more readily than the aquo dimer and the increased binding resulting from this behavior would more than offset the higher charge of the latter. The argument proposed here could be tested by observing whether the distribution coefficient of the aquo dimer increases with time, but no such experiments have been reported.

Conclusions

In the present work it was shown that *p*-dioxane is a suitable "inert probe" for the study of the second coordination sphere of complex ions. It was used to study the behavior of the octaaquobis(μ-hydroxo)dichromium(III) perchlorate acid and perchlorate salt solutions. The peak broadening of the dioxane is less for the dimer compared to that for the hexaaquochromium(III) ion under all conditions studied, and this was attributed to exclusion of the dioxane from some of the dimer faces in the second coordination sphere. The difference between the calculated and expected broadening for the dimer due to the exclusion is probably due to the longer correlation time for rotation of the dimer compared to that for the monomer. The magnetic susceptibility of the dimer gives a spin-only value for the chromium ions, indicating no interaction across the bis(μ-hydroxo) birdges. It is postulated that, at high acid concentrations, the color change of the dimer is due to an inner-sphere perchlorato complex.

Registry No. Cr, 7440-47-3; *p*-dioxane, 123-91-1; $Cr_2(H_2O)_8(OH)_2^{4+}$, 23852-05-3.

Contribution from the Institute of Inorganic Chemistry, University of Basel, CH-4056 Basel, Switzerland

Metal Ion Promoted Hydrophobic Interactions between Nucleotides and Amino Acids. Mixed-Ligand Adenosine 5'-Triphosphate/Metal Ion(II)/L-Leucinate Systems and Related Ternary Complexes[1,2]

HELMUT SIGEL,* BEDA E. FISCHER, and ETELKA FARKAS[3]

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Mixed-ligand complexes of the type $M(ATP)(Aa)^{3-}$, where $M^{2+} = Mn^{2+}$, Cu^{2+}, Zn^{2+}, Cd^{2+}, or Pb^{2+} and $Aa^- =$ alaninate, 2-aminopropionate (α-aminobutyrate), norvalinate, norleucinate, leucinate (leu), or isoleucinate, have been studied by potentiometric pH titrations and ^1H NMR; some earlier results on $M(ATP)(tryptophanate)^{3-}$ have been included for comparison. The potentiometric measurements (with Mn^{2+}, Cu^{2+}, and Zn^{2+}) reveal a slightly higher formation tendency, expressed as $\Delta \log K_M = \log K^{M(ATP)}_{M(ATP)(Aa)} - \log K^{M}_{M(Aa)}$, for the systems with leucinate compared to those with alaninate. This increase in stability is attributed to an intramolecular hydrophobic ligand–ligand interaction between the purine moiety of ATP^{4-} and the isopropyl residue of leucinate. The position of the intramolecular isomeric equilibrium between an "open" and "closed" form, in which the hydrophobic interaction occurs, was estimated: of the ternary $M(ATP)(leu)^{3-}$ complexes with Mn^{2+}, Cu^{2+}, and Zn^{2+} about 41, 21, and ~5% (to ~30%) exist in the folded, i.e. closed, form. The intramolecular aromatic-ring stacking interactions between the purine moiety of ATP^{4-} and the indole residue of tryptophanate (trp^-) in the $M(ATP)(trp)^{3-}$ complexes is (as expected) more pronounced: of the complexes with Mn^{2+}, Cu^{2+}, and Zn^{2+} about 52, 35, and 74% exist in the stacked form. ^1H NMR shift measurements of the mentioned ATP/amino acid systems in the absence and presence of Zn^{2+}, Cd^{2+}, or Pb^{2+} confirmed that such hydrophobic ligand–ligand interactions exist and that they are effectively promoted by the formation of a metal ion bridge between the two reactants; to some extent a promotion can also occur by the polar interactions between the ammonium group of the amino acid and the phosphate chain of the nucleotide. For the metal ion facilitated interaction: the longer the side chain of the aliphatic amino acid, the larger is the upfield shift of the terminal methyl group(s) of the amino acid side chain, resulting from the interaction with the aromatic purine moiety within the ternary complex. The $\Delta G°$ values calculated from the equilibrium constants agree well with the theoretical predictions for such interactions. It is also shown that mixed-ligand complexes of the mentioned kind exist in the physiological pH range and that the formation of a metal ion bridge increases the probability for "recognition" between two species; this result is fascinating regarding the specificity and selectivity observed in nature.

The essential relationships between nucleic acids and amino acids in biological systems are their binding and recognition interactions.[4] The aim of various studies was therefore to characterize amino acid– or protein–DNA interactions and to clarify the binding of nucleotides, which occur as coenzymes in enzymic reactions, to the protein part of the enzymes. Among the possible kinds of interactions are (i) hydrogen bonding between the amino acids and the nucleic acid bases,[5] (ii) polar interactions[6,7] between positively charged ammonium groups of amino acids and negatively charged phosphate groups of nucleotides, (iii) aromatic-ring stacking between the purine or pyrimidine bases and suitable aromatic side-chain residues of the amino acids, like the indole[7-11] or imidazole groups,[12] and (iv) the related hydrophobic interactions.[13-15]

It was also shown that two such interactions may occur simultaneously,[7,10] leading thus to cooperativity. Furthermore, the polar interactions, i.e. the ionic bridge, may be replaced by a metal ion,[7,9,11] leading thus to the formation of ternary

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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:		
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.

complexes.[16,17] In these studies it was shown that the formation of such a metal ion bridge promotes the aromatic-ring stacking and that therefore distinct structures are favored in solution, in connection with a slight increase of the overall stability of the ternary complexes. It became thus obvious that working with monomeric species in aqueous solution permits the effects of individual factors to be assessed and especially to show that selectivity is already observed on this level; clearly, this will help to understand the more complex interactions between larger molecules.

After studying the intramolecular aromatic-ring stacking interaction in mixed-ligand complexes,[9,11,18] it was logical to see whether a hydrophobic interaction between an aromatic-ring system and, e.g., an isopropyl group might also occur in suitable ternary complexes.[19] This is indeed the case: there is a hydrophobic adduct formation between the aromatic rings of 2,2′-bipyridyl or 1,10-phenanthroline and the side chain of aliphatic amino acids, like leucine, which may be enhanced in ternary systems also containing a metal ion.[20,21] This result has led us now to study systems with ligands occurring in nature: we used ATP[22] and the various amino acids shown in Figure 1.

As glycinate complexes are known to be sometimes exceptional in their stability,[23] L-alaninate was selected as the basis for comparisons because in stability studies it behaves normally[23] and it also shows only a very limited tendency for hydrophobic interactions.[15] Therefore the data measured for systems containing this amino acid were compared with the data obtained for the other ternary systems formed with the amino acids having a longer aliphatic side chain.

The ternary systems containing ATP^{4-}, alaninate, or tryptophanate and the metal ion Mn^{2+}, Cu^{2+}, or Zn^{2+} had already been studied earlier.[9] For reasons of comparison we now studied the corresponding systems with leucinate. Indeed, the $M(ATP)(leu)^{3-}$ complexes are somewhat more stable than the $M(ATP)(ala)^{3-}$ complexes, indicating an intramolecular hydrophobic interaction in the former species, but these are slightly less stable than the corresponding trp^- complexes for which a stacking interaction between the purine system of ATP^{4-} and the indole moiety of trp^- has been shown to occur.

To confirm the intramolecular hydrophobic interactions in the ternary $M(ATP)(leu)^{3-}$ complexes by an independent method, we carried out ^1H NMR shift measurements with the diamagnetic metal ion Zn^{2+}, Cd^{2+}, or Pb^{2+}, ATP^{4-}, and the aliphatic amino acids shown in Figure 1. It became obvious that the hydrophobic ligand–ligand interaction in such ternary complexes depends on the kind and size of the side chain.

Experimental Section

Materials. $Mn(ClO_4)_2$ (purum) was obtained from Fluka AG, Buchs, Switzerland. $Mg(NO_3)_2$, $Cd(NO_3)_2$, $Pb(NO_3)_2$, and $La(NO_3)_3$ (all of p.A. grade) were from Merck AG, Darmstadt, FRG. The disodium salt of adenosine 5′-triphosphate was purchased from Serva Feinbiochemica GmbH, Heidelberg, FRG, and from Boehringer GmbH, Mannheim, FRG. All the other materials were the same as used recently.[20]

Determination of Equilibrium Constants by Potentiometric Titrations. The potentiometric pH titrations were carried out with a Metrohm E 536 potentiograph and a Metrohm EA 121 glass electrode (see also ref 2).

The stability constant $K^{Mn}_{Mn(leu)}$ was determined in the same way as described[9] for $K^{Mn}_{Mn(trp)}$, but the ratios of Mn^{2+}:leu were 10:1, 15:1, 20:1, and 25:1, where $[H(leu)] = 1.2 \times 10^{-3}$ M. The conditions used for the titrations of the mixed-ligand systems with leucine were exactly as described[9] for the corresponding systems with tryptophan. Under these conditions the metal ion facilitated self-association of ATP^{4-} is small and about 90% or more of $M(ATP)^{2-}$ is present in the desired monomeric form;[24] hereby, it is assumed that the self-stacking tendency of ATP^{4-} is promoted by Mn^{2+} similarly as by Mg^{2+}.

The evaluation of the titration data was performed by following procedure II of ref 9: in this procedure the assumption of complete formation of $M(ATP)^{2-}$ in a 1:1 M^{2+}:ATP^{4-} mixture is made; it has been proven[9] that this is a good approximation and is preferable to a procedure where all species are considered.[2,9] This procedure allows the calculation of the equilibrium constant $K^{M(ATP)}_{M(ATP)(leu)}$ considering the species H^+, $H_2(leu)^+$, H(leu), leu^-, $M(ATP)^{2-}$, and $M(ATP)(leu)^{3-}$.

