings are often orientated in a butterfly-like way (*e.g.*, [39, 45]). (ii) Interactions are also possible between edges of aromatic-ring systems, as suggested [46], *e.g.*, for Cu(1,10-phenanthroline)(benzoate)$^+$; pertinent solid-state interactions have been described [39, 47, 48]. In fact, (iii) the majority of the interacting phenylalanines in proteins have their ring-planes perpendicular on each other [12, 49]. That hydrophobic interactions between aliphatic side-chains are less stable than aromatic-ring stacks (Table III) is expected and was also already indicated in Section 3.

How is the situation regarding the intramolecular ligand-ligand interaction in organic solvent-water mixtures? The bell-shaped curves of Fig. 7 representing the formation of $Cu(AA)_{2/cl}$ for Val^-, Leu^-, and Nva^- in dependence on the mol fraction of dioxane added to water can only result, if at first the increasing amounts of dioxane promote the intramolecular interaction in the concentration-independent equilibrium (7a), while at higher concentrations inhibition occurs. However, the same effect, though less pronounced, must also occur with $Cu(Trp)_2$ and $Cu(Phe)_2$, because the inhibition of intramolecular stack formation, *e.g.*, in 50% dioxane is only very minor (Fig. 7) compared with the effect of the same solvent composition on metal ion unbridged stacks (Table IV). The observation at $Cu(AA)_{2/cl}$ species should not be confused with results given in Fig. 5: the overall stability of $Cu(AA)^+$ and $Cu(AA)_2$ complexes increases with the increasing mol fraction of dioxane.

How may the solvent composition influence the position of equilibrium (7a)? A tentative but appealing explanation is that at low mol fractions the ethylene units of the dioxane molecules do not associate well with the separated alkyl or aryl amino acid side-chains in the open $Cu(AA)_2$ isomer. Instead, the already larger intramolecular hydrophobic adducts or stacks formed by the mentioned side-chains in $Cu(AA)_{2/cl}$ are preferably solvated, stabilizing therefore the $Cu(AA)_{2/cl}$ species. At high concentrations of the organic solvent the hydrophobic solvation by dioxane of the individual side-chain residues in $Cu(AA)_{2/op}$ is enforced and thus the parts forming the adducts are separated; *i.e.*, now $Cu(AA)_{2/op}$ is favored and the formation of $Cu(AA)_{2/cl}$ is inhibited. These indicated opposing solvent effects are contrary to the experience with simple unbridged stacks which are destabilized already by small amounts of organic solvents (Table IV) [15, 18].

A more detailed discussion of the solvent effects, including the role of the aqueous solvation shell of the metal ion, on the position of equilibrium (7a) is given in [34] and [50] and shall not be repeated here. However, three points must be emphasized: (i) In $Cu(AA)_{2/cl}$ the intramolecular aromatic/aliphatic adduct present in water will act, due to its lipophilicity, upon addition of an organic solvent as a germ attracting the aliphatic parts of these organic solvent molecules forming a micelle-like unit close to the

Table IV. Stability constants $K^{(A)}_{(A)(B)}$ for the equilibrium A + B $\rightleftharpoons$ [A][B] of binary adducts in dependence on the amount of dioxane added to an aqueous solution[a–c].

No.	[A][B]	Solvent	$K^{(A)}_{(A)(B)}$ (M^{-1})
1	[Phen][ATP^{4-}]	D_2O	38 ± 8
2	[Phen][ATP^{4-}]	30% (v/v) D_6-Diox./D_2O	4.8 ± 1.1
3	[Phen][ATP^{4-}]	50% (v/v) D_6-Diox./D_2O	1.8 ± 0.7
4	[H(Phen)$^+$][H_2(Trp)$^+$]	H_2O	25.1
5	[H(Phen)$^+$][H_2(Trp)$^+$]	60% (v/v) Dioxane/H_2O	0.6
6	[$Zn(Phen)^{2+}$][BzOH]	H_2O	2.2 ± 0.2
7	[$Zn(Phen)^{2+}$][BzOH]	50% (v/v) Dioxane/H_2O	0.42 ± 0.09
8	[$Zn(Phen)^{2+}$][BzOH]	80% (v/v) Dioxane/H_2O	<0.1

