180. On the Position of the Intramolecular Equilibrium between Opened and Aromatic-Ring Stacked Forms in Ternary Complexes Composed of Adenosine 5′-triphosphate, Mg^{2+} or Zn^{2+}, and L-Tryptophanate, and in Related Ternary Systems[1])

by **Paul R. Mitchell, Bernhard Prijs,** and **Helmut Sigel**

Institut für Anorganische Chemie der Universität Basel, Spitalstrasse 51, CH-4056 Basel, Switzerland

Herrn Professor Dr. *Edgardo Giovannini* zum 70. Geburtstag gewidmet

(30.V.79)

Summary

The intramolecular and dimensionless equilibrium constant, K', for the equilibrium between open and aromatic-ring stacked isomers of ternary complexes formed between adenosine 5′-triphosphate, inosine 5′-triphosphate, or uridine 5′-triphosphate, Mg^{2+} or Zn^{2+}, and L-tryptophanate, 2,2′-bipyridyl (bipy), or 1,10-phenanthroline (phen) have been estimated by ^{1}H-NMR. shift measurements in D_2O (I = 0.1 M, $NaNO_3$; 27°). The approximate equilibrium constants K' are ≥ 20, ≥ 20, 1.2, 0.9, 0.7, 0.5, and 0.3 for the ternary complexes Zn(phen)(ATP)$^{2-}$, Mg(phen)(ATP)$^{2-}$, Zn(bipy)(ATP)$^{2-}$, Zn(bipy)(ITP)$^{2-}$, Zn(bipy)(UTP)$^{2-}$, Zn(trp)(ATP)$^{3-}$, and Mg(trp)(ATP)$^{3-}$, respectively. The percentages of the stacked isomers decrease in the order Zn(phen)(ATP)$^{2-}$ ($\geq 95\%$) $\gg$ Zn(bipy)(ATP)$^{2-}$ (55%) > Zn(bipy)(ITP)$^{2-}$ (48%) > Zn(bipy)(UTP)$^{2-}$ ($\sim 40\%$), which coincides with the order of stability of the binary metal-free adducts (phen)(ATP)$^{4-}$ > (bipy)(ATP)$^{4-}$ $\simeq$ (bipy)(ITP)$^{4-}$ > (bipy)(UTP)$^{4-}$; the corresponding stability constants are $K^{A}_{AB} = 28.2 \pm 4.7$, 8.1 ± 2.6, 8.8 ± 1.8, and ~ 1 (M^{-1}), respectively (D_2O; I = 0.1 M, $NaNO_3$; 27°): these series reflect the decreasing size of the aromatic-ring systems forming the stacks. The indole moiety of tryptophan resembles 2,2′-bipyridyl rather than 1,10-phenanthroline and $K^{(trp)}_{(trp)(ATP)} = 6.2 \pm 1.8$ M^{-1} for (trp)(ATP)$^{4-}$. Implications with respect to the stability of ternary complexes, and to biological systems are indicated briefly.

1. Introduction. – As under biological conditions solutions of metal ions and ligands exist as multiligand-multimetal ion equilibria [2], studies of the equilibria involved in mixed-ligand complex formation [3] have been stimulated and those factors which govern the stability of ternary metal ion complexes containing different kinds of ligands are now well understood [4–7]. Although in these ternary complexes the metal ion usually links the two ligands together, and the interactions

[1]) Part XXXII of the series 'Ternary Complexes in Solution'; for part XXXI see [1].

0018-019X/79/6/1723-13$01.00/0

between the metal ion and the ligands determine the stability of these complexes, in some cases a ternary complex results from an aromatic-ring stacking between two suitable ligands with a metal ion coordinated to only one of them [5] [8] [9]; such adducts are usually rather unstable. However, in systems where both of the stacked moieties also have suitably oriented donor groups, the concentration of the stacked adduct may be increased tremendously by the formation of a metal ion bridge. In favourable cases this situation may lead to cooperative effects, *i.e.* the metal ion bridge favours stacking and the stack favours the metal ion ligand interaction; this results in an enhanced stability of the ternary complex as observed[2]) for Mg(phen)(ATP)$^{2-}$ and Ca(phen)(ATP)$^{2-}$ [7].

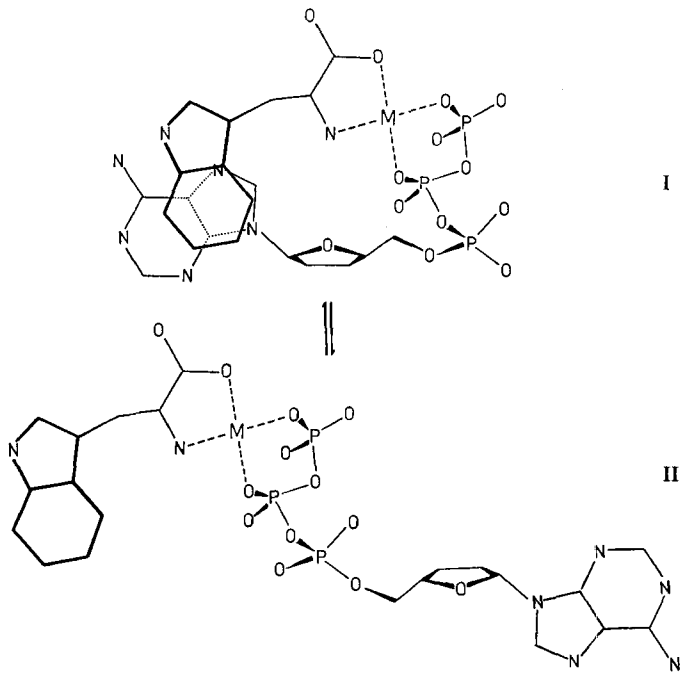

Fig. 1. *Intramolecular stacking equilibrium* between the stacked isomer (I) and the open isomer (II) of M(ATP)(trp)$^{3-}$

An intramolecular ligand-ligand interaction within a mixed ligand complex has been observed [7] [9–12] by UV. or ^{1}H-NMR. spectroscopy in a number of systems including the natural combinations [13–16] ATP^{4-}/L-tryptophanate/M^{2+}, Cu^{2+} or Zn^{2+}. For the closed stacked form of M(ATP)(trp)$^{3-}$, I of *Figure 1* has been tentatively suggested [14] in equilibrium with the open isomer indicated by II. As

[2]) Abbreviations: ala = alaninate; ATP = adenosine 5′-triphosphate; bipy = 2,2′-bipyridyl; ITP = inosine 5′-triphosphate; $M^{2+} = Mg^{2+}$ or Zn^{2+}; NTP = ATP, ITP or UTP; phen = 1,10-phenanthroline; trp = tryptophan (its ionic form follows from the overall charge of the complexes); UTP = uridine 5′-triphosphate.

this intramolecular equilibrium (1) is independent of the total concentration of the ternary complex formed, the corresponding constant K'

open isomer $\rightleftharpoons$ stacked isomer

$$K' = [\text{stacked isomer}]/[\text{open isomer}] \quad (1)$$

is dimensionless and thus difficult to determine. Rough guesses of the position of the intramolecular equilibrium (1), based on UV. absorption data [10] [14] or on an enhanced stability of the ternary complexes [7] [12] have been made in a number of cases. However, there are so far no reliable values of the intramolecular and dimensionless equilibrium constant K' for any system.

