Solvent Effects on Intramolecular Hydrophobic Ligand–Ligand Interactions in Binary and Ternary Complexes*

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Abstract

The stability constants of mixed ligand complexes of the type M(Phen)(ACA)$^+$, where M = Cu^{2+} or Zn^{2+}, Phen = 1,10-phenanthroline and ACA^- = propionate, valerate and 2-cyclohexylacetate, were determined by potentiometric pH titration in 50% (v/v) dioxane–water and were compared with the stabilities of the corresponding ternary complexes formed with formate and acetate. The ternary complexes containing the alkanecarboxylates (ACA^-) are significantly more stable, due to intramolecular hydrophobic interactions between the alkyl residue of the ACA^- ligands and the 1,10-phenanthroline molecule. For Zn(Phen)(valerate)$^+$ this intramolecular ligand-ligand interaction was confirmed by ^1H NMR shift measurements. The formation degree of the intramolecular adducts in the ternary Cu^{2+} and Zn^{2+} complexes was calculated and the position of the intramolecular equilibrium between the opened and closed isomer was determined: the closed isomer occurs between about 10 to 35 percent. Comparisons with related data show that the extent of this interaction is about the same in water and in 50% aqueous dioxane; this contrasts with the experience made with simple unbridged adducts, which are destabilized by the addition of dioxane (or other organic solvents). Furthermore, evaluation of the available stability data for the Cu^{2+}/leucinate (Leu^-) system shows that addition of some dioxane to an aqueous solution (in which the closed isomer exists to about 20%) *favors* the intramolecular interaction between the two isopropyl residues in $Cu(Leu)_2$ considerably: in 40 to 50% aqueous dioxane the formation degree of the closed isomer reaches about 80%. Higher concentrations of the organic solvent destabilize the hydrophobic interaction. The overall stability of Cu(Leu)$^+$ and $Cu(Leu)_2$, as well as of Cu(alaninate)$^+$ and $Cu(alaninate)_2$, is governed by the polarity of the solvent while the extent of the intramolecular ligand-ligand interaction is influenced by the hydrophobic properties of the solvent molecules. Based on stability data it is shown that intramolecular ligand–ligand interactions are quite a common feature for many binary and ternary amino acid complexes: *e.g.*, $M(norvalinate)_2$, $M(phenylalaninate)_2$, $M(tyrosinate)_2$ [M = Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}] or $Cu(tryptophanate)_2$ and M(phenylalaninate)(norvalinate) or M(phenylalaninate)(tyrosinate) [M = Co^{2+}, Ni^{2+}, Cu^{2+}]. In addition, evidence is presented that direct M^{2+}–aromatic interactions are of no significance in these amino acid complexes in solution. The relevance of the present results with regard to biological systems is indicated.

Introduction

The associations between nucleic acids and proteins involve mainly electrostatic interactions, hydrogen bonding, aromatic-ring stacking and hydrophobic interactions [3]. Among these noncovalent interactions aromatic-ring stacking and hydrophobic interactions have recently received much attention because the forces governing the corresponding associations are not always evident [4–6]. Even in adducts of low molecular weight these interactions can be crucial and it has been shown [7] that metal ions are able to promote hydrophobic and stacking interactions between suitable side chains of amino acids and the nucleic base residues of nucleotides in mixed ligand complexes.

As structural conditions cannot easily be varied in mixed ligand complexes of nucleotides and/or amino acids, we have studied the structurally more simple systems containing 1,10-phenanthroline, Cu^{2+} or Zn^{2+}, and a phenylalkanecarboxylate. The optimal conditions for the formation of intramolecular stacks have been described [1] and the influence of organic solvents on the formation degree of these stacks was quantified [8]. In contrast to the general experience made with simple

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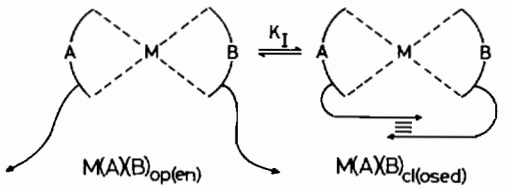
and unbridged binary stacking adducts the addition of *e.g.* dioxane to an aqueous solution favors the formation of the intramolecular stacks: the highest formation degree is reached in aqueous solutions which consist of about 50% of dioxane [8].

Hydrophobic interactions are related to stacking interactions [4–6], and it was therefore interesting to see whether organic solvents could also favor intramolecular hydrophobic interactions in mixed ligand complexes. As the polarity of water in the active-site cavity of enzymes is reduced [9], this question is of general interest. In accord with our previous studies [1, 8] we again used 1,10-phenanthroline, Cu^{2+} and Zn^{2+}, but instead of phenylalkanecarboxylates we employed now only alkanecarboxylates (ACA^-), *i.e.* propionate, valerate, and 2-cyclohexylacetate.

From a previous ^1H NMR shift study [10] in aqueous solution it is known that the alkyl residue of valerate (Va^-)* and the ring system of 1,10-phenanthroline (Phen)* undergo in the ternary $Zn(Phen)(Va)^+$ complex a hydrophobic interaction [11]. We have now determined the extent of this interaction for all the mentioned $M(Phen)(ACA)^+$ complexes in 50% aqueous dioxane by calculating the dimensionless equilibrium constant K_I (eqn. 2) of the intramolecular equilibrium schematically represented in 1:



(1)

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

Indeed, the results show that even in 50% aqueous dioxane the extent of the intramolecular hydrophobic interaction is considerable. Moreover, the recently published [12] stability constants of the Cu^{2+}–leucinate complexes, $Cu(Leu)^+$ and $Cu(Leu)_2$, which had been measured in aqueous solutions containing between 0 and 70% dioxane, allowed a quantitative evaluation of the influence of dioxane on the hydrophobic interaction between the isopropyl moieties in $Cu(Leu)_2$. That the isopropyl residue of leucinate is suited for hydrophobic interactions had been observed before [6, 7, 13]. In addition, these results offer a solid basis to evaluate other literature data and provide evidence for intramolecular ligand-ligand interactions in many metal ion complexes of amino acids with aliphatic and/or aromatic side chains.

*Abbreviations: Aa^-, amino acid anion; Abu^-, α-aminobutyrate; Ac^-, acetate; ACA^-, alkanecarboxylate (= Pr^-, Va^-, $CHAc^-$); Ala^-, α-alaninate; ATP, adenosine 5'-triphosphate; Bpy, 2,2'-bipyridyl; CA^-, carboxylate ligand; $CHAc^-$, 2-cyclohexylacetate; Gly, glycinate; L, general ligand; Leu^-, leucinate; M^{2+}, general divalent metal ion; Nle, norleucinate; Nva, norvalinate; PAc^-, 2-phenylacetate; Phe, phenylalaninate; $PheCa^-$, phenylalkanecarboxylate; Phen, 1,10-phenanthroline; PPr^-, 3-phenylpropionate; Pr^-, propionate; $TMSPr^-$, 3(trimethylsilyl)propionate; Trp, tryptophanate; Tyr, tyrosinate; Va^-, valerate.

Experimental Section

Propionic acid (*puriss.*), valeric acid (*puriss.*) and 2-cyclohexylacetic acid (*purum*) were obtained from Fluka AG, Buchs, Switzerland. All other materials were the same as used earlier [1, 2]. The stock solutions were also prepared as described [2].

The concentrations of the solutions used for the potentiometric pH titrations (under nitrogen; I = 0.1, $NaClO_4$, 25 °C) were the same as before [1, 2], and the evaluation of the data was done as described [2]: this means a pair of titration curves obtained by titrating corresponding solutions in the presence and absence of ligand was always evaluated. In other words, the titration curve of the strong acid ($HClO_4$) was not calculated but was experimentally determined each time. The direct pH meter readings were used in the calculations for the acidity constants of the ligands; no 'corrections' were applied for the change in solvent to 50% aqueous dioxane, though correction factors have been published [14].

The ^1H NMR spectra of the valeric acid systems were recorded on a Varian Anaspect EM-360 spectrometer (60 MHz) at 34 °C and the signals assigned according to the literature [15]. The experiments and their evaluations were done as before [2, 8].

Results and Discussion

1. Stability of the Binary $M(CA)^+$ and Ternary $M(Phen)(CA)^+$ Complexes

By potentiometric pH titrations we determined in 50% (v/v) dioxane-water the acidity constants of several carboxylic acids and the stability constants of the corresponding binary and ternary carboxylate complexes. These results are listed in Table I; all equilibrium constants are defined as usual [1, 2, 8].