^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-90 FT spectrometer (90.025 MHz) at 27 °C in D_2O solutions, using the center peak of the tetramethylammonium triplet as reference;[25] all chemical shifts were converted to a trimethylsilyl propanesulfonate reference by adding 3.188 ppm.[18] The pD values were obtained by adding 0.4 to the pH meter reading.[26]

In the ^1H NMR measurements we followed the change in the chemical shift of the terminal methyl group of the amino acid side chain ([Aa] = 0.02 M) as a function of the second reagent.[20] With all amino acids shown in Figure 1, measurements could be carried out with Cd^{2+} (0.225 M, pH 7.0, I = 0.7), $Cd(ATP)^{2-}$ ($[Cd^{2+}]$ = [ATP] = 0.225 M, pH 7.0, I = 1.13), $Pb(ATP)^{2-}$ ($[Pb^{2+}]$ = [ATP] = 0.16 M, pH 7.5, I = 0.8),[27] and $Zn(ATP)^{2-}$ ($[Zn^{2+}]$ = [ATP] = 0.225 M, pH 7.0, I = 1.13), and the influence of these reagents on the position of the resonance signal of the terminal methyl group of the amino acid could be determined. Always, the center positions of the multiplets corresponding to these methyl groups were evaluated.

The position of the resonance signals of the amino acid anions and the effects of protonation and Zn^{2+} coordination have been measured

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Table I. Logarithms of the Stability Constants of Binary $M(Aa)^+$ and Ternary $M(ATP)(Aa)^{3-}$ Complexes As Determined by Potentiometric pH Titrations (25 °C; $I = 0.1$, $NaClO_4$)[a]

amino acid anion (Aa^-)	M^{2+}	$\log K^{M}_{M(Aa)}$	$\log K^{M(ATP)}_{M(ATP)(Aa)}$[b]	$\Delta \log K_M$
L-alaninate[c]	Mn^{2+}	2.67 ± 0.05	1.36 ± 0.09	−1.31
	Cu^{2+}	8.25 ± 0.03	6.75 ± 0.05	−1.50[d]
	Zn^{2+}	4.51 ± 0.03	3.71 ± 0.03	−0.80
L-leucinate[e]	Mn^{2+}	2.49 ± 0.03	1.41 ± 0.20	−1.08
	Cu^{2+}	8.32 ± 0.01[f]	6.92 ± 0.05	−1.40
	Zn^{2+}	4.56 ± 0.02[f]	3.78 ± 0.05	−0.78
DL-tryptophanate[g]	Mn^{2+}	2.50 ± 0.03	1.51 ± 0.02	−0.99
	Cu^{2+}	8.27 ± 0.05	6.96 ± 0.05	−1.31
	Zn^{2+}	4.69 ± 0.02	4.48 ± 0.04	−0.21

[a] The errors given are 3 times the standard error of the mean value or the sum of the probable systematic errors, whichever is larger. [b] The stabilities of the binary $M(ATP)^{2-}$ complexes are $\log K^{M}_{M(ATP)} = 4.81$ for Mn^{2+}, 6.21 for Cu^{2+}, and 5.16 for Zn^{2+} (average of the results given in ref 36). [c] From ref 9 and 31 and: Griesser, R.; Prijs, B.; Sigel, H.; Föry, W.; Wright, L. D.; McCormick, D. B. *Biochemistry* 1970, *9*, 3285–93. [d] Jatsimirskij et al.[37] determined for the Cu^{2+}/ATP^{4-}/glycinate system $\Delta \log K_{Cu} = -1.71$. [e] $pK^{H}_{H_2(leu)} = 2.32 \pm 0.01$, $pK^{H}_{H(leu)} = 9.66 \pm 0.01$. [f] From ref 20. [g] From ref 9.

by us before in a different connection; these results[20] were also used now. The binary Pb^{2+}/Aa systems could not be studied because at pH values above 4.5 a precipitate forms in 0.225 M Pb^{2+} solutions. Attempts to include the ternary La^{3+}/- and $Mg^{2+}/ATP/Aa$ systems also failed, because in the La^{3+} systems a precipitate is formed in the pH range 3–12.5 (cf. also ref 28) and with Mg^{2+} the coordination tendency toward Aa is too low at pH 6.0 and hydrolysis and precipitation occur at pH > 10.

The chemical shifts, $\delta_{Cd(Aa)}$, were calculated with eq 1 of ref 20 and the concentrations of $Cd(Aa)^+$. These latter concentrations were calculated with the acidity constants of the amino acids (see ref 20) and $\log K^{Cd}_{Cd(Aa)} = 4.0$, which was used for all the amino acid systems. This constant is the average of the values determined for several $Cd(Aa)^+$ complexes;[29,30] as the formation degree of $Cd(Aa)^+$ is high under the given experimental conditions, the calculations are not very sensitive to the value of $\log K^{Cd}_{Cd(Aa)}$. The results are listed in Table III.

The ternary Cd^{2+}/- and $Pb^{2+}/ATP/Aa$ systems were treated as binary ones; i.e., it was assumed that $Cd(ATP)^{2-}$ and $Pb(ATP)^{2-}$ form completely. This assumption is justified as the corresponding stability constants are rather large: $\log K^{Cd}_{Cd(ATP)} \simeq 4.9$ (ref 31) and $\log K^{Pb}_{Pb(ATP)} = 7.02$ (ref 32). The constants $K^{M(ATP)}_{M(ATP)(Aa)}$ used in these simplified calculations were determined from NMR experiments (vide infra and Table II): $\log K^{Cd(ATP)}_{Cd(ATP)(Aa)} = 3.51$ and $\log K^{Pb(ATP)}_{Pb(ATP)(Aa)} = 2.7$. The chemical shift of the methyl group of the amino acid in the $M(ATP)(Aa)^{3-}$ complexes, $\delta_{M(ATP)(Aa)}$, was calculated according to eq 1. For reasons of uniformity the ternary

$$\delta_{M(ATP)(Aa)} = \frac{\delta_{obsd}[Aa]_{tot} - (\delta_{H(Aa)}[H(Aa)] + \delta_{Aa}[Aa^-])}{[M(ATP)(Aa)^{3-}]} \quad (1)$$

Zn^{2+} systems (constants from Table I) were treated in the same way; the results are listed in Table III.

To be sure that the above assumptions (eq 1) are justified, we have in addition evaluated all the ternary $Zn^{2+}/ATP/Aa$ systems according to eq 2. This extended evaluation was possible with Zn^{2+} because

$$\delta_{Zn(ATP)(Aa)} = \frac{\delta_{obsd}[Aa]_{tot} - (\delta_{H(Aa)}[H(Aa)] + \delta_{Aa}[Aa^-] + \delta_{Zn(Aa)}[Zn(Aa)^+])}{[Zn(ATP)(Aa)^{3-}]} \quad (2)$$

from the potentiometric pH titrations of the L-alanine and L-leucine systems all needed equilibrium constants for the corresponding binary and ternary systems (Table I) are known with the necessary accuracy; for the other amino acids the average constants of the ala and leu systems could be used (see ref 20). Indeed, the agreement between the results obtained with eq 1 and 2 is excellent: the values of $\delta_{Zn(ATP)(Aa)}$ differ only slightly at the third position after the decimal point.

Another series of measurements were carried out with constant concentrations of L-leucine (0.01 or 0.02 M) and increasing concentrations of ATP^{4-}, $Zn(ATP)^{2-}$, $Cd(ATP)^{2-}$, or $Pb(ATP)^{2-}$. The experimental details are given in the caption of Figure 2. From these experiments the equilibrium constants for the association of the mentioned species with leu can be computed by the curve-fitting procedure used earlier.[20,33] The evaluation of the ternary systems was done with eq 4 of ref 20; the results are listed in Table II. However, it must be pointed out that the stability constants determined in this way can only be taken as estimations because under the high concentrations necessary for such experiments considerable self-association of ATP^{4-} or $M(ATP)^{2-}$ occurs.[24]

Results and Discussion

1. Potentiometric Determination of the Stability of Ternary Complexes Consisting of L-Leucinate, ATP^{4-}, and Mn^{2+}, Cu^{2+}, or Zn^{2+}. Our first goal was to see whether a possible intramolecular ligand–ligand interaction in the ternary $M(ATP)(leu)^{3-}$ complexes is reflected in their stability. Therefore we have measured the formation constants of eq 3 and 4. These constants may now be compared according to

$$M^{2+} + Aa^- \rightleftharpoons M(Aa)^+ \qquad K^{M}_{M(Aa)} = [M(Aa)]/([M][Aa]) \quad (3)$$

$$M(ATP)^{2-} + Aa^- \rightleftharpoons M(ATP)(Aa)^{3-} \qquad K^{M(ATP)}_{M(ATP)(Aa)} = [M(ATP)(Aa)]/([M(ATP)][Aa]) \quad (4)$$

eq 5. The value of $\Delta \log K_M$ characterizes the coordination

$$\Delta \log K_M = \log K^{M(ATP)}_{M(ATP)(Aa)} - \log K^{M}_{M(Aa)} = \log K^{M(Aa)}_{M(Aa)(ATP)} - \log K^{M}_{M(ATP)} \quad (5)$$

tendency of the amino acid anion toward $M(ATP)^{2-}$ (eq 4) relative to that toward $M(aq)^{2+}$ (eq 3), and factors that arise through direct[9,18,20] or indirect (i.e. metal ion mediated)[16,17] ligand–ligand interactions in ternary complexes should show up in this description.

