$$-\frac{d\text{Cu(oxac)}_{keto}}{dt} = (k_4[H^+] + k_5 + k_{-CO_2}) \times [\text{Cu(oxac)}_{keto}] - (k_{-4}[H^+] + k_{-5}) \times f_{\text{Cu(oxac),enol}}[\text{Cu(oxac)}_{enol}]_{tot}$$

$$\frac{d[\text{Cu(oxac)}_{enol}]_{tot}}{dt} = (k_4[H^+] + k_5)(\text{Cu(oxac)}_{keto}) - (k_{-4}[H^+] + k_{-5}) f_{\text{Cu(oxac),enol}}[\text{Cu(oxac)}_{enol}]_{tot}$$

$$\frac{d\text{Cu(pyr)}_{enol}}{dt} = k_{-CO_2}[\text{Cu(oxac)}_{keto}] - k_H[\text{Cu(pyr)}_{enolate}]$$

$$\frac{d\text{Cu(pyr)}}{dt} = k_H[\text{Cu(pyr)}_{enolate}]$$

$$[\text{Cu(oxac)}_{enol}]_{tot} \sim [\text{Cu(oxac)}_{enol}] + [\text{Cu}_2\text{H}_{-1}\text{oxac}^+]$$

A fourth-order Runge-Kutta procedure was used to perform the numerical integration

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Ternary Complexes in Solution. XXIV.[1] Metal Ion Bridging of Stacked Purine–Indole Adducts. The Mixed-Ligand Complexes of Adenosine 5′-Triphosphate, Tryptophan, and Manganese(II), Copper(II), or Zinc(II)

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Abstract. The stability constants of the mixed-ligand complexes containing ATP, tryptophan (Trp), and Mn^{2+}, Cu^{2+}, or Zn^{2+} (= M) were determined from potentiometric titrations. The stability of complexes is characterized by $\Delta \log K_M = \log K^{M(ATP)}_{M(ATP)(Trp)} - \log K^{M}_{M(Trp)}$, which corresponds to the equilibrium, $M(ATP)^{2-} + M(Trp)^{+} \rightleftharpoons M(ATP)(Trp)^{3-} + M^{2+}$. A comparison of these data with those of the corresponding complexes containing alaninate instead of tryptophanate reveals an increased stability (about 0.2 to 0.6 log unit) of the ternary complexes formed with tryptophanate. This gave the first hint of an intramolecular stacking between the indole and purine moieties in these mixed-ligand complexes; i.e., these complexes can be considered as metal ion bridged stacking adducts. In fact, uv-difference absorbance studies revealed a new absorbance in the 295-nm region which could be attributed to an indole-purine interaction in these ternary complexes by comparison with systems containing only tryptophan and either adenosine or ATP. Changes in the ^1H NMR spectrum of the Zn^{2+}-ATP-Trp system confirmed that the aromatic moieties are linked together by the coordination of a metal ion to the phosphate chain of ATP and the amino acetate part of tryptophan. The extents of formation of the ternary complexes, $M(ATP)(Trp)^{3-}$, were calculated. The factors favoring their formation (e.g., the oxidation of Mn^{2+} to Mn^{3+}) and the structure of their folded form are discussed. Possible biological implications are outlined taking into account systems in which the coenzyme and the activating metal ion bind less well to the enzyme than does the coenzyme-metal ion complex.

It is now well established[2,3] that charge-transfer interactions may play a major role in biological systems. Examples are the interactions of adenine coenzymes and glutamate dehydrogenase,[4] creatine kinase,[5] octopine dehydrogenase,[6] or myosin,[7] where stacking between the adenine moiety of the coenzyme and an aromatic amino acid residue has been suggested. Among the side chains of amino acids, the indole moiety is the most potent electron donor.[8] Indeed, charge-transfer-type interactions between tryptophan or other indole derivatives and nucleosides or nucleotides occur in aqueous solution.[9-12] However, purine and pyrimidine bases can also stack with each other,[13] and this contributes to the stability of nucleic acid helices.[14]

In general, the stability of such adducts is small, unless

one of the aromatic moieties is brought near the other by the formation of a bridge. In these cases, charge-transfer or stacking adducts of much higher stability may be formed. Such intramolecular interactions were observed in models of FAD[15-17] or NAD,[18-20] and in NAD itself,[21-23] where the π systems are connected by covalent bonds.

