52. Intramolecular Stacking in Ternary Complexes Containing Uridine 5′-Triphosphate, 2,2′-Bipyridyl, and a Divalent Metal Ion[1])

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Summary

The stability constants of the ternary complexes containing UTP, 2,2′-bipyridyl (bipy), and Co^{2+}, Ni^{2+}, Cu^{2+}, or Zn^{2+} (M^{2+}) have been determined by potentiometric titrations *(Table 1)*. Changes in stability are quantified by $\Delta\log K_M = \log K^{M(bipy)}_{M(bipy)(UTP)} - \log K^{M}_{M(UTP)}$. For the Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} systems $\Delta\log K_M$ is 0.10, −0.13, 0.36, and 0.15, respectively. All these ternary complexes are considerably more stable than would be expected on statistical grounds; indeed, for Co^{2+}, Cu^{2+}, and Zn^{2+}, UTP^{4-} binds more tightly to $M(bipy)^{2+}$ than to M^{2+}. An UV. difference spectroscopic study suggests that stacked adducts between bipyridyl and the pyrimidine moiety of uridine are formed. ^{1}H-NMR. studies of the bipy/uridine, bipy/UTP, and bipy/UTP/Zn^{2+} systems *(Figs. 1* and *2)* confirm the presence of stacking in the binary adducts and in the ternary complex. There is also evidence for the existence of the stacked protonated complex, $Zn(bipy)(HUTP)^-$, with the proton at the γ-phosphate group. The acidity constant of this ternary complex has been measured *(Fig. 3)*. The observed stability enhancement of stacked adducts by the formation of a metal ion bridge is discussed *(Fig. 4)* and biological implications are indicated.

1. Introduction. – In a ternary complex not only may *indirect* interactions between the ligands be mediated by the metal [2] [3], and competition for the available coordination sites cause displacement of some donor atoms [4], but *direct* intramolecular ligand-ligand interactions may also influence the stability and reactivity of the ternary complex considerably. For example, an intramolecular aromatic ring stacking between the purine and indole residues occurs in the mixed-ligand complexes of ATP, tryptophan, and Mn^{2+}, Cu^{2+}, or Zn^{2+} [5]. Similarly, the complexes formed by bipyridyl, Co^{2+}, Ni^{2+}, Cu^{2+}, or Zn^{2+}, and ATP or ITP can be considered as metal ion bridged stacked bipyridyl-purine adducts [6][2]).

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[1]) Part XXIX of the series 'Ternary Complexes in Solution'; for parts XXVIII and XXVII see [1] and [2], respectively.

[2]) Abbreviations: ATP, adenosine 5′-triphosphate; bipy, 2,2′-bipyridyl; ITP, inosine 5′-triphosphate; phen, 1,10-phenanthroline; UTP, uridine 5′-triphosphate.

As stacking interactions in mixed-ligand complexes have so far only been investigated with purine-nucleotides [1] [5] [6], we have now studied similar systems with pyrimidine nucleotides, the ternary UTP/bipyridyl/M^{2+} systems. We have used the unnatural ligand bipyridyl as a second ligand, because the characterization of the mixed-ligand systems is better with this ligand [6] than for example with the naturally occurring tryptophanate [5].

The stability constants in the ternary systems UTP/bipyridyl/M^{2+} were determined by potentiometric titrations. The occurrence of stacking in these systems, both with and without a metal ion, was confirmed by ^{1}H-NMR. studies, while UV. spectra of the uridine/bipyridyl system suggested a pyrimidine-bipyridyl interaction. There is also ^{1}H-NMR. evidence for the formation of phosphate-protonated complexes, M (bipy) $(HUTP)^-$, in which stacking also occurs.

2. Experimental Part. – 2.1. *Materials.* The trisodium salt of uridine 5′-triphosphate (Type III) from *Sigma Chemical Co.*, St. Louis, Mo., U.S.A., (98% pure) was used for the potentiometric titrations, for the ^{1}H-NMR. studies and for some spectrophotometric measurements. All the other materials were the same as described recently [1] [6].

2.2. *Determination of equilibrium constants by potentiometric titrations.* These were carried out as described in detail recently [6], except that the volume of solution was only 25 ml, *i.e.* the concentrations were twice those given in [6] ($I = 0.1$, $NaClO_4$; 25°). The constants were calculated from at least three independent titration curves. $\beta^{M}_{M(bipy)(UTP)}$ was computed by using the results obtained by *Anderegg* [7] for the binary M^{2+}/bipy systems.

