imidazole, and the angular deviation for this one is negative. We observe two distinct sets of ^{14}N imino nitrogen signals at 77 K. There is possibly a phase transition between room temperature and 77 K.
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Ternary Complexes in Solution. 35.[1] Intramolecular Hydrophobic Ligand–Ligand Interactions in Mixed Ligand Complexes Containing an Aliphatic Amino Acid

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Abstract: Mixed ligand complexes of the type $M(Ar)(Aa)^+$, where $M^{2+} = Cu^{2+}$ or Zn^{2+}, Ar = 2,2′-bipyridyl (bpy) or 1,10-phenanthroline (phen), and Aa^- = alaninate, 2-aminopropionate (α-aminobutyrate), norvalinate, norleucinate, valinate, leucinate (leu), or isoleucinate, have been studied by potentiometric pH titrations and ^1H NMR. The potentiometric measurements reveal a slightly higher formation tendency, expressed as $\Delta \log K_M = \log K^{M(Ar)}_{M(Ar)(Aa)} - \log K^{M}_{M(Aa)}$, for the systems with leucinate as amino acid compared to those with alaninate. This increase in stability is attributed to an intramolecular hydrophobic ligand–ligand interaction between the aromatic ring system of bpy or phen and the isopropyl group of leucine. The position of the intramolecular isomeric equilibrium between an "open" and "closed" form, in which the hydrophobic interaction occurs, was estimated; e.g., the ternary $Zn(phen)(leu)^+$ complex exists about 26% in the folded, i.e., closed, form. ^1H NMR shift measurements of the mentioned systems in the absence and presence of Zn^{2+} confirmed that such hydrophobic ligand–ligand interactions exist and that they are promoted by the formation of a metal-ion bridge between the two reactants. The longer the side chain of the aliphatic amino acid, the larger is the upfield shift of the terminal methyl group(s), resulting from the interaction with the aromatic moiety within the ternary complex. These results prompted a literature search and it became evident that, e.g., M(phenylalaninate)(tyrosinate) or M(phenylalaninate)(norvalinate) have an enhanced stability, compared with M(phe)(ala) or M(phe)(gly), and this is now attributed to intramolecular aromatic-ring stacking and hydrophobic ligand–ligand interactions. For these and other systems (in total about 40) the percentages of the "closed" isomer were calculated; they cover the whole range till up to about 90%. The $\Delta G°$ values calculated from the equilibrium constants agree well with the theoretical predictions for such interactions. Possible biological implications are shortly discussed.

Hydrophobic interactions are known to occur among other noncovalent interactions in biomolecules;[2] they contribute, e.g., in proteins through the interaction of the side chains of the amino acid residues to the formation of distinct structural features.[3] Such hydrophobic interactions occur between two aliphatic groups, or between aliphatic and aromatic moieties; the ring stacking between two aromatic systems is also often listed within this category. With regard to metal ion complexes of low molecular weight, Pettit and Hefford[4] have recently described the situation rather precisely: "Hydrophobic interactions cannot be classified as bonds but they are important, not well understood, and often misused. Although the existence of the interactions is not in doubt,[5] their relevance to the complexes of organic molecules in aqueous solution is not clear". To help to clarify this unsatisfactory situation the present study on ternary complexes was undertaken.

Aromatic ring stacking has been observed in ternary complexes[6,7] formed by two different ligands which contain aromatic rings.[1b,8-11] At least one of these rings has to be incorporated in a flexible side chain, as in trytophanate[1b,8,12] or adenosine 5′-triphosphate,[8-10,12] that a stack may be formed; the other ring may also be of the flexible type, or it may be rigidly fixed to the metal ion as it is the case with 2,2′-bipyridyl or 1,10-phenanthroline.[9,10] Such aromatic ring stacking interactions may enhance the stability[8,10,11] of the ternary complex, but more important they create specific structures,[6,12] because the whole species may also be viewed as a metal ion bridged stacking adduct. In addition these species may be considered as relatively simple models for ternary enzyme/metal ion/ATP[13,14] or enzyme/metal ion/inhibitor[15] complexes.

Hydrophobic ligand–ligand interactions between the aliphatic side chains in ternary complexes containing functionalized cyclodextrins[16] have been studied with the aim to enhance the reactivity of the system. There is so far, to our knowledge, only a single study[17] which considers the stability and the structure of such mixed ligand complexes simultaneously: it was shown that an association of the aliphatic chains of carboxylate and sulfonate ligands with the aromatic rings of bpy or phen[14] within ternary metal ion complexes may occur. But it should also be noted in this connection that for binary (metal-free) systems a hydrophobic interaction between the aromatic moiety of phenols and the aliphatic residue of carboxylic acids has been described.[18] Moreover, it has been suggested "that hydrophobic bonds may be responsible for the increasing stabilization with chain length of the carboxylic acid dimers".[19]

The mentioned results stimulated us to examine the influence of the hydrophobic interaction between an aliphatic and an aromatic group on the stability of ternary Cu^{2+} and Zn^{2+} complexes more in detail, with the aim to quantify the extent of this interaction at least partly. As the hydrophobic interaction is important in nature, amino acids with aliphatic side chains were chosen; as aromatic ligands bpy and phen were taken, because these ligands[20] and their binary complexes[21] are well characterized, and most important the structure of this half of the mixed ligand complex which contains these aromatic ligands is unequivocally defined owing to the rigidity of bpy and phen when coordinated. The amino acids were selected

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such that the side chain varied in length and volume from a methyl group, as in L-alanine, to an *n*-butyl group, as in L-norleucine, or an isopropyl group, as in L-leucine. All the amino acids used are shown in Figure 1.

It is obvious that the methyl side chain in L-alanine is too short to allow any interaction with the aromatic rings within the square-planar Cu^{2+} complexes, and for the octahedral Zn^{2+} complexes it is expected to be insignificant, if it does occur at all; indeed, the results do confirm this. The reason why L-alanine and not glycine was selected as the basis for the comparisons is that Martin[22] has shown recently that the stability of the glycinate complexes is often somewhat exceptional—the alaninate complexes are therefore a better basis for comparisons. In order to obtain reliable equilibrium constants for the mentioned ternary complexes potentiometric pH titrations have been carried out, and the hydrophobic interactions within the ternary complexes were characterized by ^1H NMR shift measurements.

Experimental Section

Materials. 2,2'-Bipyridyl, L-alanine, L-2-aminobutyric acid, L-norvaline, L-norleucine, L-valine, L-leucine, and L-isoleucine (all purissimum) were obtained from Fluka AG, Buchs, Switzerland. 1,10-Phenanthroline hydrate (p.a.), $NaClO_4$ (p.a.), $NaNO_3$ (p.a.), D_2O (99.75%), and 10% tetramethylammonium hydroxide solution were from Merck AG, Darmstadt, Germany. $Cu(ClO_4)_2$ (purum) was from Fluka AG; $Zn(ClO_4)_2$ was from K&K Laboratories, Cleveland, Ohio; $Zn(NO_3)_2$ (p.a.) was from Merck AG. The concentrations of the M^{2+} stock solutions were determined by titration from EDTA (Merck AG).

Determination of Equilibrium Constants by Potentiometric Titrations. The potentiometric titrations were performed with a Metrohm potentiograph E 536 and a Metrohm EA 121 glass electrode. The acidity constants $K^{H}_{H(Aa)}$ of L-alanine and L-leucine were determined from pairs of automatic titrations under N_2 of 10-mL aqueous solutions containing 0.045 M $HClO_4$ and $NaClO_4$ ($I = 0.1$) in the presence and absence of 0.025 M amino acid with 0.5 M NaOH (25 °C). The acidity constants $K^{H}_{H(Aa)}$ were determined from pairs of titrations of 50-mL aqueous solutions containing 2×10^{-4} M $HClO_4$ and $NaClO_4$ ($I = 0.1$) in the presence and absence of 1.2×10^{-3} M amino acid with 0.1 M NaOH.

The conditions of the measurements for the determination of the stability constants $K^{M}_{M(Aa)}$ of the binary ala and leu complexes were the same as for $K^{H}_{H(Aa)}$, but a part of $NaClO_4$ was replaced by $Cu(ClO_4)_2$ or $Zn(ClO_4)_2$ with the ratio $[Cu^{2+}]:[Aa] = 1:1$ and $[Zn^{2+}]:[Aa] = 5:1$, 10:1, and 17.5:1. Titrations of solutions without ligand were used as a basis for the evaluation. The calculation[23] of $K^{M}_{M(Aa)}$ was done by taking into account the species H^+, $H_2(Aa)^+$, $H(Aa)$, Aa^-, M^{2+}, $M(Aa)^+$, and $M(Aa)_2$; for $M^{2+} = Zn^{2+}$ also $M(Aa)_3^-$ was included. To enable this calculation procedure, previously titrations had been carried out with the amino acid in excess over the metal ion; i.e., $M(ClO_4)_2$ was 6×10^{-4} M and the ratios of $[M^{2+}]:[Aa]$ were 1:3, 1:4, and 1:5 for Cu^{2+} and 1:5, 1:10, 1:16, 1:20, and 1:40 for Zn^{2+}. From these data ($K^{M}_{M(Aa)}$), $K^{M(Aa)}_{M(Aa)_2}$ and $K^{M(Aa)_2}_{M(Aa)_3}$ were calculated in the usual way.

The conditions for the determinations of the stability constants of the ternary complexes were similar to those used for the binary complexes, i.e., $[HClO_4] = 2 \times 10^{-4}$ M, $[Aa] = 1.2 \times 10^{-3}$ M, but Cu^{2+} and Zn^{2+} were replaced by Cu^{2+}/phen, Cu^{2+}/bpy, Zn^{2+}/phen, or Zn^{2+}/bpy. The Zn^{2+}/phen-containing ternary systems were titrated in a nitrate medium as in the presence of perchlorate with $[Zn^{2+}] = [phen] = 0.03$ M a precipitate forms. The overall stability constant $\beta^{M}_{M(Ar)(Aa)}$ of the ternary complex $M(Ar)(Aa)^+$ was computed[23] by taking into account the species H^+, $H_2(Ar)^{2+}$, $H(Ar)^+$, Ar, $M(Ar)^{2+}$, $M(Ar)_2^{2+}$, $H_2(Aa)^+$, $H(Aa)$, Aa^-, $M(Aa)^+$, $M(Aa)_2$, M^{2+}, and $M(Ar)(Aa)^+$. The acidity constants[21,24] of bpy and phen and the stability constants[21] of their Cu^{2+} and Zn^{2+} complexes were taken from earlier work.