It must be added that the value of $10^{\Delta \log K_M}$ is the equilibrium constant of eq 6 and that the values expected on statistical

$$M(ATP)^{2-} + M(Aa)^+ \rightleftharpoons M(ATP)(Aa)^{3-} + M^{2+} \quad (6)$$

grounds[16] for $\Delta \log K_M$ are *negative*.[34] For example, for two

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(34) A recent claim[35] about "high positive $\Delta \log K$ values" only arose because in this work[35] $\Delta \log K$ was "reversely" defined, i.e. for the equilibrium $M(ATP)(Aa) + M \rightleftharpoons M(ATP) + M(Aa)$. Hence, in the above definition according to eq 5 the values of ref 35 would be strongly *negative*, i.e. between −2.06 and −3.91; as a consequence there is so far *no* indication for a "*high ability* of Mg(II), Ca(II), and Mn(II) to form mixed-ligand complexes with ATP^{4-} and histidinate". That these $\Delta \log K_M$ values are more negative than those of Table I is to be expected because histidinate is a tridentate ligand while the amino acid anions of Table I coordinate only in a bidentate fashion.

different bidentate ligands $\Delta \log K_M$ is between −0.4 for an octahedral coordination sphere and about −0.9 for the distorted coordination sphere of Cu^{2+}; hence, smaller values are expected for the ternary Cu^{2+} systems than for those with Mn^{2+} or Zn^{2+}.

The results of the $M(ATP)^{2-}$/leu system are listed in Table I together with related data[36,37] and the constants of the $M(ATP)^{2-}$/ala$^-$ and -/trp$^-$ systems, which have been determined earlier.[9] A comparison of the $\Delta \log K_M$ values of the ternary leucinate and alaninate complexes reveals that the $M(ATP)(leu)^{3-}$ complexes of Mn^{2+}, Cu^{2+}, and Zn^{2+} are, on the average, about 0.1 log unit more stable. This increase in stability is rather small, but still it is a first hint that an intramolecular hydrophobic interaction exists in these ternary complexes between the isopropyl moiety of leucinate and the purine system of ATP^{4-}. As aromatic-ring stacking interactions are in general more pronounced,[15] it is not surprising that the analogous comparison for the $M(ATP)(trp)^{3-}$ complexes reveals a stability increase between 0.2 and 0.6 log units. Indeed, since this intramolecular stack was first described,[9,38] it has now been repeatedly confirmed,[11,39,40] and X-ray structure studies[41] of related ternary complexes also show the presence of an intramolecular stack.

As indicated, the increase in stability observed for the $M(ATP)(leu)^{3-}$ complexes is very small, in fact it is just at the limit of significance. Therefore it seemed desirable to confirm the postulated ligand–ligand interaction in these ternary nucleotide–amino acid complexes by another independent method; this was done by ^1H NMR shift measurements.

2. ^1H NMR Measurements of Binary Adducts between ATP^{4-} and L-Leucine. 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.[42] Our first intention was therefore to see whether any indication for an interaction between the isopropyl residue of L-leucine and the purine moiety of ATP^{4-} could be obtained in the absence of metal ions. Indeed, from the left part of Figure 2[43-45] it is obvious that increasing concentrations of ATP^{4-} shift the resonance of the methyl groups of leucine increasingly upfield. Certainly, the observed upfield shifts are small and the resulting line through the measured points shows no curvature, but for such hydrophobic adducts a low stability must be expected.[15,20]

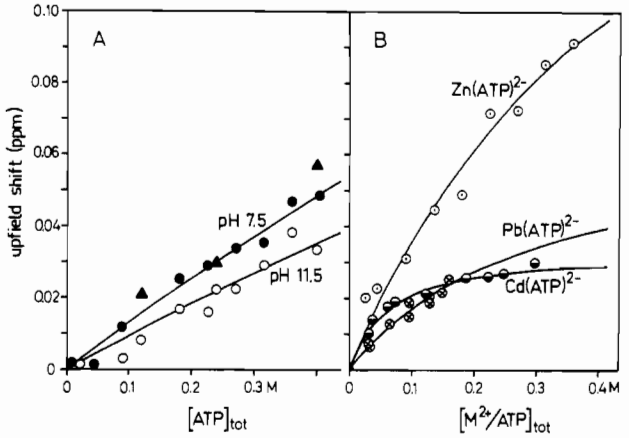

Figure 2. (A) Upfield shift of the resonance of the methyl groups of L-leucine (the center of the doublet was evaluated) in dependence on $[ATP^{4-}]$ at pH 7.5 (●; here still about 10% of the total ATP are present as $H(ATP)^{3-}$) or 11.5 (○). The lines drawn are the computer-calculated best fits (eq 3 of ref 20) assuming an upfield shift of $\Delta\delta = 0.5$.[43] The individual conditions are as follows. For pH 7.5 (=pD 7.9): 90.025 MHz, T = 27 °C, D_2O, I = 0.1–2.4 (KNO_3), $[leu]_{tot}$ = 0.01 M, $\delta_0 = 0.964$, $\delta_\infty = 0.464$, $K = 0.27 \pm 0.02$ M^{-1} [▲: for comparison in H_2O at 60 MHz (34 °C) under the same conditions but with $[leu]_{tot}$ = 0.02 M]. For pH 11.5: 60 MHz, T = 34 °C, H_2O, I = 0.1–2.2 (KNO_3), $[leu]_{tot}$ = 0.02 M, $\delta_0 = 0.926$, $\delta_\infty = 0.426$, $K = 0.19 \pm 0.04$ M^{-1}. (B) Upfield shift of the methyl resonance signal of leu in dependence on $[M^{2+}/ATP^{4-}]_{tot}$,[44] where $M^{2+} = Zn^{2+}$ (⊙), Cd^{2+} (⊖), or Pb^{2+} (⊗), at 60 MHz and T = 34 °C in H_2O. The curves shown are the computer-calculated best fits (eq 4 of ref 20) of the experimental data with the parameters given below for the complex formation between leu$^-$ and $M(ATP)^{2-}$. The individual conditions are as follows. For $Zn(ATP)^{2-}$: pH 7.0, $[leu]_{tot}$ = 0.02 M, I = 0.1–1.8 (KNO_3), $\delta_0 = 0.978$, $\delta_\infty = 0.764 \pm 0.055$, $\Delta\delta = 0.214$, $K_{app} = 2.06 \pm 0.81$ M^{-1} (see ref 45), $\log K^{Zn(ATP)}_{Zn(ATP)(leu)} = (0.31 + 2.66) \pm 0.34 = 2.97 \pm 0.34$. For $Pb(ATP)^{2-}$: pH 7.5, $[leu]_{tot}$ = 0.02 M, I = 0.1–0.8 (KNO_3), $\delta_0 = 0.974$, $\delta_\infty = 0.906 \pm 0.025$, $\Delta\delta = 0.068$, $K_{app} = 3.59 \pm 1.93$ M^{-1},[45] $\log K^{Pb(ATP)}_{Pb(ATP)(leu)} = (0.56 + 2.16) \pm 0.23 = 2.72 \pm 0.23$. For $Cd(ATP)^{2-}$: pH 7.5, $[leu]_{tot}$ = 0.02 M, I = 0.1–1.5 (KNO_3), $\delta_0 = 0.974$, $\delta_\infty = 0.941 \pm 0.007$, $\Delta\delta = 0.033$, $K_{app} = 19.98 \pm 3.31$ M^{-1},[45] $\log K^{Cd(ATP)}_{Cd(ATP)(leu)} = (1.30 + 2.16) \pm 0.07 = 3.46 \pm 0.07$.

As an interaction between leucine and ATP^{4-} can in principle be hydrophobic and/or polar, experiments at two different pH values were carried out: at pH 7.5 the amino acid is present as the zwitterion H(leu) (i.e., the amino group is protonated and therefore positively charged), while at pH 11.5 the amino acid exists in its anionic form leu$^-$. In the monoprotonated form H(leu) the positively charged ammonium group may interact with the negatively charged triphosphate chain of ATP^{4-}, thus forming an ion pair. In addition to this, a hydrophobic interaction between the isopropyl group of leu and the purine ring of ATP^{4-} is also possible. This hydrophobic interaction is still possible at pH 11.5, but an ion pair can no longer be formed because $pK^H_{H(leu)} = 9.66$; i.e., the amino acid is now present as anion.

The observation of an upfield shift in both experiments (Figure 2A) verifies the occurrence of a hydrophobic interaction at both pH values. Furthermore, the larger upfield shift at the lower pH at constant concentrations of ATP^{4-} indicates that at pH 7.5 the hydrophobic interaction is stabilized by a polar interaction. Similar observations have been made before with AMP^{2-} or ATP^{4-} and aromatic amino acids[7] or catecholamines,[46] where, besides the polar interaction, ring stacking occurs.