[a] Entries **1–3** are from [15]. I = 0.1 M ($NaNO_3$); 27 °C. The constants were determined by ^{1}H NMR shift measurements; [b] entries **4** and **5** are from [35]. I = 0.4 M (HCl); 23 °C. The constants were determined by UV spectrophotometry; [c] entries **6–8** (BzOH = benzyl alcohol) are from [18]. I = 0.25 to 0.5 (in **6**), to 1.3 (in **7**) and to 0.6 (in **8**) ($NaNO_3$); 34 °C. The constants were determined by ^{1}H NMR shift measurements under the assumption that Zn^{2+} and 1,10-phenanthroline react practically completely to $Zn(Phen)^{2+}$; there is no Zn^{2+}-bridge formed between the two interacting aromatic-ring systems.

metal ion. (ii) For this micelle-like unit in $Cu(AA)_{2/cl}$ it is to be expected that different hydrophobic solvation degrees mean somewhat different structures for the actual adduct; it appears well possible that organic solvent molecules intercalate between the aryl/aryl or alkyl/alkyl side-chain residues; hence, there is probably a whole series of intramolecular adducts existing, but there is presently no way to identify these with certainty, and therefore the whole observed stability increase is attributed to a (single) so-called "closed" species (eq. (7a)). (iii) The proximity of the micelle-like unit in $Cu(AA)_{2/cl}$ at one side of Cu^{2+} is expected to reduce the *effective* dielectric constant in the close vicinity of the metal ion, and this should favor the M^{2+}/O^- binding strength; in fact, this may well be the actual "mechanism" leading to the increased stability of $Cu(AA)_{2/cl}$. It is evident that at high concentrations of the organic solvent the situations in $Cu(AA)_{2/op}$ (where AA^- = Trp^-, Phe^-, Val^-, Nva^-, and Leu^-) and in $Cu(Ala)_2$ will become quite alike and the different dielectric-constant effect will thus diminish.

5. Some conclusions regarding side-chain interactions of amino acids

To evaluate the solvent influence on hydrophobic and stacking interactions somewhat further, the following consideration may be helpful. The solvent influence on binary and unbridged adducts formed between amino acid residues has so far not been studied, but stability constants of related adducts have been determined in several solvent mixtures; some results on the effect of dioxane are given in Table IV.

It is well-known that addition of an organic solvent like dioxane (or ethanol) to an aqueous solution of binary and unbridged stacking adducts, [A][B], will strongly reduce their concentration [18, 51, 52]. The same conclusion is borne out from the results summarized in Table IV. It should further be emphasized that coordination of a metal ion either to the reactant A or B does not alter this situation, as long as no metal ion-bridge between A and B is formed: This conclusion is supported by entries No. 6 to 8 in Table IV, which show the influence of dioxane on the stack formation between $Zn(Phen)^{2+}$ and benzyl alcohol (BzOH). All these results are easily rationalized: addition of an organic solvent leads to a solvation of the aromatic-ring systems by the alkyl parts of the organic solvent molecules; hence, this "hydrophobic" solvation competes with the formation of the stacking adducts and reduces thus their formation degree [18]. For example, the stability of such binary adducts is drastically decreased if the solvent is changed from water to 50% (v/v) dioxane–water: the stability of the adducts is reduced by factors of about ⅕ to 1/40. This observation contrasts strongly with the present results summarized in Fig. 7: It is obvious that the solvent influence on metal ion-bridged adducts as present in $Cu(AA)_2$ complexes is much less destructive, in fact even promotion may occur. In other words, a metal ion-bridge may promote hydrophobic and stacking interactions also under conditions of a reduced solvent polarity.