We have now measured the upfield shift in the stacked binary complexes, *e.g.* (trp) $(ATP)^{4-}$, and the upfield shift in the ternary mixture. *e.g.* M^{2+}/trp/ATP^{4-}. Using the known or estimated stability constants for all the species present in the ternary mixture, we have calculated the degree of formation of the ternary complexes and hence the upfield shifts for the ternary complexes. From the ratio of the upfield shift in the ternary complex to the upfield shift in the binary complex, we have estimated K' (equation (1)) for several ternary complexes composed of ATP, ITP, or UTP, Mg^{2+} or Zn^{2+}, and tryptophanate, 2,2'-bipyridyl, or 1,10-phenanthroline.

2. Experimental Section. – 2.1. *Materials.* The disodium salts of ATP and ITP were from *Serva Feinbiochemica GmbH,* Heidelberg, Germany; the trisodium salt of UTP (Type III) was from *Sigma Chemical Co.,* St. Louis, Missouri, U.S.A., and 2,2'-bipyridyl *(purissimum)* was from *Fluka AG,* Buchs, Switzerland. 1,10-Phenanthroline hydrate, magnesium and zinc nitrate hexahydrates (all *p.a.)* and L-tryptophan (99%) were from *Merck AG,* Darmstadt, Germany. The exact metal concentrations in the stock solutions were determined by titration with EDTA, and for the Zn^{2+} salt in addition gravimetrically as $Zn(C_5H_5N)_2(SCN)_2$. All other chemicals were of the best purity available from *Merck AG.*

2.2. *Apparatus.* The pH was measured with a *Metrohm* potentiometer E 510 with a micro glass electrode; the pD was obtained [17] by adding 0.4 to the pH meter reading. ^{1}H-NMR. spectra were recorded in D_2O solutions on a *Bruker* WH-90 FT spectrometer (90.025 MHz), using the centre peak of the resonance of the tetramethylammonium ion (0.002 to 0.004M) as reference; chemical shifts were converted to a trimethylsilylpropanesulfonate reference by adding 3.188 ppm. Calculations were carried out using a *Hewlett-Packard* 9821 A calculator connected to a 9862 A calculator plotter.

2.3. *Measurement of the stability constants of aromatic-ring stacking by ^{1}H-NMR.* The stability constant of (trp)$(ATP)^{4-}$ was calculated from the variation of the upfield shift of the resonances of H–C(1'), H–C(2), and H–C(8) of ATP^{4-} (0.01M; I = 0.1, $NaNO_3$ in D_2O; pD 8.3) as the concentration of L-tryptophan was increased from 0.01M to 0.067M in a similar manner to that used earlier [7] for the phen/ATP^{4-} and bipy/ATP^{4-} systems. The stability constant of (bipy)$(ITP)^{4-}$ was calculated from measurements using ITP^{4-} (0.01M; pD 8.0) and 2,2'-bipyridyl (0.01 to 0.05M). For the $Zn(phen)^{2+}$/adenosine system, the concentration of adenosine was 0.01M (pD 6.6) and the concentrations of Zn^{2+} and phenanthroline (1:1 mixture) were varied from 0.015 to 0.12M; it was thus not possible to keep the ionic strength constant, which varied in this series from 0.1 ($NaNO_3$) to 0.36.

2.4. *Measurement of the upfield shifts of the ternary complexes.* The upfield shifts of H–C(1'), H–C(2) and H–C(8) in the ternary system Zn^{2+}/phen/ATP^{4-} have already been reported [7] and the upfield shifts of the other ternary systems listed in *Table 2* were measured using [NTP] = 0.01M; [phen], [bipy], or [trp] = 0.01M; and $[M^{2+}] = 0.008$ and 0.01M.

The calculation of the degree of formation of the ternary complexes was carried out by considering for example the species M^{2+}, $M(phen)^{2+}$, $M(phen)_2^{2+}$, $M(phen)_3^{2+}$, $M(NTP)^{2-}$, $M(NTP)(OH)^{3-}$, $M(NTP)_2^{6-}$, $M_2(NTP)$, $M(phen)(NTP)^{2-}$, $M(phen)_2(NTP)^{2-}$, $(phen)(NTP)^{4-}$, and $(phen)_2(NTP)^{4-}$. Some constants had to be estimated as described for table III in [7]; the other constants were taken from

the literature: Zn^{2+}/phen/ATP^{4-} and Zn^{2+}/bipy/ATP^{4-} systems, *cf.* table III in [7]; Mg^{2+}/phen/ATP^{4-}, *cf.* table I in [7]; Zn^{2+}/bipy/UTP^{4-} and Zn/bipy/$HUTP^{3-}$ [9]; Zn^{2+}/bipy/ITP^{4-} and Zn^{2+}/bipy/$HITP^{3-}$ [10]; Zn^{2+}/trp^{-}/ATP^{4-} [14]. The stability constants of the binary $Mg(trp)^{+}$ and the ternary $Mg(trp)(ATP)^{3-}$ complexes were estimated as follows: as the stability constants of the 1:1 complexes of Mn^{2+}, Cu^{2+} and Zn^{2+} with trp^{-} are essentially the same as with ala^{-} [14], despite the small difference in basicity between these two ligands, we estimate $\log K^{Mg}_{Mg(trp)} \simeq \log K^{Mg}_{Mg(ala)} = 1.96$ ($I = 0.075$ M; 25°) [18]. Furthermore, as $\Delta\log K_{Zn} = \log K^{Zn(ATP)}_{Zn(ATP)(trp)} - \log K^{Zn}_{Zn(trp)} = -0.18$ [14] we estimate $\log K^{Mg(ATP)}_{Mg(ATP)(trp)} \simeq \log K^{Mg}_{Mg(trp)} + \Delta\log K_{Zn} = 1.96 - 0.18 \simeq 1.8$. The value for $\log K^{Mg}_{Mg(ATP)}$ (4.24) was taken from [7]. As under the conditions employed the degree of formation of the ternary Zn^{2+} complexes is high (70–80%), the calculated degree of formation is affected very little by possible errors in the stability constants. The degree of formation of the ternary Mg^{2+} complexes is, however, much lower (~ 30%).