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(42) Jackman, L. M.; Sternhell, S. "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", 2nd ed.; Pergamon Press: Oxford, 1969; p 94.
(43) This value is the average of the upfield shifts usually observed in related systems; see section 2.
(44) The stability constant $\log K^M_{M(ATP)}$ of these complexes is rather large [Zn^{2+}, 5.16 (footnote *b* of Table I); Cd^{2+}, ~4.9;[31] Pb^{2+}, 7.02[32]], and therefore complex formation is rather complete in 1:1 mixtures of ATP^{4-} and M^{2+} under the given conditions.
(45) (a) The constant K_{app} is an *apparent* constant valid only at the pH of its determination. So that the pH-independent stability constant can be obtained, the competition by the proton must be considered; this is done with the relation[45b,c] $\log K^{M(ATP)}_{M(ATP)(Aa)} = \log K_{app} + \log (1 + [H^+]/K^H_{H(Aa)})$. (b) Sigel, H.; McCormick, D. B. *Acc. Chem. Res.* **1970**, *3*, 201–8. (c) Farkas, E.; Fischer, B. E.; Griesser, R.; Rheinberger, V. M.; Sigel, H. *Z. Naturforsch., B: Anorg. Chem., Org. Chem.* **1979**, *34B*, 208–16.
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An exact evaluation of these binary systems, i.e. the calculation of stability constants for the $(ATP)(H(leu))^{4-}$ and $(ATP)(leu)^{5-}$ adducts, is difficult to achieve for several reasons: (i) ATP^{4-} shows self-stacking,[24,47] and (ii) the graphs of Figure 2A show no curvature. This means that no limiting shift and thus no stability constant can be calculated directly. However, by use of the following procedure an estimate is possible:

From earlier studies[7,11,25,48] 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-ring aromatic systems is not smaller than 0.2 ppm and not larger than 0.8 ppm. Using these limits, one obtains the formation constants $K^{(ATP)}_{(ATP)(leu)} = 0.48$ and $0.12\ M^{-1}$ and $K^{(ATP)}_{(ATP)(H(leu))} = 0.68$ and $0.17\ M^{-1}$ (see also caption of Figure 2A); hence, $K^{(ATP)}_{(ATP)(leu)} = 0.30 \pm 0.18\ M^{-1}$ and $K^{(ATP)}_{(ATP)(H(leu))} = 0.43 \pm 0.25\ M^{-1}$. By also taking into account the mentioned self-association[47] of ATP^{4-}, we find that the formation constants increase by a factor of about 1.3; therefore, $K^{(ATP)}_{(ATP)(leu)} = 0.4 \pm 0.2\ M^{-1}$ and $K^{(ATP)}_{(ATP)(H(leu))} = 0.6 \pm 0.3\ M^{-1}$. These values are of the same order as earlier estimations[20] for the hydrophobic interactions between H(leu) and 2,2′-bipyridyl or 1,10-phenanthroline: $K^{(bpy)}_{(bpy)(H(leu))} = 0.6 \pm 0.4\ M^{-1}$ and $K^{(phen)}_{(phen)(H(leu))} = 1.4 \pm 0.9\ M^{-1}$.

It should be added that the estimated formation constant $K^{(ATP)}_{(ATP)(H(leu))}$ is actually an *overall* formation constant, in contrast to $K^{(ATP)}_{(ATP)(leu)}$, because the adduct $(ATP)(H(leu))^{4-}$ may exist in three isomeric forms. There is an intramolecular and hence concentration-independent equilibrium among an isomer that is formed by purely hydrophobic interactions, a second species with only ionic interactions, and a third adduct in which both interactions are present.[49]

3. ^1H NMR Studies of Ternary Systems Containing ATP^{4-}, L-Leucinate, and Zn^{2+}, Cd^{2+}, or Pb^{2+}. From the results described in section 2 it is obvious that an ionic bridge can promote a hydrophobic interaction. One may now formally exchange the proton at the amino group against a bivalent metal ion and thus obtain a ternary complex; as the association tendency of a triphosphate chain toward the metal ion coordinated to an amino acid is expected to be larger than toward the ammonium group, the hydrophobic interaction between an amino acid and a nucleotide may possibly be further enhanced by the presence of a metal ion. Therefore the ternary $ATP^{4-}/leu^-/Zn^{2+}$, $-/Cd^{2+}$, and $-/Pb^{2+}$ systems have now been studied (Figure 2B).

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 shifts the resonances of the aliphatic protons upfield, if compared to the shift positions measured for the signals of the free amino acid system.[42] In contrast, protonation or coordination of a metal ion shifts the signals of protons close to the binding site in the ligand downfield.[20,50] NMR is therefore the ideal method to trace the coordination of diamagnetic metal ions, like Zn^{2+}, Cd^{2+}, or Pb^{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 the present experiments the concentration of leucine (0.02 M) and the pH were kept constant and the dependence of the chemical shift of the terminal methyl groups of leu on $[M(ATP)^{2-}]$ was measured[44] as shown in Figure 2B. From these experiments one may deduce an equilibrium constant for the association between leu^- and $M(ATP)^{2-}$, i.e. $K^{M(ATP)}_{M(ATP)(leu)}$ (see Experimental Section and caption of Figure 2B); the results are listed in Table II together with related data.[29,30,51]

Table II. Logarithms of the Stability Constants $K^{M(ATP)}_{M(ATP)(leu)}$ As Determined by ^1H NMR Shift Experiments (34 °C; $I = 0.1$–2, KNO_3; 60 MHz), together with the Chemical Shifts $\delta_{M(ATP)(leu)}$ and the Upfield Shifts $\Delta\delta$ of the Terminal Methyl Groups of L-Leucinate in These Ternary Complexes, and Some Related Data[a]

M^{2+}	$\log K^{M}_{M(leu)}$	$\log K^{M(ATP)}_{M(ATP)(leu)}$[b]	$\delta_{M(ATP)(leu)}$[c]	$\Delta\delta$[d]
Zn^{2+}	4.56 ± 0.02[e]	3.23 ± 0.25 (3.78 ± 0.05[e])	0.84 ± 0.03	0.14
Cd^{2+}	4.0[f,g]	3.51 ± 0.15	0.94 ± 0.01	0.04
Pb^{2+}	5.2[f,h]	2.7 ± 0.7	0.91 ± 0.03	0.07

[a] The errors given are 3 times the standard deviations. [b] See Experimental Section and caption of Figure 2B about the calculation of these values. Experiments carried out at different pH values lead within experimental error to the same stability constant. [c] These values correspond to those given for δ_∞ in the caption of Figure 2B, but they are usually not identical with those because here the average results of several experiments are listed. The values were measured against TMA as internal reference and converted to values downfield from trimethylsilyl propanesulfonate by adding 3.188 ppm.[18] [d] $\Delta\delta = \delta_{H(leu)} - \delta_{M(ATP)(leu)}$ whereby $\delta_{H(leu)} = 0.98$. [e] Determined by potentiometric pH titrations; from Table I. [f] Average of the values given in the literature for several $M(Aa)^+$ complexes. [g] From ref 29 and 30. [h] From ref 51.

A comparison of the stability constants of the ternary complexes with those of the corresponding binary complexes shows that the coordination tendency of leu^- toward $M(ATP)^{2-}$ is lower than toward $M(aq)^{2+}$, i.e. the values of $\Delta \log K_M$ (eq 5) are *negative*; this result agrees with those of the potentiometric pH titrations described in section 1. Also, the stability constant determined for $Zn(ATP)(leu)^{3-}$ by ^1H NMR is of the same order as the one determined for $Zn(ATP)(leu)^{3-}$ by potentiometric titration. That the present value is somewhat lower may partly be due to the different ionic strength and the somewhat higher temperature (34 °C instead of 25 °C); the main reason is, however, that under the conditions of the NMR experiments the self-association of $M(ATP)^{2-}$ is considerable,[24] and this has not been considered in the evaluation; hence, the constants given in Table II are rather lower limits. In conclusion, the stability constants determined by the ^1H NMR shift measurements are less accurate than those determined by the potentiometric titrations in diluted solutions; the important result of the NMR experiments is that they provide *direct* evidence for the intramolecular hydrophobic ligand–ligand interactions in these ternary $M(ATP)(leu)^{3-}$ complexes.

The $\delta_{M(ATP)(leu)}$ values, which correspond to the chemical shift of the methyl protons in the ternary complexes, and consequently the amount of the upfield shifts $\Delta\delta$ (Table II) are markedly different for the three $M(ATP)(leu)^{3-}$ complexes. This result seems surprising at first sight. However, it originates either in a different orientation of the hydrophobic moieties in the ternary complexes or in a different extent of the intramolecular ligand–ligand interaction (see section 5); both reasons would lead to different limiting shifts and both reasons could originate in the differences of the coordination spheres of Zn^{2+}, Cd^{2+}, and Pb^{2+}.