2.3. *Determination of equilibrium constants by spectrophotometric measurements.* UV. difference spectra were measured at 25° ($I = 0.1$, $NaClO_4$) using a *Varian Techtron* spectrophotometer (Model 635) as described in detail recently [1]: [bipy] $= 2 \times 10^{-4}$M; the uridine concentration was varied from 0.01 to 0.15M. The stability constant of the stacked adduct between uridine and bipy was calculated by the *Benesi-Hildebrand* method [8]. Some preliminary experiments on the UTP^{4-}/bipy, Zn^{2+}/UTP/bipy and Cu^{2+}/UTP/bipy systems were carried out as described recently [6].

2.4. ^{1}*H-NMR. measurements.* Spectra were recorded in D_2O on a *Bruker* WH-90 FT spectrometer (90.025 MHz) using the center peak of the resonance of the tetramethylammonium ion (0.002 to 0.004M) as reference: chemical shifts were converted to a trimethylsilylpropane sulfonate reference by adding 3.188 ppm. The pD was adjusted to the desired value ±0.05 by 'dotting' with a fine glass rod; quoted pD values were obtained by adding 0.40 to the pH meter reading [9].

3. Results and Discussion. – 3.1. *Stability of the binary and ternary UTP metal ion complexes.* The stability constants of the binary systems with UTP and Co^{2+}, Ni^{2+}, Cu^{2+} or Zn^{2+}, and of the ternary systems which contained bipyridyl were determined by potentiometric titrations and are given in *Table 1.* Our stability constant of $Co(UTP)^{2-}$ is in excellent agreement with $\log K^{Co}_{Co(UTP)} = 4.55$ as determined by *Walaas* [11] from ion exchange studies. The present values also compare well with the corresponding constants of other nucleoside 5′-triphosphates [12]; this is expected as the basicity of the terminal phosphate group is, to a first approximation, independent of the base moiety.

The change in stability of such mixed-ligand complexes can be quantified by comparison of the stabilities of the binary and ternary complexes [3]:

$$\begin{aligned} \Delta \log K_M &= \log K^{M(bipy)}_{M(bipy)(UTP)} - \log K^{M}_{M(UTP)} \\ &= \log K^{M(UTP)}_{M(UTP)(bipy)} - \log K^{M}_{M(bipy)} \end{aligned} \qquad (1)$$

e.g. comparing the difference in stability, for the reaction of UTP^{4-} with $M(bipy)^{2+}$ or $M(aq)^{2+}$; $\Delta\log K_M$ is identical with the constant of equilibrium (2):

$$M(UTP)^{2-} + M(bipy)^{2+} \rightleftharpoons M(bipy)(UTP)^{2-} + M^{2+} \qquad (2)$$

In general one expects *negative* values for $\Delta\log K_M$, because usually $K^M_{ML} > K^{ML}_{ML_2}$. The statistical values for the coordination of two different bidentate ligands to a regular and to a distorted octahedral coordination sphere are $\Delta\log K_{oh} = -0.4$ and $\Delta\log K_{do} \sim -0.9$, respectively [3]. Thus we conclude from the results of *Table 1* that all these ternary complexes are more stable than expected; indeed the mixed-ligand complexes are by far the dominant complexes in solution [12].

Table 1. *Logarithms of the stability constants of the ternary complexes $M(bipy)(UTP)^{2-}$ and of the corresponding binary complexes $M(bipy)^{2+}$ and $M(UTP)^{2-}$ determined by potentiometric titrations*[a])

M^{2+}	$\log K^M_{M(UTP)}$[b])	$\log K^M_{M(bipy)}$[c])	$\log \beta^M_{M(bipy)(UTP)}$	$\log K^{M(UTP)}_{M(UTP)(bipy)}$	$\log K^{M(bipy)}_{M(bipy)(UTP)}$	$\Delta\log K_M$
Co^{2+}	4.53 ± 0.01	6.06 [7][d])	10.69 ± 0.02	6.16	4.63	0.10
Ni^{2+}	4.29 ± 0.01	7.13 [7][d])	11.29 ± 0.03	7.00	4.16	−0.13
Cu^{2+}	5.53 ± 0.02	8.00 [7][d])	13.89 ± 0.02	8.36	5.89	0.36
Zn^{2+}	4.75 ± 0.01	5.30 [7][d])	10.20 ± 0.02	5.45	4.90	0.15

a) 25°; $I = 0.1$, $NaClO_4$. The errors given are three times the standard error of the mean value, or the sum of the probable systematic errors, whichever is the larger.

b) Acidity constant of $H(UTP)^{3-}$: $pK^H_{H(UTP)} = 6.45 \pm 0.01$.

c) Acidity constants of $H_2(bipy)^{2+}$ and $H(bipy)^+$: $pK^H_{H_2(bipy)} = -0.2$ [10] and $pK^H_{H(bipy)} = 4.49$[d]).

