Influence of Dioxane on the Extent of Intramolecular Hydrophobic Ligand–Ligand Interactions in the Binary Cu^{2+} 1:2 Complexes of *L*-Leucinate, *L*-Valinate and *L*-Norvalinate

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Abstract

The stability constants of the binary $Cu(AA)^+$ and $Cu(AA)_2$ complexes, where $AA^- = L$-alaninate (Ala^-), *L*-leucinate (Leu^-), *L*-valinate (Val^-) or *L*-norvalinate, have been determined by potentiometric pH titrations in water, and in 30, 50, 70 and 80% (*v*/*v*) dioxane–water mixtures ($I = 0.1$ M, $NaNO_3$; 25 °C). The overall stability of $Cu(AA)^+$ and $Cu(AA)_2$ is governed for all four amino acetates (AA^-) by the polarity of the solvent, while the extent of the intramolecular hydrophobic ligand–ligand interaction between the aliphatic side-chains in $Cu(Val)_2$, $Cu(Leu)_2$ and $Cu(Nva)_2$ is obviously influenced by the hydrophobic solvation properties of the organic solvent molecules. Based on the stability difference $\Delta \log K^*_{AA} = \log K^{Cu(AA)}_{Cu(AA)_2} - \log K^{Cu}_{Cu(AA)}$ it is shown that $Cu(Val)_2$, $Cu(Leu)_2$ and $Cu(Nva)_2$ are more stable than $Cu(Ala)_2$, and this increased stability is taken as evidence for hydrophobic side-chain interactions in $Cu(Val)_2$, $Cu(Leu)_2$ and $Cu(Nva)_2$; such interactions are not possible in $Cu(Ala)_2$ due to the small size of the methyl side-chain. By using the stability data of the Cu^{2+}/Ala^- system as a basis for the evaluation, the extent of the hydrophobic ligand–ligand interaction (= closed form) in the other three $Cu(AA)_2$ complexes is calculated: the percentages of the closed forms vary between about 10 and 30% (based on $Cu(AA)_{2/tot}$). The formation degree of the closed species is influenced by the solvent: addition of some dioxane to an aqueous solution favors their formation, contrary to the experience with simple unbridged hydrophobic adducts which are destabilized. Such a destabilization of the closed $Cu(AA)_2$ species occurs only at high concentrations of the organic solvent (usually more than 70%). The general relevance of the present results, especially with regard to biological systems, is indicated.

Introduction

There is significant evidence that many of the specific interactions in biological systems are achieved by side-chain groups of proteins [1–3]. However, such interactions may also be important in low-molecular-weight species; indeed, hydrophobic and stacking interactions in aqueous solutions of binary and ternary complexes of amino acids with suitable side-chains are now well established [4, 5]. In aqueous solution, where most studies up to now were done, the extent of hydrophobic and stacking interactions depends on the structure of the amino acids [4, 5]; the intensity of the side-chain interaction increases in the series aliphatic–aliphatic < aliphatic–aromatic < aromatic–aromatic.

Our knowledge on aliphatic–aliphatic side-chain interactions in metal ion complexes rests only on some few examples [4, 5], and the influence of organic solvents on these interactions has hardly been studied [5]. This latter type of influence is meaningful with regard to biological systems, because the solvent polarity is expected to be reduced at the surface of proteins and in active-site cavities of enzymes; indeed, there is now good evidence that the 'equivalent solution' or 'effective' dielectric constant is lower in such cavities than in water [6, 7].

To see how organic solvents influence intramolecular hydrophobic ligand–ligand interactions the stability of the binary $Cu(AA)_2$ complexes, where $AA^- = L$-alaninate (Ala^-)†, *L*-valinate (Val^-), *L*-norvalinate (NVa^-) or *L*-leucinate, was measured in aqueous solution and in several dioxane–water

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†Abbreviations: AA^-, amino acetate (= amino acid anion); Ala^-, *L*-alaninate, L, general ligand; Leu^-, *L*-leucinate; M^{2+}, general divalent metal ion; Nva^-, *L*-norvalinate, Val^-, *L*-valinate.

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L-AMINO ACIDS

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

Fig. 1. *L*-Amino acids (AA) used in this study, together with the structure of their aliphatic side-chain.

mixtures. The structures of the mentioned amino acids are shown in Fig. 1. The evaluation of the experimental data regarding the extent of the intramolecular aliphatic side-chain interactions is based on the results obtained for $Cu(Ala)_2$; the methyl side-chains in this binary complex are too short to allow an interaction between these residues of the two equatorially coordinated alaninates. It may be mentioned in this connection that $Cu(glycinate)_2$ appears as unsuitable for the comparisons, as $M(glycinate)_2$ complexes often show [8, 9] exceptional stability. In the $Cu(AA)_2$ complexes with the three amino acetates of Fig. 1 containing a longer aliphatic residue an intramolecular ligand–ligand interaction occurs; the extent of this interaction is solvent dependent.