4. ^1H NMR Studies of Ternary Zn^{2+}, Cd^{2+}, and Pb^{2+} Complexes Composed of ATP^{4-} and Amino Acids with Aliphatic

(47) This self-association can be taken into account[20] by calculating the concentration of the sum of particles, $[\sum_n (ATP^{4-})_n]$, and by using this sum on the x axis instead of the analytical ATP^{4-} concentration. However, this procedure increases only the slope of the line; a curvature is still not observed as the association between ATP and leu is too weak.
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(49) For a discussion of such equilibria see ref 7.
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Table III. Chemical Shifts of the Terminal Methyl Groups[a] (δ_{Aa}) of Various Amino Acid Anions (Measured at pH 12.4) and the Differences in Experimentally Determined [$\delta_{H(Aa)}$ (Measured at pH 6.3)] or Extrapolated[b] ($\delta_{M(Aa)}$, $\delta_{M(ATP)(Aa)}$) Chemical Shifts for Several Amino Acid Containing Species in H_2O at 34 °C[c,d]

Aa	δ_{Aa}	$\delta_{Aa} - \delta_{H(Aa)}$	$\delta_{Aa} - \delta_{Zn(Aa)}$	$\delta_{Aa} - \delta_{Cd(Aa)}$	$\delta_{Zn(Aa)} - \delta_{Zn(ATP)(Aa)}$	$\delta_{Cd(Aa)} - \delta_{Cd(ATP)(Aa)}$	$\delta_{av} - \delta_{Pb(ATP)(Aa)}$[e]
ala	1.233	−0.255	−0.196	−0.213	−0.041	−0.060	−0.068
abu	0.893	−0.099	−0.104	−0.101	0.041	0.006	−0.004
nva	0.917	−0.040	−0.034	−0.032	0.079	0.022	0.049
nle	0.900	−0.017	−0.015	−0.008	0.129	0.042	0.067
leu	0.920	−0.059	−0.037	−0.040	0.086	0.027	0.075
ile	0.923	−0.095	−0.100	−0.087	0.075	0.010	0.031

[a] Always the midpoint of the multiplet is given, relative to trimethylsilyl propanesulfonate. [b] See Experimental Section. [c] The values for δ_{Aa}, $\delta_{Aa} - \delta_{H(Aa)}$, and $\delta_{Aa} - \delta_{Zn(Aa)}$ are taken from our earlier work.[20] The value of δ_{leu} was incorrectly listed as 0.964 in Table V of ref 20, this given value corresponds to the lower field peak of the doublet; the value for the center is $\delta_{leu} = 0.920$; all the other values listed in Table V of ref 20 are correctly given. [d] Positive values correspond to upfield shifts. [e] A value for $\delta_{Pb(Aa)}$ could not be determined (see Experimental Section). Therefore the average value (δ_{av}) of $\delta_{Zn(Aa)}$ and $\delta_{Cd(Aa)}$ was calculated and taken as a basis for comparison with $\delta_{Pb(ATP)(Aa)}$. This is justified as Zn^{2+} and Cd^{2+} show very similar effects on the position of the resonance signal, and a corresponding behavior can be expected for Pb^{2+}. It may be added that the effect of H^+ is also very similar to the one of Zn^{2+} [20,50] and Cd^{2+}.

Side Chains of Different Lengths and Sizes. It is obvious that the extent of the intramolecular hydrophobic interaction in the ternary $M(ATP)(Aa)^{3-}$ 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 amino acids shown in Figure 1. The effects on the side chain of the amino acid through protonation and Zn^{2+}, Cd^{2+}, or $M(ATP)^{2-}$ 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^+, M^{2+}, and $M(ATP)^{2-}$. The experimentally determined shifts of Aa^- and the relative shifts of H(Aa), as well as the extrapolated shifts (see Experimental Section) of $M(Aa)^+$ and $M(ATP)(Aa)^{3-}$ are compiled in Table III. The upfield shifts $\delta_{M(Aa)} - \delta_{M(ATP)(Aa)}$ given for $Zn(ATP)(Aa)^{3-}$, $Cd(ATP)(Aa)^{3-}$, and $Pb(ATP)(Aa)^{3-}$ must be considered rather as lower limits, because under the experimental conditions considerable amounts of $M(ATP)^{2-}$ will be self-stacked.[24,52]

In Figure 3 the chemical shifts of the methyl group(s) of the amino acids are plotted, relative to the signal of the amino acid anions, for the protonated form H(Aa) and the complexes $Zn(Aa)^+$ and $Cd(Aa)^+$; as the values for these three systems are very similar,[53] the data points are connected only with a single line for reasons of clarity (left side in Figure 3). As expected, protonation, as well as Zn^{2+} and Cd^{2+} complexation, leads to downfield shifts, and the shift differences decrease with increasing distance between the observed methyl group and the site of coordination, i.e. with increasing chain length.

The last three columns of Table III and the corresponding lines in Figure 3 (those at the right side) show the influence of the M^{2+}-coordinated ATP^{4-} on the methyl resonance of the also coordinated amino acids. Under the influence of ATP^{4-} the resonances of the methyl signals of the amino acids are shifted clearly upfield; i.e., the signals of the ternary $M(ATP)(Aa)^{3-}$ complexes appear at higher fields than those of the binary $M(Aa)^+$ complexes. This is direct evidence that the aliphatic side chain of the amino acids interacts with the purine system of ATP within the ternary complexes.

Such an interaction seems to be completely absent in the ternary alaninate complexes, but with increasing chain length of the amino acids the shift differences became larger in accordance with the more favorable conditions for a hydrophobic interaction. That the shift difference is mainly determined by the chain length is also seen from the results with the branched-chain amino acids (lower part of Figure 3): it is evident that the shift differences for the ternary complexes of leu correspond much better to those of nva, which has the same chain length, than to those of nle, which has the same number of carbon atoms.[54] Moreover, ile behaves more as abu than as nva, nle, or leu; i.e., it appears to react as an ethyl-substituted aminobutyric acid, which indicates that steric restrictions exist in this type of interaction. That the observed upfield shifts originate in a specific interaction and are *not* due to a simple random interaction is also confirmed by the absence of an upfield shift for the $M^{2+}/ATP^{4-}/ala^-$ system, in fact ala^- with the shortest side chain would be most favored for this.

From Figure 3 it is further obvious that the upfield shifts observed for the ternary $M(ATP)(Aa)^{3-}$ complexes depend also on the metal ion. The upfield shift is smallest with $Cd(ATP)^{2-}$, increases for $Pb(ATP)^{2-}$, and is largest for $Zn(ATP)^{2-}$. This confirms the results of section 3 and indicates again that the coordination sphere of the metal ion affects the

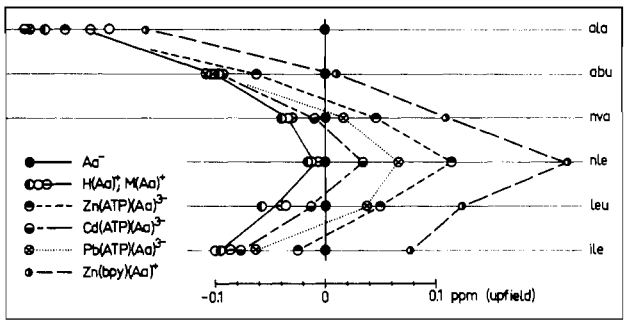


Figure 3. Chemical shift of the methyl group (always the midpoint of the multiplets was measured) of straight-chain amino acids (ala, abu, nva, nle) and of branched-chain amino acids (leu, ile, see Figure 1) for the protonated (◐) and Zn^{2+}- (○), Cd^{2+}- (⊖), $Zn(ATP)^{2-}$- (⊜), $Cd(ATP)^{2-}$- (⊖), or $Pb(ATP)^{2-}$-coordinated (⊗) amino acids, relative to the resonance position of the Aa anions (●). The values of Aa^-, relative to trimethylsilyl propanesulfonate, are listed in Table III. The corresponding data for $Zn(bpy)(Aa)^+$ (◑) are taken from ref 20 and are given for comparison.

(52) For example, in a 0.2 M $Zn(ATP)^{2-}$ solution the actual particle concentration is only about 0.11 M (see ref 24). As the concentration of the amino acid was only 0.02 M in these experiments, i.e. about 10% of $[ATP]_{tot}$, the amount of self-association will not be strongly disturbed by the formation of the ternary complexes; hence, the upfield shifts of $Zn(ATP)(Aa)^{3-}$ may actually be larger by a factor up to about 1.8 (see eq 1).

(53) That the influences on chemical shifts of H^+ and M^{2+}, like Zn^{2+} or Cd^{2+}, are very similar despite the different charge of these cations has been observed before.[20,50]

(54) The fact that the upfield shift is largest for the longest side chain, i.e. where the observed methyl group is furthest removed from the metal ion, is a good proof that the upfield shift is not due to a metal ion transmitted effect of the aromatic ligand on the amino acid but that it is due to a direct interaction between the side chains of the two ligands: in other words, an association of the amino acid side chain with the purine ring of ATP^{4-}, where the aliphatic group lies above or below the aromatic ring.

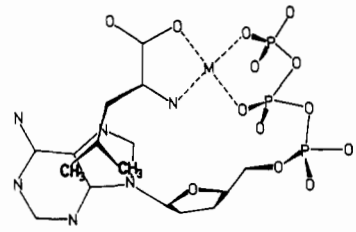


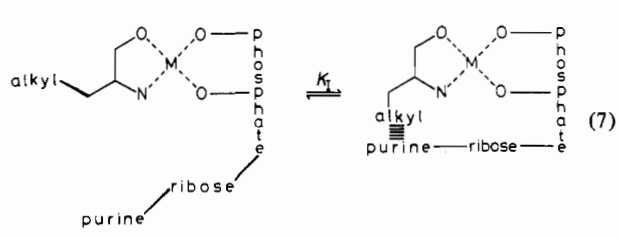
Figure 4. Tentative and simplified structure of the ternary complex formed by M^{2+}, ATP^{4-}, and leu^-, showing the interaction of the isopropyl group of leu^- with the aromatic purine moiety of ATP^{4-}.

arrangement of the ligands and hence the intramolecular ligand–ligand interaction. In addition, all the upfield shifts observed under the influence of $M(ATP)^{2-}$ are smaller than those determined for $Zn(bpy)^{2+}$. This could be the result of the greater flexibility in the ternary $M(ATP)(Aa)^{3-}$ complexes compared with that in $M(bpy)(Aa)^+$, leading thus to a more favored hydrophobic interaction in the latter complexes. A more favored intramolecular ligand–ligand interaction has also been observed[11] in the corresponding ternary complexes with tryptophanate, i.e. in $Zn(bpy)(trp)^+$ compared with $Zn(ATP)(trp)^{3-}$, where stacks involving the indole moiety are formed.