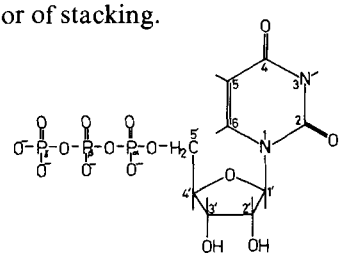
d) 20°; $I = 0.1$, $NaNO_3$. See [7] for the constants of the corresponding 1:2 complexes.

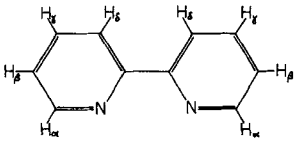
3.2. *Spectrophotometric evidence for the formation of stacking adducts between uridine and 2,2'-bipyridyl.* The formation of stacked adducts between the purine moiety of nucleosides or nucleotides and bipyridyl or phenanthroline is accompanied by a slight shift of the UV. absorption to lower energy [1] [6]. A similar change may be expected with a pyrimidine. Indeed, the spectrum of a uridine/bipy mixture, compared with that of the components, shows a slight shift to lower energy, which can be measured by recording difference spectra. The evaluation of such spectrophotometric measurements for this binary system by *Benesi-Hildebrand* plots [8] results in straight lines, indicating 1:1 complex formation. The stability constant K_{St} for the adduct between uridine and bipyridyl $\log K_{St} = 0.5 \pm 0.2$. As in the binary system no interaction other than aromatic stacking is likely, the results suggest the formation of stacked complexes between the pyrimidine moiety of uridine and bipyridyl.

Some of the experiments with the uridine/bipy system were also carried out in the presence of Ni^{2+}, Cu^{2+}, or Zn^{2+} with $[bipy] = [M^{2+}]$. The stability constants are, within the rather large experimental error, the same as for the binary adduct[3]); *i.e.* coordination of a metal ion to bipyridyl does not significantly alter the stability of its stacking adduct with uridine. Attempts to determine the stability constant for the bipy/UTP^{4-} system spectrophotometrically were unsuccessful.

3) The values obtained in the presence of Ni^{2+}, Cu^{2+}, and Zn^{2+} were $\log K_{St} = 0.4$, 0.7 and 0.0 (±0.4), respectively (25°; $I = 0.1$, $NaClO_4$).

3.3. *^{1}H-NMR. studies of the bipy/uridine and bipy/UTP^{4-} systems.* As we wished to use ^{1}H-NMR. to confirm the presence of stacking in the binary systems such as bipy/UTP^{4-} and in the ternary systems with diamagnetic metal ions, we measured the concentration dependence of the resonances of uridine and of UTP^{4-}; the self-association causes no shift up to the concentration (10^{-2} M) used in this study. The ^{1}H-NMR. spectrum of bipyridyl is more concentration dependent [13], and this limits the usefulness of shifts of the bipyridyl resonances as a quantitative indicator of stacking.





Bipyridyl shifts the pyrimidine-ring protons H–C(5) and H–C(6) and the ribose protons H–C(1′) and H–C(2′) of uridine and of UTP^{4-} *upfield (Table 2).* It is not surprising that the chemical shifts observed now are much smaller than those found earlier for purine/phen and purine/bipy systems [1] as the aromatic system of a pyrimidine is much smaller than that of a purine and the binary complex with the pyrimidine is therefore less stable.

Although the small upfield shifts observed in the bipy/uridine and bipy/UTP^{4-} systems, $\leqslant 0.04$ and $\leqslant 0.02$ ppm respectively, precluded determination of the stability constants, the results are consistent with $\log K_{St} \sim 0.3$ for bipy/uridine (*cf.* the spectrophotometric value, $\log K_{St} = 0.5 \pm 0.2$) and $\log K_{St} \sim 0$ for bipy/UTP^{4-}: this latter stability constant could not be determined spectrophotometrically (*cf.* 3.2).