3. Results and Discussion. – The binary adducts between the ligands were first studied by ^{1}H-NMR. as the extrapolated chemical shifts in the stacked binary complexes (the 'limiting shifts') are needed for comparison with the chemical shifts of the ternary metal-containing systems, in order to estimate the intramolecular constant K' of equilibrium (1).

3.1. L-*Tryptophan/ATP^{4-} and other binary systems.* The H–C(2) and H–C(8) and the ribose H–C(1′) of ATP^{4-} (a displayed formula with numbered atoms is shown in [7]) are shifted upfield in the presence of L-tryptophan. This enantiomer was used in the present study as it is more than six times as soluble as DL-trp used earlier [14], and the lower solubility (0.013 M) would make a NMR. study difficult. The ^{1}H-NMR. spectra of ATP^{4-} and of ATP^{4-}/trp are shown in *Figure 2.* The shift of H–C(2), H–C(8) and H–C(1′) is much less than in the presence of the same concentrations of phenanthroline, but is similar to that with bipyridyl [7]. The variation of the upfield shifts of H–C(1′), H–C(2), and H–C(8) of ATP^{4-} with increasing L-tryptophan concentration is shown in *Figure 3.* Computer fitting of these data gives a stability constant for $(trp)(ATP)^{4-}$ of $K^{(ATP)}_{(ATP)(trp)} = 6.2 \pm 1.8\ M^{-1}$ *(Table 1)*, and the extrapolated upfield shifts of $(trp)(ATP)^{4-}$ which are given in *Table 2.*

Table 1. *Stability constants,* K^{A}_{AB}, *of some binary stacked adducts of nucleotides*[a])

Complex (AB)	^{1}H-NMR.			Spectrophotometric (25°)		
	Ref.	pD	K^{A}_{AB}	K^{A}_{AB}	I/pH	Ref.
$(phen)(ATP)^{4-}$	[7]	8.5	28.2 ± 4.7[c])	15.5[d])	0.1 ($NaClO_4$)/~8	[7]
$(bipy)(ATP)^{4-}$	[7]	8.5	8.1 ± 2.6	8.1 ± 4.1[e])	0.04–0.8/~7	[19]
$(bipy)(ITP)^{4-}$	[b])	8.0	8.8 ± 1.8	27.5 ± 6.3	0.03–0.7/~7	[19]
$(bipy)(UTP)^{4-}$	[9]	8.3	~1[f])	-[g])	-	[9]
$(trp)(ATP)^{4-}$	[b])	8.3	6.2 ± 1.8	117 ± 76	0.5 ($NaClO_4$)/8.0	[14]
				19	0.15–0.4/8	[20]

[a]) The constants were determined by ^{1}H-NMR. in D_2O at 27° ($I = 0.1$ M, $NaNO_3$); for comparison the corresponding constants determined spectrophotometrically are also given.

[b]) This work. All the errors given are three times the standard error of the mean value.

[c]) For comparison Zn(phen)(adenosine)$^{2+}$ was also studied: $K^{Zn(phen)}_{Zn(phen)(aden)} = 14.1 \pm 3.1$ (pD 6.6; $I = 0.1 - 0.36$ M; 27°)[b,d]).

[d]) $K^{(phen)}_{(phen)(aden)} = 21.4 \pm 2.5$ (pH ~ 8; $I = 0.1$ M, $NaClO_4$; 25°) [7].

[e]) $K^{Zn(bipy)}_{Zn(bipy)(aden)} = 12.9 \pm 8.0$ (pH ~ 6; $I = 0.1$ M, $NaClO_4$; 25° [10].

[f]) $K^{(bipy)}_{(bipy)(uridine)} \simeq 2.5$ (pD 7.9; $I = 0.1$ M, $NaNO_3$; 27°) [9][g]).

[g]) $K^{(bipy)}_{(bipy)(uridine)} \simeq 3$ (pH 7.5; $I = 0.1$ M, $NaClO_4$; 25°) [9]; $K^{Zn(bipy)}_{Zn(bipy)(uridine)} \simeq 1.0$ (pH 7; $I = 0.1$ M, $NaClO_4$; 25°) [9].