Experimental

Materials

L-Alanine, *L*-valine, *L*-norvaline and *L*-leucine (all puriss.) were obtained from Fluka AG, Buchs, Switzerland. The disodium salt of ethylenediamine-*N,N,N',N'*-tetraacetic acid (Na_2H_2EDTA), potassium hydrogen phthalate, HNO_3, NaOH (Titrisol), $Cu(NO_3)_2 \cdot 3H_2O$, $NaNO_3$ (all pro analysi), and 1,4-dioxane (extra pure) were purchased from Merck AG, Darmstadt, F.R.G.

All solutions were prepared with distilled and CO_2-free water. The titer of the NaOH used for the titrations was determined with potassium hydrogen phthalate. The exact concentrations of the amino acid solutions were measured by titrations with NaOH. The concentration of the stock solution of copper(II) nitrate was determined with EDTA.

Potentiometric pH Titrations

The pH titrations were carried out with a Metrohm potentiograph E536, dosimat E535 and macro EA 121 glass electrodes. The buffer solutions (pH 4.64, 7.00 and 9.00) used for calibration were also from Metrohm AG, Herisau, Switzerland. The direct pH-meter readings were used in the calculations for the acidity constants; no 'corrections' were applied for the change in solvent from water to the dioxane–water mixtures, though correction factors have been published [10]. The titration speed of the potentiograph was varied between 15 and 30 min; the titration speed for the dioxane–water mixtures was longer compared with that for the aqueous solutions, as the response time of the electrode is larger at higher concentrations of the organic solvent. The mixed solvents (*v/v*) were prepared by taking, e.g. 25 ml aqueous solution and adding 25 ml dioxane.

The calculations were done with a Hewlett-Packard 9825A calculator, which was connected with a plotter 7470A and a printer 82905B. Some of the calculations were also carried out in the 'Universitäts-rechenzentrum' on a Univac 1100/81.

Determination of the Acidity Constants

As the difference between $pK^H_{H_2(AA)}$ and $pK^H_{H(AA)}$ is large (>5 log units), the buffer regions between $H_2(AA)^+$ and $H(AA)^\pm$ are not overlapping and $K^H_{H_2(AA)}$ and $K^H_{H(AA)}$ can therefore be determined independently.

The acidity constant $K^H_{H_2(AA)}$ of $H_2(AA)^+$ was determined by titrating 50 ml of solutions which were 4 mM in HNO_3 and which contained also $NaNO_3$ ($I = 0.1$; 25 °C) in the presence and absence of 5 mM $H(AA)^\pm$ (the aqueous stock solutions had been adjusted, when necessary, with NaOH to pH 5.9) under N_2 with 2 ml of 0.1 M NaOH, and by using the differences in NaOH consumption between two such titrations for the calculations. Values for $K^H_{H_2(AA)}$ were calculated from four independent pairs of titrations by taking into account the species H^+, $H_2(AA)^+$ and $H(AA)^\pm$ with the mentioned Hewlett-Packard 9825 A calculator by a curve-fit procedure using a Newton-Gauss non-linear least-squares program within the pH range determined by the lowest point of neutralization reached under the experimental conditions and about 97% neutralization for the equilibrium $H_2(AA)^+/H(AA)^\pm$.

The determination of $K^H_{H(AA)}$ for $H(AA)^\pm$ was done by titrating under N_2 50 ml of solutions which were 0.15 mM in HNO_3 ($I = 0.1$ M, $NaNO_3$; 25 °C) in the presence and absence of 0.6 mM $H(AA)^\pm$ (the aqueous stock solutions had also been adjusted with NaOH to pH 5.9, when necessary) with 1 ml of 0.05 M NaOH. Values for $K^H_{H(AA)}$ were computed from at least six independent pairs of titrations as described above (between 3% and 97% neutralization for the equilibrium $H(AA)^+/AA^-$) by taking into account the species H^+, $H(AA)^\pm$ and AA^-.