Recently, it has been demonstrated that such stacked or charge-transfer adducts could also be stabilized by an ionic bridge in which a metal ion coordinates to the two constituents of the adduct. The metal ion used was Cu^{2+}, and the ligands were 2,2'-bipyridyl and the 5'-triphosphates of adenosine,[24,25] inosine,[24,25] or guanosine.[26] In the resulting ternary complexes, Cu^{2+} is coordinated to the nitrogen donors of 2,2'-bipyridyl and to the phosphate chain of the nucleotides, and the dominant configuration is that which allows stacking between the aromatic amine and the purine moiety.

Such stacked metal ion bridged complexes may occur in many biological systems including metal-ion-dependent enzyme systems, nucleic acid–protein interactions, or synaptosomes[27] (which contain biogenic aromatic amines, ATP, and several metal ions). However, so far stabilities have been measured only for the examples containing 2,2'-bipyridyl.[25] Therefore, we felt it desirable to do a model study using the naturally occurring constituents, tryptophan and ATP (cf. Scheme I).[17]

Firstly, the simple systems containing only tryptophan and ATP were studied in the pH range from 1 to 8 by means of uv-difference spectra: the occurrence of stacking was demonstrated (cf. also ref 11). We prefer here the term "stacking" because it is a neutral expression which states nothing about the forces holding the two aromatic moieties together. Other terms often used to name such interactions are, besides "charge-transfer",[28] "pseudo-charge-transfer" or "charge-transfer type" interactions,[11] "dipole-induced dipole interactions",[29] "hydrophobic",[30,31] "electrostatic",[31] or "ring current" effects.[32] In other words, at present it is our single intention to demonstrate an interaction between an indole and purine moiety in ternary metal ion complexes but not to define its physical type. Indeed, experiments carried out in the presence of Mn^{2+}, Cu^{2+}, or Zn^{2+} revealed the formation of stacked adducts.

The stability of the metal ion complexes was determined by potentiometric titrations, while the occurrence of stacking between the indole residue of tryptophan and the purine moiety of ATP was demonstrated by uv-difference spectra and by ^1H NMR measurements. For comparison, a number of experiments were performed replacing tryptophan by alanine or ATP by adenosine.

Experimental Section

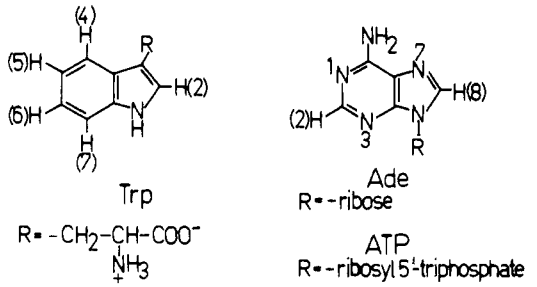
Materials. Manganese(II), copper(II), zinc(II) (all purum) and sodium (purissimum) perchlorates, adenosine, DL-tryptophan,[33] L-leucine, L-alanine (all purissimum), and D_2O (99.75%) were obtained from Fluka AG, Buchs, Switzerland. The disodium salt of adenosine 5'-triphosphate (pro analysi) was purchased from Serva Feinbiochemica GMBH, Heidelberg, Germany, who stated that the ATP contained about 1% ADP + AMP; in addition, we determined the content of inorganic phosphate[37] and found it to be less than 3%.

Technical sodium tripolyphosphate (Chemische Fabrik Schweizerhalle, Basel, Switzerland) was purified by the method of Watters et al.[38] The reprecipitation by methanol was repeated three times; the content of inorganic phosphate was then less than 1.2%.

The exact metal(II) ion concentration of the perchlorate stock solutions was determined with ethylenediamine-*N,N,N',N'*-tetraacetate.