Plots of log $K^M_{M(L)}$ *vs.* $pK^H_{H(L)}$ for a series of structurally related ligands should result in straight lines [16, 17]. Indeed, from Fig. 1 it is evident that for the binary Cu^{2+} and Zn^{2+} complexes the plots of log $K^M_{M(CA)}$ *versus* $pK^H_{H(CA)}$ result in straight lines. However, for the ternary complexes the situa-

TABLE I. Negative Logarithms of the Acidity Constants of Several Carboxylic Acids and Logarithms of the Corresponding Binary $M(CA)^+$ and Ternary $M(Phen)(CA)^+$ Complexes in 50% (v/v) Dioxane–Water (corresponding to a mol fraction of 0.175) at I = 0.1 ($NaClO_4$) and 25 °C. The Resulting Values for $\Delta \log K_M$ (eqn. 3) are also listed.[a]

CA^-	$pK^H_{H(CA)}$	$\log K^{Cu}_{Cu(CA)}$	$\log K^{Cu(Phen)}_{Cu(Phen)(CA)}$	$\Delta \log K_{Cu}$	$\log K^{Zn}_{Zn(CA)}$	$\log K^{Zn(Phen)}_{Zn(Phen)(CA)}$	$\Delta \log K_{Zn}$
$HCOO^-$	4.73 ± 0.02	2.79 ± 0.02	2.82 ± 0.02	0.03 ± 0.03	1.96 ± 0.01	1.82 ± 0.02	−0.14 ± 0.02
Ac^-	5.97 ± 0.01	3.31 ± 0.02	3.35 ± 0.01	0.04 ± 0.02	2.31 ± 0.01	2.15 ± 0.01	−0.16 ± 0.01
Pr^-	6.24 ± 0.01	3.41 ± 0.02	3.51 ± 0.02	0.10 ± 0.03	2.40 ± 0.01	2.29 ± 0.02	−0.11 ± 0.02
Va^-	6.34 ± 0.01	3.44 ± 0.02	3.61 ± 0.02	0.17 ± 0.03	2.44 ± 0.02	2.42 ± 0.02	−0.02 ± 0.03
$CHAc^-$	6.48 ± 0.02	3.45 ± 0.02	3.70 ± 0.01	0.25 ± 0.02	2.46 ± 0.01	2.51 ± 0.01	0.05 ± 0.01

[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 $\Delta \log K_M$ were calculated according to the error propagation after Gauss. The above values for $HCOO^-$ and Ac^- are taken from our earlier work [1].

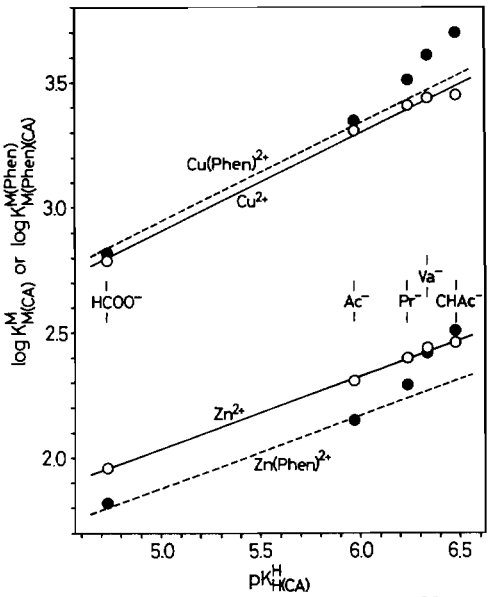


Fig. 1. Relationship between $\log K^M_{M(CA)}$ or $\log K^{M(Phen)}_{M(Phen)(CA)}$ and $pK^H_{H(CA)}$ for the binary complexes, $M(CA)^+$ (○), or the ternary complexes, $M(Phen)(CA)^+$ (●), with *simple* carboxylates ($HCOO^-$, Ac^-) and several *alkane*-carboxylates (Pr^-, Va^-, $CHAc^-$). The data are from Table I and those fo the binary complexes fit on straight lines (*full* lines; regression: m_{Cu} = 0.393 ± 0.020 (1σ) and m_{Zn} = 0.291 ± 0.007); the reference lines for the ternary complexes (*broken* lines) are drawn with the corresponding slopes but only through the points of $HCOO^-$ and Ac^- (see text).

tion is quite different: only the values for the Cu(Phen)$(CA)^+$ and Zn(Phen)$(CA)^+$ complexes with formate and acetate fit (within experimental error) on straight lines (*i.e.* the broken lines in Fig. 1) parallel to the reference lines of the binary complexes (solid lines). The points due to the ternary *alkane*carboxylate complexes with propionate, valerate and 2-cyclohexylacetate for both metal ions are significantly above the corresponding broken line; hence, these ternary complexes are more stable than expected on the basis of the basicity of the

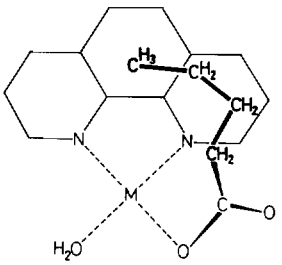


Fig. 2. Possible (schematic) structure of $M(Phen)(Va)^+$ for the isomer with the intramolecular hydrophobic ligand–ligand interaction between the alkyl residue of valerate and the aromatic ring system of 1,10-phenanthroline.

carboxylate groups, suggesting that some additional interaction must occur. This, and the fact that the extent of the increase in stability obviously depends on the size of the alkyl residue of the carboxylate ligand, indicate that a hydrophobic ligand–ligand interaction is the source of this enhanced stability. A schematic structure of a possible ligand arrangement in such a complex is shown in Fig. 2.

A common way [18] to quantify the stability of ternary complexes is defined in eqn. 3:

$$\Delta \log K_M = \log K^{M(Phen)}_{M(Phen)(CA)} - \log K^M_{M(CA)} \qquad (3)$$

The constant $10^{\Delta \log K_M}$ determines the position of equilibrium 4:

$$M(Phen)^{2+} + M(CA)^+ \rightleftharpoons M(Phen)(CA)^+ + M^{2+} \qquad (4)$$

which contains on both sides species of the same charge type, minimizing any electrostatic contribution to the equilibrium constant (eqn. 3). Statistical considerations for $\Delta \log K_M$, assuming an octahedral (O_h) coordination sphere for Zn^{2+}, lead to $\Delta \log K_{oh}$ = −0.18; should the coordination sphere of Zn^{2+} be tetrahedral (th) then $\Delta \log K_{th}$ = −0.3 [1]. For the tetragonal or Jahn–Teller distorted octahedral coordination sphere of Cu^{2+} one estimates

[1] $\Delta \log K_{Cu/statist} \simeq -0.5$. A comparison of these statistical values with the experimentally determined values for $\Delta \log K_M$, which are listed in Table I, shows that even for the ternary Cu^{2+} systems with formate and acetate $\Delta \log K_{Cu}$ is slightly *positive* and hence significantly larger than the statistical estimate. In case of the Zn^{2+} complexes, the stability enhancement is somewhat smaller, but the situation with both metal ions corresponds to previous experience [1, 2, 11, 18, 19] and has been discussed before [18, 19]. Here it is important to note that also with the $\Delta \log K_M$ quantification the ternary Cu^{2+} and Zn^{2+} complexes containing the alkanecarboxylates are significantly more stable than are those with formate and acetate. This confirms the above reasoning made with regard to Fig. 1: the ternary $M(Phen)(ACA)^+$ complexes show an increased stability which may be attributed to an intramolecular ligand–ligand interaction.

2. ^1H NMR Shift Study of the $Zn(Phen)(Va)^+$ Complex

With the aim to obtain a direct proof of the intramolecular ligand–ligand interaction we recorded the ^1H NMR spectra for the Zn^{2+}/Phen/valerate system in 50% (v/v) dioxane–water. The variation of the chemical shift of the methyl protons of Va^- with increasing concentrations of Zn^{2+}/Phen is shown in Fig. 3. The resulting stability constant, $\log K^{Zn(Phen)}_{Zn(Phen)(Va)} = 2.0 \pm 0.2$ ($I = 0.25$–0.5, $NaNO_3$; 34 °C), is in fair agreement with the value determined by potentiometric pH titration, 2.42 ± 0.02 ($I = 0.1$, $NaClO_4$; 25 °C), considering the differences in

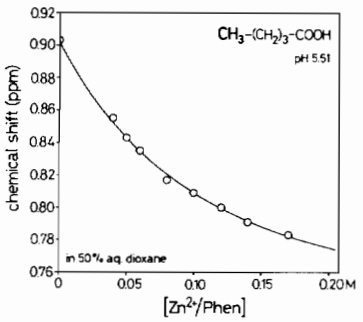


Fig. 3. Variation of the chemical shift of the resonance of the methyl protons of valeric acid (4×10^{-2} M) in dependence on increasing concentrations of Zn^{2+}/Phen (1:1) in 50% (v/v) dioxane–water at pH 5.51 ($I = 0.25$–0.5, $NaNO_3$; 34 °C). The spectra were measured relative to internal $(CH_3)_4N^+$ and converted to values relative to sodium (trimethylsilyl)propane sulfonate by adding 3.163 ppm. The curve shown is the computer-calculated best fit of the experimental data [8, 13]: $\delta_o = 0.903 \pm 0.001$ ppm, $\delta_\infty = 0.717 \pm 0.007$ ppm (1σ). The apparent stability constant, $\log K_{app} = 1.113 \pm 0.043$ (1σ), was transformed into the pH independent stability constant as described in [8, 13] by using $pK^H_{H(Va)} = 6.34$ of Table I ($I = 0.1$; 25 °C)*: $\log K^{Zn(Phen)}_{Zn(Phen)(Va)} = 2.0 \pm 0.2$ (3σ).

temperature and ionic strength. Both these effects are expected to lower the complex stability; in fact, the size of the effect observed now corresponds well with previous experience [8].