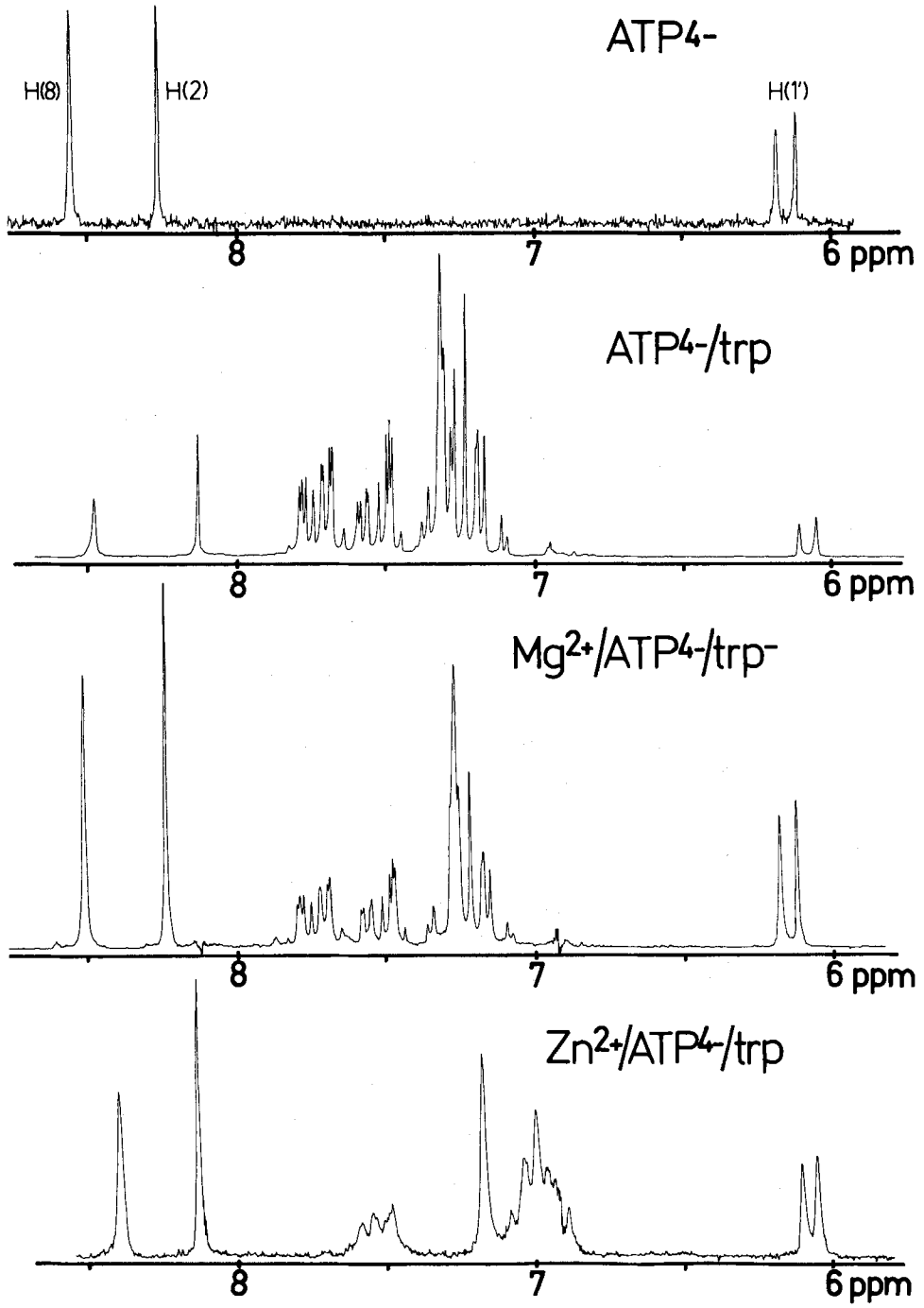

Fig. 2. *^{1}H-NMR. spectra of tryptophan/ATP^{4-} systems:* ATP^{4-} (0.01M) at pD 8.2; ATP^{4-} (0.01M) and L-tryptophan (0.05M) at pD 8.3; Mg^{2+} (0.01M), ATP^{4-} (0.01M), and L-tryptophan (0.01M) *at* pD 10.4; Zn^{2+} (0.01M), ATP^{4-} (0.01M), and L-tryptophan (0.01M) at pD 9.3. The spectra were measured relative to $(CH_3)_4N^+NO_3^-$ (0.002M) and converted to ppm relative to sodium trimethylsilylpropanesulfonate by adding 3.188 ppm (90.025 MHz; 27°; I = 0.1M, $NaNO_3$ in D_2O)

This constant is considerably lower (see *Table 1*) than the values found in two spectrophotometric studies [14] [20] which were hampered by the high absorption of the system. Moreover, at the high ionic strength used in one study [14], the ATP is predominantly present as $Na(ATP)^{3-}$, and at the high concentrations required to give a significant change in the absorption, considerable self-stacking of $Na(ATP)^{3-}$ occurs [21] [22]. Under the conditions of the present ^{1}H-NMR. experiment ($[ATP^{4-}] = 0.01$ M) less than 2% of the ATP^{4-} is self-stacked [21], and no evidence could be found in the ^{1}H-NMR. spectrum for self-stacking of L-tryptophan under the conditions used.

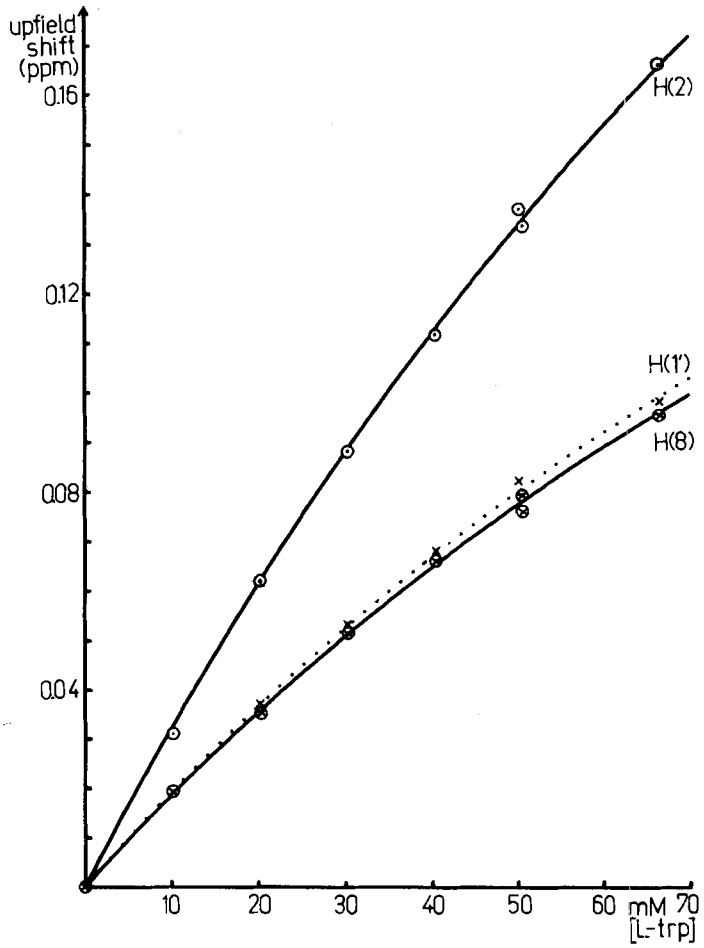

Fig. 3. *Upfield shifts of the resonances of H–C(1')* (×), *H–C(2)* (⊙), *and H–C(8)* (⊗) *of* ATP^{4-} (0.01 M) *in the presence of an increasing concentration of* L-*tryptophan*, compared with the resonance positions in the same concentration of ATP^{4-} itself (90.025 MHz; 27°; I = 0.1 M, $NaNO_3$ in D_2O; pD = 8.3). The curves shown are the computer calculated best fit of the experimental data

For the binary purine systems phen/ATP^{4-} and bipy/ATP^{4-}, it has previously [7] been possible not only to determine the stability constants (see *Table 1*) of the stacked complexes by ^{1}H-NMR., but also to determine the limiting shifts of H–C(1′), H–C(2), and H–C(8). These shifts are listed in *Table 2* together with those of the other binary systems. For the pyrimidine systems, bipy/UTP^{4-} and bipy/uridine, the stability constant of the stacked complexes is much lower [9] and the accuracy of the limiting shift is much less than with the purine systems.

The variation of the upfield shift of H–C(1′), H–C(2) and H–C(8) of ITP^{4-} with increasing bipyridyl concentration closely resembles that found earlier [7] for (bipy)$(ATP)^{4-}$, and the stability constants determined for these two binary adducts are also the same *(cf. Table 1)*. However, the upfield shifts and the extrapolated shifts for the completely formed (bipy)$(ITP)^{4-}$ are significantly smaller than those for (bipy)$(ATP)^{4-}$ even though the aromatic system responsible for the shift, that of bipyridyl, is the same in both systems.