^1H NMR Measurements. ^1H NMR spectra were recorded on a Varian Anaspect EM-360 spectrometer (60 MHz) at 34 °C in H_2O solutions or on a Bruker WH-90FT spectrometer (90.025 MHz) at 27 °C in D_2O solutions, using the center peak of the tetramethylammonium triplet as reference;[17] all chemical shifts were converted to a trimethylsilylpropanesulfonate reference by adding 3.188 ppm.[10]

L-AMINO ACIDS

		R–CH(NH_3^+)COO^- R–
Straight Chain:		
Alanine	(ala)	CH_3–
α-Aminobutyric Acid	(abu)	CH_3–CH_2–
Norvaline	(nva)	CH_3–CH_2–CH_2–
Norleucine	(nle)	CH_3–CH_2–CH_2–CH_2–
Branched Chain:		
Valine	(val)	$(CH_3)_2$CH–
Leucine	(leu)	$(CH_3)_2$CH–CH_2–
Isoleucine	(ile)	CH_3–CH_2–CH(CH_3)–

Figure 1. List of L-amino acids used in this study, together with the structure of their aliphatic side chains.

The pD values were obtained by adding 0.4 to the pH meter reading.[25]

In the ^1H NMR measurements we followed the change in the chemical shift of the terminal methyl group of the amino acid as a function of the second reagent concentration. With all amino acids, a set of five different measurements was performed in order to see the influence of protonation and of the coordination of Zn^{2+}, $Zn(bpy)^{2+}$, or $Zn(phen)^{2+}$ on the resonance signal of the terminal methyl group. Always the center position of the multiplets corresponding to these methyl groups was evaluated. The experimental conditions with [Aa] = 0.02 M were (1) pH 12.4, $I \simeq 0.1$; (2) pH 6.3, $I = 0.1$, $NaNO_3$; (3) $[Zn^{2+}] = 0.225$ M, pH 6.0, $I = 0.7$; (4) $[Zn^{2+}] = [bpy] = 0.225$ M, pH 6.0, $I = 0.7$; (5) $[Zn^{2+}] = [phen] = 0.225$ M, pH 6.0, $I = 0.7$. All measurements involving Zn^{2+} had to be carried out below pH 6.2 owing to the tendency of Zn^{2+} to form hydroxo complexes.

Based on the shifts measured in these experiments and on the concentrations of the various amino acid species the extrapolated shifts for $Zn(Aa)^+$ (see eq 1), $Zn(bpy)(Aa)^+$, and $Zn(phen)(Aa)^+$ (eq 2) were calculated (cf. Table V) by using the equilibrium constants determined from potentiometric pH titrations. In the case of the systems containing L-alanine or L-leucine the constants reported now (Tables I and II) were used for this calculation. For all the other amino acids given in Figure 1 the average values of the constants determined for the ala and leu systems were taken; a comparison of published data[26-28] as well as of our own results (Tables I and II) shows that this is justified.

The chemical shift of the methyl group of the amino acid in the $Zn(Aa)^+$ complex, $\delta_{Zn(Aa)}$, was calculated according to eq 1 from the observed value of δ_{Aa} (experiment 1; see above), $\delta_{H(Aa)}$ (experiment 2), and the observed value of experiment (3), δ_{obsd}.

$$\delta_{Zn(Aa)} = \frac{\delta_{obsd}[Aa]_{tot} - (\delta_{H(Aa)}[H(Aa)] + \delta_{Aa}[Aa^-])}{[Zn(Aa)^+]} \quad (1)$$

Calculations where the species $Zn(Aa)_2$ was also included under the assumption that the shift of the methyl resonance of the amino acid in this 1:2 species is the same as in $Zn(Aa)^+$ showed that $\delta_{Zn(Aa)}$ did not change significantly; therefore, $Zn(Aa)_2$ was omitted.

With δ_{Aa}, $\delta_{H(Aa)}$, and $\delta_{Zn(Aa)}$ the shifts for the ternary species $Zn(bpy)(Aa)^+$ and $Zn(phen)(Aa)^+$ (see Table V) were calculated from the mentioned experiments (4) and (5) by using the equation

$$\delta_{Zn(Ar)(Aa)} = \frac{\delta_{obsd}[Aa]_{tot} - (\delta_{H(Aa)}[H(Aa)] + \delta_{Aa}[Aa^-] + \delta_{Zn(Aa)}[Zn(Aa)^+])}{[Zn(Ar)(Aa)^+]} \quad (2)$$

Additional measurements (Table IV and Figure 2) were carried out with L-alanine and L-leucine, keeping the amino acid concentration constant (0.01, 0.015, or 0.02 M) and varying $[Zn^{2+}] = 0$–0.27

M, $[Zn^{2+}/bpy]$ = 0–0.55 M, $[Zn^{2+}/phen]$ = 0–0.25 M, [bpy] = 0–0.4 M, or [phen] = 0–0.25 M. These latter two systems were measured in 25% aqueous methanol as solvent for reasons of solubility. From these experiments equilibrium constants for the complex formation between the mentioned species and ala or leu can be calculated. In Figure 2 representative examples with L-leucine are shown. The data measured for the metal ion free systems, i.e., the (Ar)(H·leu) adducts, were evaluated with the equation

$$\delta_{obsd} = \frac{\delta_{H(Aa)}[H(Aa)] + \delta_{(Ar)(H\cdot Aa)}[(Ar)(H\cdot Aa)]}{[Aa]_{tot}} \quad (3)$$

The data of the binary Zn^{2+}/Aa systems were treated as described,[29] but the monoprotonated amino acid species, H(Aa), was also taken into account. Hence the equation used for the calculations is analogous to eq 4. The ternary systems were evaluated according to eq 4, i.e., by treating the systems as binary ones which consist only of $Zn(Ar)^{2+}$ and leu.

$$\delta_{obsd} = \frac{\delta_{Aa}[Aa] + \delta_{H(Aa)}[H(Aa)] + \delta_{Zn(Ar)(Aa)}[Zn(Ar)(Aa)^+]}{[Aa]_{tot}} \quad (4)$$

However, a computed curve best fitting the experimental data of the ternary systems may also be obtained by including into the calculation the species Zn^{2+}, $Zn(Ar)^{2+}$, $Zn(Ar)_2{}^{2+}$, $Zn(Ar)_3{}^{2+}$, $Zn(leu)^+$, $Zn(Ar)(leu)^+$, and $Zn(Ar)_2(leu)^+$. The concentrations of these species were calculated by using the constants from Table I for the binary $Zn(Ar)_n{}^{2+}$ complexes, and $K^{Zn(Ar)}_{Zn(Ar)(leu)}$ from the preceding evaluation but enhanced by 0.1 log unit;[17b] in addition $K^{Zn}_{Zn(leu)} = K^{Zn(Ar)}_{Zn(Ar)(leu)} - \Delta \log K_{Zn}$ (from the potentiometric experiments; see eq 11) was used, and $K^{Zn(Ar)_2}_{Zn(Ar)_2(leu)}$ was assumed to be smaller than $K^{Zn(Ar)}_{Zn(Ar)(leu)}$ by a statistical factor of 5.[10] The curve is then of the following form:

$$\delta_{obsd} = \frac{\delta_{Aa}[Aa] + \delta_{H(Aa)}[H(Aa)] + \delta_{Zn(Aa)}[Zn(Aa)^+] + \delta_{Zn(Ar)(Aa)}[Zn(Ar)(Aa)^+]}{[Aa]_{tot}} + \frac{(2\delta_{Zn(Ar)(Aa)} - \delta_{H(Aa)})[Zn(Ar)_2(Aa)^+]}{[Aa]_{tot}} \quad (5)$$

The values for δ_{Aa}, $\delta_{H(Aa)}$, and $\delta_{Zn(Aa)}$ were taken from Table V and $\delta_{Zn(Ar)(Aa)}$ is then calculated as 0.694 ppm for $Zn(bpy)(leu)^+$ and as 0.756 ppm for $Zn(phen)(leu)^+$. The extrapolated values of δ_∞ ($=\delta_{Zn(Ar)(Aa)}$), given in the legend to Figure 2, are a little bit larger than the two mentioned values; i.e., the shift difference resulting from the calculation with eq 5 is a bit smaller. This is understandable because by taking into account also $Zn(leu)^+$ a small part of the shift in the ternary system is then determined by this species, which results in a slight downfield contribution.