Despite the indicated lack of data for amino acid systems some comparisons are possible: Assuming ideal fits the following stability constants for binary amino acid adducts in aqueous solution have been derived [11]: $K^{(Phe)}_{(Phe)(Leu)} = 2\ M^{-1}$, $K^{(Phe)}_{(Phe)_2} = 10.7\ M^{-1}$, and $K^{(i\text{-}Leu)}_{(i\text{-}Leu)_2} = 12.5\ M^{-1}$. For related systems the following values have been estimated: $K^{(Bpy)}_{(Bpy)(H\cdot Leu)} = 0.6 \pm 0.4\ M^{-1}$ [30], $K^{(Phen)}_{(Phen)(H\cdot Leu)} = 1.4 \pm 0.9\ M^{-1}$ [30], and $K^{(ATP)}_{(ATP)(H\cdot Trp)} = 6.2\ M^{-1}$ [36]. Taking a value of $10\ M^{-1}$ (which is rather an upper limit) for the stability constant of a hydrophobic adduct between two amino acids in water one calculates for a 2×10^{-3} M reactant solution that about 4% of the amino acid molecules are present in the form of a binary adduct [13]. These few percent should be compared with the results shown in Fig. 8, which apply for solutions containing Cu^{2+} (10^{-3} M) and phenylalanine or tryptophan (2×10^{-3} M) at pH 7. In these two systems in aqueous solution about 55 and 72%, respectively, of the amino acids are present as $Cu(AA)_{2/cl}$, and similar high concentrations of the metal ion-bridged adducts still exist in 50% (v/v) dioxane–water, while under these conditions the concentration of the unbridged stacks is certainly below 1%.

The results of Fig. 7 and 8 are instructive as they reveal the structuring forces inherent in the side-chains of phenylalanine, tryptophan, and aliphatic amino acids. These forces were certainly important during evolution of life and they are still participating in the formation of the three-dimensional structures of proteins, as well as in the determination of selectivity as it occurs in bio-systems, *e.g.* with regard to nucleotides [7, 8].

Taking everything together it appears that the most important results of this study are: (i) the

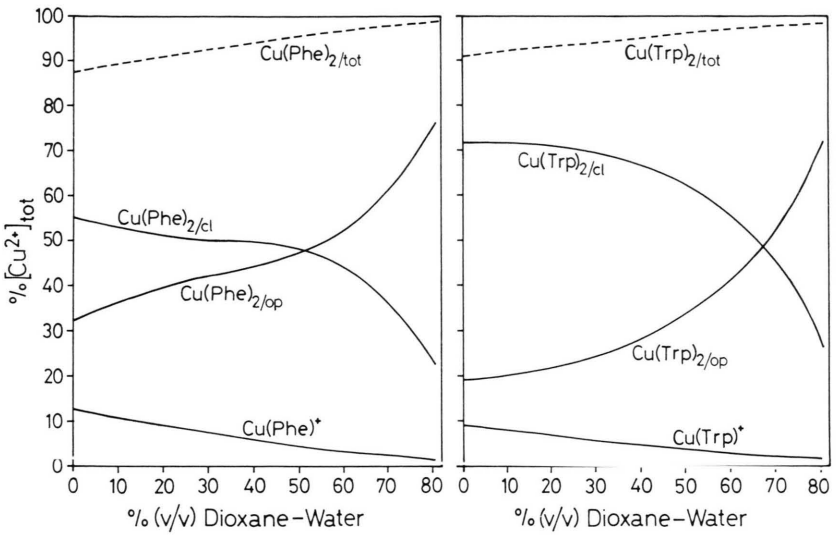


Fig. 8. Effect of the amount of dioxane added to an aqueous solution of Cu^{2+} and L-phenylalanine (left part) or L-tryptophan (right part) at pH 7.00 on the concentration of the species present (full lines). The broken lines represent the total concentration of the $Cu(AA)_2$ complexes. The results are given as the percentage of the total Cu^{2+} concentration ($= 10^{-3}$ M; $[AA]_{tot} = 2 \times 10^{-3}$ M) present; computed with constants listed in Tables I and II; I = 0.1 M ($NaNO_3$) and 25 °C. The concentration of the uncomplexed Cu^{2+} is in water $< 0.2\%$ and in 80% dioxane $\leq 0.001\%$; hydroxo-complex formation of Cu^{2+} is insignificant under these conditions.

stabilization of intramolecular stacks and hydrophobic adducts by metal ion-bridging, and (ii) the observation that a reduced polarity of the solvent, *i.e.* the presence of hydrophobic groups from an organic solvent, does not prevent the formation of intramolecular stacks or hydrophobic adducts in $M(AA)_2$ complexes. Considering the reduced polarity in active-site cavities of enzymes [16, 17] this latter observation is important, because the type of interactions described here are expected to create cooperativity [34, 50] and thus also selectivity.