Apparatus. The absorbance spectra were recorded with a Beckman spectrophotometer DB, connected to a W+W Electronic H-

Scheme I



Speed Recorder 202. The pH measurements were performed with a Metrohm potentiometer E 353 B with type U micro glass electrodes. The potentiometric titrations were carried out with a Metrohm potentiograph E 336 and Metrohm UX glass electrode. The ^1H NMR spectra were taken with a Bruker WH-90 FT spectrometer at 90 MHz.

Determination of Equilibrium Constants by Potentiometric Titrations. The acidity constant K^H_{HL} of tryptophan was determined from automatic titrations of aqueous solutions (50 ml) containing 3×10^{-4} *M* $HClO_4$ and $NaClO_4$ ($I = 0.1$) in the presence and absence of 1.2×10^{-3} *M* H(Trp) under N_2 with 0.1 *M* NaOH (25°C). The differences between such a pair of titrations were measured, and the constant was calculated from the pH range which corresponded to a degree of neutralization between 0.1 and 0.9.

The conditions of measurements for the determination of the stability constants, K^M_{ML}, were the same as for the acidity constant, but $NaClO_4$ was partly replaced by $M(ClO_4)_2$ to give the ratios Mn^{2+}:Trp = 10:1 and 20:1, Zn^{2+}:Trp = 10:1 and 20:1, and Cu^{2+}:Trp = 2:1, 3:1, and 5:1. Titrations of solutions without ligand were used as a basis for the evaluation. The calculation of K^{Mn}_{MnL} and K^{Zn}_{ZnL} was done by taking into account the species $H_2(Trp)^+$, H(Trp), Trp^-, M^{2+}, and $M(Trp)^+$.[39] $Cu(Trp)_2$, which occurs in minor concentrations (up to 4%) under these experimental conditions, was also taken into account. The value of the difference $\log K^{Cu}_{CuL} - \log K^{CuL}_{CuL_2} = 1.2$ was used, based on results obtained for L-tryptophan[40] and other α-substituted amino acids;[41] the iteration procedure was carried out as described.[42] The constants for the Cu^{2+} system have recently been determined by Weber;[40] the present results agree well with his.

The conditions used for the titrations of the mixed-ligand systems were the same as for the binary ones (and for the determination of the acidity constant), but the solutions contained, in addition, ATP^{4-}. The ratios were Mn^{2+}:ATP:Trp = 10:10:1 and 15:15:1, Zn^{2+}:ATP:Trp = 3:3:1 and 5:5:1, and Cu^{2+}:ATP:Trp = 1:1:1 and 2:2:1. The stock solutions of ATP^{4-} were always freshly prepared by rapidly titrating the dissolved disodium salt to the equivalence point with NaOH. Also, reaction solutions containing metal ions were titrated immediately after mixing to prevent dephosphorylation.[37] The experiments with alanine instead of tryptophan were carried out in the same way, but the concentration of $HClO_4$ was 1.6×10^{-4} *M*. In all cases, at least three independent titration curves were measured.

Evaluation Procedures of the Potentiometric Data of the Mixed-Ligand Systems. The following constants, which were used in the calculations, were taken from the literature: acidity constant $K^H_{H_2L}$ of tryptophan,[43] acidity constants and stability constants of the binary complexes of alanine[41] and of ATP;[44] $K^H_{H_2ATP}$ and $K^{Mn}_{Mn(ATP)}$ were from ref 45.

Procedure I (Pr. I). The overall stability constant $\beta^M_{M(ATP)(Trp)}$ was computed[39] by taking into account the species H^+, $H_2(ATP)^{2-}$, $H(ATP)^{3-}$, ATP^{4-}, $M(ATP)^{2-}$, $H_2(Trp)^+$, H(Trp), Trp^-, $M(Trp)^+$, M^{2+}, and $M(ATP)(Trp)^{3-}$; in the Cu^{2+}-containing systems, $Cu(Trp)_2$ was also considered. The differences in consumed NaOH used in the calculations were those between titrations of a solution containing only $HClO_4$ and one containing also the ternary system.