Results and Discussion

Stacking interactions between the aromatic rings of two ligands coordinated within a ternary complex may result in an enhancement of the stability of this species relative to the stability of the corresponding binary complexes.[8,10,11] Similar results may be expected for ternary systems composed of M^{2+}, 2,2′-bipyridyl, or 1,10-phenanthroline, and an amino acid with an aliphatic side chain allowing a hydrophobic interaction with the aromatic rings within the ternary complexes. In order to characterize such a possible increase in stability, one has to define first the stability of the binary (eq 6 and 7) and ternary (eq 8) complexes, as is done by the following equations:[14]

$$M^{2+} + Aa^- \rightleftharpoons M(Aa)^+$$

$$K^M_{M(Aa)} = \frac{[M(Aa)^+]}{[M^{2+}][Aa^-]} \quad (6)$$

$$M^{2+} + Ar \rightleftharpoons M(Ar)^{2+}$$

$$K^M_{M(Ar)} = \frac{[M(Ar)^{2+}]}{[M^{2+}][Ar]} \quad (7)$$

$$M^{2+} + Ar + Aa^- \rightleftharpoons M(Ar)(Aa)^+$$

$$\beta^M_{M(Ar)(Aa)} = \frac{[M(Ar)(Aa)^+]}{[M^{2+}][Ar][Aa^-]} \quad (8)$$

With these experimentally determined constants is connected, for example, the following one:

$$M(Ar)^{2+} + Aa^- \rightleftharpoons M(Ar)(Aa)^+$$

$$K^{M(Ar)}_{M(Ar)(Aa)} = \frac{[M(Ar)(Aa)^+]}{[M(Ar)^{2+}][Aa^-]} \quad (9)$$

$$\log K^{M(Ar)}_{M(Ar)(Aa)} = \log \beta^M_{M(Ar)(Aa)} - \log K^M_{M(Ar)} \quad (10)$$

One way to quantify the stability of a ternary complex relative to the stability of the corresponding binary complexes is according to the equation[6]

$$\Delta \log K_M = \log K^{M(Ar)}_{M(Ar)(Aa)} - \log K^M_{M(Aa)} = \log K^{M(Aa)}_{M(Aa)(Ar)} - \log K^M_{M(Ar)} \quad (11)$$

The value of $\Delta \log K_M$ characterizes the coordination tendency of the amino acid toward $M(Ar)^{2+}$ (eq 9) relative to M^{2+} (eq 6) and is the logarithm of the equilibrium constant due to the equation

$$M(Ar)^{2+} + M(Aa)^+ \rightleftharpoons M(Ar)(Aa)^+ + M^{2+} \quad (12)$$

Depending on the geometry of the complex and the denticity of the ligands the statistically expected value for $\Delta \log K_M$ will vary:[6] for bidentate ligands it is between −0.4 for an octahedral coordination sphere and about −0.9 for the distorted coordination sphere of Cu^{2+}.

Another way to describe the stability of a ternary complex is by the "anti disproportionation" according to the equation

$$M(Ar)_2{}^{2+} + M(Aa)_2 \rightleftharpoons 2M(Ar)(Aa)^+$$

$$X = \frac{[M(Ar)(Aa)^+]^2}{[M(Ar)_2{}^{2+}][M(Aa)_2]} \quad (13)$$

Log X is calculated with the equation

$$\log X = 2 \log \beta^M_{M(Ar)(Aa)} - (\log \beta^M_{M(Ar)_2} + \log \beta^M_{M(Aa)_2}) = (\log K^{M(Ar)}_{M(Ar)(Aa)} - \log K^{M(Aa)}_{M(Aa)_2}) + (\log K^{M(Aa)}_{M(Aa)(Ar)} - \log K^{M(Ar)}_{M(Ar)_2}) \quad (14)$$

Although this description has the advantage of a firm statistical basis ($\log X_{stat} = 0.6$), it suffers from the disadvantage that the stability of the ternary complex is compared to the stability of the binary 1:2 parent complexes, which are not on the way of formation of the ternary complex.[6,30]

The reason why we prefer for the characterization of the stability of a ternary complex $\Delta \log K_M$ (eq 11 and 12), and in part also $\log X$ (eq 13 and 14), over $\log \beta^M_{M(Ar)(Aa)}$ (eq 8) or $\log K^{M(Ar)}_{M(Ar)(Aa)}$ (eq 9 and 10) is that the first two constants quantify the stability of the ternary complex relative to the binary complexes; i.e., stability contributing factors from the binary systems cancel and a stability increase or decrease due to indirect[6,7] or direct[8,10,11] ligand–ligand interactions appears in these descriptions.

Potentiometric Determination of the Stability of the Ternary Complexes Consisting of ala or leu, bpy or phen, and Cu^{2+} or Zn^{2+}. The acidity constants of the ligands and the stability constants of the corresponding binary complexes are listed in Table I. Although the constants of the Cu^{2+} and Zn^{2+} amino acid systems had been determined before,[26-28,31] we have remeasured these constants as it is preferable to determine the binary and ternary constants under the same conditions because experimental differences might otherwise show up in $\Delta \log K_M$.[32] However, the present results agree well with those from the literature.[26-28,31]

Table II shows the equilibrium constants for the mixed ligand systems. The $\Delta \log K_M$ values are in general negative, as expected,[6] and they are more negative for the Cu^{2+} systems than for the corresponding Zn^{2+} systems. This observation is

Table I. Negative Logarithms of the Acidity Constants of ala, leu, bpy, and phen and Logarithms of the Stability Constants of Their Binary Cu^{2+} and Zn^{2+} Complexes (25 °C; I = 0.1, $NaClO_4$)[a] as Determined by Potentiometric pH Titrations

ligand (L)	$pK^{H}_{H_2L}$	pK^{H}_{HL}	$\log K^{Cu}_{CuL}$	$\log K^{CuL}_{CuL_2}$	$\log K^{Zn}_{ZnL}$	$\log K^{ZnL}_{ZnL_2}$
L-ala	2.34 ± 0.01	9.82 ± 0.01	8.33 ± 0.02	6.94 ± 0.02	4.62 ± 0.04	4.23 ± 0.04[b]
L-leu	2.32 ± 0.01	9.66 ± 0.01	8.32 ± 0.01	7.02 ± 0.03	4.56 ± 0.02	4.18 ± 0.02[c]
bpy[d]	−0.2[e]	4.49	8.00	5.60	5.30	4.53
phen[d]	−1.6[e]	4.95	9.25	6.75	6.55	5.80

[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. [b] Log $K^{Zn(ala)_2}_{Zn(ala)_3}$ = 3.31 ± 0.22. [c] Log $K^{Zn(leu)_2}_{Zn(leu)_3}$ = 3.12 ± 0.15. [d] t = 20 °C; I = 0.1, $NaNO_3$ (cf. ref 21). [e] From ref 24.

Table II. Logarithms of the Stability Constants (25 °C; I = 0.1, $NaClO_4$) of the Ternary $M(Ar)(Aa)^+$ Complexes as Determined by Potentiometric pH Titrations[a]

M^{2+}	Ar	Aa^-	$\log \beta^{M}_{M(Ar)(Aa)}$ (eq 8)	$\log K^{M(Ar)}_{M(Ar)(Aa)}$ (eq 9, 10)	$\Delta \log K_M$ (eq 11, 12)	$\log X$ (eq 13, 14)
Cu^{2+}	bpy	ala	16.05 ± 0.01	8.05	−0.28	3.23
	phen	ala	17.17 ± 0.03	7.92	−0.41	3.07
	bpy	leu	16.03 ± 0.02	8.03	−0.29	3.12
	phen	leu	17.22 ± 0.01	7.97	−0.35	3.10
Zn^{2+}	bpy	ala	9.72 ± 0.03	4.42	−0.20	0.76
	phen	ala	11.15 ± 0.03[b]	4.60	−0.02	1.10
	bpy	leu	9.71 ± 0.05	4.41	−0.15	0.85
	phen	leu	11.22 ± 0.03[b]	4.67	+0.11	1.35

[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. [b] I = 0.1, $NaNO_3$.

in agreement with the different coordination geometries of these metal ions as was already indicated by the mentioned statistical values. Contrary to $\Delta \log K_M$ the values of $\log X$ are much higher for Cu^{2+} than for Zn^{2+}; this is due to the relatively small value of $\log K^{Cu(Ar)}_{Cu(Ar)_2}$ (Table I). As mentioned above, the binary 1:2 complex formation constants influence the value of $\log X$ (cf. eq 14). The value of $\log X$ and $\Delta \log K_M$ of the bpy/ala^- systems agree well with an earlier determination,[31] and they are also comparable to those of the ternary systems containing bpy and glycinate.[33]

As our aim is to see whether a hydrophobic interaction between the aromatic rings and the amino acid side chain shows up in the stability of the ternary complex, the values of $\Delta \log K_M$ and $\log X$ of those ternary systems are now compared that differ only in the amino acid. With the aliphatic side chain of leucinate a hydrophobic interaction seems possible, while this is not the case with alaninate; therefore the values for the leu-containing systems *minus* the values for the corresponding ala-containing systems were calculated. These differences are given in Table III. With the exception of Cu(bpy)(leu/ala) (see below), all these differences turn out to be positive, which means that the ternary complexes containing leu are more stable than those containing ala. This holds for the $\Delta \log K_M$ as well as for the $\log X$ characterization; both descriptions show a parallel trend. Hence, even though the mentioned differences are small, they may be taken as a first hint that in Cu(phen)(leu)$^+$, Zn(bpy)(leu)$^+$, and Zn(phen)(leu)$^+$ an intramolecular hydrophobic interaction occurs. In other words, the positive values in Table III indicate that equilibria 15 ($\Delta\Delta \log K_M$) and 16 ($\Delta \log X$) are displaced toward their right sides—i.e., selectivity is observed.

$$M(leu)^+ + M(Ar)(ala)^+ \rightleftharpoons M(Ar)(leu)^+ + M(ala)^+ \quad (15)$$

$$M(leu)_2 + 2M(Ar)(ala)^+ \rightleftharpoons 2M(Ar)(leu)^+ + M(ala)_2 \quad (16)$$

The $\Delta\Delta \log K_M$ value of the first entry in Table III indicates that there is no or only a very weak such interaction in Cu(bpy)(leu)$^+$. Moreover, the corresponding value of $\Delta \log X$ is negative (=−0.11), which is the result of the relatively low value of $\log X$ for Cu(bpy)(leu)$^+$, and this in turn results from the somewhat increased value of $\log K^{Cu(leu)}_{Cu(leu)_2}$, compared with $\log K^{Cu(ala)}_{Cu(ala)_2}$ (Table I). This hints at a hydrophobic interaction between the aliphatic side chains in $Cu(leu)_2$. This interpretation is in accordance with an X-ray structural analysis[34] of this complex: "Coordination of the polar ends of the amino acids with Cu^{II} allows the nonpolar side chains to align, creating hydrophobic regions". This reasoning shows in addition that the characterization based on $\Delta \log K_M$ is preferable because here no extraneous factors are introduced into the comparison.