It has been observed earlier [9] [10] by spectrophotometric measurements that the stability of the binary adducts (bipy)(adenosine) and (bipy)(uridine) is only slightly altered by the coordination of a metal ion to bipyridyl (*cf.* also footnotes of *Table 1*). However, as the ring current of bipyridyl or phenanthroline might be affected by such a coordination, we have now also studied by ^{1}H-NMR. the Zn(phen)$^{2+}$/adenosine system. Although its stability constant, $K^{Zn(phen)}_{Zn(phen)(adenosine)} = 14.1 \pm 3.1$, is smaller than that for (phen)$(ATP)^{4-}$ ($K^{(phen)}_{(phen)(ATP)} = 28.2 \pm 4.7$; *Table 1*) the limiting shifts *(Table 2)* are fairly similar, averaging 17% more for Zn(phen)-(adenosine)$^{2+}$ than for (phen)$(ATP)^{4-}$. Hence we may conclude that the shifts determined for the binary stacked adducts are probably approximately the same as for a completely stacked ternary complex and may thus be used for comparison with those of the ternary systems. For each of the binary systems studied, the shifts of H–C(8), H–C(2) and H–C(1′) give, within experimental error, the same stability constant.

An overall view on the stability constants listed in *Table 1* reveals that the phenanthroline adducts are more stable than those of bipyridyl, and that the purines ATP and ITP give much more stable adducts than the pyrimidine UTP. As might be expected from the size of the aromatic ring system, tryptophan resembles bipyridyl rather than phenanthroline. It may be added in this connection that in the complexes (bipy)$(ATP)^{4-}$ and (bipy)$(ITP)^{4-}$ the resonances of the protons of bipyridyl are also shifted upfield by the ring current of the nucleotide: the shift with ITP^{4-} is only ~60% of that with ATP^{4-}, in good agreement with the ratio of the calculated [23] ring current effects for the nucleic bases adenine and hypoxanthine, 1:0.59.

3.2. *Zn^{2+} or Mg^{2+}/L-tryptophanate/ATP^4 and other ternary systems.* For ^{1}H-NMR. studies of the upfield shift due to stacking only diamagnetic metal ions are suitable and we have selected Zn^{2+} and Mg^{2+} for two reasons: (i) these two metal ions are of biological interest, and (ii) the stability constants necessary for the calculation of the formation degree of the complexes are available for the ternary systems [7] [9] [10] [14] [24]. As we wanted to concentrate on the position of the intramolecular equilibrium (1), we selected the ternary systems containing

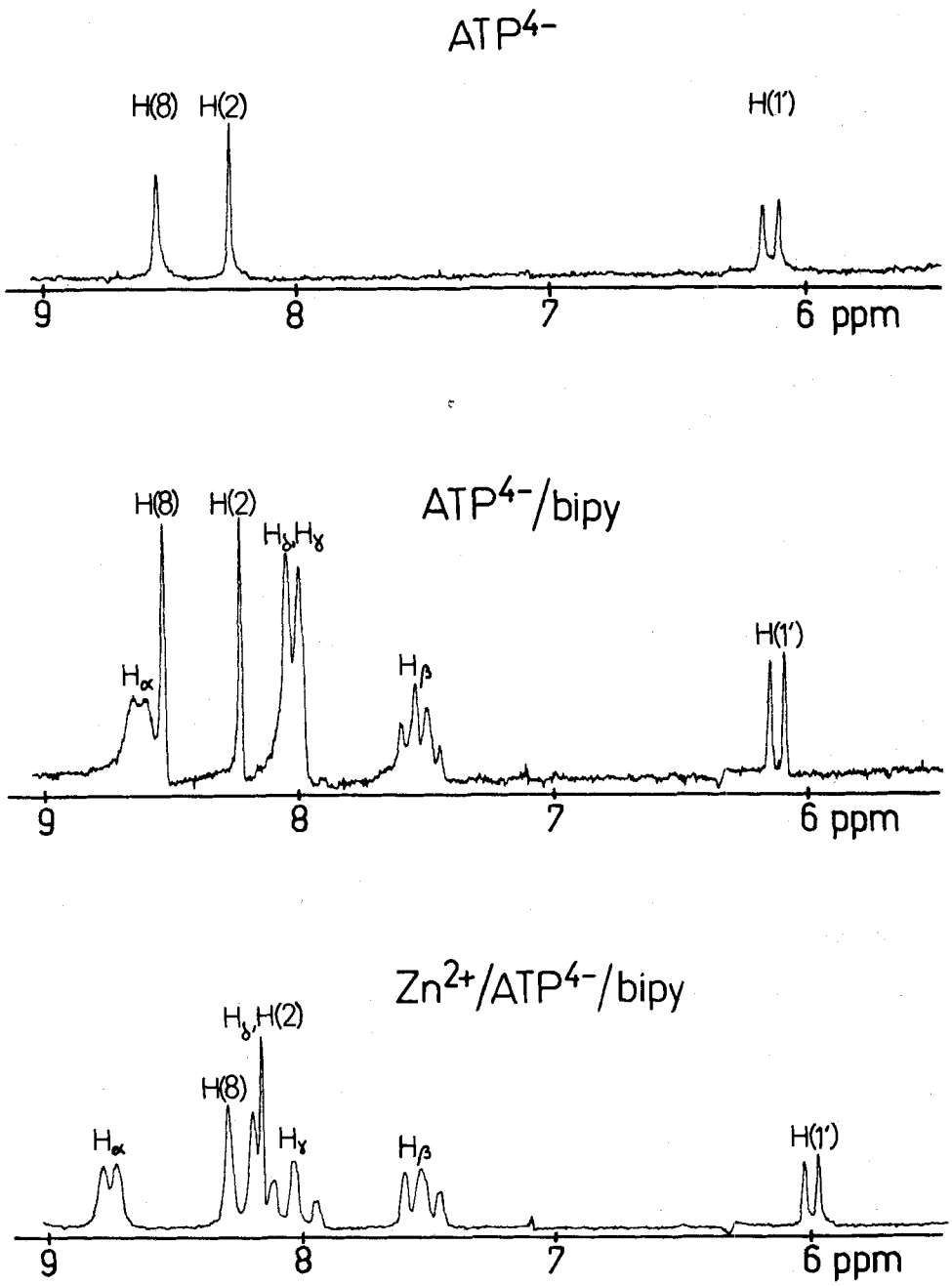

Fig. 4. *^{1}H-NMR. spectra of 0.01 M solutions of ATP^{4-}, of ATP^{4-}/2,2'-bipyridyl, and of Zn^{2+}/ATP^{4-}/2,2'-bipyridyl*, measured relative to $(CH_3)_4N^+NO_3^-$ (0.004 M) and converted to ppm relative to sodium trimethylsilylpropanesulfonate by adding 3.188 ppm (90.025 MHz; 27°; I = 0.1 M, $NaNO_3$ in D_2O; pD = 8.0)

Zn^{2+}/bipy, Mg^{2+}/phen, Zn^{2+}/phen, and Zn^{2+}/trp, for which an aromatic-ring stacking, analogous to I of *Figure 1*, had been shown to occur: the natural Mg^{2+}/trp/ATP^{4-} system was expected to resemble the corresponding Zn^{2+} system.