Table 2. *Upfield shifts* (ppm)[a], *observed in the ^{1}H-NMR. spectrum, for the stacking between the pyrimidine moiety of uridine or of UTP^{4-} and 2,2′-bipyridyl*

Proton	uridine/bipy[b]	UTP^{4-}/bipy[c]
Pyrimidine moiety		
H–C(5)	0.046	0.022
H–C(6)	0.041	0.018
Ribose moiety		
H–C(1′)	0.038	0.021
H–C(2′)	0.03	[d]
H–C(3′)	0.02	[d]
H–C(4′)	0.01	[d]
H–C(5′)	~0	[d]

[a] [Uridine] or [UTP^{4-}] = 0.01 M; [bipy] = 0.05 M, in D_2O; 27°; $I = 0.1$, $NaNO_3$; measured using $(CH_3)_4NNO_3$ (0.002 M) as reference, compared with the resonances of uridine or of UTP^{4-} alone under the same conditions.

[b] pD = 7.9.

[c] pD = 8.3.

[d] Owing to the more complicated nature of the 2′,3′,4′, and 5′ resonances of ribose in UTP^{4-} exact measurements are difficult; however the upfield shifts are not greater than 0.01 ppm.

The relative sizes of the upfield shifts of the different protons of uridine and UTP^{4-}, H–C(6) ~ H–C(5) ~ H–C(1′) > H–C(2′) > H–C(3′) > H–C(4′) ~ H–C(5′), further confirm the presence of orientated stacking between bipyridyl and these pyrimidine derivatives, although it seems that the bipyridyl/pyrimidine adducts are somewhat less stable than the bipyridyl/purine adducts studied earlier [1] [6].

3.4. *^{1}H-NMR. study of the Zn^{2+}/bipy/UTP system.* Of the metal ions for which the stability constants of the ternary $M(bipy)(UTP)^{2-}$ complexes are known *(Table 1)*, only the diamagnetic Zn^{2+} is suitable for a ^{1}H-NMR. study. Coordination of a diamagnetic metal ion usually shifts the resonances of nearby groups downfield, whereas in aromatic systems the ring current shifts those protons which lie above an aromatic ring upfield [14]; *e.g.* the upfield shift of the ATP resonances in bipy/ATP [6] or phen/ATP [1] mixtures on addition of Zn^{2+} confirmed the presence of stacking in the ternary complexes $Zn(bipy)(ATP)^{2-}$ and $Zn(phen)(ATP)^{2-}$.

In the binary complex $Zn(UTP)^{2-}$ the resonances of H–C(1′) and H–C(5) are unchanged although that of H–C(6) is shifted 0.067 ppm upfield. However in the ternary system Zn^{2+}/bipy/UTP^{4-} (0.01M) at pD 7.9 the resonances of H–C(1′), H–C(5), and H–C(6) of UTP are shifted upfield from those of free

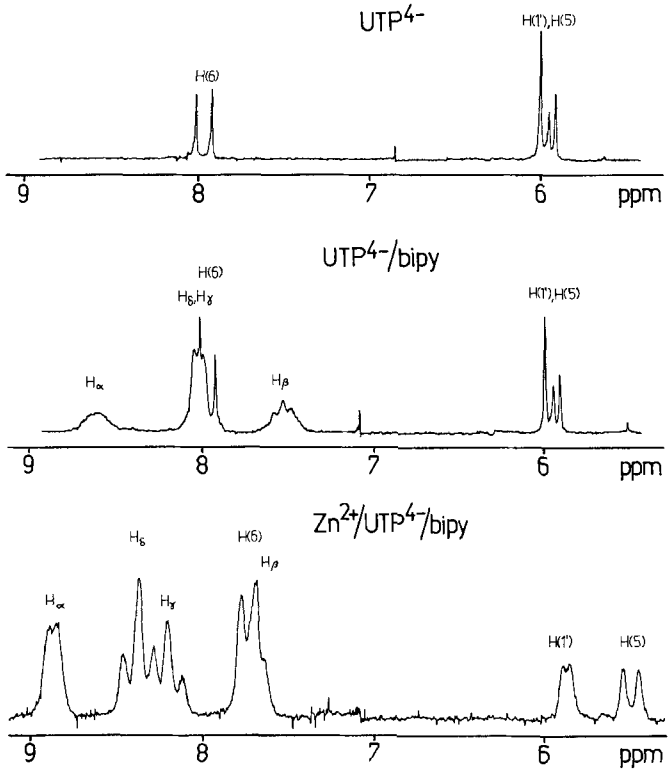


Fig. 1. *^{1}H-NMR. spectra of UTP^{4-}* (0.01M); *of UTP^{4-} and bipy* (each 0.01M); *and of UTP^{4-}, bipy, and Zn^{2+}* (each 0.01M, pD = 7.9).