In conclusion, the results of this section confirm the occurrence of an intramolecular hydrophobic interaction in the ternary $M(ATP)(leu)^{3-}$ complexes, for which first hints and evidence had been obtained already in sections 1 and 3, respectively. Furthermore, there is now evidence that such a ligand–ligand interaction exists also in the corresponding ternary complexes of Zn^{2+}, Cd^{2+}, or Pb^{2+} with nva, nle, or ile, as well as to some extent also in $Zn(ATP)(abu)^{3-}$. A tentative structure of such a ternary complex is shown in Figure 4 for the $M(ATP)(leu)^{3-}$ complexes.

5. Isomeric Equilibria of $M(ATP)(Aa)^{3-}$ 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 (section 1) and for the observed upfield shifts in the ternary complexes (sections 3 and 4), does not mean that all of the $M(ATP)(Aa)^{3-}$ species exist in this folded form. This is already indicated, despite the shortcomings,[52] by the size of the upfield shifts observed for the $M(ATP)(leu)^{3-}$ and the other $M(ATP)(Aa)^{3-}$ complexes: these upfield shifts are smaller than the shift expected for the binary (metal ion free) hydrophobic $(ATP)(leu)^{5-}$ adduct, which is on the order of 0.5 ± 0.3 ppm (see section 2). The observation that the upfield shift is larger in the metal-free system has also been made in a related study[11] dealing with stacking adducts.

All this indicates that in solution an intramolecular equilibrium exists between an "open" and a "closed" form, i.e. between two isomers as indicated in equilibrium 7. If these



(7)

two isomers are designated as $M(ATP)(Aa)^{3-}(op)$ and $M(ATP)(Aa)^{3-}(cl)$, the constant of this intramolecular and therefore concentration-independent equilibrium is defined by eq 8.

$$K_I = [M(ATP)(Aa)^{3-}(cl)]/[M(ATP)(Aa)^{3-}(op)] \quad (8)$$

Unless the dimensionless equilibrium constant K_I is very large, there are appreciable amounts of the isomer present in which the two side chains of the ligands do not interact. Thus, while the limiting shift, δ_∞, of the metal-free adduct between the ligands is a measure for the upfield shift of the leucinate protons in the binary $(ATP)(leu)^{5-}$ complex, in which all leu^- undergo a hydrophobic interaction with ATP^{4-}, $\delta_{M(ATP)(leu)}$ $(=\delta_\infty)$ for the ternary $M(ATP)(leu)^{3-}$ complexes is also a function of K_I, because only the isomer with a ligand–ligand interaction contributes to the upfield shift. Clearly, a comparison of $\Delta\delta = \delta_0 - \delta_\infty$ of the binary adduct with the difference $\delta_{M(Aa)} - \delta_{M(ATP)(Aa)}$ of the metal ion complexes will allow an estimation[11] of the percentage of the "closed" isomer. Unfortunately, δ_∞ of the binary adduct is only very roughly known (Figure 2A; section 2). Despite this handicap, we have made an estimation based on $\Delta\delta = 0.5 \pm 0.3$ ppm for the $M(ATP)(leu)^{3-}$ complexes, because for these some data based on the potentiometric titrations are also available. The estimations are summarized in Table IV.

Another approach to determine K_I and hence the percentage of the "closed" isomer rests on the stability constants obtained from the potentiometric pH titrations (Table I). This means, K_I may be calculated according to eq 9 (for details see ref 20).

$$K_I = \frac{10^{\Delta \log K_{M/expt}}}{10^{\Delta \log K_{M/op}}} - 1 \quad (9)$$

In this equation $\Delta \log K_{M/expt}$ is the experimentally determined value from eq 5, while $\Delta \log K_{M/op}$ is the corresponding value for a related system without an intramolecular ligand–ligand interaction.

In the present case, the values of $\Delta \log K_M$ are available for some ternary $M^{2+}/ATP^{4-}/leu^-$ systems (Table I). As in the $M(ATP)(ala)^{3-}$ complexes the intramolecular interaction is very small or even nonexistent (section 4), we may use the corresponding values of $\Delta \log K_M$ as $\Delta \log K_{M/op}$, because the coordination spheres of M^{2+} in $M(ATP)(leu)^{3-}$ and $M(ATP)(ala)^{3-}$ are identical. Hence, K_I for the $M(ATP)(leu)^{3-}$ complexes may be obtained, and the K_I values in turn then allow the calculation of the percentage of the "closed" isomer. These data are assembled in Table IV, together with the related data for the ternary $M(ATP)(trp)^{3-}$ complexes, which we now also have calculated.

It must be emphasized that the values of K_I and the resulting percentages given in Table IV can be considered only as rough estimates, especially in case of the leucinate complexes, because they are derived from differences between rather large constants that are connected with a certain experimental error. It has been shown though[20] that with use of the $\Delta \log K_M$ values for calculations of this type systematic errors cancel to a large part. Indeed, the trends that are indicated by the results are most reasonable: for each metal ion where both complexes, $M(ATP)(leu)^{3-}$ and $M(ATP)(trp)^{3-}$, have been studied the percentage of $M(ATP)(Aa)^{3-}(cl)$ is larger for the tryptophanate complexes. This result is expected because usually stacks are more stable than hydrophobic adducts.[15] However, most important, both methods[55] used for the estimations given in Table IV reveal that eq 7 is a truly existing intramolecular equilibrium.

General Conclusions

Such intramolecular interactions also have been observed between *covalently* linked suitable groups; for example, nicotinamide adenine dinucleotide (NAD^+) and dihydronicotinamide adenine dinucleotide (NADH) exist in neutral frozen

(55) In comparing the potentiometric pH titrations with the ^1H NMR shift measurements, one has in general to conclude that the first method gives the more reliable stability constants but only indirect hints for an intramolecular ligand–ligand interaction, while the NMR method provides direct evidence for the intramolecular interaction but only estimations for the constants. A combination of both methods is therefore to be recommended for such studies.

Table IV. Estimations of the Intramolecular Dimensionless Equilibrium Constant K_I for the Ternary M(ATP)(leu)$^{3-}$ and M(ATP)(trp)$^{3-}$ Complexes and of the Percentage of the "Closed" Isomer for the Same Systems in Aqueous Solution

M(ATP)(Aa)$^{3-}$	$\Delta \log K_{M/exptl}$ (eq 5)[a]	$\Delta \log K_{M/op}$[a,b]	K_I (eq 8, 9)	% M(ATP)(Aa)$^{3-}$(cl) (eq 7) from K_I[c]	from NMR[d]
Mn(ATP)(leu)$^{3-}$	−1.08	−1.31	0.70	41	
Cu(ATP)(leu)$^{3-}$	−1.40	−1.50 (−1.71)	0.26 (1.04)	21 (51)	
Zn(ATP)(leu)$^{3-}$	−0.78	−0.80	~0.05	~5	~30 (20/75)[e]
Cd(ATP)(leu)$^{3-}$					~10 (5/25)[e]
Pb(ATP)(leu)$^{3-}$					~25 (15/70)[e]
Mg(ATP)(trp)$^{3-}$					~30[f]
Mn(ATP)(trp)$^{3-}$	−0.99	−1.31	1.09	52	
Cu(ATP)(trp)$^{3-}$	−1.31	−1.50 (−1.71)	0.55 (1.50)	35 (60)	
Zn(ATP)(trp)$^{3-}$	−0.21	−0.80	2.89	74	~45[f]

[a] Data from Table I (25 °C; I = 0.1, $NaClO_4$). [b] As basis for $\Delta \log K_{M/op}$ the data of the M^{2+}/ATP^{4-}/ala$^-$ systems were used; for the justification of this see the text. The value in parentheses is $\Delta \log K_{Cu}$ of the Cu^{2+}/ATP^{4-}/glycinate system (see footnote *d* of Table I); this value was also used as a basis for reasons of comparison. [c] Calculated from the listed K_I values. [d] Calculated from ^1H NMR shift experiments. [e] These values were calculated by multiplying the shift differences $\delta_{M(Aa)} - \delta_{M(ATP)(Aa)}$ of Table III (34 °C) with a factor of 1.8 (see ref 52) and assuming an upfield shift $\Delta\delta = \delta_0 - \delta_\infty$ of 0.5 for the binary (ATP)(leu)$^{3-}$ adduct (see sections 2 and 5). The values in parentheses give the approximate lower and upper limits calculated with $\Delta\delta$ = 0.8 and 0.2, respectively. [f] Results at 27 °C in D_2O from ref 11; the average percentage of the results obtained from the shifts of the resonances of H-8, H-2, and H-1′ of ATP is given.