However, the main point of these measurements are the following conclusions. Protonation or metal ion coordination shift the signals of ligand protons close to the binding site *down*field [10, 11]; indeed, a small downfield shift upon Zn^{2+} coordination has been shown earlier for an aqueous solution [10]. Similarly, protonation in 50% aqueous dioxane results also in a small downfield shift: $\Delta\delta = 0.015 \pm 0.003$ ppm ($\delta_{Va} = 0.890$ ppm; $\delta_{H(Va)} = 0.905$ ppm). Such a small effect is expected, because the distance in valerate between the protons of the methyl group and the basic carboxylate group is quite large. However, in a complex in which the aliphatic residue of a carboxylate is located above or below the plane of an aromatic ring, the signals of the aliphatic protons should be shifted upfield relative to those of the free carboxylate [11]; this is exactly observed as is seen in Fig. 3: $\Delta\delta = 0.173 \pm 0.022$ (3σ) ppm ($\delta_{Va} = 0.890$ ppm; $\delta_{Zn(Phen)(Va)} = 0.717$ ppm).

It is interesting to note that this upfield shift of 0.173 ppm measured for a 50% dioxane solution is quite significant, but is clearly smaller than the value of 0.31 ppm measured earlier [10] in aqueous solution**. This is an indication that the hydrophobic methylene groups of the dioxane molecules present in the solvent mixture participate in the solvation of the ternary complex; this could lead *e.g.* to a different orientation of the methyl group of valerate on the phenanthroline ring system (Fig. 2) and thus result in a different upfield shift. The corresponding observation has been made recently [8] for 2-phenylacetate (PAc^-) in the $Zn(Phen)(PAc)^+$ complex in a series of solvent mixtures. It is important to emphasize, also based on these earlier observations [8], that a smaller upfield shift does *not* a priori mean that the extent of the hydrophobic interaction is smaller; it is an indication only for a different solvation and/or orientation of the aliphatic and aromatic systems.

*It may be mentioned here that the influence of some variation in temperature (25 to 35 °C) and ionic strength (0.1 to 1.0 M) on the acidity constants of carboxylic acids is small (*i.e.* within 0.02 log unit) [20]; hence, the use of the acidity constant of Table I for the calculations indicated in the legend of Fig. 3 is justified.

**A preliminary experiment [21], carried out analogously to the one shown in Fig. 3, with *iso*-valerate (i-Va) and Zn^{2+}/Phen in aqueous solution at pH 5.8 (I = 0.3, $NaNO_3$; 34 °C) gave an upfield shift for the two terminal methyl groups of i-Va ($\Delta\delta \simeq 0.35$ ppm) and the following estimate for the stability constant: $\log K^{Zn(Phen)}_{Zn(Phen)(i\text{-}Va)} \simeq 0.7$ (calculated with $pK^H_{H(i\text{-}Va)} = 4.7$).

TABLE II. Extent of the Intramolecular Ligand–Ligand Interaction (see *e.g.* Fig. 2) in Ternary Cu^{2+} and Zn^{2+} Complexes Containing 1,10-Phenanthroline and an Alkanecarboxylate (ACA^-): Intramolecular and Dimensionless Equilibrium Constant K_I and Percentage of the Isomer $M(Phen)(ACA)^+_{cl}$ with the Hydrophobic Interaction in 50% (v/v) Dioxane–Water at I = 0.1 ($NaClO_4$) and 25 °C. The Corresponding Data Determined in Water for Some Related Systems are Given for Comparison.

No. Complex	Δ log K_M (eqn. 3)[a]		ΔΔ log K (eqn. 6)[c]	K_I (eqn. 2, 5)[c]	% $M(Phen)(ACA)^+_{cl}$ (eqn. 1)[c]
in 50% Dioxane:					
1 $Cu(Phen)(HCOO)^+$	0.03 ± 0.03	0.04 ± 0.02[b]			
2 $Cu(Phen)(Ac)^+$	0.04 ± 0.02				
3 $Cu(Phen)(Pr)^+$	0.10 ± 0.03		0.06 ± 0.03(0.03)	0.15 ± 0.07(0.09)	13 ± 6(7)
4 $Cu(Phen)(Va)^+$	0.17 ± 0.03		0.13 ± 0.03(0.03)	0.35 ± 0.09(0.10)	26 ± 5(6)
5 $Cu(Phen)(CHAc)^+$	0.25 ± 0.02		0.21 ± 0.02(0.03)	0.62 ± 0.08(0.11)	38 ± 3(4)
6 $Zn(Phen)(HCOO)^+$	−0.14 ± 0.02	−0.15 ± 0.01[b]			
7 $Zn(Phen)(Ac)^+$	−0.16 ± 0.01				
8 $Zn(Phen)(Pr)^+$	−0.11 ± 0.02		0.04 ± 0.02(0.03)	0.10 ± 0.06(0.07)	9 ± 5(5)
9 $Zn(Phen)(Va)^+$	−0.02 ± 0.03		0.13 ± 0.03(0.03)	0.35 ± 0.09(0.10)	26 ± 5(5)
10 $Zn(Phen)(CHAc)^+$	0.05 ± 0.01		0.20 ± 0.01(0.02)	0.58 ± 0.05(0.07)	37 ± 2(3)
in Water:					
11 $Cu(Phen)(HCOO)^+$	−0.04 ± 0.10	−0.02 ± 0.06[b]			
12 $Cu(Phen)(Ac)^+$	−0.01 ± 0.05				
13 $Cu(Phen)(Pr)^+$	0.02 ± 0.03		0.04 ± 0.03(0.07)	0.10 ± 0.08(0.17)	9 ± 7(14)
14 $Cu(Phen)(TMSPr)^+$	0.16 ± 0.03		0.18 ± 0.03(0.06)	0.51 ± 0.10(0.22)	34 ± 4(10)
15 $Zn(Phen)(HCOO)^+$	−0.17 ± 0.06	−0.19 ± 0.04[b]			
16 $Zn(Phen)(Ac)^+$	−0.21 ± 0.03				
17 $Zn(Phen)(TMSPr)^+$	0.02 ± 0.05		0.21 ± 0.05(0.06)	0.62 ± 0.20(0.24)	38 ± 8(9)

[a]These values and their error ranges (*three times* the standard error) are from Table I (entries No. 1–10), [2] (No. 11–13; I = 0.1, KNO_3; 25 °C, [10] (No. 14, 17; I = 0.1, $NaNO_3$; 35 °C) and [1] (No. 15, 16; I = 0.1, KNO_3; 25 °C). [b]This value corresponds to Δ log K_{op} of eqn. 6. [c]The error limits given with these data correspond to the errors of the individual values of Δ log K_M. The error limits in parentheses include also the error in Δ log K_{op}; these error limits should be used in external comparisons. For internal comparisons, *i.e.* within the same series of data, the errors based only on the individual Δ log K_M value are more appropriate, because Δ log K_{op} is the same within a series and any error in this value will influence the calculations in the same (systematic) way.