Table III. Indication of an Intramolecular Hydrophobic Interaction in M(Ar)(leu)$^+$ Complexes, Based on the Comparison of the Stability of the M(Ar)(leu)$^+$ with the M(Ar)(ala)$^+$ Species[a]

systems	$\Delta\Delta \log K_M$[b]	$\Delta \log X$[c]
Cu(bpy)(leu/ala)	−0.01	−0.11
Cu(phen)(leu/ala)	0.06	0.03
Zn(bpy)(leu/ala)	0.05	0.09
Zn(phen)(leu/ala)	0.13	0.25

[a] Calculated with the data of Table II. [b] $\Delta\Delta \log K_M = \Delta \log K_{M/Ar/leu} - \Delta \log K^{M}_{M/Ar/ala}$. [c] $\Delta \log X = \log X_{Ar/leu} - \log X_{Ar/ala}$.

The values for the 2,2′-bipyridyl-containing systems in Table III are smaller than for the corresponding 1,10-phenanthroline systems, which indicates that with the larger phenanthroline the hydrophobic interaction is somewhat more effective, a result one might have expected. The larger differences for the Zn^{2+} systems, compared to the Cu^{2+} systems, are probably due to the different geometries of the coordination spheres of these two metal ions: the side chain in leu is still fairly short and an interaction with the coordinated ligand is much more likely in an arrangement, where the amino acid has the possibility to occupy an axial and an equatorial position in an octahedral coordination sphere, than when both ligands occupy four equatorial positions. As Cu^{2+} has a preference for the second type of coordination it is understandable that the ligand–ligand interaction is somewhat reduced in the Cu^{2+} complexes.

Taking everything together, one must conclude that the discussed trends in complex stability are all in favor of an intramolecular hydrophobic interaction between the isopropyl side chain of leucine and the aromatic rings of bpy or phen

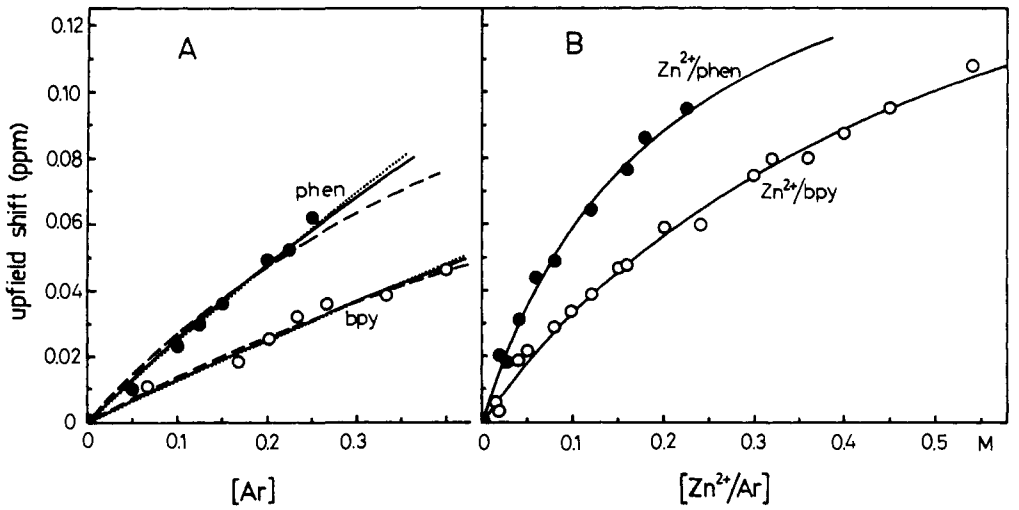


Figure 2. Upfield shift of the resonance of the methyl groups of L-leucine (the center of the doublet was evaluated) in dependence on increasing concentrations of bpy (○) or phen (●) (part A) and Zn^{2+}/bpy (○) or Zn/phen (●) (part B), compared with the corresponding resonance *position* of L-leucine alone. The curves shown are the computer-calculated best fits of the experimental data with the parameters given below for the complex formation between leu and one of the above given second reactants. Part A (eq 3): bpy (○) 60 MHz; 34 °C; 25% CH_3OH in H_2O; I = 0.1, TMA; [leu] = 0.02 M; pH 7.50. δ_0 0.975 ppm; for $\Delta\delta$ = 0.2 ppm (cf. text) δ_∞ 0.775 ppm (broken line) and K = 0.76 ± 0.07 M^{-1}; for $\Delta\delta$ = 0.5 ppm δ_∞ 0.475 ppm (full line) and K = 0.27 ± 0.02 M^{-1}; for $\Delta\delta$ = 0.8 ppm δ_∞ 0.175 ppm (dotted line) and K = 0.16 ± 0.01 M^{-1}. phen (●) 90.025 MHz; 27 °C; 25% CD_3OD in D_2O; I = 0.1, TMA; [leu] = 0.01 M; pD 7.60. δ_0 0.954; for $\Delta\delta$ = 0.2 ppm, δ_∞ 0.754 ppm (broken line) and K = 1.58 ± 0.21 M^{-1}; for $\Delta\delta$ = 0.5 ppm, δ_∞ 0.454 ppm (full line) and K = 0.53 ± 0.04 M^{-1}; for $\Delta\delta$ = 0.8 ppm, δ_∞ 0.154 ppm (dotted line) and K = 0.32 ± 0.02 M^{-1}. Part B (eq 4): Zn^{2+}/bpy (○) 60 MHz; 34 °C; H_2O; I = 0.1-1.65, $NaNO_3$; [leu] = 0.015 M; pH 5.95. δ_0 0.972 ppm, δ_∞ 0.768 ± 0.014 ppm, and K_{app} = 1.96 ± 0.21 M^{-1} (see text). Zn^{2+}/phen (●) 60 MHz; 34 °C; H_2O; I = 0.1-0.7, $NaNO_3$; [leu] = 0.015 M; pH 6.05. δ_0 0.972 ppm, δ_∞ 0.800 ± 0.015 ppm, and K_{app} = 5.48 ± 0.84 M^{-1} (see text).

within the three ternary complexes $Cu(phen)(leu)^+$, $Zn(bpy)(leu)^+$, and $Zn(phen)(leu)^+$. Certainly, as said before, the differences in the $\Delta \log K_M$ and $\log X$ values between the leu- and ala-containing systems are small and just at the limit of significance. Therefore it seemed desirable to study the ligand-ligand interaction in these ternary amino acid containing complexes in addition by another independent method; this may be done by ^1H NMR measurements.

^1H NMR Studies of the Binary Adducts between Leucine and bpy or phen. In an association where the aliphatic side chain of an amino acid is located above or below the plane of an aromatic ring the signals of the aliphatic protons should be shifted upfield, relative to the signals obtained for the free amino acid, owing to the ring current of the aromatic system.[35] Our first intention was therefore to see if any indication for an interaction between the isopropyl residue of L-leucine and the aromatic bpy or phen ligands in the absence of metal ions could be obtained. Indeed, from Figure 2 (left part) it is obvious that increasing concentrations of bpy or phen do increasingly shift the methyl resonance of leucine upfield. Certainly, the observed upfield shifts are small,[36] but for such hydrophobic adducts as (bpy)(H·leu) or (phen)(H·leu) a low stability must be expected.[3,37]

These measurements on the binary metal free systems were carried out in 25% aqueous methanol as solvent for reasons of solubility.[38] An exact evaluation of these binary systems, i.e., the calculation of a stability constant for these hydrophobic adducts, is only hard to achieve for several reasons: (i) the aromatic partners show self-stacking,[20] and (ii) the association tendency of two aromatic rings within these systems is somewhat larger than the association tendency between an aromatic ring and the aliphatic moiety of leu, a behavior which had to be expected.[3] Hence, the analytical concentration of the aromatic species does not correspond to the active concentration, because through the mentioned self-association a part of the aromatic rings is no longer available for a hydrophobic interaction with leu. Taking this into account and replacing the analytical concentration by the active particle concentration, $\Sigma_n [A_n]$, which can be calculated with the known constants of the self-association process,[41] one is still left with a graph (corresponding to those of Figure 2A) that shows no curvature. This means that no limiting shift and thus no stability constant can directly be calculated.

We have therefore used the following procedure for an estimation. From earlier studies[1b,12,17] it is evident that the limiting or maximum shift (i.e., the shift obtained for the complete formation of a species) that may be achieved from two- and three-ring aromatic systems is certainly not smaller than 0.2 ppm and not larger than 0.8 ppm. Using these limits (see Figure 2A) one obtains the formation constants $K^{(bpy)}_{(bpy)(H\cdot leu)}$ = 0.76 and 0.16 M^{-1}, and $K^{(phen)}_{(phen)(H\cdot leu)}$ = 1.58 and 0.32 M^{-1}, respectively. Hence, by taking also into account the mentioned self-association of bpy and phen we may conclude[42] that $K^{(bpy)}_{(bpy)(H\cdot leu)} = 0.6 \pm 0.4\ M^{-1}$ and $K^{(phen)}_{(phen)(H\cdot leu)} = 1.4 \pm 0.9\ M^{-1}$. These results, which have been obtained for 25% aqueous methanol as solvent, are in good agreement with theoretical calculations[3,37] on the formation of hydrophobic bonds, especially if one takes into account that hydrophobic interactions are somewhat reduced in mixed solvents.[39,40] Nemethy and Scheraga[3,37] calculated for an interaction between leucine and phenylalanine a value of about 2 M^{-1} for $K^{(phe)}_{(phe)(leu)}$ (25 °C) in aqueous solution.

^1H NMR Studies of the Ternary Leucine/Zn^{2+}/bpy or phen Systems. As mentioned before and as shown in the left part of Figure 2, an association between the aliphatic side chain of an amino acid and an aromatic ring system does shift the resonances of the aliphatic protons upfield, if compared to the shift positions measured for the signals of the free amino acid.[35] In contrast, protonation or coordination of a metal ion shifts the signals of protons close to the binding site in the ligand downfield. NMR is therefore the ideal method to trace the coordination of the diamagnetic Zn^{2+} and especially to monitor the ligand-ligand interaction within a ternary complex containing one ligand with an aromatic group and another one with an aliphatic side chain.