Table V. Effect of the Formation of a Metal Ion Bridge on the Concentration of the Hydrophobic or Aromatic-Ring Stacking Ligand–Ligand Adduct in the Physiological pH Range,[a] the Concentrations of (ATP)(H(Aa))$^{4-}$ or M(ATP)(Aa)$^{3-}$(cl) Being Given as the Percentage of the Total Reactant Concentrations[b]

M^{2+}	$[reactant]_{tot}$	% (ATP)(H(leu))$^{4-}$ pH 7–7.5	% M(ATP)(leu)$^{3-}$(cl) pH 7	% M(ATP)(leu)$^{3-}$(cl) pH 7.5	% (ATP)(H(trp))$^{4-}$ pH 7–7.5	% M(ATP)(trp)$^{3-}$(cl) pH 7	% M(ATP)(trp)$^{3-}$(cl) pH 7.5
	[ATP] = [Aa] = 10^{-3} M	0.06			0.6		
	= 10^{-2} M	0.6			5.5		
	= 5×10^{-2} M	2.8			20		
Mn^{2+}	[Mn] = [ATP] = [Aa] = 10^{-3} M		0.002	0.006[c]		0.006	0.02[c]
	= 10^{-2} M		0.02	0.07[c]		0.06	0.19[c]
	= 5×10^{-2} M		0.11	0.35[c]		0.32	0.96
Zn^{2+}	[Zn] = [ATP] = [Aa] = 10^{-3} M		0.23	0.66		6.3	14
	= 10^{-2} M		2.0	4.3		29	41
	= 5×10^{-2} M		5.9	9.3		48	56
Cu^{2+}	[Cu] = [ATP] = [Aa] = 10^{-3} M		11	12		20	21
	= 10^{-2} M		16	16		27	28
	= 5×10^{-2} M		17	17		30	31

[a] If the comparison would be based on (ATP)(Aa)$^{5-}$ instead of (ATP)(H(Aa))$^{4-}$, i.e. if the polar interaction between the ammonium group and the phosphate chain would be eliminated, the effect of the metal ion bridge would even be more pronounced, because $K^{(ATP)}_{(ATP)(H(leu))} \simeq 0.6\ M^{-1} > K^{(ATP)}_{(ATP)(leu)} \simeq 0.4\ M^{-1}$ (section 2) and $K^{(ATP)}_{(ATP)(H(trp))} = 6.2 \pm 1.8\ M^{-1}$ (ref 11) $\simeq K^{(AMP)}_{(AMP)(H(trp))} = 6.83 \pm 0.81\ M^{-1} > K^{(AMP)}_{(AMP)(trp)} = 2.24 \pm 0.29\ M^{-1}$ (ref 7). [b] The percentages were calculated for (ATP)(H(Aa))$^{4-}$ with $K^{(ATP)}_{(ATP)(H(leu))} = 0.6\ M^{-1}$ (section 2) and $K^{(ATP)}_{(ATP)(H(trp))} = 6.2\ M^{-1}$ (ref 11) on the assumption that all adducts with a polar interaction also undergo a hydrophobic or a stacking interaction; for M(ATP)(Aa)$^{3-}$(cl) the concentrations of M(ATP)(Aa)$^{3-}$(cl) were first calculated with the constants given in Table I and in the caption of Figure 5 (for M(ATP)(trp)$^{3-}$ in addition the values of ref 9 were used); then the K_I values listed in Table IV were applied; the only exception is the Zn(ATP)(leu)$^{3-}$ system for which K_I = 0.25 was used (which corresponds to 20% Zn(ATP)(leu)$^{3-}$(cl) to make a compromise between the potentiometric titrations and the NMR results. [c] Already at pH 8.5 the values for % M(ATP)(leu)$^{3-}$(cl) correspond to the values given above for % (ATP)(H(leu))$^{4-}$. The corresponding situation is reached with trp at pH ~9.4.

aqueous solution to about 95–100% in the folded form.[56] In neutral aqueous solution at 25 °C (and I = 0.15 M) the amount for NAD^+ (and most probably also for NADH) is, as one would expect, somewhat smaller (~44%),[57] but still remarkably large, while in neutral propylene glycol, in the absence of water, the intramolecular association is weak.[56]

It is a general observation that the addition of organic solvents to aqueous solutions reduces stacking and hydrophobic interactions,[58] and this must also be expected for the systems studied here. As the water activity certainly changes, e.g. between biological fluids and the surface of membranes, the described intramolecular associations are subtle tools to favor selectively certain structures under certain conditions; indeed, one may say with Tanford[14] "the hydrophobic effect is a unique organizing force".

Energetic Considerations. The energies involved with hydrophobic and aromatic-ring stacking are small: the differences in complex stability between systems with and without hydrophobic interactions range for the M(ATP)(leu)$^{3-}$ complexes from 0.02 to 0.23 log unit (Table IV), which corresponds to $\Delta G°$ = −0.11 to −1.3 kJ/mol. Scheraga calculated[15] for the hydrophobic interaction between the phenyl residue of phenylalanine and the isopropyl moiety of leucine $\Delta G°$ = −1.7 kJ/mol, also for an aqueous solution at 25 °C. Hence, the agreement between the theoretical and the experimental data is excellent, especially if one considers that the theoretical calculation is based on a maximal interaction of the hydrophobic residues, while the mobility of the ligands in the ternary complexes is obviously restricted and therefore very often the interaction will be sterically not ideal. Actually, for mixed-ligand complexes a gradual tailing off of the interaction toward zero is expected and also indicated by the present observations.

For the stacking interactions in the ternary M(ATP)(trp)$^{3-}$ complexes $\Delta G°$ ranges from −1.1 to −3.4 kJ/mol, corresponding to stability differences of 0.19–0.59 log unit (Table IV). These results are also within Scheraga's calculations,[15]

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where for several types of interactions $\Delta G°$ values between −1.3 and −6.3 kJ/mol were obtained. Calorimetric measurements by Rizzarelli et al.[59] for several M(ATP)(amino acid)$^{3-}$ complexes revealed that $\Delta G°$ contains a negative contribution of $\Delta H°$, which means that the formation of the stacking adduct is not solely entropy driven. Similar results were obtained by Sóvágó and Martin[57] for the intramolecular stack in NAD^+, where $\Delta H° = -13$ kJ/mol.

Extent of Complex Formation in the Physiological pH Range. As a demonstration that ternary complexes of the discussed kind exist also in the physiological pH range Figure 5 has been designed. Even at 10^{-3} M reactant concentrations Cu(ATP)(leu)$^{3-}$ is formed in rather large amounts (Figure 5A), but Zn(ATP)(leu)$^{3-}$ also extends its existence under these conditions into the pH range around 7; clearly, at 10-fold higher concentrations this complex is formed in more appreciable amounts (Figure 5B). The rather unstable Mn(ATP)(leu)$^{3-}$ complex (see Table I) occurs in a 5×10^{-2} M reactant solution at pH ~7 only in traces, but at pH >8 its formation increases rapidly (Figure 5C). Of course, the increase in concentration of one of the ligands will favor the formation of the mixed-ligand complexes even more; hence, such species must be considered in any discussion about the composition of biological fluids. Moreover, the mentioned concentrations are well within the concentration range occurring in biological systems: for example, the chromaffin granules, which store catecholamines in the adrenal medulla, contain both substantial amounts of metal ions and high concentrations of ATP (~0.1 M).[63]

To mediate a realistic feeling of how the formation of a metal ion bridge between two ligands influences the formation of ligand–ligand associations, we have calculated the data of Table V. It is evident from a comparison of the binary ATP/amino acid systems with the corresponding systems containing also Zn^{2+} or Cu^{2+} that the weak hydrophobic and stacking interactions may be dramatically favored by the formation of metal ion bridges: e.g., at pH 7 and 10^{-3} M reactant concentrations the hydrophobic interaction between the isopropyl residue of leucine and the purine moiety of ATP is favored by factors of about 4 (Zn^{2+}) and 200 (Cu^{2+}), while the aromatic-ring stacking involving the indole residue of tryptophan is increased by factors of about 10 and 35. Consequently, the probability for "recognition" between a nucleotide and an amino acid may be metal ion promoted.

However, the examples with Mn^{2+} in Table V reveal also that there are conditions under which no favored adduct formation via the ternary complex occurs; i.e., the ligand–ligand interaction in the metal ion free system is more pronounced. In such a case a larger formation degree of the ternary complex must be obtained to gain a "positive" effect; this may often be achieved by raising the pH. Indeed at pH 8.5 the adduct formation is already equal in both systems, and at pH about 9 the ligand–ligand interaction is also dominating in Mn(ATP)(leu)$^{3-}$ compared with ATP^{4-}/H(leu); the corresponding crucial pH for the trp system is about 9.4 (Table V, footnote *c*). Moreover, at large reactant concentrations one also may reach conditions under which no promotion is possible anymore, because even under complete formation of the ternary complex only a certain percentage exists as the "closed" isomer; in other words, the *inter*molecular interactions may then become more important.