UTP^{4-} by 0.121, 0.474, and 0.254 ppm, respectively, thus clearly confirming the presence of stacking in $Zn(bipy)(UTP)^{2-}$. The shift for the ternary system is very much greater than observed for the binary bipy/UTP^{4-} system (0.01M), in which the upfield shifts of H–C(1′), H–C(5) and H–C(6) are all ~0.005 ppm *(Fig. 1)*. This increase is due to the higher stability of the ternary stacked adduct than of the binary bipy/UTP^{4-} system. The variation of the upfield shift of the UTP resonances in a bipy/UTP^{4-} mixture as $[Zn^{2+}]$ is increased is shown in *Figure 2*. Addition of Zn^{2+} to a uridine/bipy mixture has no effect on the size of the upfield shifts of H–C(6), H–C(5), H–C(1′) or H–C(2′); indeed the stability of the stacking adducts uridine/$Zn(bipy)^{2+}$ and uridine/bipy are very similar.

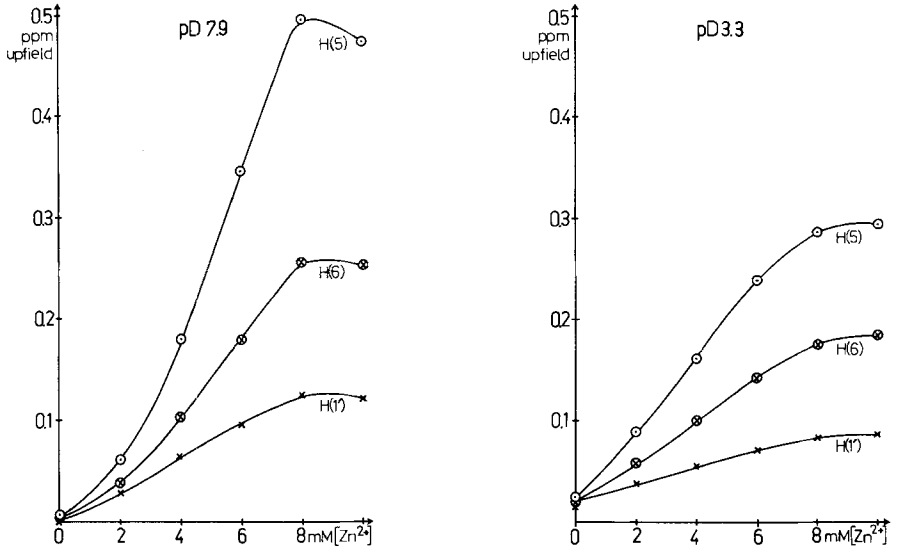


Fig. 2. *Upfield shifts of the resonances of* H–C(1′) (×) H–C(5) (⊙) *and* H–C(6) (⊗) *of* UTP^{4-} *in a mixture of* UTP^{4-} *and bipyridyl* (each 0.01M) compared with the resonance positions of the protons in UTP itself, with increasing $[Zn^{2+}]$ (90.025 MHz, 27°; $I = 0.1$, $NaNO_3$):
a) pD 7.9; b) pD 3.3

The resonances of H_β, H_γ and H_δ of bipyridyl are also shifted upfield by stacking although less than those of UTP. Compared with the Zn^{2+}/bipy system, stacking in $Zn(bipy)(UTP)^{2-}$ causes upfield shifts in the ternary system of 0.014, 0.064, and 0.105 ppm for H_β, H_γ and H_δ; H_α is more influenced by the changing environment of the metal ion, and is shifted downfield by ~0.26 ppm.