3. Extent of the Intramolecular Hydrophobic Ligand–Ligand Interaction in the Ternary Complexes Containing Alkanecarboxylates

The ^1H NMR measurements prove only that a hydrophobic interaction occurs in $Zn(Phen)(Va)^+$, but this does not mean that the formation of a species similar to the one shown in Fig. 2 is dominating. In fact, one has to expect an equilibrium between an 'open' and a 'closed' form as indicated already in eqn. 1. With eqn. 5 (for details see [8, 13]):

$$K_I = 10^{\Delta\Delta \log K} - 1 \qquad (5)$$

where*:

$$\Delta\Delta \log K = \Delta \log K_{(M/Phen/ACA)} - \Delta \log K_{(M/Phen/ACA)_{op}} \qquad (6)$$

*Usually the difference of eqn. 3 is referred to as Δ log K_M; only in those cases where further identification is needed are additional subscripts given, like Δ log $K_{(M/Phen/ACA)}$.

information about the position of equilibrium 1 may be calculated. The values to be used for Δ log $K_{(M/Phen/ACA)}$ correspond to those given for Δ log K_M in Table I; the problem at first sight seems to be to obtain values for Δ log $K_{(M/Phen/ACA)_{op}}$. However, from Fig. 1 it is evident that the stability of the open isomers is well represented by the ternary complexes containing formate or acetate; with formate no hydrophobic interaction is possible and, in accord with Fig. 1, for acetate so far no such interaction has been detected [1, 8, 10, 11]. Hence, Δ log $K_{(M/Phen/ACA)_{op}}$ is obtained by averaging the Δ log K_M values of the formate and acetate systems.

The results of the calculations for the ternary complexes of the alkanecarboxylates using eqn. 5 and the constants listed in Table I are summarized in Table II. The assumption about the existence of equilibrium 1 is confirmed; both isomers occur in appreciable concentrations in 50% aqueous dioxane, although the percentage of the open form is always somewhat larger. It is interesting to note that the percentage of the closed isomer for a given

alkanecarboxylate is similar in the Cu(Phen)(ACA)$^+$ and Zn(Phen)(ACA)$^+$ complexes indicating that the interaction is not very dependent on the geometry of the coordination sphere of the metal ion. This is different with phenylalkanecarboxylates as ligands [1, 2, 8]. However, in the present cases the percentage of the closed isomer depends clearly on the size of the aliphatic residue of the carboxylate ligand. Furthermore, a comparison with previous results [1, 2] obtained for the corresponding ternary complexes with phenylalkanecarboxylates, indicates that aromatic-ring stacking interactions are somewhat more stable than hydrophobic interactions: *e.g.*, of Cu(Phen)(PAc)$^+$ about 60% exist in the stacked form in 50% aqueous dioxane.

4. Solvent Influence on the Hydrophobic Interaction in M(Phen)(ACA)$^+$ Complexes

Although the systems with the alkanecarboxylates (entries No. 1–10 of Table II) have only been studied in 50% (v/v) dioxane-water, some conclusions about the solvent influence on M(Phen)(ACA)$^+$ are possible by taking into account the entries No. 11–17 of Table II. The size of the aliphatic residue of 3(trimethylsilyl)propionate (TMSPr$^-$) is somewhere between that of valerate (Va$^-$) and 2-cyclohexylacetate (CHAc$^-$), so that these three ligand systems may be compared with each other. The extent of the hydrophobic interaction in Cu(Phen)(TMSPr)$^+$ and in [Zn(Phen)(TMSPr)$^+$ is (within experimental error) the same in aqueous solution, confirming that the influence of the geometry of the coordination sphere of the metal ion seems to be small (see Section 3). The interesting point is however that the approximately 35% determined for the closed isomers, M(Phen)(TMSPr)$^+_{cl}$, are within the error limits equal to the percentages determined for M(Phen)(Va)$^+$ and especially M(Phen)(CHAc)$^+$ in 50% aqueous dioxane.

Despite all shortcomings due to the structural differences of the alkyl residues of the carboxylate ligands, it is clear that the hydrophobic interaction in water is not significantly more pronounced than in 50% aqueous dioxane; a conclusion which is also in accord with entries No. 3, 8 and 13 of Table II. In other words, the addition of up to 50% dioxane to an aqueous solution does *not* diminish the extent of the intramolecular hydrophobic interaction. This result corresponds to our previous observations [8] made with phenylalkanecarboxylates (PheCA$^-$) and the stack formation in M(Phen)(PheCA)$^+$ complexes. However, for any hydrophobic interaction in binary, *i.e.* metal ion-free systems, a strong decrease in the stability of the adducts must be expected [8, 22] by the addition of organic solvents, like dioxane or ethanol, to an aqueous solution of the reactants. One may conclude that the continuing existence of hydrophobic interactions (or even their promotion; see Section 6) in mixed aqueous/organic solvents is closely connected with the formation of a metal ion-bridge between the two ligands carrying the interacting residues.

5. Evidence for an Intramolecular Hydrophobic Interaction in Cu(Leucinate)$_2$

With the described results in mind we read the recent publication of Zelano *et al.* [12], a 'Potentiometric Study of Copper(II) Complexes of *L*-Leucine in Water-Dioxane Mixtures'. The results of this study as far as they are pertinent for the present considerations are summarized in Table III. If one compares the differences according to eqn. 7 (which is analogous to eqn. 3)*:

$$\Delta \log K^*_M = \log K^{M(Aa)}_{M(Aa)_2} - \log K^{M}_{M(Aa)} \qquad (7)$$

*All stability differences, such as $\Delta \log K^*_M$, or intramolecular constants, K^*_I, which refer to binary complexes are marked with an asterisk (*). In addition, the statements made in footnote * p. 155 apply correspondingly here.

TABLE III. Negative Logarithms of the Acidity Constants of *L*-Leucine and Logarithms of the Corresponding Binary Cu^{2+} Complexes, together with the Stability Difference $\Delta \log K^*_{Cu}$ (eqn. 7), in Dependence on the Amount of Dioxane Added to Water (I = 0.1, $NaClO_4$; 25 °C).[a]

% (v/v) Dioxane)	$pK^{H}_{H_2(Leu)}$	$pK^{H}_{H(Leu)}$	$\log K^{Cu}_{Cu(Leu)}$	$\log K^{Cu(Leu)}_{Cu(Leu)_2}$	$\Delta \log K^*_{Cu}$ [b]
0	2.35	9.51	8.19	6.94	−1.25
10	2.46	9.59	8.39	7.14	−1.25
20	2.65	9.57	8.57	7.45	−1.12
30	2.79	9.60	8.81	7.79	−1.02
40	2.99	9.56	9.11	8.13	−0.98
50	3.20	9.70	9.40	8.32	−1.08
60	3.45	9.68	9.65	8.41	−1.24
70	3.92	9.77	10.01	8.47	−1.54

[a]These data are from the work of Zelano, Roletto, and Vani [12]. [b]See eqn. 7.

TABLE IV. Negative Logarithms of the Acidity Constants of Alanine and Logarithms of the Corresponding Binary Cu^{2+} Complexes, together with the Stability Difference $\Delta \log K^{*}_{Cu}$ (eqn. 7), in Dependence on the Amount of Dioxane Added to Water (25 °C).[a]

No.	% (v/v)[b] Dioxane	I	Inert Salt	$pK^{H}_{H_2(Ala)}$	$pK^{H}_{H(Ala)}$	$\log K^{Cu}_{Cu(Ala)}$	$\log K^{Cu(Ala)}_{Cu(Ala)_2}$	$\Delta \log K^{*}_{Cu}$
1	0	0.1	[c]	2.30	9.69	8.13	6.79	−1.34
2	0	0.5	[c]	2.29	9.66	8.14	6.76	−1.38
3	0	0.2	$NaClO_4$	2.31	9.73	8.18	6.82	−1.36
4	0	0.2	KCl	2.35	9.69	8.07	6.72	−1.35
5	19.4(20)	0.2	KCl	2.65	9.78	8.45	6.88	−1.57
6	34.2(35)	0.2	KCl	2.89	9.78	8.74	7.13	−1.61
7	49.1(50)	0.2	KCl	3.25	9.91	9.27	7.46	−1.81
8	64.2(65)	0.2	KCl	3.65	10.11	9.64	7.69	−1.95
9	64.2(65)	0.1	KNO_3			10.06	8.12	−1.94

[a]Entries No. 4–9 are from the work of Gergely and Kiss [23], No. 1 and 2 are from [24] and No. 3 from [25]. The Cu^{2+} complexes of the *L*-, *D*- and *DL*-isomers had the same values (see [24]). [b]The numbers in parenthesis are the weight percentages given in [23]. [c]The entries of No. 1 and 2 are averages of data obtained from solutions containing $NaClO_4$ or KNO_3 (see [24]).