Procedure II (Pr. II). The evaluation was done assuming that in the binary system the complex $M(ATP)^{2-}$ is completely formed; in the pH range of question this holds to a large part.[37,46] This permits calculation of the stability constant, $K^{M(ATP)}_{M(ATP)(Trp)}$, for the ternary complexes by the usual method for binary complexes considering only the species H^+, $H_2(Trp)^+$, H(Trp), Trp^-, $M(ATP)^{2-}$,

Table I. Logarithms of the Stability Constants of the Binary and Ternary DL-Tryptophanate- and L-Alaninate-M^{2+}-ATP^{4-} Systems as Determined by Potentiometric Titrations (25°C; I = 0.1, $NaClO_4$)[a,52]

Amino acetate (Aa)	M^{2+}	Log $K^{M}_{M(Aa)}$	Log $K^{M}_{M(ATP)}$	Log $\beta^{M}_{M(ATP)(Aa)}$ Pr. I[c]	Log $\beta^{M}_{M(ATP)(Aa)}$ Pr. II[c]	Log $K^{M(ATP)}_{M(ATP)(Aa)}$ Pr. I[c]	Log $K^{M(ATP)}_{M(ATP)(Aa)}$ Pr. II[c]	Log $K^{M}_{M(Aa)(ATP)}$[d]	Δ log K_M[d]
Tryptophanate	Mn^{2+}	2.50 ± 0.03	4.78[b]	6.35 ± 0.07	6.29	1.57	1.51 ± 0.02	3.82	−0.96
	Cu^{2+}	8.27 ± 0.05[e]	6.38[f]	13.35 ± 0.04	13.34	6.97	6.96 ± 0.05	5.08	−1.30
	Zn^{2+}	4.69 ± 0.02	5.21[f]	9.75 ± 0.04	9.69	4.54	4.48 ± 0.04	5.03	−0.18
Alaninate	Mn^{2+}	2.67[g]	4.78[b]		6.14		1.36 ± 0.09	3.47	−1.31
	Cu^{2+}	8.25[g,h]	6.38[f]	13.07 ± 0.07	13.13	6.69	6.75 ± 0.05	4.85	−1.53
	Zn^{2+}	4.51[g]	5.21[f]		8.92		3.71 ± 0.03	4.41	−0.80

[a] Acidity constants of the ligands: $pK^{H}_{H_2(Trp)}$ = 2.39 (I = 1),[43] $pK^{H}_{H(Trp)}$ = 9.41 ± 0.01; $pK^{H}_{H_2(Ala)}$ = 2.26,[41] $pK^{H}_{H(Ala)}$ = 9.83;[41] $pK^{H}_{H_2(ATP)}$ = 4.06,[b] $pK^{H}_{H_3(ATP)}$ = 6.42.[44] [b] Cf. ref 45 (I = 0.1, KNO_3). [c] See Experimental Section. [d] Average from the results of Pr. I and Pr. II. [e] Log $K^{Cu(Trp)}_{Cu(Trp)_2}$ = 7.07. [f] Cf. ref 44. [g] Cf. ref 41. [h] Log $K^{Cu(Ala)}_{Cu(Ala)_2}$ = 7.05.[41]

and $M(ATP)(Trp)^{3-}$.[37,39b] Here the difference used in the calculations was obtained from a pair of titrations of solutions with, and without, tryptophan (ternary system and binary system, respectively).

Obviously, procedure I is more attractive because all species are considered, while procedure II has the advantage that possible errors due to the hydrolysis[46] and dephosphorylation[37,47] of the M^{2+}-ATP system (which is present in *both* solutions of a titration pair) is largely eliminated. However, the agreement between the results obtained with the two evaluation procedures is excellent for the mixed-ligand systems containing tryptophan, even though different pairs of experiments were evaluated. For the ternary systems with Mn^{2+} or Zn^{2+}, and alanine only, procedure II led to satisfying results (with procedure I, the constants varied over the pH range and the standard deviation was high).