A similar experiment in which Zn^{2+} was added to a bipy/UTP mixture was carried out at pD 3.3. These results are also shown in *Figure 2*. Although the upfield shifts are smaller than observed at pD 7.9, H–C(1′), H–C(5) and H–C(6), shift 0.086, 0.295, and 0.186 ppm upfield, respectively, clearly showing that an intramolecular stacking also exists in the protonated complex $Zn(bipy)(HUTP)^{-}$. The smaller upfield shift is probably the result of the smaller degree of formation of this protonated complex. Such protonated complexes are known also for other nucleotides [12].

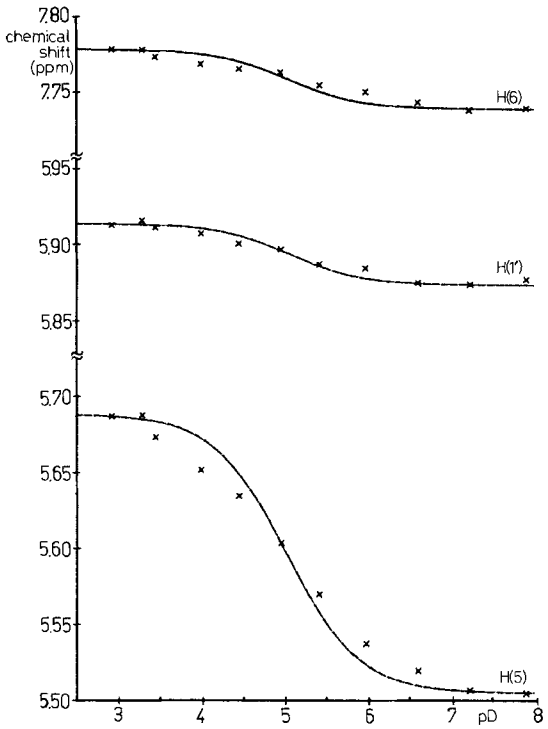


Fig. 3. *Chemical shifts of the resonances of* H–C(1'), H–C(5), *and* H–C(6) *of UTP in a mixture of UTP, bipyridyl, and* Zn^{2+} (each 0.01 M) *in dependence on pD* (27°, $I = 0.1$, $NaNO_3$)

From an experiment with $[Zn^{2+}] = [bipy] = [UTP] = 0.01$ M, in which the pD was increased from 3 to 8 *(Fig. 3)*, the acidity constant of Zn(bipy)(DUTP)$^-$, $pK^D_{Zn(bipy)(DUTP)} \simeq 5.04$, was estimated. Bearing in mind the isotope effect[4]) this value agrees reasonably with rough estimates made from preliminary spectrophotometric measurements on the ternary systems M^{2+}/bipy/UTP which indicate that for both Zn^{2+} and Cu^{2+} $pK^H_{M(bipy)(HUTP)} \sim 4.4$. These values are of the same order as those observed for other protonated nucleoside 5'-triphosphate complexes; in all these cases the site of protonation is the γ-phosphate group [12].

4. Conclusions. – From the ^{1}H-NMR. studies *(Figs. 2* and *3)* that it is not only clear stacking adducts are formed in the binary UTP/bipy system but that intramolecular stacking also occurs in Zn(bipy) $(UTP)^{2-}$ and Zn(bipy) $(HUTP)^-$. As the stability of the corresponding complexes with Co^{2+}, Ni^{2+}, and Cu^{2+} is similar *(cf. Table 1)*, it seems probable that stacking also occurs in these ternary complexes.

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[4]) The primary isotope effect typically causes pK^D_a to be 0.4 to 0.7 log units greater than the corresponding pK^H_a: for the deprotonation $H_2PO_4^- \rightleftharpoons HPO_4^{2-} + H^+$ pK^D_a is 0.58 log units higher than pK^H_a [15], and we have found that for the reaction $HATP^{3-} \rightleftharpoons ATP^{4-} + H^+$ pK^D_a is 0.54 log units higher than pK^H_a, *i.e.* $pK^H_{H(ATP)} = 6.42$ ([1]; 25°; $I = 0.1$, $NaClO_4$) and $pK^D_{D(ATP)} = 6.96$ (this work; 25°; $I = 0.1$, $NaClO_4$; D_2O content > 96%). Thus from the value found for $pK^D_{Zn(bipy)(DUTP)}$, it can be estimated that $pK^H_{Zn(bipy)(HUTP)} \simeq 4.5$.