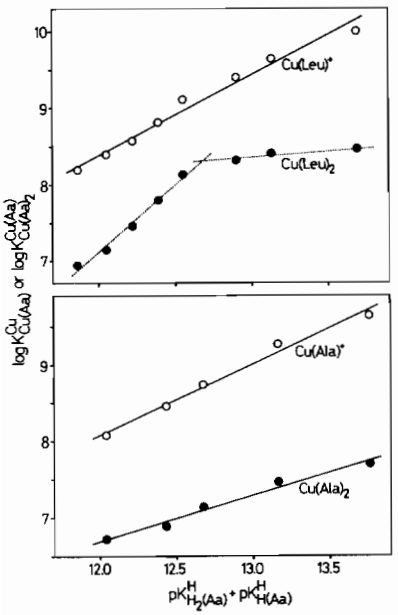


Fig. 4. Relationship between $\log K^{Cu}_{Cu(Aa)}$ or $\log K^{Cu(Aa)}_{Cu(Aa)_2}$ and $pK^{H}_{H_2(Aa)} + pK^{H}_{H(Aa)}$ for binary $Cu(Aa)^{+}$ (○) and $Cu(Aa)_2$ (●) complexes, where Aa^{-} = *L*-leucinate (Leu^{-}; upper part) or *D,L*-alaninate (Ala^{-}, lower part). The data are from Tables III and IV. The slopes of the straight (regression) lines are $m_{Cu(Leu)} = 1.038 \pm 0.063$ (1σ), $m_{Cu(Ala)} = 0.935 \pm 0.065$, $m_{Cu(Ala)_2} = 0.594 \pm 0.052$ (see text).

one recognizes an initial increase and then a decrease with increasing amounts of dioxane. An evaluation of this alteration of $\Delta \log K^{*}_{Cu}$ becomes possible by considering also some earlier results of Gergely and Kiss [23], who describe the influence of dioxane on the Cu^{2+}/alaninate system (Table IV).

By plotting, similar to Fig. 1 (see Section 1), the stability constants *versus* the acidity constants for the systems of Tables III and IV, the resulting Fig. 4 allows the following conclusions:

(i) For $Cu(Leu)^{+}$ and $Cu(Ala)^{+}$ straight lines are obtained which have (within experimental error) an identical slope ($m \simeq 1$). Hence, the properties of both complexes are normal.

(ii) For $Cu(Ala)_2$ a straight line is also observed, but with a somewhat smaller slope ($m = 0.6$). This property is expected, because the decreasing polarity of the solvent favors here only a +/− interaction, while in point *i* it is a 2+/− interaction.

(iii) The properties of $Cu(Leu)_2$ are obviously anormal, because this complex is clearly more stable than expected in several of the solvent mixtures. Hence, it is this complex which is responsible for the 'irregular' order of the $\Delta \log K^{*}_{Cu}$ values (Table III).

As $Cu(Ala)_2$ shows the expected stabilities in the solvent mixtures and as the coordination sphere around Cu^{2+} is the same in $Cu(Ala)_2$ and $Cu(Leu)_2$, this means that the larger alkyl residue of leucinate has to be responsible for the increased stabilities of $Cu(Leu)_2$. In addition, it is only the *second* leucinate which leads to an increased stability. We believe that the only way to explain these observations is by an intramolecular hydrophobic interaction between the two isopropyl residues of the two leucinates in $Cu(Leu)_2$.

This conclusion is further supported by the following points: (i) A direct Cu^{2+}-isopropyl interaction is *not* expected in the Cu^{2+}/leucinate system, in accord with the recent study of Kim and Martin [26]: a Pd^{2+}–aliphatic interaction, if it occurs at all, does not contribute to complex stability in aqueous solution. Moreover, the hypothetical Cu^{2+}–isopropyl interaction should already be possible in $Cu(Leu)^{+}$ and not only in $Cu(Leu)_2$ (see also Sec-

tion 8). In contrast, (ii), hydrophobic interactions between aromatic-ring systems and the isopropyl group of leucinate are already known [7, 13] to be responsible for the increased stability of several mixed ligand complexes in water. (iii) Scheraga [6] concluded, assuming ideal fits, that the strength (ΔG°) of the hydrophobic interaction in aqueous solution between the aliphatic residues of two isoleucines is comparable to the phenyl–phenyl interaction between two phenylalanines. (iv) A crystal structure study by Schugar *et al.* [27] of bis(*L*-leucinate)copper(II) revealed an N_2O_2 *trans*-ligation and in addition showed that coordination of the polar ends of Leu^- to Cu^{2+} allows the nonpolar side chains to align in an intermolecular fashion, thus creating regions with hydrophobic interactions.

6. Influence of Dioxane on the Extent of the Hydrophobic Interaction in Cu(Leu)$_2$

The occurrence of a hydrophobic interaction in $Cu(Leu)_2$ does not of course mean that in each individual complex molecule this interaction is present; again an intramolecular equilibrium between two isomeric species has to be expected (eqn. 1 with A = B). A quantitative evaluation of the situation is possible with eqn. 5, but $\Delta\Delta \log K^*$ is now defined by eqn. 8:

$$\Delta\Delta \log K^* = \Delta \log K^*_{(M/2\,Aa)} - \Delta \log K^*_{(M/2\,Aa)_{op}} \quad (8)$$

Evidently, $\Delta \log K^*_{(Cu/2Leu)}$ corresponds to $\Delta \log K^*_{Cu}$ of eqn. 7, while $\Delta \log K^*_{(Cu/2Leu)_{op}}$ is expected to be well represented by $\Delta \log K^*_{Cu}$ of the Cu^{2+}/alaninate system.

The problem encountered is that the Cu^{2+}/leucinate system has been studied in the presence of $NaClO_4$ at an ionic strength of 0.1 M (Table III), while the Cu^{2+}/alaninate values refer to an ionic strength of 0.2 M which was adjusted with KCl (Table IV). As Cl^- has a weak affinity [20, 28] toward Cu^{2+}, one expects [29] that the formation degrees and hence the stability constants of the Cu^{2+}/alaninate systems are somewhat affected by the presence of Cl^- ions. A comparison of the constants for entries No. 1–3 with No. 4 of Table IV indicates that this is indeed the case. However, the values given in the last column of Table IV (No. 1–4) demonstrate that such an influence is *not* manifested in the differences $\Delta \log K^*_{Cu}$; furthermore, this is also true for mixed solvents as follows from entries No. 8 and 9 in Table IV. Hence, these values of the Cu^{2+}/alaninate system provide indeed a basis to determine $\Delta \log K^*_{op}$ for the Cu^{2+}/leucinate system.

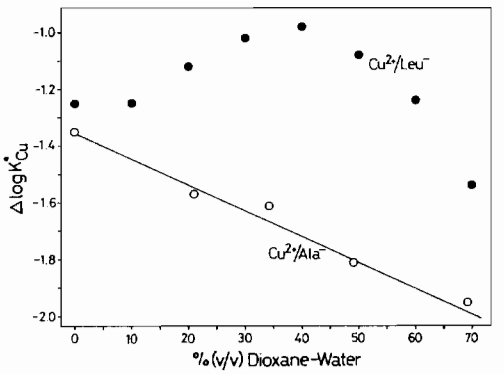


Fig. 5. Dependence for $\Delta \log K^*_{Cu}$ (eqn. 7) of the Cu^{2+}/leucinate (•; Table III) and Cu^{2+}/alaninate (○; Table IV) systems on the amount of dioxane added to water. The data for the straight (regression) line of the Cu^{2+}/Ala^- system are $m = -0.00912 \pm 0.00080$ and $y_o = -1.354 \pm 0.032$ (1σ); the interpolations based on these values are used in Table V.

In Fig. 5 $\Delta \log K^*_{Cu}$ (eqn. 7) of Cu^{2+}/Ala^- and Cu^{2+}/Leu^- is plotted in dependence on the per-

TABLE V. Extent of the Intramolecular Ligand–Ligand Interaction in the Binary $Cu(Leu)_2$ Complexes in Dependence on the Amount of Dioxane Added to Water: Intramolecular and Dimensionless Equilibrium Constant K_I^* and Percentage of the Isomer $Cu(Leu)_{2/cl}$ with the Hydrophobic Interaction in Different Solvents at $I = 0.1$ ($NaClO_4$) and 25 °C.