Addition of one equivalent of Mg^{2+} to a 1:1 solution of ATP^{4-} and tryptophanate (0.01 M) also leads to a small, but significant upfield shift of these resonances *(Fig. 2)*. This upfield shift is considerably more pronounced with Zn^{2+}, as $Zn(trp)(ATP)^{3-}$ is much more stable than $Mg(trp)(ATP)^{3-}$. For comparison, the corresponding ^{1}H-NMR. spectra of ATP^{4-} with bipy and Zn^{2+}/bipy are shown in *Figure 4*. The overall picture resembles closely the one with trp, but the increased formation of a stacked species by linking the two ligands together by coordinating a metal ion is even more evident.

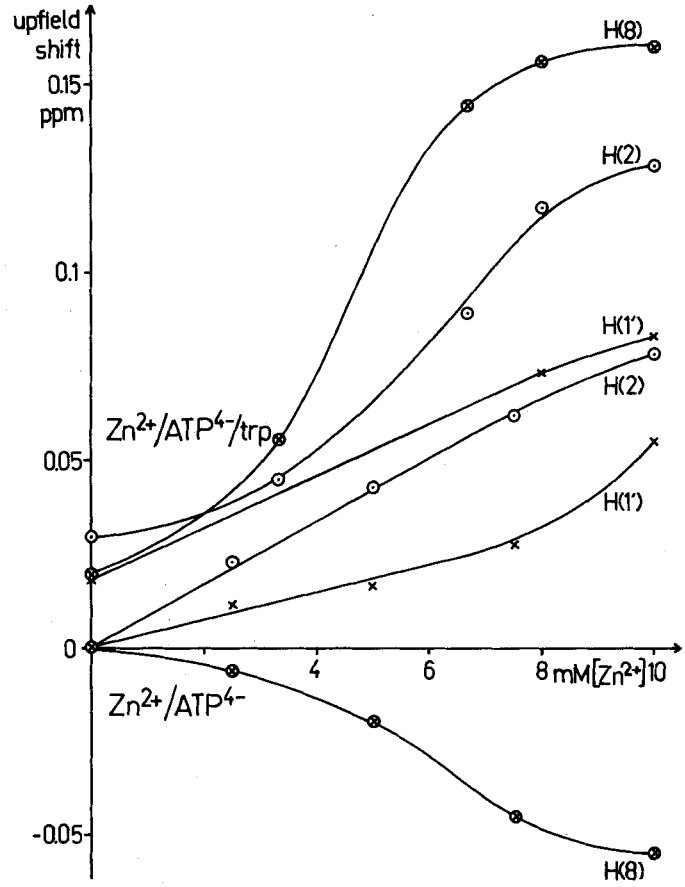

Fig. 5. *Upper curves: upfield shift of the resonances of H–C(1')* (×), *H–C(2)* (⊙), *and H–C(8)* (⊗) *of* ATP^{4-} *in* ATP^{4-}/L-*tryptophan* (each 0.01 M; pD 9.5) compared with those of the same protons in ATP^{4-} itself, with increasing [Zn^{2+}]. *Lower curves: upfield shift of the resonances of H–C(1')* (×) *and H–C(2)* (⊙), *and downfield shift of the resonance of H–C(8)* (⊗) *of* ATP^{4-} (0.01 M; pD 7.4) *with increasing* [Zn^{2+}] (90.025 MHz; 27°; I = 0.1 M, $NaNO_3$ in D_2O)

The variation of the chemical shift of the ATP^{4-} resonances in a trp/ATP^{4-} 1:1 mixture as the Zn^{2+} concentration is increased is shown in the upper part of *Figure 5*. The increasing upfield shifts confirm the stacking within the Zn(trp)-$(ATP)^{3-}$ complex, because in aromatic systems the ring current shifts those protons which lie above an aromatic ring upfield [25], whereas coordination of a diamagnetic metal ion usually shifts the resonances of nearby groups downfield: in the binary complex $Zn(ATP)^{2-}$ the resonance of H–C(8) is shifted downfield and those of H–C(2) and H–C(1′) upfield (*Fig. 5*, lower part)[3]).

It was recently proposed [16] that the Zn^{2+}/N(7) interaction, present [27–29] in $Zn(ATP)^{2-}$, is maintained in the stacked isomer of Zn(trp)$(ATP)^{3-}$. This suggestion clearly conflicts with the fact that in the ternary Zn/trp/ATP^{4-} system the resonance of H–C(8) is the one which is *most* strongly shifted upfield (*cf.* top curve in *Fig. 5*). The formation of the stacked isomer of Zn(trp)$(ATP)^{3-}$ is obviously connected with a disruption of the Zn^{2+}/N(7) interaction, otherwise the signal of H–C(8) should be shifted either downfield, or not at all. Thus the ^{1}H-NMR. spectrum further confirms the structure suggested earlier [14] in which there is no interaction between the metal ion and N(7) for the *stacked* isomer of Zn(trp)-$(ATP)^{3-}$ (*cf.* I in *Fig. 1*).

3.3. *The intramolecular stacking equilibrium.* Experimental results such as those depicted in the upper part of *Figure 5*, together with the computed formation degree of the corresponding ternary complexes (see Experimental Part), allow accurate calculation of the shifts due to the resonances of H–C(1′), H–C(2) and H–C(8) of ATP^{4-} and ITP^{4-}, and of H–C(1′), H–C(5) and H–C(6) of UTP^{4-}, in the ternary complexes M(phen)$(ATP)^{2-}$, Zn(bipy)$(NTP)^{2-}$, and M(trp)$(ATP)^{3-}$. These shifts, together with those calculated for the signals of the corresponding protons in the binary stacked adducts (phen)$(ATP)^{4-}$, (bipy)$(NTP)^{4-}$, and (trp)$(ATP)^{4-}$, are listed in *Table 2*.