In order to confirm the hydrophobic intramolecular ligand-ligand interaction within the ternary Zn^{2+} complexes the methyl resonance signals of leu were monitored as a

Table IV. Comparison of the Logarithms of the Formation Constants of Several Zn^{2+} Complexes as Determined by ^1H NMR (60 MHz; 34 °C) or Potentiometric pH Titrations (25 °C; I = 0.1)[a]

complex	eq	log K	^1H NMR	pot. titr.
$Zn(ala)^+$	(6)	$\log K^{Zn}_{Zn(ala)}$	4.8 ± 0.1[b]	4.62
$Zn(leu)^+$	(6)	$\log K^{Zn}_{Zn(leu)}$	[c]	4.56
$Zn(bpy)(leu)^+$	(9)	$\log K^{Zn(bpy)}_{Zn(bpy)(leu)}$	4.0 ± 0.3[d]	4.41
$Zn(phen)(leu)^+$	(9)	$\log K^{Zn(phen)}_{Zn(phen)(leu)}$	4.4 ± 0.2[e]	4.67

[a] The errors given are three times the standard error or the sum of the probable systematic errors, whichever is larger. The potentiometric results are of Tables I and II. Attempts to determine by NMR also $\log K^{Zn(Ar)}_{Zn(Ar)(ala)}$ failed, as the measurements were too inaccurate. However, the shift data of the ternary bpy containing system could still be explained using the formation constant from the potentiometric titration and the δ values of Table V. In the phen-containing ternary system the shift difference between H(ala) and the $Zn(phen)(ala)^+$ complex is only 0.023 ppm (cf. Figure 3 and Table V), and this is too small for a meaningful result. [b] I = 0.1–0.85, $NaNO_3$. [c] The addition of Zn^{2+} has practically no effect on the position of the methyl resonances of leu (cf. Figure 3 and the text); therefore, this constant could not be determined. [d] I = 0.1–1.65, $NaNO_3$. [e] I = 0.1–0.7, $NaNO_3$.

function of increasing concentrations of added Zn^{2+}/bpy or Zn^{2+}/phen. Using these reagents in excess over the amino acid and considering only the reaction between $Zn(bpy)^{2+}$ or $Zn(phen)^{2+}$ and leu the experimentally obtained curve, δ_{obsd} as a function of the reagent concentration, should provide a formation constant for equilibrium 9.[43] It should be added that the addition of Zn^{2+} has practically no effect on the position of the signal of leucine; this is understandable because (i) the distance of the methyl group from the ligating site is relatively large, and (ii) the experiments had to be performed under conditions where the amino acid is present mainly as the zwitterion (see Experimental Section) and this species has about the same chemical shift as the Zn^{2+} complex (cf. also Table V).

The measured chemical shifts, δ_{obsd}, in dependence on [Zn^{2+}/bpy] or [Zn^{2+}/phen] are plotted in the right part of Figure 2. Computer fitting of these data gives the limiting shifts, δ_∞ (cf. legend to Figure 2),[44] for the methyl resonances in the ternary $Zn(bpy)(leu)^+$ and $Zn(phen)(leu)^+$ complexes, as well as the apparent stability constants, K_{app}, of these ternary complexes. The values of K_{app} are valid only at the pH of the experiment, but by taking into account with the equation

$$\log K^{M(Ar)}_{M(Ar)(Aa)} = \log K_{app} + \log (1 + [H^+]/K^H_{H(Aa)}) \quad (17)$$

the competition[11,45] between the proton and $Zn(Ar)^{2+}$ for coordinating at the basic site of leu, the pH-independent stability constants $\log K^{Zn(Ar)}_{Zn(Ar)(leu)}$ are obtained. These constants are listed in Table IV, together with information on the ala-containing systems. The formation constants obtained from the NMR measurements agree well with those from the potentiometric pH titrations; this is especially true if one takes also into account that temperature and ionic strength were different in these experiments.

However, the most important result of this NMR study is that two main conclusions may directly be read from Figure 2 without any assumptions and calculations: (i) the shifts due to phen are larger than due to bpy, and (ii) the shifts due to $Zn(bpy)^{2+}$ and $Zn(phen)^{2+}$ are larger than due to bpy or phen alone. The first point means that the larger π system exerts the larger shift, while the more important second point shows that the weak hydrophobic interaction between the isopropyl group of leu and the aromatic ligands is enhanced dramatically by the formation of a metal ion bridge.

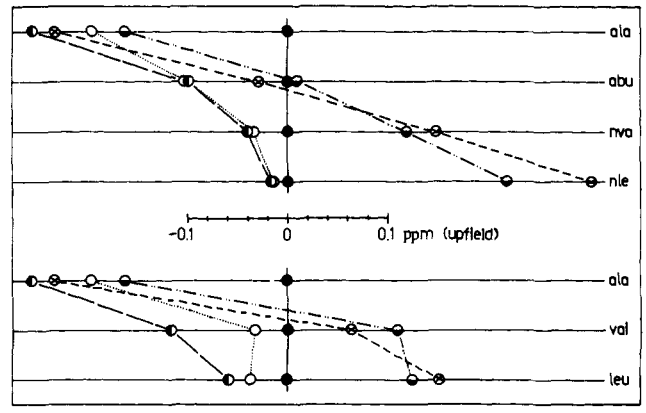


Figure 3. Chemical shift of the methyl group (always the midpoint of the multiplets was measured) of straight-chain amino acids (upper part) and of branched-chain amino acids (lower part) for the protonated (◐) and Zn^{2+} (○), $Zn(bpy)^{2+}$ (◒), or $Zn(phen)^{2+}$ (⊗) coordinated amino acids, relative to the resonance position of the Aa anions (●). The values of the amino acid anions, relative to trimethylsilylpropanesulfonate, are listed in Table V.

^1H NMR Studies of Ternary Zn^{2+} Complexes Composed of bpy or phen and Amino Acids with Aliphatic Side Chains of Different Length and Size. It is obvious that the extent of the intramolecular hydrophobic interaction in the ternary $Zn(Ar)(Aa)^+$ complexes should be dependent on the length and possibly also on the volume of the aliphatic side chain of the amino acid. We have therefore studied several systems with the straight-chain and branched-chain amino acids shown in Figure 1. The effects on the side chain of the amino acid through protonation and Zn^{2+}, $Zn(bpy)^{2+}$, or $Zn(phen)^{2+}$ coordination were measured by monitoring the relative position of the signal of the terminal methyl group(s) of the amino acid as a function of H^+, Zn^{2+}, and $Zn(Ar)^{2+}$. The experimentally determined shifts of Aa^- and the relative shifts of H(Aa), as well as the extrapolated shifts (see Experimental Section) of $Zn(Aa)^+$, $Zn(bpy)(Aa)^+$, and $Zn(phen)(Aa)^+$, are compiled in Table V. The assigned δ values of the methyl groups of the amino acids which commonly occur in proteins agree well with those of the literature.[46]

In the upper part of Figure 3 the data are plotted for the straight-chain amino acids ala, abu, nva, and nle, and in the lower part for the two branched-chain amino acids val and leu. As expected, protonation leads to a downfield shift and the shift difference decreases with increasing distance between the observed methyl group and the site of protonation, i.e., with increasing chain length. The same general picture holds for the coordination of Zn^{2+}. Despite the different charge of H^+ and Zn^{2+}, the two cations have—with the possible exception of valine[47]—a very similar effect on the position of the methyl resonance signals.

The last two columns of Table V and the corresponding lines in Figure 3 (those at the right side) show the influence of the Zn^{2+} coordinated aromatic ligands on the methyl resonance of the also coordinated amino acids. Under the influence of bpy and phen the resonances of the methyl signals of the amino acids are shifted clearly to higher field; i.e., the aliphatic chain interacts with the aromatic ring within the ternary complex. This interaction is very poor or even completely absent in the case of the ala complexes, but with increasing chain length the shift differences become larger as the methyl group reaches further over the aromatic ligand. That the shift difference is determined by the chain length is also seen from the results with the branched-chain amino acids (lower part of Figure 3). Furthermore, by comparing the values of the straight-chain with those of the branched-chain amino acids, it becomes evident that the shift differences of leu correspond much better

Table V. Chemical Shift of the Terminal Methyl Groups[a] (δ_{Aa}) of Various Amino Acid Anions (Measured at pH 12.4) and the Differences in Experimentally Determined ($\delta_{H(Aa)}$)[b] or Extrapolated[c] ($\delta_{Zn(Aa)}$, $\delta_{Zn(bpy)(Aa)}$, $\delta_{Zn(phen)(Aa)}$) Chemical Shifts for Several Amino Acid Containing Species (ppm)[d]

Aa	δ_{Aa}	$\delta_{Aa} - \delta_{H(Aa)}$	$\delta_{Aa} - \delta_{Zn(Aa)}$	$\delta_{Zn(Aa)} - \delta_{Zn(bpy)(Aa)}$	$\delta_{Zn(Aa)} - \delta_{Zn(phen)(Aa)}$
ala	1.233	−0.255	−0.196	0.034	−0.036
abu	0.893	−0.099	−0.104	0.114	0.075
nva	0.917	−0.040	−0.034	0.143	0.182
nle	0.900	−0.017	−0.015	0.234	0.318
val	0.912	−0.117	−0.033	0.142	0.096
leu	0.964	−0.059	−0.037	0.161	0.188
ile	0.923	−0.095	−0.100	0.177	0.165

[a] Always the midpoint of the multiplet is given, relative to trimethylsilylpropanesulfonate. [b] Measured at pH 6.3. [c] See Experimental Section (eq 1 and 2). [d] Positive values correspond to upfield shifts.