% (v/v) Dioxane	Mol Fraction	$\Delta \log K^*_{Cu}$ (eqn. 7)[a]	$\Delta \log K^*_{op}$ (see[b])	$\Delta\Delta \log K^*$ (eqn. 8)[c]	K_I^* (eqns. 2, 5)[c]	% $Cu(Leu)_{2/cl}$ (eqn. 1; A = B)[c]
0	0	−1.25	−1.35	0.10	0.26 ± 0.14	21 ± 9(18)
10	0.023	−1.25	−1.45	0.20	0.58 ± 0.18	37 ± 7(15)
20	0.050	−1.12	−1.54	0.42	1.63 ± 0.30	62 ± 4(9)
30	0.083	−1.02	−1.63	0.61	3.07 ± 0.47	75 ± 3(6)
40	0.124	−0.98	−1.72	0.74	4.50 ± 0.63	82 ± 2(4)
50	0.175	−1.08	−1.81	0.73	4.37 ± 0.62	81 ± 2(4)
60	0.241	−1.24	−1.90	0.66	3.57 ± 0.53	78 ± 3(5)
70	0.331	−1.54	−1.99	0.45	1.82 ± 0.32	65 ± 4(8)

[a]From Table III. [b]Calculated on the basis of the data plotted for Cu^{2+}/Ala^- in Fig. 5. [c]The estimate of the average error of $\Delta\Delta \log K^*$ is ±0.05 log unit; the errors given with K_I^* and % $Cu(Leu)_{2/cl}$ are calculated on this basis. The error limits given in the last column in parenthesis are based on the (rather extreme) error limit of ±0.10 log unit for $\Delta\Delta \log K^*$.

centage of dioxane present in the aqueous solvent mixtures. As the values for the Cu^{2+}/Ala^{-} system fit a straight line, the necessary $\Delta \log K^{*}_{(Cu/2Leu)_{op}}$ values can easily be calculated for all needed dioxane/water ratios. The resulting values are listed in the fourth column of Table V.

We are now in the position to calculate K_I^* for equilibrium 1 (with A = B) and hence the percentage of the closed isomer of $Cu(Leu)_2$. The results summarized in Table V show that in water about 20% of the total $Cu(Leu)_2$ formed are present in the closed form. The formation degree of this closed isomer increases with increasing amounts of dioxane and reaches its maximum with about 80% at high concentrations of dioxane (>60%) the extent of the hydrophobic interaction decreases again.

7. Two Opposing Solvent Effects Govern the Formation Degree of $Cu(Leu)_{2/cl}$

This conclusion is evident from Fig. 6 where the percentage of the closed isomer according to equilibrium 1 is plotted *vs.* the mole fraction of dioxane. The two further examples [8] given in this figure refer to the ternary complexes formed between $Cu(Phen)^{2+}$ and 2-phenylacetate (PAc^{-}) or 3-phenylpropionate (PPr^{-}); in these cases intramolecular aromatic-ring stacking occurs. This shows that the present result on hydrophobic interactions is part of a more general phenomenon.

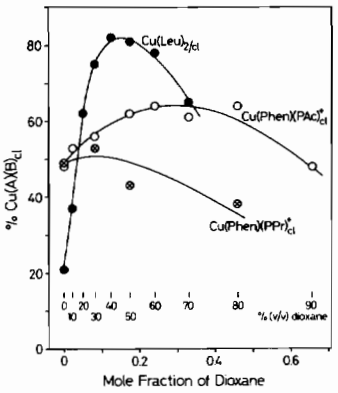


Fig. 6. Formation degree of the isomer with the intramolecular ligand–ligand interaction, $Cu(A)(B)_{cl}$ (see eqn. 1), for the complexes $Cu(Leu)_2$ (•), $Cu(Phen)(PAc)^{+}$ (○) and $Cu(Phen)(PPr)^{+}$ (⊛) in dependence on the mole fractions of dioxane; the second solvent component is water. The plotted data are taken from Table V and [8] (I = 0.1; 25 °C).

The bell-shaped curves of Fig. 6 can only result if at first the increasing amounts of dioxane promote the intramolecular interaction in the concentration-independent equilibrium 1, while at higher concentrations inhibition occurs. This result should not be confused with the observations summarized in Fig. 7: The overall stability of the complexes

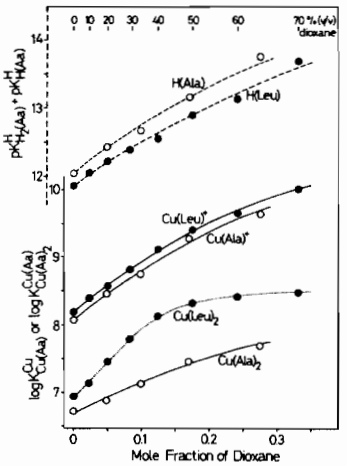


Fig. 7. Sum of the negative logarithms (broken lines) of the acidity constants of protonated alanine or leucine and logarithms (full or dotted lines; lower part) of the stability constants of the Cu^{2+} 1:1 or 1:2 complexes with alaninate or leucinate in dependence on the mole fractions of dioxane; the second solvent component is water. The plotted data are from Tables III and IV.

increases with the increasing mole fraction of dioxane; this is also true [8] for Cu(Phen)[phenylalkanecarboxylates]$^{+}$, as well as for systems without a ligand–ligand interaction, like Cu^{2+}/Ala^{-} (see Fig. 7).

How may the solvent composition influence the position of equilibrium 1? A tentative but appealing explanation is that at low mol fractions (ascending parts of the curves in Fig. 6) the ethylene moieties of the dioxane molecules do not associate well with the separated alkyl residues of the open isomer, probably due to the closeness of the aqueous solvation shell of the bridging metal ion. Instead, the already larger intramolecular alkyl adducts formed by the isopropyl residues of the coordinated leucinates are preferably solvated, stabilizing in this way $Cu(Leu)_{2/cl}$ (or correspondingly $Cu(Phen)(PheCa)^{+}_{cl}$) over $Cu(Leu)_{2/op}$. At high concentrations of the organic solvent (descending parts of the curves in Fig. 6) a hydrophobic solvation by dioxane of the individual isopropyl residues in $Cu(Leu)_2$ is enforced and thus the parts forming the adducts are separated; *i.e.*, now $Cu(Leu)_{2/op}$ is favored and the formation of the closed isomer is inhibited. An additional facilitation of the intramolecular ligand–ligand interaction may occur at mid-solvent ratios (peaks in Fig. 6) which may lead to a reduction in the size of the hydration shell of the metal ion, allowing thus a sterically less strained and more extensive hydrophobic overlap between the isopropyl groups in $Cu(Leu)_2$, or of corresponding groups in other complexes with a hydrophobic ligand–ligand interaction (for a more detailed discussion of this problem see [8]).

TABLE VI. Evidence from Stability Data for Intramolecular Hydrophobic and Aromatic-ring Stacking Interactions in Some Binary M^{2+}/Amino Acid Complexes in Aqueous Solution, with Estimations for K_I^* and for the Percentage of the 'closed' Isomer (I = 0.05–0.1; 25 °C).

No.	Complex $M(Aa)_2$	$\Delta \log K_M^*$ (eqn. 7)[a]	$\Delta \log K_{op}^*$	$\Delta\Delta \log K^*$ (eqn. 8)	K_I^* (eqns. 2, 5)	% $M(Aa)_{2/cl}$ (eqn. 1; A = B)
1	$Co(Gly)_2$	−0.82				
2	$Co(Ala)_2$	−0.84	−0.83			
3	$Co(Nva)_2$	−0.77	−0.83	0.06	0.15	13
4	$Co(Nle)_2$	−0.77	−0.83	0.06	0.15	13
5	$Co(Phe)_2$	−0.59	−0.83	0.24	0.74	43
6	$Co(Phe)_2$	−0.54	−0.83	0.29	0.95	49
7	$Co(Tyr)_2$	−0.48	−0.83	0.35	1.2	55
8	$Co(Tyr)_2$	−0.22	−0.83	0.61	3.1	76
9	$Ni(Gly)_2$	−0.95				
10	$Ni(Ala)_2$	−0.99	−0.96			
11	$Ni(Ala)_2$	−0.95				
12	$Ni(Nva)_2$	−0.97	−0.96	~0.01	~0.023	~2
13	$Ni(Nle)_2$	−0.98	−0.96	~0.02	~0.047	~4
14	$Ni(Phe)_2$	−0.79	−0.96	0.17	0.48	32
15	$Ni(Phe)_2$	−0.71	−0.96	0.25	0.78	44
16	$Ni(Tyr)_2$	−0.77	−0.96	0.19	0.55	35
17	$Ni(Tyr)_2$	−0.74	−0.96	0.22	0.66	40
18	$Cu(Ala)_2$	−1.39				
19	$Cu(Ala)_2$	−1.37	−1.38			
20	$Cu(Ala)_2$	−1.39				
21	$Cu(Nva)_2$	−1.30	−1.38	0.08	0.20	17
22	$Cu(L\text{-}Leu)_2$	−1.30	−1.38	0.08	0.20	17
23	$Cu(L\text{-}Leu)_2$	−1.25	−1.35[a]	0.10	0.26	21 ± 9
24	$Cu(Phe)_2$	−1.00	−1.38	0.38	1.4	58
25	$Cu(Phe)_2$	−0.95	−1.38	0.43	1.7	63
26	$Cu(L\text{-}Phe)_2$	−1.03	−1.38	0.35	1.2	55
27	$Cu(Tyr)_2$	−0.93	−1.38	0.45	1.8	64
28	$Cu(Tyr)_2$	−0.88	−1.38	0.50	2.2	69
29	$Cu(L\text{-}Trp)_2$	−0.48	−1.38	0.90	6.9	87
30	$Zn(Ala)_2$	−0.53				
31	$Zn(Phe)_2$	−0.22	−0.53	0.31	1.0	50
32	$Zn(Phe)_2$	−0.23	−0.58[a]	0.35	1.2	55
33	$Zn(Tyr)_2$	−0.05	−0.53	0.48	2.0	67