It is noticeable that for the binary complexes H–C(2) is shifted more than H–C(8) whereas the reverse is true for the ternary complexes. This change in the relative size of the shifts of H–C(2) and H–C(8) on formation of the ternary complexes is to be expected because in the binary adducts the two aromatic moieties are free to interact in the orientation that results in the highest stability of the adduct. This flexibility is considerably reduced once these two ligands are bridged by coordination to a metal ion, and thus the orientation of the two aromatic moieties forming the stack is expected to be somewhat different in the ternary complexes: this affects the chemical shifts of the protons. H–C(2) and H–C(8) are affected most (see *Table 2*) as these protons are directly bound to the stacked

---

[3]) It was recently observed [21] [22] that $Mg(ATP)^{2-}$ self-stacks somewhat better than ATP^{4-}, *i.e.* Mg^{2+} promotes the self-stacking of ATP^{4-}. These observed upfield shifts of H–C(2) and H–C(1′) in $Zn(ATP)^{2-}$ (see also [7] [10] [26]) would thus be due to the enhancement of the self-stacking of ATP^{4-} by Zn^{2+}, while the downfield shift of H–C(8) results from the known [27–29] interaction between Zn^{2+} and N(7) of the adenine moiety in $Zn(ATP)^{2-}$. Furthermore, in an experiment [7] with Mg^{2+}/ATP^{4-}, under the same conditions as the one shown in the lower part of *Figure 5*, H–C(1′), H–C(2) *and* H–C(8) are shifted upfield [21] which is in accordance with the Mg^{2+} promoted self-stacking [21] [22] of ATP^{4-} and the *absence* [27–29] of a significant Mg^{2+}/N(7) interaction.

Table 2. *Calculated upfield shifts* (in ppm) *for complete complexation, approximate percentages of the stacked isomer of the ternary Zn^{2+} and Mg^{2+} complexes, and intramolecular constant K′ of equilibrium (1)*

Complex	Upfield shifts of				% stacked isomer[d]	K'
	H–C(2)[b]	H–C(8)[b]	Average[b][c]	H–C(1′)[b]		
(phen)(ATP)$^{4-}$	0.80[e]	0.47[e]	0.64	0.46[e]	–	–
Zn(phen)(ATP)$^{2-}$	0.46	0.64	0.55	0.45	⩾95	⩾20
	(58%)	(136%)	(86%)	(98%)		
Mg(phen)(ATP)$^{2-}$	0.59	~0.8[f]	0.69	0.48	100	⩾20
	(74%)	(~170%)	(108%)	(104%)		
(bipy)(ATP)$^{4-}$	0.50	0.27	0.38	0.36	–	–
Zn(bipy)(ATP)$^{2-}$	0.11	0.31	0.21	0.20	55	1.2
	(22%)	(115%)	(55%)	(56%)		
(bipy)(ITP)$^{4-}$	0.31	0.14	0.22	0.24	–	–
Zn(bipy)(ITP)$^{2-}$	0.08	0.15	0.11	0.11	48	0.9
	(26%)	(107%)	(50%)	(46%)		
(bipy)(UTP)$^{4-}$	0.39[g]	0.37[h]	–[i]	0.39	–	–
Zn(bipy)(UTP)$^{2-}$	0.59[g]	0.31[h]	–[i]	0.15	~40	~0.7
	(151%)	(84%)		(38%)		
Zn(bipy)(HUTP)$^{-}$	0.56[g]	0.35[h]	–[i]	0.16	~40	~0.7
	(144%)	(95%)		(42%)		
(trp)(ATP)$^{4-}$	0.59	0.34	0.47	0.35	–	–
Zn(trp)(ATP)$^{3-}$	0.18	0.23	0.20	0.11	35	0.5
	(31%)	(68%)	(43%)	(31%)		
Mg(trp)(ATP)$^{3-}$	0.11	0.18	0.14	0.055	~20	~0.3
	(19%)	(53%)	(30%)	(16%)		

a) The measurements were carried out in D_2O at 27° (I = 0.1 M, $NaNO_3$).
b) The shifts in the ternary systems, as a percentage of the shift in the corresponding binary metal-free system, are given in parentheses.
c) The average shift of H–C(2) and H–C(8) is discussed in the text.
d) The values given above are our best estimates of the percentage of the stacked isomer, based mainly on the shift of H–C(1′): for the systems with phen/ATP, bipy/ATP and bipy/ITP, the *average* of the shifts of H–C(2) and H–C(8) gives the same result; for the bipy/UTP systems the shifts of H–C(5) and H–C(6) are anomalous and do not give meaningful results.
e) The limiting shifts for Zn(phen)(adenosine)$^{2+}$ are 0.52, 1.01 and 0.52 ppm for H–C(1′), H–C(2) and H–C(8), respectively.
f) The resonance of H–C(8) was broadened (see [7]).
g) Shift of H–C(5) of the pyrimidine moiety.
h) Shift of H–C(6) of the pyrimidine moiety.
i) The average shifts of H–C(5) and H–C(6) in the ternary systems, 0.45 ppm, gave no meaningful results.

purine moiety; the ribose proton H–C(1′) which is much further away seems to be little influenced by the different orientation of the aromatic rings in the stack. This is confirmed by the similarity between the shifts of H–C(1′) for the binary (phen)(ATP)$^{4-}$ and the ternary Zn(phen)(ATP)$^{2-}$ and Mg(phen)(ATP)$^{2-}$ complexes.

The percentage of the stacked isomer present in the ternary complexes can therefore be estimated from a comparison of the relative shifts of H–C(1′) in the

ternary complexes with those in the corresponding binary complexes *(Table 2)*. Owing to the change in orientation, the shifts of H–C(2) and H–C(8) cannot be used directly in this way. However it seems plausible that a larger influence of the ring current of the second ligand on H–C(8) is accompanied by a smaller influence on H–C(2), and *vice versa,* as the two rings in the stack are moved over each other or rotated; the average shift of H–C(2) and H–C(8) should therefore be less sensitive to different orientations of the stack in the binary adduct and in the ternary complexes. Indeed, the percentages of the stacked isomer of the ternary complexes calculated using the average shift of H–C(2) and H–C(8) agree reasonably well with those based on the shifts of H–C(1′) *(Table 2)*.

From the percentage of the stacked isomer (I in *Fig. 1* or its analogues) present in equilibrium with the open form (II, or analogues) the dimensionless equilibrium constant K' for the intramolecular equilibrium (1) between the two isomers of $M(trp)(ATP)^{3-}$ and the other ternary complexes can be estimated. The corresponding equilibrium constants K' are listed in *Table 2*. It is interesting to note that the order of stability of the binary metal-free stacked adducts $(phen)(ATP)^{4-} > (bipy)(ATP)^{4-} \simeq (bipy)(ITP)^{4-} > (bipy)(UTP)^{4-}$ (see *Table 1*) coincides with the percentage of the ring closed isomer in the corresponding ternary complexes *(Table 2) i.e.* with the trend in the size of K'. This is not unexpected as the equilibria between the two free aromatic ligands and the binary stacked complexes are very similar to those between the two coordinated aromatic ligands in the open form of the ternary complexes giving the stacked ternary complexes.