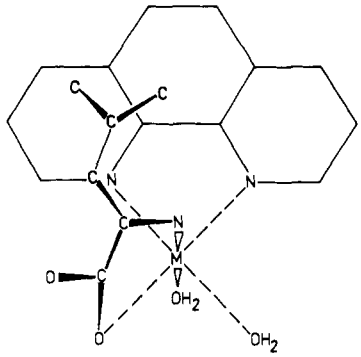


Figure 4. Tentative and simplified structure of the ternary complex formed between M^{2+}, 1,10-phenanthroline, and leucinate, showing the interaction of the isopropyl group of leu^- with the aromatic ring system of phen.

to those of nva, which has the same chain length, than to those of nle, which has the same number of carbon atoms.

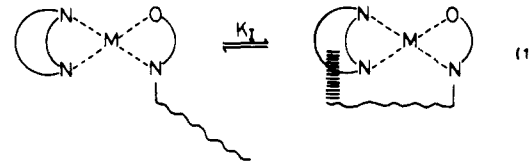
Another aspect is also nicely seen from Figure 3. In those cases of the coordinated amino acids where the methyl group reaches far enough over the aromatic ligands, the shift due to phen is larger than due to bpy. This result may again be rationalized with the larger π system of phen and thus its larger ring current, compared to that of bpy, which shifts the signal of the methyl group located above this ring system to even higher field than with bpy. To conclude, all these results confirm the occurrence of an intramolecular hydrophobic interaction in those ternary $M(Ar)(Aa)^+$ complexes which contain a long enough aliphatic side chain.

General Conclusions

The determined stability constants of the ternary complexes, i.e., especially the values of $\Delta \log K_M$ (Tables II and III), give hints into the direction of an intramolecular hydrophobic ligand-ligand interaction in $M(Ar)(leu)^+$ complexes. Unequivocally confirmed is this indication by the results described in the ^1H NMR sections: here is shown that a hydrophobic interaction does exist in mixed ligand complexes containing a heteroaromatic N ligand and an amino acid provided that the aliphatic amino acid side chain is long enough to reach the aromatic system.

A tentative structure of such a ternary complex is shown in Figure 4 for the $M(phen)(leu)^+$ complexes. In this structure the isopropyl group of the amino acid is located above the aromatic ring of 1,10-phenanthroline, and the two ligands are linked together by the metal ion; in other words, the metal ion promotes the hydrophobic interaction (Figure 2).

The Isomeric Equilibria of $M(Ar)(leu)^+$ Complexes. It is clear that the occurrence of a complex species with a structure similar to the one shown in Figure 4, i.e., the structure which is responsible for the slight increase in stability (Table III) and for the observed upfield shifts in the ternary complexes (Table V and Figure 3), does not mean that all of the $M(Ar)(Aa)^+$ species exist in this folded form. In solution there is certainly an intramolecular equilibrium between an "open" and a "closed" form, i.e., between two isomers as indicated in equilibrium 18. If these two isomers are designated as $M(Ar)(Aa)_{op}^+$ and $M(Ar)(Aa)_{cl}^+$, the dimensionless constant of this intramolecular equilibrium is defined by



(18)

$$K_I = [M(Ar)(Aa)_{cl}^+]/[M(Ar)(Aa)_{op}^+] \quad (19)$$

Values of K_I may be determined, based on the following reasoning. If the ternary complex of equilibrium 12 exists in the two mentioned isomeric forms, then the experimentally measured value of $\Delta \log K_{M/exp}$ may be defined by

$$10^{\Delta \log K_{M/exp}} = \frac{[M(Ar)(Aa)_{op}^+ + M(Ar)(Aa)_{cl}^+][M^{2+}]}{[M(Ar)^{2+}][M(Aa)^+]} \quad (20)$$

For a system without such an intramolecular interaction eq 21 holds:

$$10^{\Delta \log K_{M/op}} = \frac{[M(Ar)(Aa)_{op}^+][M^{2+}]}{[M(Ar)^{2+}][M(Aa)^+]} \quad (21)$$

A combination of eq 19-21 results in

$$K_I = \frac{10^{\Delta \log K_{M/exp}}}{10^{\Delta \log K_{M/op}}} - 1 \quad (22)$$

With eq 22 we may now calculate values for K_I provided that the values of $\Delta \log K_{M/exp}$ and $\Delta \log K_{M/op}$ are known.[48]

For the present cases we have determined values of $\Delta \log K_{M/exp}$ for the ternary complexes of M^{2+}, leu^-, and bpy or phen (Table II). As in the $M(Ar)(ala)^+$ complexes the intramolecular interaction will be very small, in fact is most probably nonexistent as all our results indicate, we may use the corresponding values of $\Delta \log K_M$ as $\Delta \log K_{M/op}$ and hence calculate K_I for the $M(Ar)(leu)^+$ complexes. The K_I values in turn allow then the calculation of the percentage of the "closed" isomer; this means of the complex with the intramolecular hydrophobic interaction. These data are assembled in Table VI.

It must be emphasized that the values of K_I and the resulting percentages given in Table VI can only be considered as rough estimates because they are derived from differences between rather large constants which are connected with a certain experimental error. But even so, the trends which are indicated by these results are most reasonable, i.e., a larger percentage

of the closed isomer in the Zn^{2+} than in the Cu^{2+} complexes, and larger percentages for the phen systems compared with the bpy systems. Moreover, if one recalls that the shift one may expect for a complete interaction is in the order of 0.5 ppm,[1b,12,17] one may compare this shift with the shifts determined for the Zn(Ar)(leu)$^+$ complexes (Table V) and estimate[12] again the percentage of the closed isomers; this is now in the order of 35%. Considering the crudeness of the estimation, the agreement with the percentages given in Table VI is good; but more important, both estimations reveal that eq 18 is a truly existing equilibrium.

Hydrophobic Interactions in Mixed Ligand Amino Acid Complexes. With the described results in mind we carried out a search of the literature for data on the stability of ternary complexes containing two different amino acids, because one may expect that in a number of cases enhanced stabilities have been observed. Indeed, after the early work of Martin and Pâris[50-52] on ternary complexes containing simple amino acids, the groups of Gergely,[53-57] Pettit,[4,58-61] and Yamauchi[62,63] had studied mixed ligand complexes with the structurally more complicated amino acids, and in several instances they had observed an increased stability which was attributed to a number of effects. We have used these carefully determined results[53,61,63,64] to calculate the information which is now assembled in Table VII.

By viewing the values of $\Delta \log K_{M/exp}$ in Table VII it is quite evident, right from the beginning, that always in those cases where an intramolecular hydrophobic interaction or an aromatic-ring stacking within the ternary complex is possible, an increased stability is observed (see also the $\Delta\Delta \log K_M$ values). Moreover, for the Cu^{2+}/D-his$^-$/L-trp$^-$ and Cu^{2+}/L-bzh$^-$/D-trp$^-$ systems even *positive* values for $\Delta \log K_M$ are observed (no. 52 and 62 in Table VII). As in the $\Delta \log K_M$ consideration[6] an *increase* in the stability of a ternary complex cannot originate from steric restrictions (which may, however, lead to stereoselectivity), a *cooperative* effect between the two different ligands bound to the same metal ion must be responsible for the stability enhancement. There are two such cooperative effects possible: (i) an *indirect* effect resulting from the π-accepting imidazole group of his which leads to a preferred coordination of O donors in 3d metal ion complexes.[1a,6,7] This effect is, however, with a single imidazole group involved, not large enough[1a,6,7] to account for the observed high $\Delta \log K_M$ values, a conclusion which is confirmed by the results of the Cu^{2+} systems with his/bpy, his/ethylenediamine, and his/oxalate.[61] The other possibility is (ii) a *direct* intramolecular interaction between the two ligands bound to the same metal ion. Indeed, aromatic-ring stacking as observed, e.g., in M(ATP)(trp)$^{3-}$ (cf. ref 8) may be responsible for positive $\Delta \log K_M$ values as was shown for the Mg^{2+} and Ca^{2+}/phen/ATP^{4-} systems.[10] It may be added that there is also evidence that the imidazole moiety of his may participate in stacking interactions.[70] Hence, we conclude—in accordance with our described results and the theoretical calculations of Scheraga (vide infra)[3,37]—that the observed stability enhancements in the ternary complexes of Table VII are due to intramolecular hydrophobic or aromatic-ring stacking interactions.

Moreover, by using the $\Delta \log K_M$ values of complexes in which no such interaction is likely or possible, the values of the intramolecular equilibrium constants, K_I, and the percentages of the "closed" isomers (eq 18, 19, and 22)[71] may be calculated—or, to be more specific, estimated owing to the restrictions already discussed in connection with eq 22 and the results of Table VI. However, at the same time it should also be noted that the K_I values calculated from the data obtained by different authors[61,63] are in excellent agreement (cf. no. 57 and 72); it is quite obvious that systematic errors cancel by forming the difference $\Delta\Delta \log K_M$.

It is interesting to note (Table VII) that an intramolecular interaction is most strongly favored in those cases where an aromatic-ring stacking is possible, as, e.g., in M(tyr)(phe) (no. 7, 14, 26, 32, 45, and 48). This interaction is most pronounced in those ternary complexes which contain tryptophanate as a second ligand (no. 52, 53, 62, and 63). This is expected[12] because the interaction is more pronounced with large aromatic-ring systems; i.e., the indole moiety is favored over the phenyl residue. However, the percentage of the "closed" isomer with only a hydrophobic interaction between an aliphatic side chain and an aromatic ring is also still quite remarkable as, e.g., the data of the M(tyr)(nva) complexes (no. 6, 24, and 44) clearly indicate. Taking all the examples together (no. 20, 40-42), there are even indications for a hydrophobic interaction between the two aliphatic side chain residues in, e.g., M(nva)(abu) complexes.