[a]Most of the values for $\Delta \log K_M$ have been collected from the work of Gergely *et al.* (I = 0.05, KCl): [39] (entries No. 1, 3, 6, 8, 9, 11–13, 15, 17, 19, 21, 25 & 28), [40] (No. 2, 5, 7, 10, 14, 16, 18, 24, 27, 30, 31 & 33) and [41] (No. 4). The potentiometric studies of these systems were carried out with the *D,L* mixtures of the amino acids; Professor Dr. A. Gergely pointed out [42] that with these simple amino acids no differences in the stability constants of the complexes with the *D* or *L* amino acids were observed. The other values are from [13] (No. 20 & 22; I = 0.1, $NaClO_4$), Table V (No. 23), [43] (No. 26 & 29; I = 0.1, KNO_3), and [32] (No. 32; I = 0.1; the given value for $\Delta \log K_{op}^*$ is also from this reference and corresponds to $\Delta \log K_{Zn/2Ala}^*$).

8. Intramolecular Ligand–Ligand Interactions in Some Other Binary and Related Mixed Amino Acid Complexes

In Table VI the stability differences $\Delta \log K^*$ (eqn. 7) for several binary metal ion/amino acid systems are summarized. These data provide evidence that hydrophobic and aromatic-ring stacking interactions in complexes of amino acids with suitable side chains are quite common. However, before the estimates of the percentages of the closed isomers (which are also given in Table VI) can be discussed, some general considerations are appropriate.

It was in 1961 and 1970 that the second stepwise formation constants for the Cu^{2+}/phenylalanine [30] and Cu^{2+}/tryptophan [31] systems, respectively, were reported as being rather large in comparison with other Cu^{2+}/amino acid systems. These observations have been repeatedly confirmed [20, 24, 32]. In conjunction with further studies (see the refs. listed in [33]) they have led in the comments to a

recent compilation [33] of formation constants for the complexes of phenylalanine, tyrosine, tryptophan and some other amino acids to the conclusion that 'under certain circumstances amino acids containing aromatic side chains can behave as tridentate ligands'. This means it is suggested 'that the bonding is glycine-like in the mono-complexes but that at least one aromatic ring is associated with Cu(II) in the bis-complexes'. In the crystal structure analysis [34] of a bis(*L*-tyrosinato)copper(II) complex one of the phenolic rings was found about 3.1 Å beneath the base of the copper coordination pyramid, thus giving support to "the hypothesis of a weak interaction between the π-electron system of the phenolic ring and the copper(II) ion'. Similar orientations of aromatic rings have been observed in other Cu^{2+} complexes of substituted amino acids [35] and peptides [36]. However, that the Cu^{2+}/aromatic-ring interaction (if it exists at all) is weak follows from two different crystal structure analyses of the bis-(*L*-phenylalaninato)copper(II) complex: in one case [37] the phenyl ring is located below Cu^{2+}, while in the other it is not [38].

In this context the results obtained recently by Kim and Martin [26] with (dipeptide)Pd(II) complexes in aqueous solution are of interest. These authors used several tridentate dipeptides with different side chains thus leaving one binding site available in the planar coordination sphere of Pd^{2+} for coordination of a series of unidentate amines carrying different residues. Those results pertinent here may be summarized regarding the order of decreasing interaction energies in the complexes: phenyl-aromatic > phenyl-propyl (or larger alkyl residue) > ... > Pd–aromatic $\gg$ Pd–aliphatic ~ 0. It is important to note that the Pd–aromatic interactions are already weaker than the intramolecular hydrophobic or stacking interactions, and a Cu^{2+}-aromatic interaction is expected to be even weaker than the Pd^{2+}–aromatic one. Indeed, in our recent studies [1, 2, 8, 9] of Cu^{2+} complexes with phenylalkanecarboxylates we could not discover any hint for such an interaction in solution.

In addition, the following points which are partly based on the data of Table VI argue also *against* a significant Cu^{2+}–aromatic interaction in solution but *for* intramolecular ligand–ligand interactions in many complexes: (i) It is always the stability constant for the addition of the second ligand which is larger than expected. The postulation of an intramolecular stack explains this situation convincingly, because this stack may only form once a second aromatic ring is present. (ii) The increased stability cannot result from an influence of the second ligand on Cu^{2+}, making it more suitable for a Cu^{2+}–aromatic interaction as suggested in [33] because, *e.g.*, M(Phe)-(Gly) or M(Phe)(Ala) show no increased stability [13], while M(Phe)(Nva) or M(Phe)(Tyr) do so (*vide infra*; Table VII). (iii) Detailed thermodynamic results are also in accord with the postulation of intramolecular ligand–ligand interactions: ΔH_1 for the reaction between Cu^{2+} and Trp^- or Ala^- are very similar, while ΔH_2 for the addition of the second Trp^- to $Cu(Trp)^+$ is about 5 kJ/mol more exothermic than for the reaction $Cu(Ala)^+ + Ala^- \longrightarrow Cu(Ala)_2$ [31]. This agrees with other studies [44] in which it was shown that ΔG° contains a negative contribution of ΔH°, which means that the formation of stacking adducts is not solely entropy driven. (iv) The Cu^{2+}/leucinate results could not be explained without a ligand–ligand interaction (see Section 5). (v) The data of Table VI show clearly that the increased binding tendency of the second ligand is a general feature and occurs with many other metal ions aside from Cu^{2+}: for the complexes with Co^{2+}, Ni^{2+} and Zn^{2+} the increased stability can hardly be attributed to metal ion–aromatic or metal ion–hydrophobic interactions. (vi) A comparison of the data in Table VI reveals that the situation is indeed governed by the ligands and *not* by the metal ions. The extent of the intramolecular ligand–ligand interaction is for a given ligand independent of the metal ion (though the geometry of the coordination sphere has of course some influence). (vii) The results of Table VI show the expected decrease in the intensity of the interaction: aromatic-ring stacks are more stable than aliphatic adducts (see also Section 3).*

It is evident that all points discussed now for binary amino acid complexes should also be correct for mixed amino acid complexes. Indeed, in a different connection we have already concluded earlier

TABLE VII. Estimations for the Percentage of the 'closed' Isomer with the Intramolecular Hydrophobic or Aromatic-ring Stacking Interaction (eqn. 1) for Several Mixed Amino Acid Complexes in Aqueous Solution at 25 °C (I = 0.05, KCl).[a]

Complex M(A)(B)	% $M(A)(B)_{cl}$ for		
	Co^{2+}	Ni^{2+}	Cu^{2+}
M(Nva)(Abu)		~5	9
M(Phe)(Abu)		~2	~2
M(Phe)(Nva)	21	~5	11
M(Phe)(Tyr)	50	26[b]	37[c]
M(Tyr)(Abu)		11	~5
M(Tyr)(Nva)	22	9	24
M(Tyr)(Phe)		24[b]	35[c]

[a]These data are abstracted from entries No. 1–48 of Table VII in [13]. [b,c]Note: the pairwise agreement of these data is excellent.

* See footnote * p. 162.

TABLE VIII. Influence of the Solvent Composition on the Intramolecular Stack Formation in Some Binary Cu^{2+}/Amino Acid Complexes (I = 0.2, KCl; 25 °C).

Complex $Cu(Aa)_2$	$\Delta \log K^*_{Cu}$ (eqn. 7)[a]	$\Delta \log K^*_{op}$	$\Delta\Delta \log K^*$ (eqn. 8)	K^*_I (eqns. 2, 5)	% $Cu(Aa)_{2/cl}$ (eqn. 1; A = B)
in Water:					
$Cu(Ala)_2$	−1.32				
$Cu(Phe)_2$	−0.92	−1.32	0.40	1.5	60
$Cu(Tyr)_2$	−0.87	−1.32	0.45	1.8	64
in 50% (w/w)[b] Dioxane–Water					
$Cu(Ala)_2$	−1.82				
$Cu(Phe)_2$	−1.63	−1.82	0.19	0.55	35
$Cu(Tyr)_2$	−1.64	−1.82	0.18	0.51	34

[a]The differences are taken from the work of Gergely *et al.* (Table II in [46]). [b]See column 2 in Table IV.