Determination of the Stability Constants

The determination of $K^{Cu}_{Cu(AA)}$ and $K^{Cu(AA)}_{Cu(AA)_2}$ was done by titrating under N_2 50 ml solutions of HNO_3, $Cu(NO_3)_2$ and $NaNO_3$ ($I = 0.1$ M; 25 °C) in the presence and absence of AA (the ratios of $[Cu^{2+}]$: [AA] were 1:2, 1:2.5 and 1:3) with 1 ml of 0.05 M

NaOH. The concentrations of the solutions were: $[HNO_3] = 3.75 \times 10^{-4}$ M, $[Cu^{2+}] = 3.0 \times 10^{-4}$ M, and $[H(AA)] = 6.0 \times 10^{-4}$ M, 7.5×10^{-4} M or 9.0×10^{-4} M. The titrations without AA were used as a basis for the evaluations. Values for $K^{Cu}_{Cu(AA)}$ and $K^{Cu(AA)}_{Cu(AA)_2}$ were computed from 9 independent pairs of titrations by taking into account the species H^+, $H_2(AA)^+$, $H(AA)^{\pm}$, AA^-, Cu^{2+}, $Cu(AA)^+$ and $Cu(AA)_2$. The pH range used in the calculations was from the beginning of complex formation (usually more than 10% were already present at the onset of the titration) to the point where hydrolysis started. The resulting stability constants obtained from the different $[Cu^{2+}]:[AA]$ ratios agreed well.

The determination of the stability constant $K^{Cu}_{Cu(AA)}$ was repeated by titrating under N_2 50 ml solutions ($I = 0.1$ M, $NaNO_3$; 25 °C) which were 0.375 mM in HNO_3 and 0.6 or 1.2 mM in $Cu(NO_3)_2$ in the presence and absence of 0.6 mM $H(AA)^{\pm}$ (ratios of $[Cu^{2+}]:[AA] = 1:1$ and 2:1) with 1 ml of 0.05 M NaOH. Although the formation of $Cu(AA)_2$ is small under these conditions, this species was taken into account in the calculations by using the constant $K^{Cu(AA)}_{Cu(AA)_2}$ obtained from the above described determinations with AA in excess over Cu^{2+}. Values for $K^{Cu}_{Cu(AA)}$ were computed from six independent pairs of titrations by considering the species H^+, $H_2(AA)^+$, $H(AA)^{\pm}$, AA^-, Cu^{2+}, $Cu(AA)^+$ and $Cu(AA)_2$. The pH range used in the calculations was from 5% (or 10%) $Cu(AA)^+$ formation to the beginning of hydrolysis of Cu^{2+}_{aq}; the latter was evident from the titrations without AA. The resulting values for $K^{Cu}_{Cu(AA)}$ are independent of both, the pH (every 0.1 pH unit was evaluated) and the concentration of Cu^{2+}, and the values are also in excellent agreement with those obtained from the experiments with AA in excess over Cu^{2+}. The final results given in the Tables are always the averages from all determinations.

Results and Discussion

1. Stability of the Binary $Cu(AA)^+$ and $Cu(AA)_2$ Complexes

The stability constants of the Cu^{2+} complexes of the amino acids shown in Fig. 1 were determined by potentiometric pH titrations in water and in 30, 50, 70 and 80% (*v/v*) dioxane–water. All experimental data could be satisfactorily explained by considering the following equilibria:

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

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

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

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

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

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

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

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

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

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

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

The corresponding equilibrium constant (eqn. (5b)) can be calculated with eqn. (6):

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

The results for the four amino acid systems (Fig. 1) are summarized in Table 1, where the mole fractions of dioxane in the solvent mixtures and the corresponding dielectric constants (ϵ) [11] are also listed. It is interesting to note already here, that the values for $\Delta \log K^{*}_{AA}$ are all negative, as one would expect in accordance with the general rule [12] that $K^{M}_{M(L)} > K^{M(L)}_{M(L)_2}$. However, it is further evident from the values of $\Delta \log K^{*}_{AA}$ for the complexes with *L*-valinate, *L*-norvalinate and *L*-leucinate that these are somewhat less negative than the corresponding values for *L*-alanine (Table 1). That this is not an effect of a reduced stability of the 1:1 complexes, $Cu(AA)^+$, is obvious from plots of $\log K^{Cu}_{Cu(AA)}$ *versus* $pK^{H}_{H_2(AA)} + pK^{H}_{H(AA)}$; the values for all four amino acid systems fit on the same straight line for a given solvent. This means, the 1:2 complexes of Val^-, Nva^- and Leu^- are relative to their corresponding 1:1 complexes more stable; in other words, equilibrium (5a) is for the three latter amino acids somewhat more on its right side than with Ala^- (this conclusion should not be confused with the fact that in equilibrium (5a) $Cu(AA)^+$ is still for all four systems the dominating species). This simple comparison is already indicative for the occurrence of an intramolecular ligand–ligand interaction in the $Cu(AA)_2$ complexes of Val, Nva and Leu. A more quantitative evaluation of these data is given in Section 2.