Determination of the Stability Constants of Indole–Purine Adducts by Spectrophotometric Measurements. Stacking complexes formed between two aromatic systems in aqueous solution often exhibit a detectable absorbance.[28] In our experiments a new absorbance is observed in the 295-nm region. However, to obtain a clear-cut situation, uv-difference spectra had to be recorded (25°C; I = 0.5, $NaClO_4$) The same was done very recently by Morita,[11] and, indeed, the shape and position of the spectra shown in Figure 1 of his paper correspond well with our observations.

The uv-difference spectra were taken in 1-cm quartz cells by placing in the reference beam one cell with tryptophan and a second one with adenosine or ATP; the sample beam contained one cell with the mixed system and one with the solvent. As the absorbance of the reference solutions was high, the spectrophotometer was in general used on "manual" with the slit adjusted to 2.0 mm. As a consequence, there is some stray light present and the molar absorptivity (ϵ) depends somewhat on the slit width; however, the determined stability constants are independent of the latter. The relative values of the molar absorptivities obtained here may be compared even though the absolute values are probably too low.

The experimental procedures and the evaluation of the data by Benesi-Hildebrand plots were carried out as described.[25] All straight lines were drawn according to the least-squares method. The weak self-association of adenosine (K = 4.5)[13] or ATP[48] is negligible.

Spectrophotometric Measurements of Indole–Purine Adducts in the Presence of Metal Ions. In general, the experiments were done as described in the preceding section and the uv-difference spectra had the same shape. However, in M^{2+}-ATP systems the absorbance of ATP at 260 nm is slightly altered[49] by the coordination of M^{2+} to N(7). On addition of tryptophan to an ATP-M^{2+} system, this interaction will be suppressed by the coordination of Trp to $M(ATP)^{2-}$. This behavior can be imitated with a simple amino acid having a bulky side chain and a similar coordination tendency. By using leucine, we eliminated all alterations that might possibly extend into the 295-nm region from this interaction by using the following setup. In the reference beam was one cell with ATP (or adenosine), M^{2+}, and leucine and a second one with tryptophan, while the sample beam contained one cell with ATP (or adenosine), M^{2+}, and tryptophan and one with leucine. Leucine was shown to be without effect on the stability constants or molar absorptivities of the binary adducts.

However, there was one further problem. Apparently a binary M^{2+}-Trp interaction (see Results and Figure 5) contributes also somewhat to the absorbance in the 295-nm region, at least in case of Cu^{2+}. To compensate for this we used a further ligand, triphosphate. This is possible because $M(HTP)^{2-}$ is practically of the same stability as $M(ATP)^{2-}$,[50] and therefore the following setup could be used. In the reference beam was one cell with ATP, Cu^{2+}, and Leu and a second one with TP, Cu^{2+}, and Trp, while the sample beam contained one cell with ATP, Cu^{2+}, and Trp and one with TP, Cu^{2+}, and Leu.

^{1}H NMR Measurements. D_2O was used as solvent and sodium 3-(trimethylsilyl)propane as an internal standard (25°C). The pD of the solutions was adjusted to the desired value ± 0.05 (pD = pH meter reading + 0.4)[51] by dotting with a glass stick and concentrated NaOD.

The change in the chemical shift, i.e., Δ shift (Hz), was obtained by subtracting the chemical shift of the proton under consideration observed in the presence of complexing agents from the chemical shift measured for the free ligands. This means a positive value of Δ shift implies an upfield shift of the ^{1}H NMR signal.

Results

Stability of the Binary and Ternary Metal Ion Complexes. The formation of stacked adducts between nucleosides or nucleotides and the indole group of tryptophan is well known.[9c,11,32] Hence, our first aim was to see if in mixed-ligand metal ion systems, where such a stacking is possible, the observed stability of the complexes is altered. Therefore we determined the stability constants of the systems containing ATP^{4-} with Mn^{2+}, Cu^{2+}, or Zn^{2+}, and tryptophanate or alaninate by potentiometric titrations. Stacking is obviously not possible in the ternary systems with alaninate, while in those with tryptophanate it could occur and might affect complex stability.