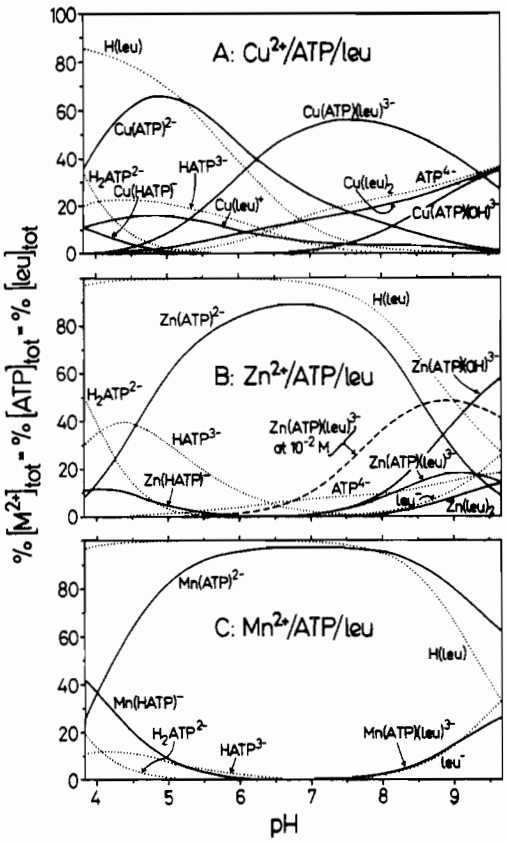

Figure 5. Effect of pH on the concentrations of the species present in an aqueous solution of (A) Cu^{2+}/ATP/leu, (B) Zn^{2+}/ATP/leu, or (C) Mn^{2+}/ATP/leu. Results are given as the percentage of the total M^{2+} (=total ATP or leu) present ($I = 0.1$ M; 25 °C), computed with the potentiometrically determined constants of Table I and the additional constants given below. The dotted lines indicate the free-ligand species and the solid lines M^{2+} or its complexes. (A) Cu^{2+}, ATP, and leu: calculated for $[Cu^{2+}]_{tot} = [ATP]_{tot} = [leu]_{tot} = 10^{-3}$ M with $pK^{H}_{H_2(ATP)} = 4.06$,[60] $pK^{H}_{H(ATP)} = 6.42$,[36] $\log K^{Cu}_{Cu(HATP)} = 3.12$,[60] $pK^{H}_{Cu(ATP)(H_2O)} = 8.17$,[61] $\log K^{Cu(leu)}_{Cu(leu)_2} = 7.02$,[20] and the estimated value $\log K^{Cu(leu)}_{Cu(leu)(HATP)} \simeq \log K^{Cu}_{Cu(HATP)} + \Delta \log K_{Cu} = 3.12$ (cf. ref 60) − 1.40 (Table I) = 1.72; $[Cu(HATP)(leu)^{2-}] < 0.2\%$, $[H_2(leu)^+] \leq 2.2\%$, $[leu^-] < 0.3\%$. (B) Zn^{2+}, ATP, and leu: calculated for $[Zn^{2+}]_{tot} = [ATP]_{tot} = [leu]_{tot} = 10^{-3}$ M with $\log K^{Zn}_{Zn(HATP)} = 2.67$,[60] $pK^{H}_{Zn(ATP)(H_2O)} = 8.87$,[62] $\log K^{Zn(leu)}_{Zn(leu)_2} = 4.18$,[20] the estimated value $\log K^{Zn(leu)}_{Zn(leu)(HATP)} \simeq \log K^{Zn}_{Zn(HATP)} + \Delta \log K_{Zn} = 2.67$ (cf. ref 60) − 0.78 (Table I) = 1.89, and the constants given under (A); $[Zn(HATP)(leu)^{2-}] < 0.01\%$, $[H_2(leu)^+] < 2.6\%$, $[Zn(leu)^+] < 5.4\%$. The broken line for $Zn(ATP)(leu)^{3-}$ was calculated for reasons of comparison with $[Zn^{2+}]_{tot} = [ATP]_{tot} = [leu]_{tot} = 10^{-2}$ M. (C) Mn^{2+}, ATP, and leu^-: calculated for $[Mn^{2+}]_{tot} = [ATP]_{tot} = [leu]_{tot} = 5 \times 10^{-2}$ M with $\log K^{Mn}_{Mn(HATP)} = 2.39$,[60] $pK^{H}_{Mn(ATP)(H_2O)} = 10.7$,[62] the estimated values $\log K^{Mn(leu)}_{Mn(leu)(HATP)} \simeq \log K^{Mn}_{Mn(HATP)} + \Delta \log K_{Mn} = 2.39$ (cf. ref 60) − 1.08 (Table I) = 1.31 and $\log K^{Mn(leu)}_{Mn(leu)_2} \simeq 2.1$, and the constants given under (A); $[Mn(HATP)(leu)^{2-}] < 0.01\%$, $[H_2(leu)^+] < 3\%$, $[ATP^{4-}] < 5.4\%$, $[Mn(ATP)(OH)^{3-}] < 6\%$, $[Mn(leu)^+] < 1.8\%$, $[Mn(leu)_2] < 3.5\%$.

From a comparison of the systems with Mn^{2+}, Cu^{2+}, and Zn^{2+} it is evident that the crucial point for the achievement of a promotion of the ligand–ligand interaction in the physiological pH range via an *intra*molecular "mechanism" is a certain minimum amount of ternary complex formation at pH 7 (cf. Figure 5 and Table V).

The present results confirm the earlier claim[4a] that working with monomeric species in aqueous media should permit an

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assessment of individual factors so that the more complex interactions between nucleic acids and amino acids can be understood. Clearly, even on the monomer level the interactions between amino acids and nucleotides are sufficiently quantifiable;[8c] if polar interactions are included, a rudimentary "preferential" scheme can be seen: the stability of the adducts decreases within the series $(ATP)(H(trp))^{4-} > (ATP)(H(leu))^{4-} > (ATP)(H(ala))^{4-}$.

In addition, the stability of the ternary $M(ATP)(Aa)^{3-}$ complexes differs not very much for a given metal ion (Table I), but the results of Table V show that the ability to form specific and distinct structures differs; especially the differences for the ternary Zn^{2+} complexes are quite pronounced: in 10^{-3} M solution at pH 7 the "closed" form of $Zn(ATP)(trp)^{3-}$ is favored by a factor of about 30 over the "closed" isomer of $Zn(ATP)(leu)^{3-}$. Hence, these data suggest that evolutionary selectivity in nucleotide/metal ion/amino acid systems is probably not so much achieved, at least for a given metal ion, by differences in complex stability but rather by the ability to form specific and distinct structures.

In summary, via the formation of mixed-ligand complexes certain ligand–ligand associations may be favored and thus distinct structures may be created in a way that involves only small changes from an energetic point of view. Regarding the specificity and selectivity observed in nature, this seems to be the most fascinating point of the present results.

Acknowledgment. We thank Rita Baumbusch for technical assistance, K. Aegerter for recording the 90-MHz ^1H NMR spectra, and the CIBA-Stiftung Basel for support toward the costs of these spectra. The computers were made available by the Rechenzentrum der Universität Basel (UNIVAC 1100/81). These supports and a research grant from the Swiss National Science Foundation are gratefully acknowledged.

Registry No. ATP, 56-65-5; Mn, 7439-96-5; Cu, 7440-50-8; Zn, 7440-66-6; Cd, 7440-43-9; Pb, 7439-92-1; leucine, 61-90-5; norleucine, 327-57-1; norvaline, 6600-40-4; α-aminobutyric acid, 80-60-4; isoleucine, 73-32-5; alanine, 56-41-7; tryptophan, 73-22-3.

Contribution from the Departments of Chemistry, University of Denver, Denver, Colorado 80208, and University of Colorado at Denver, Denver, Colorado 80202

Metal–Nitroxyl Interactions. 27. Comparison of Electron–Electron Spin–Spin Coupling Constants for Urea and Amide Linkages in Spin-Labeled Copper Porphyrins

KUNDALIKA M. MORE, GARETH R. EATON,* and SANDRA S. EATON

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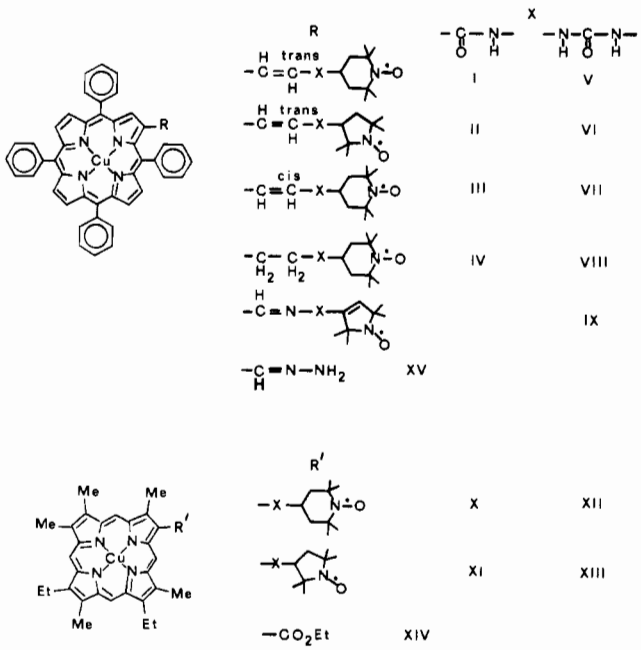
A series of spin-labeled copper porphyrins has been prepared with urea linkages between the porphyrin ring and the nitroxyl ring. The electron–electron coupling constants, J, have been obtained from the solution EPR spectra. The observed values of J are lower by factors of about 2 to >40 for the compounds with urea linkages than for analogous compounds with amide linkages. The decreased values of J for the urea linkages are consistent with a largely σ-bonding pathway for the copper–nitroxyl interaction. In several cases two components were observed in the EPR spectra. These are attributed to different conformations of the porphyrin–nitroxyl linkage.

Introduction

The ability to determine patterns and pathways of electron spin delocalization is important in areas of chemistry ranging from molecular orbital theory and electronic structure to mechanisms of reaction. Recent results from these laboratories have demonstrated that EPR studies of electron–electron spin–spin coupling constants, J, in spin-labeled metal complexes can be used to monitor changes in electron spin delocalization due to changes in coordinated metal,[1,2] changes in metal–nitroxyl linkages,[3-7] and changes in ligand conformation.[4-6] In a study of spin-labeled pyridine adducts of vanadyl and copper(II) bis(hexafluoroacetylacetonates) it was found that the change in J that resulted when an amide linkage between the pyridine and nitroxyl rings was replaced by a urea linkage correlated with the relative importance of σ- and π-delocalization pathways in the metal–nitroxyl interaction.[7] Our previous studies of the values of J for the amide-linked spin-labeled copper porphyrins I–IV indicated that σ-bonding pathways were largely responsible for the electron–electron interaction.[6] We have therefore prepared the analogous urea-linked complexes V–VIII to further explore the interaction pathway in these porphyrins. The spectral data for the amide-linked porphyrins X and XI indicated little π interaction between the porphyrin ring and the amide side chain.[4] Com-



*To whom correspondence should be addressed at the University of Denver.

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