Two of the amino acid systems listed in Table 1 have already been studied earlier (1): the Cu^{2+}/Ala system was investigated in several dioxane–water systems by Gergely and Kiss [13]; their values for $\Delta \log K^{*}_{AA}$ are somewhat *more* negative than the present ones, with the exception of the value for water which agrees. This discrepancy could be due to the use of KCl ($I = 0.2$ M; 25 °C) as background electrolyte in the work of Gergely and Kiss. (2) The

TABLE 1

Negative Logarithms of the Acidity Constants of *L*-Alanine, *L*-Valine, *L*-Norvaline and *L*-Leucine (eqns. (1) and (2)) and Logarithms of the Corresponding Binary $Cu(AA)^+$ (eqn. (3)) and $Cu(AA)_2$ (eqn. (4)) Complexes, Together with the Stability Differences $\Delta \log K^*_{AA}$ (eqns. (5) and (6)), in Dependence on the Amount of Dioxane Added to Water at $I = 0.1$ M ($NaNO_3$) and 25 °C[a]

% (*v*/*v*) Dioxane	Mole fraction	ϵ[b]	$pK^H_{H_2(AA)}$	$pK^H_{H(AA)}$	$\log K^{Cu}_{Cu(AA)}$	$\log K^{Cu(AA)}_{Cu(AA)_2}$	$\Delta \log K^*_{AA}$
L-Alanine							
0	0	78.5	2.40 ± 0.01	9.84 ± 0.01	8.22 ± 0.02	6.84 ± 0.02	−1.38 ± 0.03
30	0.083	52.7	2.79 ± 0.01	9.95 ± 0.02	8.94 ± 0.02	7.48 ± 0.04	−1.46 ± 0.04
50	0.175	35.2	3.18 ± 0.02	10.03 ± 0.01	9.51 ± 0.02	8.00 ± 0.03	−1.51 ± 0.04
70	0.331	18.6	3.61 ± 0.02	10.12 ± 0.01	10.16 ± 0.02	8.65 ± 0.02	−1.51 ± 0.03
80	0.459	11.6	3.98 ± 0.01	10.07 ± 0.01	10.56 ± 0.02	8.95 ± 0.02	−1.61 ± 0.03
L-Valine							
0	0	78.5	2.41 ± 0.02	9.66 ± 0.02	8.15 ± 0.02	6.84 ± 0.02	−1.31 ± 0.03
30	0.083	52.7	2.82 ± 0.01	9.77 ± 0.02	8.88 ± 0.02	7.52 ± 0.02	−1.36 ± 0.03
50	0.175	35.2	3.21 ± 0.02	9.86 ± 0.01	9.49 ± 0.02	8.12 ± 0.06	−1.37 ± 0.06
70	0.331	18.6	3.64 ± 0.02	9.86 ± 0.02	10.07 ± 0.03	8.72 ± 0.04	−1.35 ± 0.05
80	0.459	11.6	3.84 ± 0.02	9.82 ± 0.01	10.45 ± 0.02	8.99 ± 0.03	−1.46 ± 0.04
L-Norvaline							
0	0	78.5	2.41 ± 0.02	9.78 ± 0.01	8.20 ± 0.01	6.86 ± 0.01	−1.34 ± 0.01
30	0.083	52.7	2.81 ± 0.01	9.87 ± 0.02	8.91 ± 0.01	7.49 ± 0.03	−1.42 ± 0.03
50	0.175	35.2	3.23 ± 0.01	9.97 ± 0.01	9.51 ± 0.01	8.08 ± 0.03	−1.43 ± 0.03
70	0.331	18.6	3.65 ± 0.01	10.02 ± 0.01	10.12 ± 0.02	8.71 ± 0.02	−1.41 ± 0.03
80	0.459	11.6	4.03 ± 0.01	9.97 ± 0.01	10.71 ± 0.03	9.15 ± 0.03	−1.56 ± 0.04
L-Leucine							
0	0	78.5	2.44 ± 0.01	9.72 ± 0.01	8.19 ± 0.01	6.86 ± 0.02	−1.33 ± 0.02
30	0.083	52.7	2.86 ± 0.01	9.82 ± 0.01	8.88 ± 0.02	7.48 ± 0.02	−1.40 ± 0.03
50	0.175	35.2	3.29 ± 0.01	9.91 ± 0.02	9.52 ± 0.02	8.12 ± 0.03	−1.40 ± 0.04
70	0.331	18.6	3.72 ± 0.01	9.94 ± 0.01	10.20 ± 0.02	8.83 ± 0.02	−1.37 ± 0.03
80	0.459	11.6	4.07 ± 0.01	9.89 ± 0.01	10.73 ± 0.03	9.17 ± 0.03	−1.56 ± 0.04

[a]The errors given are three times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. The values of the error limits for $\Delta \log K^*_{AA}$ were calculated according to the error propagation after Gauss. [b]The dielectric constants for the dioxane/water mixtures are from ref. 11.