The support of this research by a grant from the Swiss National Science Foundation and a fellowship to G.L. from the University of Basel within the exchange program between the People's Republic of China and Switzerland are gratefully acknowledged.

[1] a) P. K. Warme and R. S. Morgan, J. Mol. Biol. **118,** 273 (1978);
b) P. K. Warme and R. S. Morgan, J. Mol. Biol. **118,** 289 (1978).
[2] S. K. Burley and G. A. Petsko, Science **229,** 23 (1985).
[3] D. G. Hangauer, A. F. Monzingo, and B. W. Matthews, Biochemistry **23,** 5730 (1984).
[4] D. N. Arvidson, C. Bruce, and R. P. Gunsalus, J. Biol. Chem. **261,** 238 (1986).
[5] M. I. Khamis, J. R. Casas-Finet, and A. H. Maki, J. Biol. Chem. **262,** 1725 (1987).
[6] T. Ishida, K. Kamiichi, A. Kuwahara, M. Doi, and M. Inoue, Biochem. Biophys. Res. Commun. **136,** 294 (1986).
[7] H. Sigel, Chimia **41,** 11 (1987).
[8] H. Sigel, B. E. Fischer, and E. Farkas, Inorg. Chem. **22,** 925 (1983).
[9] H. Sigel, in D. Banerjea (ed.): Coordination Chemistry – 20, p. 27, Pergamon Press (IUPAC), Oxford, New York (1980).
[10] a) C. F. Naumann and H. Sigel, FEBS Lett. **47,** 122 (1974);
b) H. Sigel and C. F. Naumann, J. Am. Chem. Soc. **98,** 730 (1976);
c) P. R. Mitchell, B. Prijs, and H. Sigel, Helv. Chim. Acta **62,** 1723 (1979).
[11] H. A. Scheraga, Acc. Chem. Res. **12,** 7 (1979).
[12] J. Singh and J. M. Thornton, FEBS Lett. **191,** 1 (1985).
[13] H. Sigel, R. Tribolet, and K. H. Scheller, Inorg. Chim. Acta **100,** 151 (1985).
[14] M. Tabata and M. Tanaka, Inorg. Chem. **27,** 3190 (1988).
[15] R. Tribolet, R. Malini-Balakrishnan, and H. Sigel, J. Chem. Soc. Dalton Trans. **1985,** 2291.
[16] H. Sigel, R. B. Martin, R. Tribolet, U. K. Häring, and R. Malini-Balakrishnan, Eur. J. Biochem. **152,** 187 (1985).
[17] N. K. Rogers, G. R. Moore, and M. J. E. Sternberg, J. Mol. Biol. **182,** 613 (1985).