Table VI. Estimations of the Intramolecular Dimensionless Equilibrium Constant K_I for the Ternary M(Ar)(leu)$^+$ Complexes and of the Percentage of the "Closed" Isomer for the Same Systems

complex	K_I (eq 19)[a]	%M(Ar)(leu)$_{cl}^+$ (eq 18)[b]
Cu(bpy)(leu)$^+$	~0	~0
Cu(phen)(leu)$^+$	0.15	13
Zn(bpy)(leu)$^+$	0.12	11
Zn(phen)(leu)$^+$	0.35	26

[a] Calculated with eq 22 from the data listed in Table II (cf. also Table III) under the assumptions given in the text. [b] Calculated with the listed K_I values and eq 19.

Furthermore, it is instructive to compare the results of Table VII also from an energetic point of view with the theoretical calculations of Scheraga.[3] He calculated for the hydrophobic interaction between the phenyl residue of phenylalanine and the isopropyl moiety of leucine in aqueous solution at 25 °C $\Delta G° = -1.7$ kJ/mol. The systems of Table VII which are most similar to the mentioned one with regard to the amino acids involved are M(phe)(nva) (no. 13, 30, and 47: $\Delta\Delta \log K_{M/av} = 0.06$), M(tyr)(nva) (no. 6, 24, and 44: $\Delta\Delta \log K_{M/av} = 0.09$), and M(his)(val) (no. 56 and 57: $\Delta\Delta \log K_{M/av} = 0.10$; see also no. 66 and 67), and for these one calculates in average for the ligand-ligand interaction which is responsible for the enhanced stability of these ternary complexes $\Delta G° = -0.34$, -0.51, and -0.57 kJ/mol, respectively. This means that the agreement between the theoretical and experimental data is most reasonable, especially if one considers that in the first two of our examples the aliphatic amino acid is not branched and that the theoretical calculation of Scheraga is based on a maximal interaction of the hydrophobic residues; it is obvious that in the ternary complexes the mobility of the ligands is restricted and that therefore very often the interaction will be sterically not ideal. This is also borne out if the whole range of the $\Delta\Delta \log K_M$ values (0.02-1.1 log units) listed in Table VII is considered: one calculates $\Delta G° = -0.11$ to -6.3 kJ/mol for the intramolecular ligand-ligand interactions in the ternary complexes; compared with Scheraga's calculations for the unrestricted amino acid/amino acid interactions which he considered, $\Delta G° = -1.2$ to -6.3 kJ/mol, the agreement is excellent and the expected gradual tailing off of the interaction toward zero in the ternary complexes is nicely seen.

In summary, the intramolecular hydrophobic interaction is obviously more widely occurring then one was aware so far—especially as far as low molecular weight complexes are concerned. At this stage we should point out that we use the term "hydrophobic" interaction for the association of lipophilic groups in an aqueous, i.e., hydrophilic, medium in accordance with others.[2,3] However, it has been noted recently[72] that from the energetic viewpoint the term "lipophilic" would be more appropriate; also others[73] prefer "lipophobic". Moreover, there

Table VII. Evidence for Intramolecular Hydrophobic and Aromatic-Ring Stacking Interactions in Ternary M(A) (B) Amino Acid Complexes. Estimations of the Intramolecular Dimensionless Equilibrium Constant K_I of the Ternary Complexes and of the Percentage of the "Closed" Isomer for the Same Systems (25 °C)

no.	complex M(A)(B) (ref 18)[a]	$\Delta \log K_{M/exp}$ (analogous to eq 20)[a]	$\Delta \log K_{M/op}$ (analogous to eq 21)	$\Delta\Delta \log K_M$ (see eq 22)[b]	K_I (analogous to eq 19)[c]	$\%M(A)(B)_{cl}$ (analogous to eq 18)[d]
1	Cu(tyr)(gly)	−0.79				
2	Cu(tyr)(ala)	−0.76	−0.78			
3	Cu(tyr)(ser)	−0.78				
4	Cu(tyr)(thr)	−0.78				
5	Cu(tyr)(abu)	−0.76	−0.78	0.02	~0.05[e]	~5[e]
6	Cu(tyr)(nva)	−0.66	−0.78	0.12	0.32[f]	24[f]
7	Cu(tyr)(phe)	−0.59	−0.78	0.19	0.55[g]	35[g]
8	Cu(phe)(gly)	−0.78				
9	Cu(phe)(ala)	−0.80	−0.79			
10	Cu(phe)(ser)	−0.79				
11	Cu(phe)(thr)	−0.80				
12	Cu(phe)(abu)	−0.78	−0.79	0.01	~0.02[e]	~2[e]
13	Cu(phe)(nva)	−0.74	−0.79	0.05	0.12[f]	11[f]
14	Cu(phe)(tyr)	−0.59	−0.79	0.20	0.58[g]	37[g]
15	Cu(ala)(gly)	−1.04				
16	Cu(abu)(gly)	−1.07				
17	Cu(abu)(ala)	−1.04	−1.04			
18	Cu(nva)(gly)	−1.04				
19	Cu(nva)(ala)	−1.02				
20	Cu(nva)(abu)	−1.00	−1.04	0.04	0.10	9
21	Ni(tyr)(gly)	−0.53	−0.51			
22	Ni(tyr)(ala)	−0.48				
23	Ni(tyr)(abu)	−0.46	−0.51	0.05	0.12[h]	11[h]
24	Ni(tyr)(nva)	−0.47	−0.51	0.04	0.10[i]	9[i]
25	Ni(tyr)(nle)	−0.45	−0.51	0.06	0.15[j]	13[j]
26	Ni(tyr)(phe)	−0.39	−0.51	0.12	0.32[g]	24[g]
27	Ni(phe)(gly)	−0.52	−0.52			
28	Ni(phe)(ala)	−0.52				
29	Ni(phe)(abu)	−0.51	−0.52	0.01	~0.02[h]	~2[h]
30	Ni(phe)(nva)	−0.50	−0.52	0.02	~0.05[i]	~5[i]
31	Ni(phe)(nle)	−0.51	−0.52	0.01	~0.02[j]	~2[j]
32	Ni(phe)(tyr)	−0.39	−0.52	0.13	0.35[g]	26[g]
33	Ni(ala)(gly)	(−0.56)				
34	Ni(abu)(gly)	(−0.59)				
35	Ni(abu)(ala)	−0.62				
36	Ni(nva)(gly)	(−0.56)	−0.64[k]			
37	Ni(nva)(ala)	−0.64				
38	Ni(nle)(gly)	(−0.61)				
39	Ni(nle)(ala)	−0.65				
40	Ni(nva)(abu)	−0.59	−0.64			
41	Ni(nle)(abu)	−0.63	−0.64	~0.02	~0.05[l]	~5[l]
42	Ni(nle)(nva)	−0.63	−0.64			
43	Co(tyr)(gly)	−0.27	−0.27			
44	Co(tyr)(nva)	−0.16	−0.27	0.11	0.29[m]	22[m]
46	Co(phe)(gly)	−0.40	−0.40			
47	Co(phe)(nva)	−0.30	−0.40	0.10	0.26[m]	21[m]
48	Co(phe)(tyr)	−0.10	−0.40	0.30	1.0[g]	50[g]
49	Cu(D- or L-his)(L-ser)	−0.77				
50	Cu(D- or L-his)(L-thr)	−0.59	−0.68[n]			
51	Cu(D- or L-his)(L-met)	−0.69				
52	Cu(D-his)(L-trp)	+0.34	−0.68	1.02	9.5	90
53	Cu(L-his)(L-trp)	−0.12	−0.68	0.56	2.6	72
54	Cu(D-his)(L-phe)	−0.34	−0.68	0.34	1.2	55
55	Cu(L-his)(L-phe)	−0.54	−0.68	0.14	0.38	28
56	Cu(D-his)(L-val)	−0.61	−0.68	0.07	0.17	15
57	Cu(L-his)(L-val)	−0.55	−0.68	0.13	0.35	26
58	Cu(D-his)(L-leu)	−0.72	−0.68	~0.0	~0	~0
59	Cu(L-his)(L-leu)	−0.69	−0.68			
60[o]	Cu(L-bzh)(D-glu)$^-$	−0.72	−0.72[p]			
61[o]	Cu(L-bzh)(L-glu)$^-$	−0.71				
62[o]	Cu(L-bzh)(D-trp)	+0.45	−0.68[p]	1.13	12.5	93
63[o]	Cu(L-bzh)(L-trp)	−0.03	−0.68[p]	0.65	3.5	78
64[o]	Cu(L-bzh)(D-phe)	−0.09	−0.68[p]	0.59	2.9	74
65[o]	Cu(L-bzh)(L-phe)	−0.40	−0.68[p]	0.28	0.91	48
66[o]	Cu(L-bzh)(D-val)	−0.61	−0.68[p]	0.07	0.17	15
67[o]	Cu(L-bzh)(L-val)	−0.55	−0.68[p]	0.13	0.35	26

Table VII (*Continued*)

no.	complex M(A)(B) (ref 18)[a]	$\Delta \log K_{M/exp}$ (analogous to eq 20)[a]	$\Delta \log K_{M/op}$ (analogous to eq 21)	$\Delta\Delta \log K_M$ (see eq 22)[b]	K_I (analogous to eq 19)[c]	% $M(A)(B)_{cl}$ (analogous to eq 18)[d]
68	Cu(L-his)(gly)	−0.86				
69	Cu(L-his)(L-ala)	−1.00	−0.93			
70	Cu(L-his)(L-ser)	−0.88				
71	Cu(L-his)(L-thr)	−0.98				
72	Cu(L-his)(L-val)	−0.81	−0.93	0.12	0.32	24