Cu^{2+}/Leu system had been studied by Zelano *et al.* [14]; their $\Delta \log K^*_{Leu}$ values are *less* negative than the present results, again with the exception of the value for the aqueous solution. The reason for this discrepancy appears less evident because Zelano *et al.* used $NaClO_4$ ($I = 0.1$ M; 25 °C) as background electrolyte; ClO_4^- and NO_3^- are not expected to differ significantly in their affinity towards Cu^{2+}, though in solvents with a low polarity this might be different.

Finally it should be pointed out that due to the mentioned discrepancies our previous evaluation [5] for the extent of the intramolecular hydrophobic ligand–ligand interaction in $Cu(Leu)_2$, which was based on the data of Zelano *et al.* [14] and Gergely and Kiss [13], differs from the present result. However, this difference is only of a quantitative nature; this means, for example, the previously calculated maximal formation degree for $Cu(Leu)_2$ with a ligand–ligand interaction was about 80% [5] while now it is only about 30% (*vide infra*). The qualitative results of the two evaluations, *i.e.* the previous [5] and the present one (Section 2), are in excellent accordance; hence, it may be emphasized that all previous interpretations and conclusions [5] are still valid.

2. Extent of the Aliphatic Side-chain Interaction in the $Cu(AA)_2$ Complexes of Valinate, Norvalinate and Leucinate

Intramolecular hydrophobic interactions in solution between suitable residues in mixed ligand complexes have been established by several methods; these include ^1H NMR shift studies in organic solvent–water mixtures [5, 15] of such interactions between an aromatic-ring system and an aliphatic residue. It is evident that any kind of ligand–ligand interaction will be reflected in an increased stability of the corresponding complex [16]. As the 2N/2O

donor-atom set bound to the equatorial part of the Cu^{2+} coordination sphere is identical in $Cu(Ala)_2$ and in $Cu(AA)_2$ with Val^-, Nva^- and Leu^-, the increased stability of the latter three complexes as expressed by $\Delta \log K^*_{AA}$ (eqn. (6)) and already discussed in Section 1, may certainly be attributed to a hydrophobic interaction between the aliphatic side chains (Fig. 1) within these complexes.

It is evident that the occurrence of a species with an intramolecular ligand–ligand interaction does not mean that all of the corresponding $Cu(AA)_2$ complexes exist in this 'closed' form. Therefore the following equilibrium must be considered

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

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

In eqn. (7) $Cu(AA)_{2/cl}$ represents the binary 1:2 complex with an intramolecular ligand–ligand interaction and $Cu(AA)_{2/op}$ represents the 'open' form without such an interaction. The dimensionless equilibrium constant K^*_I may be calculated [4, 17] with eqn. (8):

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

By employing the definition given in eqn. (9),

$$\Delta\Delta \log K^* = \Delta \log K^*_{AA} - \Delta \log K^*_{AA/op} \quad (9)$$

Eqn. (8) may be rewritten as eqn. (10):

$$K^*_I = 10^{\Delta\Delta \log K^*} - 1 \quad (10)$$

The values for $\Delta \log K^*_{AA}$ are known from the experiments (Table 1) and the stability of the open form is evidently well represented by the stability of $Cu(Ala)_2$ as in this case due to the shortness of the aliphatic residues no interaction between these residues is possible, *i.e.* one may define

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

Hence, values for K^*_I may now be calculated for the $Cu(AA)_2$ complexes with Val^-, Nva^- and Leu^-.

It is probably helpful to realize that $10^{\Delta\Delta \log K^*}$ is the ratio of two equilibrium constants and therefore $10^{\Delta\Delta \log K^*}$ itself must also be an equilibrium constant. In fact, by using the definition (11) it is easy to show that $10^{\Delta\Delta \log K^*}$ quantifies the position of equilibrium (12):

$$2Cu(AA)^+ + Cu(Ala)_2 \rightleftharpoons Cu(AA)_2 + 2Cu(Ala)^+ \quad (12)$$

The coordination spheres of Cu^{2+} on both sides of this equilibrium are identical; consequently, the values for $\Delta\Delta \log K^*$ (eqn. (9)) are a true reflection of the extent of the intramolecular ligand–ligand interaction in $Cu(AA)_2$. For the cases where AA^- represents Val^-, Nva^- or Leu^- one obtains $10^{\Delta\Delta \log K^*} > 1$, *i.e.* equilibrium (12) is displaced towards its right side.