[18] H. Sigel, R. Malini-Balakrishnan, and U. K. Häring, J. Am. Chem. Soc. **107,** 5137 (1985).
[19] G. Liang, R. Tribolet, and H. Sigel, Inorg. Chim. Acta **155,** 273 (1989).
[20] R. B. Martin, Met. Ions Biol. Syst. **9,** 1 (1979).
[21] R. B. Martin, Met. Ions Biol. Syst. **23,** 123 (1988).
[22] L. D. Pettit and R. J. W. Hefford, Met. Ions Biol. Syst. **9,** 173 (1979).
[23] T. G. Fawcett, M. Ushay, J. P. Rose, R. A. Lalancette, J. A. Potenza, and H. J. Schugar, Inorg. Chem. **18,** 327 (1979).
[24] D. Van Der Helm and C. E. Tatsch, Acta Crystallogr., Sect. B **28,** 2307 (1972).
[25] G. G. Aleksandrov, Yu. T. Struchkov, A. A. Kurganov, S. V. Rogozhin, and V. A. Davankov, J. Chem. Soc. Chem. Commun. **1972,** 1328.
[26] D. Van Der Helm, M. B. Lawson, and E. L. Enwall, Acta Crystallogr., Sect. B **27,** 2411 (1971).
[27] A. V. Ablov, I. A. Diyakon, V. Ya. Ivanova, N. N. Proskina, and L. F. Chapurina, Russ. J. Inorg. Chem. (Engl. Trans.) **10,** 339 (1965).
[28] a) B. W. Delf, R. D. Gillard, and P. O'Brien, J. Chem. Soc. Dalton Trans. **1979,** 1301;
b) P. O'Brien, J. Chem. Educ. **59,** 1052 (1982).
[29] a) See discussion in footnote 65 of [30];
b) T. V. Timofeeva, A. A. Kurganov, V. A. Davankov, and Yu. T. Struchkov, Izv. Akad. Nauk. SSSR, Ser. Khim. **1987,** 2484; Engl. Trans. **1988,** 2306.
[30] B. E. Fischer and H. Sigel, J. Am. Chem. Soc. **102,** 2998 (1980).
[31] H. Sigel, Angew. Chem. **87,** 391 (1975); Angew. Chem., Int. Ed. Engl. **14,** 394 (1975).
[32] a) G. Åkerlöf and O. A. Short, J. Am. Chem. Soc. **58,** 1241 (1936);
b) F. E. Critchfield, J. A. Gibson (Jr.), and J. L. Hall, J. Am. Chem. Soc. **75,** 1991 (1953);
c) G. Åkerlöf and O. A. Short, J. Am. Chem. Soc. **75,** 6357 (1953).
[33] R. B. Martin and H. Sigel, Comments Inorg. Chem. **6,** 285 (1988).
[34] G. Liang, R. Tribolet, and H. Sigel, Inorg. Chem. **27,** 2877 (1988).
[35] O. Yamauchi and A. Odani, J. Am. Chem. Soc. **107,** 5938 (1985).
[36] J. B. Orenberg, B. E. Fischer, and H. Sigel, J. Inorg. Nucl. Chem. **42,** 785 (1980).
[37] R. W. Gellert and R. Bau, Met. Ions Biol. Syst. **8,** 1 (1979).
[38] K. Aoki, J. Am. Chem. Soc. **100,** 7106 (1978).
[39] E. Dubler, U. K. Häring, K. H. Scheller, P. Baltzer, and H. Sigel, Inorg. Chem. **23,** 3785 (1984).
[40] H. C. Freeman, Adv. Prot. Chem. **22,** 257 (1967).
[41] D. Van Der Helm, S. E. Ealick, and J. E. Burks, Acta Crystallogr., Sect. B **31,** 1013 (1975).
[42] H. Muhonen and R. Hämäläinen, Finn. Chem. Lett. **1983,** 120.
[43] K. Aoki and H. Yamazaki, J. Chem. Soc. Dalton Trans. **1987,** 2017.
[44] W. A. Franks and D. Van Der Helm, Acta Crystallogr., Sect. B **27,** 1299 (1970).
[45] a) W. S. Sheldrick, Z. Naturforsch. B, Anorg. Chem., Org. Chem. **37b,** 863 (1982);
b) J.-P. Sauvage, personal communication (January 18th, 1988): There are examples for stacks between aromatic rings with angles of about 30°; see also C. O. Dietrich-Buchecker, J. Guilhem, A. K. Khemiss, J.-P. Kintzinger, C. Pascard, and J.-P. Sauvage, Angew. Chem., Int. Ed. Engl. **26,** 661 (1987);
c) A further example with an angle of about 38° is given in O. Yamauchi, A. Odani, T. Kohzuma, H. Masuda, K. Toriumi, and K. Saito, Inorg. Chem. **28,** 406 (1989).
[46] R. Malini-Balakrishnan, K. H. Scheller, U. K. Häring, R. Tribolet, and H. Sigel, Inorg. Chem. **24,** 2067 (1985).
[47] L. Antolini, L. Menabue, G. C. Pellacani, M. Saladini, M. Sola, L. P. Battaglia, and A. Bonamartini Corradi, J. Chem. Soc. Dalton Trans. **1984,** 2319.
[48] K. Aoki and H. Yamazaki, Nippon Kagaku Kaishi **1988,** 611.
[49] One may expect that such a perpendicular interaction of two phenyl rings is (at least) as strong as that of two isopropyl residues (*cf.* also the results in Table III and those plotted in Fig. 7).
[50] H. Sigel, Pure Appl. Chem. **61,** 923 (1989).
[51] B. Farzami, Y. H. Mariam, and F. Jordan, Biochemistry **16,** 1105 (1977).
[52] K. A. Connors and S.-R. Sun, J. Am. Chem. Soc. **93,** 7239 (1971).