The results obtained for $Mg(phen)(ATP)^{2-}$ are in excellent agreement with the observation [7] that this ternary complex is about nine times more stable than expected on a statistical basis: the ternary complex $Zn(trp)(ATP)^{3-}$ is only about four times more stable [14] [16] than $Zn(ala)(ATP)^{3-}$, and the proportion of the ring closed isomer in $Zn(trp)(ATP)^{3-}$ is much lower than with $Mg(phen)(ATP)^{2-}$. The lower values of K' for $M(trp)(ATP)^{3-}$ compared with those for $Zn(bipy)(ATP)^{2-}$ and $Zn(bipy)(ITP)^{2-}$ (the stabilities of the corresponding binary adducts $(trp)(ATP)^{4-}$, $(bipy)(ATP)^{4-}$ and $(bipy)(ITP)^{4-}$ are the same) are probably due to the larger flexibility of the indole moiety in $M(trp)(ATP)^{3-}$ compared with the rigidly coordinated bipyridyl.

4. Conclusions. - Although the results assembled in *Table 2* should only be considered as estimates for the position of the intramolecular equilibrium (1) in the ternary complexes, they clearly indicate that in all the systems, including the ternary $Mg(trp)(ATP)^{3-}$ complex, a considerable amount of the stacked isomer (I in *Fig. 1* and analogues) is present. This aromatic-ring stacking is the sole cause for the increased stability of the ternary Mg^{2+} complexes, while in the case of Zn^{2+} other factors (which have been discussed earlier [5–7] [10] [12]) may also be important. However, we observe here cooperative effects which result in an enhanced stability of the ternary complexes.

The cooperation resulting from a ligand-ligand interaction resembles that observed for enzyme/substrate/metal ion systems and indicates that stacking may be a cause of some of the cooperative effects [30] seen in natural systems [31–34]. It has recently been observed that intramolecular hydrophobic interactions

in ternary complexes [35] may occur between an aromatic moiety and aliphatic side chains of carboxylates [36] and amino acids [37], again resulting in cooperative effects. An equilibrium between an open and closed form (analogous to equilibrium (1)) also exists for these ternary complexes. As these intramolecular equilibria may be shifted to the one or to other side by a change in the hydrophilicity of the medium, nature has here a tool to expose cyclically first one and then the other part of a mixed ligand complex for a reaction with a substrate.

We thank Mr. *K. Aegerter* for recording the 90 MHz-^{1}H-NMR. spectra, and the *Swiss National Science Foundation* for a research grant.

REFERENCES

[1] *B.E. Fischer & H. Sigel,* Inorg. Chemistry *18*, 425 (1979).
[2] *D.D. Perrin & R.P. Agarwal,* Met. Ions Biol. Syst. *2*, 167 (1973).
[3] *H. Sigel,* Ed., 'Mixed-Ligand Complexes', Vol. 2 of 'Met. Ions Biol. Syst.', Marcel Dekker, Inc., New York and Basel 1973.
[4] *H. Sigel,* Met. Ions Biol. Syst. *2*, 63 (1973).
[5] *H. Sigel,* Angew. Chemie *87*, 391 (1975); Int. Ed. *14*, 394 (1975).
[6] *H. Sigel, B.E. Fischer & B. Prijs,* J. Amer. chem. Soc. *99*, 4489 (1977).
[7] *P.R. Mitchell & H. Sigel,* J. Amer. chem. Soc. *100*, 1564 (1978).
[8] *J. Granot,* J. Amer. chem. Soc. *100*, 2886 (1978).
[9] *Y. Fukuda, P.R. Mitchell & H. Sigel,* Helv. *61*, 638 (1978).
[10] *P. Chaudhuri & H. Sigel,* J. Amer. chem. Soc. *99*, 3142 (1977).
[11] *J. Granot, M.M. Werber & A. Danchin,* Bioinorg. Chemistry *8*, 81 (1978).
[12] *E. Farkas, B.E. Fischer, R. Griesser, V.M. Rheinberger & H. Sigel,* Z. Naturforsch. *34b*, 208 (1979).
[13] *C.F. Naumann & H. Sigel,* FEBS Letters *47*, 122 (1974).
[14] *H. Sigel & C.F. Naumann,* J. Amer. chem. Soc. *98*, 730 (1976).
[15] *R. Basosi, E. Gaggelli & E. Tiezzi,* J. chem. Res. (S) *1977*, 278.
[16] *J.-J. Toulmé,* Bioinorg. Chemistry *8*, 319 (1978).
[17] *P.K. Glasoe & F.A. Long,* J. phys. Chemistry *64*, 188 (1960).
[18] *C.B. Monk,* Trans. Faraday Soc. *47*, 297 (1951).
[19] *C.F. Naumann & H. Sigel,* J. Amer. chem. Soc. *96*, 2750 (1974).
[20] *F. Morita,* Biochim. biophys. Acta *343*, 674 (1974).
[21] *P.R. Mitchell & H. Sigel,* European J. Biochemistry *83*, 149 (1978).
[22] *M.P. Heyn & R. Bretz,* Biophys. Chemistry *3*, 35 (1975).
[23] *C. Giessner-Prettre & B. Pullman,* C.r. hebd. Séances Acad. Sci. *261*, 2521 (1965).
[24] *H. Sigel,* J. inorg. nucl. Chemistry *39*, 1903 (1977).
[25] *L.M. Jackman & S. Sternhell,* 'Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry', 2nd ed., Pergamon, Oxford, p.94, 1969.
[26] *T.A. Glassman, C. Cooper, G.P.P. Kuntz & T.J. Swift,* FEBS Letters *39*, 73 (1974).
[27] *M. Cohn & T.R. Hughes, Jr.,* J. biol. Chemistry *237*, 176 (1962).
[28] *J.A. Happe & M. Morales,* J. Amer. chem. Soc. *88*, 2077 (1966).
[29] *R.B. Martin & Y.H. Mariam,* Met. Ions Biol. Syst. *8*, 57 (1979).
[30] *E. Frieden,* J. chem. Educ. *52*, 754 (1975).
[31] *E. Breslow & A.W. Girotti,* J. biol. Chemistry *245*, 1527 (1970).
[32] *E. Breslow,* Met. Ions Biol. Syst. *3*, 133 (1974).
[33] *G.H. Reed & M. Cohn,* J. biol. Chemistry *247*, 3073 (1972).
[34] *D.H. Buttlaire & M. Cohn,* J. biol. Chemistry *249*, 5741 (1974).
[35] *P.R. Mitchell & H. Sigel,* Angew. Chemie *88*, 585 (1976); Int. Ed. *15*, 548 (1976).
[36] *P.R. Mitchell,* J. chem. Soc. Dalton Trans. *1979*, 771.
[37] *B.E. Fischer & H. Sigel,* Abstracts of XIXth Internat. Conf. Coord. Chem. (Prague), 42a, 1978.