The results based on eqns. (8) and (10) are summarized in Table 2. The values calculated for K^*_I confirm the preceding conclusions regarding the intramolecular hydrophobic interaction in $Cu(Val)_2$, $Cu(Nva)_2$ and $Cu(Leu)_2$. In all these cases equilibrium (7a) is indeed operating. Knowledge of K^*_I allows of course also to calculate the percentage of the closed form in equilibrium (7a) by the use of eqn. (13):

$$\% Cu(AA)_{2/cl} = \frac{K^*_I}{1 + K^*_I} \times 100 \quad (13)$$

These percentages are listed in the right hand column of Table 2.

3. Some Comments on Hydrophobic Interactions in Binary Amino Acid Complexes with Aliphatic Side-Chains

To facilitate comparisons between $Cu(Val)_{2/cl}$, $Cu(Nva)_{2/cl}$ and $Cu(Leu)_{2/cl}$ according to equilibrium (7a), the percentages of $Cu(AA)_{2/cl}$ are plotted in Fig. 2 in dependence on the mole fraction of dioxane. The resulting bell-shaped curves are part of a more general phenomenon. Corresponding observations have been made with M(Phen)(phenylalkanecarboxylates)$^+$ [17] and M(Phen)(isoalkanecarboxylates)$^+$ [15] (where $M^{2+} = Cu^{2+}$ or Zn^{2+} and Phen = 1,10-phenanthroline) under the influence of increasing amounts of ethanol or dioxane; clearly the phenylalkanecarboxylates allow the formation of intramolecular stacks and hence the percentages of the closed forms are here somewhat larger than with the isoalkanecarboxylates allowing only a 'simple' hydrophobic interaction, but the principle observations are the same. It may be added that in Cu(*L*-phenylalaninate)$_2$ and Cu(*L*-tryptophanate)$_2$ the intramolecular ligand–ligand interaction is also more pronounced than in the three mentioned $Cu(AA)_2$ complexes, though the solvent influence is also somewhat different [18].

The bell-shaped curves of Fig. 2 can only mean that opposing solvent effects govern the formation degree of $Cu(AA)_{2/cl}$. These possible solvent effects have been discussed [5] in detail, especially in refs. 15 and 17. The main point for the present is that the addition of some organic solvent to an aqueous solution favours the formation of the complexes with a hydrophobic ligand–ligand interaction; this is contrary to the experience with simple unbridged hydrophobic adducts which are destabilized [17, 19]. Such a destabilization of the closed species among the $Cu(AA)_2$ complexes occurs only at high concentrations of organic solvent, *i.e.* in >70% (*v*/*v*) dioxane–water (Fig. 2). It should be noted that the overall stability of the $Cu(AA)^+$ and $Cu(AA)_2$ com-

TABLE 2. Extent of the Intramolecular Ligand–Ligand Interaction in the Binary $Cu(AA)_2$ Complexes of *L*-Valinate, *L*-Norvalinate and *L*-Leucinate in Dependence on the Amount of Dioxane Added to Water: Intramolecular and Dimensionless Equilibrium Constant K_I^* (eqns. (7), (8), (10)) and Percentage (eqn. (13)) of the Closed Species $Cu(AA)_{2/cl}$ (eqn. (7)) in Different Solvents at I = 0.1 M ($NaNO_3$) and 25 °C[a]

% (*v*/*v*) Dioxane	$\Delta \log K^*_{AA}$	$\Delta \log K^*_{AA/op}$	$\Delta\Delta \log K^*$	K_I^*	% $Cu(AA)_{2/cl}$
L-Valinate					
0	−1.31 ± 0.03	−1.38 ± 0.03	0.07 ± 0.04	0.17 ± 0.11	15 ± 8
30	−1.36 ± 0.03	−1.46 ± 0.04	0.10 ± 0.05	0.26 ± 0.14	21 ± 9
50	−1.37 ± 0.06	−1.51 ± 0.04	0.14 ± 0.07	0.38 ± 0.23	28 ± 12
70	−1.35 ± 0.05	−1.51 ± 0.03	0.16 ± 0.06	0.45 ± 0.19	31 ± 9
80	−1.46 ± 0.04	−1.61 ± 0.03	0.15 ± 0.05	0.41 ± 0.16	29 ± 8
L-Norvalinate					
0	−1.34 ± 0.01	−1.38 ± 0.03	0.04 ± 0.03	0.10 ± 0.08	9 ± 7
30	−1.42 ± 0.03	−1.46 ± 0.04	0.04 ± 0.05	0.10 ± 0.13	9 ± 10
50	−1.43 ± 0.03	−1.51 ± 0.04	0.08 ± 0.05	0.20 ± 0.14	17 ± 10
70	−1.41 ± 0.03	−1.51 ± 0.03	0.10 ± 0.04	0.26 ± 0.12	21 ± 8
80	−1.56 ± 0.04	−1.61 ± 0.03	0.05 ± 0.05	0.12 ± 0.13	11 ± 10
L-Leucinate					
0	−1.33 ± 0.02	−1.38 ± 0.03	0.05 ± 0.04	0.12 ± 0.09	11 ± 7
30	−1.40 ± 0.03	−1.46 ± 0.04	0.06 ± 0.05	0.15 ± 0.13	13 ± 10
50	−1.40 ± 0.04	−1.51 ± 0.04	0.11 ± 0.06	0.29 ± 0.17	22 ± 10
70	−1.37 ± 0.03	−1.51 ± 0.03	0.14 ± 0.04	0.38 ± 0.13	28 ± 7
80	−1.56 ± 0.04	−1.61 ± 0.03	0.05 ± 0.05	0.12 ± 0.13	11 ± 10