However, intramolecular stacking in $M(bipy)(UTP)^{2-}$ does not exclude the presence of an opened form according to the intramolecular equilibrium (3):

$$\text{bipy-}M^{2+}\text{-UTP} \overset{K'}{\rightleftharpoons} \begin{matrix} \text{bipy} \\ | \\ \text{UTP} \end{matrix} \diagdown\!\!\diagup M^{2+} \qquad (3)$$

The corresponding constant K' is dimension less and independent of the concentration and is thus difficult to determine. Comparison of the size of the upfield shift of H–C(1′) in the ternary systems Zn^{2+}/bipy/UTP^{4-}, Zn^{2+}/bipy/ATP^{4-}, [6], and Zn^{2+}/phen/ATP^{4-} [1] indicates that about half of the Zn(bipy)$(UTP)^{2-}$ is stacked. A simplified and tentative structure of these metal ion bridged stacked adducts is given in *Figure 4*.

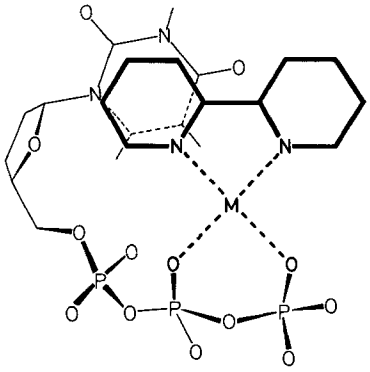

Fig. 4. *A tentative and simplified structure of the ternary stacked complex* $M(bipy)(UTP)^{2-}$

The pH dependence of complex formation for the binary Zn^{2+}/UTP system and for the ternary Zn^{2+}/bipy/UTP system is shown in *Figure 5*. In the ternary system (0.001M), the mixed-ligand complex dominates over a wide pH range, and indeed at pH 7 Zn(bipy)$(UTP)^{2-}$ is formed to about 75%: only ~1% of the stacked adduct (bipy)$(UTP)^{4-}$ is present in the binary system. Thus the coordination tendency of the phosphate groups determines the extent of formation of the ternary stacked adduct and its concentration is enormously increased by the metal ion which coordinates strongly to both ligands, forming a metal bridge.

Thus it is quite clear that the concentration of weak stacked adducts may be increased by an additional polar interaction: for example, *N*(1)-methylnicotinamide ion binds weakly to tryptophan-62 of lysozyme forming a stacked adduct [17]; however in a related isonicotinylium glycoside a polar interaction of the sugar with the enzyme occurs in addition to the stacking, and this combination of two weak interactions leads to an increased stability constant [18]. We believe that metal ions can also enhance the formation of stacking adducts by bridging two suitable ligands *in vivo*. It is already known for metal ion-free systems that pyrimidine-nucleosides interact with the aromatic moieties of tryptophan [19], tyrosine or tyramine [20]. In addition, the binding of the pyrimidine 3′-CMP to ribonuclease increases the affinity of this protein for Cu^{2+} (or Zn^{2+}), and conversely, binding of

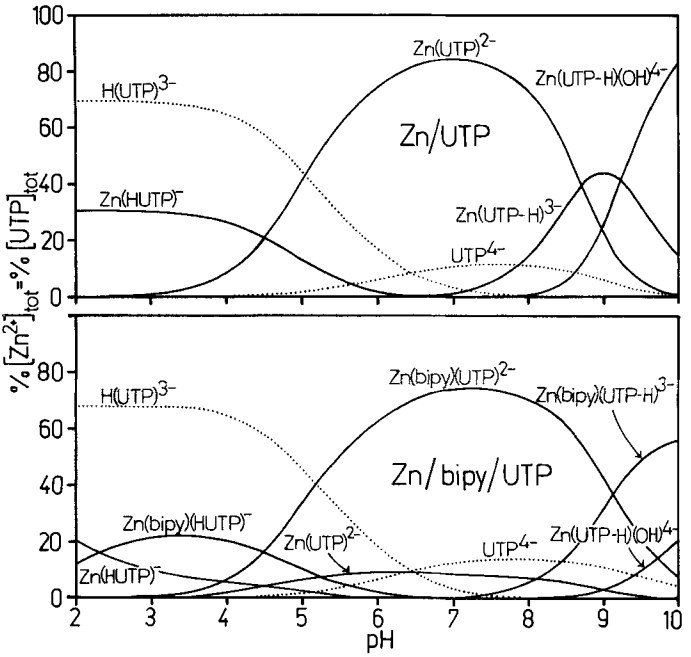

Fig. 5. *Effect of pH on the concentrations of the species present in an aqueous solution* (25°; $I=0.1$) *of* Zn^{2+}*/UTP and* Zn^{2+}*/UTP/bipy.*