[a] The constants for $\Delta \log K_{M/exp}$ were taken for entries no. 1-48 (I = 0.05, KCl) from the work of Gergely et al.,[53] for no. 49-67 (I = 0.1, KNO_3) from that of Brookes and Pettit,[61] and for no. 68-72 (I = 0.1, KNO_3) from Yamauchi et al.[63] The potentiometric studies of the systems no. 1-48 were carried out with the D,L mixtures of the amino acids;[64] Professor Dr. A. Gergely pointed out that with these simple amino acids no differences in the stability constants of the complexes with the D or L amino acids were observed; see the discussion in ref 65. [b] $\Delta\Delta \log K_M = \Delta \log K_{M/exp} - \Delta \log K_{M/op}$; i.e., this value is the decisive parameter of eq 22. [c] Calculated with eq 22. To be on the safe side, the attitude was taken to use as a basis those data of $\Delta \log K_{M/op}$ which lead rather to too small values for K_I, and hence to rather a too small percentage for the "closed" isomer; cf. footnotes *e*, *f*, *h*, *i*, *j*, *l*, *m*, and *p*. [d] Calculated with the listed K_I values and $K_I = [M(A)(B)_{cl}]/[M(A)(B)_{op}]$, corresponding to eq 19. [e] Based on $\Delta \log K_{M/op} = -1.04$ of Cu(abu)(ala) one obtains for Cu(tyr)(abu) and Cu(phe)(abu) K_I = 0.91 and 0.82, i.e., 48 and 45% for the "closed" isomer, respectively. [f] Based on $\Delta \log K_{M/op} = -1.02$ of Cu(nva)(ala) one obtains for Cu(tyr)(nva) and Cu(phe)(nva) K_I = 1.3 and 0.91, i.e., 57 and 48% for the "closed" isomer, respectively. [g] The species M(tyr)(phe) and M(phe)(tyr) are identical because all these complexes are labile; it is therefore gratifying to note that the percentages of the "closed" isomer calculated on the basis of these two corresponding cases agree well with each other. This holds for the Cu^{2+}, Ni^{2+}, and Co^{2+} systems. [h] Based on $\Delta \log K_{M/op} = -0.62$ of Ni(abu)(ala) one obtains for Ni(tyr)(abu) and Ni(phe)(abu) K_I = 0.45 and 0.29, i.e., 31 and 22% for the "closed" isomer, respectively. [i] Based on $\Delta \log K_{M/op} = -0.64$ of Ni(nva)(ala) one obtains for Ni(tyr)(nva) and Ni(phe)(nva) K_I = 0.48 and 0.38, i.e., 32 and 28% for the "closed" isomer, respectively. [j] Based on $\Delta \log K_{M/op} = -0.65$ of Ni(nle)(ala) one obtains for the Ni(tyr)(nle) and Ni(phe)(nle) K_I = 0.58 and 0.38, i.e., 37 and 28% for the "closed" isomer, respectively. [k] The $\Delta \log K_{M/exp}$ constants for the Ni(A)(gly) complexes were listed but not used for the calculation of this average value, because all the data of the Ni(A)(gly) systems appear to be slightly too high, possibly indicating that the methyl group of ala introduces a slight steric effect; if so, the Ni(A)(ala) complexes are a better basis for comparison. [l] An individual evaluation for these complexes based on the corresponding Ni(A)(ala) complexes results in K_I = 0.12, 0.05, and 0.05, i.e., in 11, 5, and 5% of the "closed" isomer for Ni(nva)(abu), Ni(nle)(abu), and Ni(nle)(nva), respectively. [m] Based on $\Delta \log K_{M/op} = -0.48$ of Co(nva)(gly) one obtains for Co(tyr)(nva) and Co(phe)(nva) K_I = 1.1 and 0.51, i.e., 52 and 34% for the "closed" isomer, respectively. [n] This value is certainly a fair basis for comparisons: ser, thr, and met behave toward Cu^{2+} in solution as bidentate ligands;[22] there is also no stereoselectivity observed in the ternary complexes with his (see the values in the above table),[4] and finally entries no. 1-4, 8-11, and 68-71 in the above table show that ser and thr behave like gly and ala. [o] Similar results have been obtained[61] for the corresponding Cu(L-dbzh)(B) complexes. [p] The value $\Delta \log K_{M/op} = -0.72$ seems actually to be a usable basis, especially as there is no stereoselectivity observed; however, to be on the safe side we used the preceding value, i.e., $\Delta \log K_{M/op} = -0.68$, for the calculations.

is also no agreement about the forces governing this effect. Tanford[74] concludes: "The hydrophobic effect is a unique organizing force, based on repulsion by the solvent instead of attractive forces at the side of organization". This is in contrast to Hildebrand's[75] statements: ". . . alkyl groups . . . in water are not forced together by "phobia" for water . . . there is no hydrophobia between water and alkanes; there is only not enough hydrophilia to pry apart the hydrogen bonds of water so that the alkanes can go into solution without assistance from attached polar groups" (cf. also ref 76). But as Némethy, Scheraga, and Kauzmann[77] pointed out "the source of immiscibility is an entropy factor, the water-hydrocarbon system differs qualitatively, and in a unique manner from most systems of low miscibility" (cf. also ref 78).

By using the term "hydrophobic" we do not imply any conclusions about the driving forces of these associations, because our experimental results do not allow this. All we may say, as long as an aqueous solution is considered, is that the described intramolecular hydrophobic ligand-ligand interaction in mixed ligand complexes emerges from the lipophilicity of one part of the ligands and from the hydrophilicity of the remainder of the complex. By reducing the polarity of the solvent and thus improving the solvation of the apolar groups a decrease in such "hydrophobic" interactions is achieved.[20,39,71]

To conclude, the general implication from our results is that weak hydrophobic interactions which exist in nature, e.g., between two side chains of amino acids in proteins, may be promoted by metal-ion bridging between the two moieties involved. This may be demonstrated by the present results: using concentrations of the reactants of 10^{-3} M one calculates with an association constant of 1.4 M^{-1} for a phen/H·leu system that 0.14% exist as an adduct, while at pH 7.5 or 8 in the ternary Zn^{2+}/phen/leu system about 3.2% [$Zn(phen)(leu)_{tot}^{+}$: 12.2%] or 6.3% [$Zn(phen)(leu)_{tot}^{+}$: 24.3%], respectively (calculated with the results of Tables I, II, and VI), exist in the "closed" isomeric form, $Zn(phen)(leu)_{cl}^{+}$; this corresponds to a promotion by a factor of about 23 or 45, respectively. There is one further aspect which should be pointed out. The most important point of the described hydrophobic interaction from our view is not so much that the formation of certain mixed ligand complexes may be somewhat favored by the intramolecular hydrophobic ligand-ligand interaction, but rather that this interaction is responsible for the creation of distinct structures. With regard to the selectivity observed in nature this seems to us the most fascinating point of these results.

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(42) From the data given above (see also Figure 2A) one obtains $K^{(bpy)}_{(bpy)(H\text{-}leu)} = (0.76 + 0.16)/2 = 0.46 \pm 0.30$; by taking into account the mentioned self-association of bpy the formation constant is increased by a factor of about 1.3, which results in the value given in the text. The value of $K^{(phen)}_{(phen)(H\text{-}leu)}$ was calculated analogously; the factor due to the self-association of phen is about 1.5.
(43) The effect of incomplete $Zn(Ar)^{2+}$ formation in a 1:1 mixture with Ar = bpy or phen has been discussed in detail recently;[17b] see also Experimental Section.
(44) The values of δ_∞ given in the legend to Figure 2 for the ternary complexes correspond to $\delta_{Zn(bpy)(leu)}$ and $\delta_{Zn(phen)(leu)}$, which may be calculated from the data in Table V. The observed differences result from the rather weak curvature (Figure 2B), which leads to a rather large error, and the fact that in a 1:1 mixture of Zn^{2+} and bpy or phen also some Zn^{2+} is still present and therefore also some $Zn(leu)^+$ forms; hence δ_∞ is also somewhat influenced by $\delta_{Zn(leu)}$ (cf. ref 43). The shift values given in Table V are the more exact ones, as these data were calculated (eq 1 and 2) by using the formation constants obtained from the potentiometric pH titrations.
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(65) It should be added that, if one assumes planar bis coordination, cis and trans isomers are possible. 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; in the meso complex the reverse will be true.[4] The cis and trans isomers of $Cu(ala)_2$ have been isolated and X-ray structure determinations were done.[66,67] The cis and trans isomers of $Cu(gly)_2$ have also been characterized in the solid state.[68] In the equilibrium *cis*-$[Cu(gly)_2]H_2O \rightleftharpoons$ *trans*-$[Cu(gly)_2]\cdot H_2O$ the trans isomer is the thermodynamically more stable and the following two values for ΔH have been measured: 1.8 (cf. ref 69) and 9.1 kJ/mol (cf. ref 68). Both these values for ΔH, which are valid for the solids, are already rather small and it seems safe to assume that the energy barrier between the cis/trans isomer of bidentate amino acids in solution is even significantly lower; hence, this isomer of the ternary complex allowing intramolecular side chain interaction is most probably very easily formed. In fact, the differences observed for the optical isomers in the systems[61] no. 52–67 may in part (i.e., aside from structural differences in the ligand–ligand interactions) be due to such a cis/trans rearrangement; this is here probably somewhat more inhibited owing to the possible terdentate coordination of his[22] and bzh. This interpretation is also in agreement with systems no. 49–51 and 60/61, in which no hydrophobic interaction is possible and therefore no reflection of the chirality of the amino acids is observed.
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(71) It should be mentioned in this connection that an equation (analogous to eq 22) for the calculation of K_i may also be derived based on the log X approach (eq 13, 14). However, even though mathematically correct, such an equation should not be used, because the stability constants of the binary 1:2 complexes (i.e., $K^{ML}_{ML_2}$) influence log X and therefore indirectly the result for K_i. For example, the difference log K^{M}_{ML} − log $K^{ML}_{ML_2}$ is unusually small for the complexes with tryptophanate, phenylalaninate, or tyrosinate (tyr^-);[1b,22,55,57,61] it was suggested[1b] that this is also due to aromatic-ring stacking within the 1:2 complexes. This view is further supported by the observation[55] that the difference in the values of log K^{Cu}_{CuL} − log $K^{CuL}_{CuL_2}$ between the alaninate and the phenylalaninate or tyrosinate complexes is nearly diminished in 50% aqueous dioxane, and it is known that dioxane does indeed inhibit stacking.[39]
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