[a]The values for $\Delta \log K^*_{AA}$ (eqn. (6)) and their error ranges (three times the standard error) are from Table 1; $\Delta \log K^*_{AA/op} = \Delta \log K^*_{Ala}$ (eqn. (11)). The error limits for $\Delta\Delta \log K^*$ (eqn. (9)), K_I^* (eqn. (10)) and % $Cu(AA)_{2/cl}$ (eqn. (13)) were calculated according to the error propagation after Gauss.

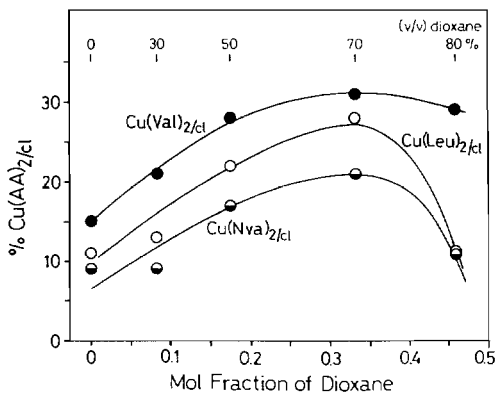

Fig. 2. Formation degree of the intramolecularly closed species (eqn. (7a)) for the binary $Cu(Val)_2$ (●), $Cu(Leu)_2$ (○) and $Cu(Nva)_2$ (◒) complexes in dependence on the mole fractions of dioxane in water. Note, % $Cu(AA)_{2/cl}$ is based on $[Cu(AA)_2]_{tot}$ = 100%. The plotted data for $Cu(AA)_{2/cl}$ are taken from Table 2.

plexes is governed by the polarity of the solvent (Table 1), *i.e.* their stability increases with decreasing solvent polarity, whereas the position of the intramolecular equilibrium (7a) is differently influenced; for the latter the hydrophobic solvation properties of the solvent molecules appear to be important.

4. Some Structural Considerations on $Cu(AA)_2$ Complexes with Intramolecular Hydrophobic Interactions

In agreement with recent discussions [15, 17] one has to assume that the closed form of $Cu(AA)_2$, *i.e.* the species with the hydrophobic interaction between the alkyl residues, is actually a whole series of species in the dioxane–water mixtures, and that these species differ in their degree of hydrophobic solvation by the ethylene units of dioxane. Figure 2 indicates that the aliphatic adduct in $Cu(Val)_2$ is especially suitable for such a hydrophobic solvation; this could further indicate that the size and proximity of the aliphatic 'micelle' neighboring the metal ion could reduce the *effective* dielectric constant at the metal ion and consequently coulombic Cu^{2+}/ligand interactions (like Cu^{2+}/O^-) would be stabilized. This would be a powerful cooperative mechanism [15, 17].

It should be pointed out that the two isopropyl residues in $Cu(Val)_2$ can get in contact with each other only in a *trans* arrangement of the two glycinate-like units to the equatorial part of the Cu^{2+} coordination sphere. Only in a *trans* arrangement are the side-chains of two amino acids with the same chirality on the same side of the complex [20]. In addition, space-filling molecular models indicate that contact between the two isopropyl residues is only

possible if there is no apical water molecule coordinated to Cu^{2+} on the same side of the complex. Hence, a penta-coordinated (and well known [21, 22]) Cu^{2+} sphere is suggested: the 2N/2O donor-atom set being equatorially coordinated in a *trans* fashion with a more distant water molecule in an apical position (opposite to the side-chains) completing the square-pyramid. Of course, in $Cu(AA)_{2/op}$ a *cis* coordination of the glycinate-like unit is possible; for amino acids of the same chirality the side-chains will then be on opposite sides of the complex.