Results are given as the percentage of the total UTP (or Zn^{2+}) present; computed with the constants of [5] [7], the present results, and estimating $\log K^{Zn}_{Zn(HUTP)} = \log K^{Zn(bipy)}_{Zn(bipy)(HUTP)} - 0.15 = 2{,}95 - 0.15 = 2.8$, for concentrations of 0.001M for each reactant. The dotted lines indicate the free UTP species and the solid lines the UTP complexes. Doubly protonated species were ignored as their constants are unknown; such species could only exist at low pH as for the primary protons of the triphosphate chain $pK_a \leqslant 2.6$ [16].

Upper Part: Zn^{2+} and UTP ($[UTP\text{-}H)^{5-}] \leqslant 1.5\%$)

Lower Part: Zn^{2+}, bipyridyl and UTP ($[UTP\text{-}H)^{5-}] \leqslant 8.5\%$ and $[Zn(UTP\text{-}H)^{3-}] \leqslant 6.3\%$)

Cu^{2+} to ribonuclease increases its affinity for 3′-CMP [21]. A stacking interaction between a suitable aminoacid side chain and the pyrimidine moiety may be in part [3] responsible for this observation. Indeed, there is evidence for a possible stacking between the purine moiety of 5′-AMP and histidine (119) of ribonuclease [22].

In conclusion, there is no doubt that stacking with a pyrimidine moiety of a nucleotide can occur, and that this may be of considerable influence in enzyme systems.

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REFERENCES

[1] *P.R. Mitchell & H. Sigel*, J. Amer. chem. Soc. *100* (1978), in press.
[2] *H. Sigel, B.E. Fischer & B. Prijs*, J. Amer. chem. Soc. *99*, 4489 (1977).
[3] *H. Sigel*, Angew. Chem. *87*, 391 (1975); Int. Ed. *14*, 394 (1975).
[4] *H. Sigel*, J. Amer. chem. Soc. *97*, 3209 (1975).
[5] *H. Sigel & C.F. Naumann*, J. Amer. chem. Soc. *98*, 730 (1976).
[6] *P. Chaudhuri & H. Sigel*, J. Amer. chem. Soc. *99*, 3142 (1977).
[7] *G. Anderegg*, Helv. *46*, 2397 (1963).
[8] *H.A. Benesi & J.H. Hildebrand*, J. Amer. chem. Soc. *71*, 2703 (1949).
[9] *P.K. Glasoe & F.A. Long*, J. phys. Chemistry *64*, 188 (1960).
[10] *R.H. Linnell & A. Kaczmarczyk*, J. phys. Chemistry *65*, 1196 (1961).
[11] *E. Walaas*, Acta chem. scand. *12*, 528 (1958).
[12] *H. Sigel*, J. inorg. nucl. Chemistry *39*, 1903 (1977).
[13] *P.R. Mitchell*, in preparation.
[14] *L.M. Jackman & S. Sternhell*, 'Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry', 2nd edn, p. 94, Pergamon Press, Oxford, 1969.
[15] *R. Gary, R.G. Bates & R.A. Robinson*, J. phys. Chemistry *68*, 3806 (1964).
[16] *R. Phillips, S.J.*, Chem. Rev. *66*, 501 (1966).
[17] *J. Verhoeven & R. Schwyzer*, Helv. *55*, 2572 (1972).
[18] *R.A. Bradshaw & D.A. Deranleau*, Biochemistry *9*, 3310 (1970).
[19] *J.-L. Dimicoli & C. Hélène*, J. Amer. chem. Soc. *95*, 1036 (1973).
[20] *C. Hélène, T. Montenay-Garestier & J.-L. Dimicoli*, Biochim. biophys. Acta *254*, 349 (1971).
[21] *E. Breslow & A.W. Girotti*, J. biol. Chemistry *245*, 1527 (1970); E. Breslow, 'Metal Ions in Biological Systems' (*H. Sigel*, ed.), Vol. 3, pp. 133–155, Marcel Dekker, New York, 1974.
[22] *P.H. Haffner & J.H. Wang*, Biochemistry *12*, 1608 (1973).