[13] that intramolecular ligand–ligand interactions occur in several ternary amino acid complexes. The pertinent results are summarized in Table VII; the data fit very neatly into the described picture. Moreover, the sum of the data from Tables VI and VII allows the conclusion that the extent of the intramolecular ligand–ligand interaction in the complexes decreases in the series aromatic–aromatic > aromatic–aliphatic > aliphatic–aliphatic. It may be added that in purely organic molecules with suitable groups *intra*molecular phenyl–alkyl interactions have also been observed [45].

Finally, many of the individual results presented here, if considered for themselves, would not lead to very convincing conclusions. It is the wealth of the data which point into the same direction and which therefore, if taken together, provide strong evidence that hydrophobic and aromatic-ring stacking interactions indeed occur in binary and ternary amino acid complexes containing suitable ligands.

Unfortunately, no final conclusions can yet be drawn regarding the influence of organic solvents on the stability of the intramolecular interaction in the amino acid complexes listed in Tables VI and VII. The data summarized in Table VIII apply only to two solvents and leave open the possibility that there is a higher formation degree of the stack in an aqueous solvent mixture containing between about 10 to 40% dioxane. That the type of the solvent-influence depends on the structure of the ligands is obvious from Fig. 6. However, the data of Table VIII still allow the conclusion that a metal ion-bridge between two aromatic moieties favors their stack; by going from water to 50% aqueous dioxane the percentage of the closed isomer decreases in these cases by a factor of about 0.5, while the decrease for unbridged stacks [8, 22] under the same conditions is more pronounced, the factor being about 0.2.

*In a recent paper [49], an interaction of Cu^{2+} with the aromatic side chains in $Cu(Phe)_2$, $Cu(Trp)_2$ and related Cu^{2+} complexes has again been proposed. It should be emphasized that equatorial coordination of two amino acids with the same chirality gives for the trans isomer (which is usually favored [27, 34, 35, 38]) a complex with both side chains on the same side of the coordination square. With this in mind, one may conclude, *e.g.*, for $Cu(Phe)_2$ that the two phenyl moieties are separated at the basis of the plane by about 5.5 Å, but a slight inclination of the two ring-planes towards each other allows easily an approach of the 'top' ring-parts to about 3.5 Å (which is the usual distance in stacking interactions; see *e.g.* [2]), and there would still be enough space for an apically coordinated water molecule, if this is still there (see also the discussion in Section 7. In addition, penta-coordinated Cu^{2+} is well known [34, 36, 37]). Two points must be further emphasized in this connection: (i) it is known that stacked aromatic rings may be orientated in a butterfly-like way; in fact, this is a rather common orientation (see [2] and references therein); (ii) interactions are also possible between edges of aromatic systems, as suggested, *e.g.*, for Cu(Phen)(benzoate)$^+$ [1]; pertinent solid-state interactions have been described [2, 50]. Clearly, in complexes of metal ions with tetrahedral or octahedral coordination spheres and amino acids of the considered type a ligand-ligand interaction is even more easily achieved. In addition, hydrophobic interactions may also become very important in amino acid complexes (as well as for other-type complexes described in [49]) lending a significant contribution to ΔG° [6] and thus to complex stability (see *e.g.* Tables VI and VII). Finally, it must be stressed that the influence of dioxane on the stability of $Cu(Leu)_2$ (Section 6) cannot be explained with the suggestions made in [49]; furthermore, the conclusions presented in Section 8 hold for binary *and* ternary complexes *not* only of Cu^{2+}, but also for Co^{2+}, Ni^{2+} and Zn^{2+} (as well as for further metal ion), and, *e.g.*, for Zn^{2+} complexes the explanations given in [49] are again not applicable.

General Conclusions

To evaluate the influence of the metal ion on ligand-ligand interactions in more detail the following considerations may be helpful. The stability constant of the hydrophobic adduct between two leucines, in aqueous solution is not known, but the stabilities of several related adducts have been estimated: *e.g.*, $K^{(Bpy)}_{(Bpy)(H\cdot Leu)} = 0.6 \pm 0.4\ M^{-1}$ [13], $K^{(Phen)}_{(Phen)(H\cdot Leu)} = 1.4 \pm 0.9\ M^{-1}$ [13], $K^{(Phe)}_{(Phe)(Leu)} = 2\ M^{-1}$ [6]*, $K^{(ATP)}_{(ATP)(H\cdot Trp)} = 6.2\ M^{-1}$ [47], $K^{(Phe)}_{(Phe)_2} = 10.7\ M^{-1}$ [6]*, and $K^{(i\text{-}Leu)}_{(i\text{-}Leu)_2} = 12.5\ M^{-1}$ [6]*. Based on these data one may assume a value of about $10\ M^{-1}$ (as an upper limit) for the hydrophobic adduct between the isopropyl residues of two leucines. This means that in a 2×10^{-3} M aqueous solution of leucine about 4% (or less) may exist in the form of the binary hydrophobic adduct.

These few percents should be compared with the data summarized in Fig. 8, which apply for

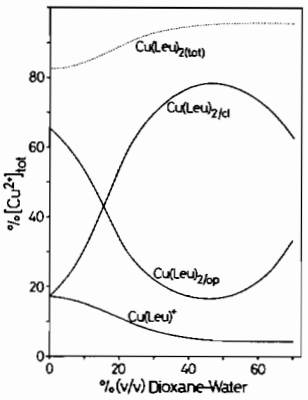


Fig. 8. Effect of the amount of dioxane added to an aqueous solution of Cu^{2+} and *L*-leucine at pH 7.00 on the concentration of the species present in solution (full lines). The dotted line represents the total concentration of the $Cu(Leu)_2$ complex. Results are given as the percentage of the total Cu^{2+} (= 10^{-3} M; $[Leu]_{tot} = 2 \times 10^{-3}$ M) present; computed with the constants listed in Tables III and V ($I = 0.1$; 25 °C). The concentration of the uncomplexed Cu^{2+} is in water 0.2% and in 70% dioxane <0.006%; any hydroxo-complex formation of Cu^{2+} is under these conditions insignificant.

solutions containing Cu^{2+} (10^{-3} M) and Leu (2×10^{-3} M) at pH 7. It becomes thus evident that the addition of 0.5 equivalent of Cu^{2+} favors the hydrophobic interaction between two isopropyl residues of Leu in an aqueous solution considerably: about 17% exist now as $Cu(Leu)_{2/cl}$. In other words, the formation of a metal ion-bridge between the two hydrophobic moieties forming the adduct increases the stability of this adduct significantly.

*These constants refer to ideal fits; from Table I of [6].

However, it is even more noteworthy that the addition of dioxane will certainly *reduce* the stability of the *binary* leucine adduct as it has been shown to occur for many related unbridged adducts [8, 22], while the formation of $Cu(Leu)_{2/cl}$ is favored (Fig. 8). This bridged adduct reaches concentrations of nearly 80% and even in 70% aqueous dioxane still about 60% exist in this form. Hence, the metal ion-bridge favors the hydrophobic interaction under these conditions by factors of (probably) more than 100. In addition, it should be pointed out that at pH 7 the total formation degree of $Cu(Leu)_2$ is not much altered by the addition of dioxane to an aqueous solution of the reactants, as is evident from the dotted curve in the upper part of Fig. 8.

The present results emphasize clearly that via the formation of metal ion-bridges certain ligand–ligand associations may be favored, and consequently distinct structures can be created. Moreover, we have seen now several examples where the addition of some organic solvent favors certain structures. Regarding the interactions (summarized in Tables VI to VIII) between the side chains in amino acid complexes, clearly more work about the influence of other solvents is needed. Such interactions are significant in determining *e.g.* the three-dimensional structure of proteins and are important for an understanding of the selectivity processes occurring in nature. That there is a mutual influence between metal ion-coordination and hydrophobic/stacking interactions is evident, for example, from the great affinity [48] of several alkanecarboxylates for carboxypeptidase A; this affinity is clearly governed by a cooperative interplay [8] between metal ion-coordination and a reduction of the intrinsic polarity in the active-site cavity by hydrophobic interactions between the alkyl moieties of the carboxylates and nearby hydrophobic residues in the active-site region.

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