It is interesting to note in this connection that Al-Ani and Olin [23] have studied in aqueous solution (0.25 M NaCl; 25 °C) the position of equilibrium (14)

$$Cu(L\text{-Val})_2 + Cu(D\text{-Val})_2 \rightleftharpoons 2Cu(L\text{-Val})(D\text{-Val}) \quad (14)$$

They claim that "a small but significant difference could be established between the extent of complexation with optically active ligand and racemate", the mixed complex being slightly disfavored. Assuming that *trans* coordination of the glycinate-like unit is dominating (which is usually the case [21, 24]) then a hydrophobic ligand–ligand interaction is possible in $Cu(L\text{-Val})_2$ and $Cu(D\text{-Val})_2$ but not in $Cu(L\text{-Val})(D\text{-Val})$; consequently the *meso* complex should be disfavored, what indeed is observed [23]. This is further in agreement with results of Davankov *et al.* [25], who studied Cu^{2+} complexes of N-substituted amino acids and noted also a destabilization of the *meso* complex relative to the complexes containing amino acids of the same chirality. It may be noted that in the *meso* complex a side-chain interaction becomes possible in the *cis* isomer; as the *cis/trans* isomerization barrier in solution is certainly low [ref. 4, footnote 65], as solution equilibration rates are rapid for Cu^{2+}, and because the energy differences between the *cis/trans* isomers in solution may be assumed as small, no large difference from the statistical case regarding equilibrium (14) can be expected, what again agrees with the experimental result [23].

General Conclusions

The results summarized in Table 2 and Fig. 2 show that intramolecular hydrophobic side-chain interactions in $Cu(AA)_2$ complexes are small. Therefore, it should be emphasized that the effect of such interactions on the structures of the complexes existing in solution is still significant. To demonstrate this, Fig. 3 has been composed with the Cu^{2+}/Leu system as an example:

(i) It is evident that the formation degree of $Cu(Leu)_{2/cl}$ is significant in all solvent mixtures at pH 7.

(ii) It is remarkable that the formation degree of $Cu(Leu)_{2/op}$ over a wide dioxane–water range alters somewhat less than the formation degree of $Cu(Leu)_{2/cl}$.

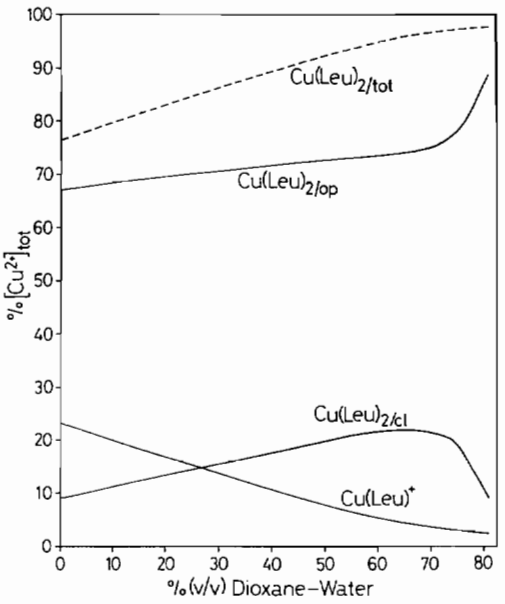

Fig. 3. Effect of the amount of dioxane added to an aqueous solution of Cu^{2+} and *L*-leucine at pH 7.00 on the concentration of the species present (full lines). The broken line represents the total concentration of the $Cu(Leu)_2$ complex. The results are given as the percentage of the total Cu^{2+} concentration ($= 10^{-3}$ M; $[Leu]_{tot} = 2 \times 10^{-3}$ M) present; computed with the constants listed in Tables 1 and 2; $I = 0.1$ M ($NaNO_3$) and 25 °C. The concentration of the uncomplexed Cu^{2+} is in water <0.33% and in 80% dioxane <0.0015%; hydroxo-complex formation of Cu^{2+} is under these conditions insignificant.

(iii) The stabilization of the metal-ion linked hydrophobic adduct, *i.e.* of $Cu(Leu)_{2/cl}$, by the addition of some dioxane is nicely seen; as indicated before, this contrasts with the observations made for unbridged adducts.

To conclude, the properties described here for amino acid complexes of Cu^{2+} hold certainly also for the corresponding complexes with other metal ions*. No doubt, the characterized interactions are weak and may therefore be easily overlooked; however, for the creation of certain three-dimensional structures in biological systems they are ideal, because the energy differences between isomeric forms are small, allowing for example an easy transformation of a substrate from an inactive into an active form.

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*For examples of other $M(AA)_{2/cl}$ complexes see Table VI in ref. 5.

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