The stability constants of the binary ATP and, in part, of the amino acid complexes had already been determined. The missing data and those of the ternary complexes have now been measured. The results[52] are assembled in Table I, and the stability constants of the mixed-ligand systems are defined by eq 1-3. The overall stability constant $\beta^{M}_{M(ATP)(Aa)}$ is connected with the constants $K^{M(ATP)}_{M(ATP)(Aa)}$ and $K^{M(Aa)}_{M(Aa)(ATP)}$ by eq 4 and 5, respectively.

$$M^{2+} + ATP^{4-} + Aa^{-} \rightleftharpoons M(ATP)(Aa)^{3-}$$
$$\beta^{M}_{M(ATP)(Aa)} = [M(ATP)(Aa)]/[M][ATP][Aa] \quad (1)$$

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

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

$$\log K^{M(ATP)}_{M(ATP)(Aa)} = \log \beta^{M}_{M(ATP)(Aa)} - \log K^{M}_{M(ATP)} \quad (4)$$

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

The common way to characterize the stability of mixed-

ligand complexes of the kind studied here is according to the equation[39,53-55]

$$\Delta \log K_M = \log K^{M(ATP)}_{M(ATP)(Aa)} - \log K^{M}_{M(Aa)} = \log K^{M(Aa)}_{M(Aa)(ATP)} - \log K^{M}_{M(ATP)} \quad (6)$$

This means by comparing the difference in stability, $\Delta \log K_M$, e.g., for the reaction between $M(ATP)^{2-}$ or $M(aq)^{2+}$ and amino acetate, Aa. In addition, $\Delta \log K_M$ is identical with the constant due to equilibrium 7.

$$M(ATP)^{2-} + M(Aa)^{+} \rightleftharpoons M(ATP)(Aa)^{3-} + M^{2+} \quad (7)$$

Even though there are a number of exceptions known,[39,54,55] one expects, in general, to observe negative values for $\Delta \log K_M$ (eq 6), since usually it holds that $K^{M}_{ML} > K^{ML}_{ML_2}$.[36] In fact, the values of $\Delta \log K_M$ measured for the mixed-ligand complexes containing tryptophanate or alaninate are negative for both systems.

A closer study of the values of $\Delta \log K_M$ in Table I reveals that the ternary complexes $M(ATP)(Trp)^{3-}$ are more stable, by about 0.2 to 0.6 log unit, than the corresponding $M(ATP)(Ala)^{3-}$ ones. This slight increase in stability of $M(ATP)(Trp)^{3-}$ could be explained by a direct interaction between the purine and indole moieties. Hence, this result can be considered as a first hint for the formation of metal ion bridged stacked adducts.

The difference in stability between the ternary complexes containing tryptophanate and those with alaninate is reflected in their formation degree. For example, the maximum degree of formation, given as the percentage of the total concentration of the reagents present ([Trp] or [Ala] = [ATP] = $[M^{2+}] = 10^{-3}$ *M*) is for $M(ATP)(Trp)^{3-}$ 2, 63, and 54% for Mn^{2+}, Cu^{2+}, and Zn^{2+}, respectively; the comparable numbers for $M(ATP)(Ala)^{3-}$ are 1, 50, and 16%. The mixed-ligand complexes exist in their highest concentrations at about pH 10.2 for Mn^{2+}, 7.5 for Cu^{2+}, and 9.1 for Zn^{2+} in both systems.

Stability of the Stacked Purine–Indole Adducts. Based on uv-difference spectra it is possible to measure the apparent stability constants, K_{St} (eq 8) for the interaction between adenosine or ATP and tryptophan as a function of pH and to evaluate the influence of the triphosphate chain of ATP on the stability of the stacked adducts.

$$Trp + A \rightleftharpoons (Trp)(A) \qquad K_{St} = [(Trp)(A)]/[Trp][A] \quad (8)$$

A = Ade or ATP

The apparent stability constants, K_{St}, were determined by Benesi–Hildebrand plots. The concentration of tryptophan was kept constant while increasing concentrations of adenosine or ATP were used. Plots of 1/[Ade] or 1/[ATP] vs. $1/\Delta E_{293}$ resulted in straight lines, thus indicating that 1:1 adducts are formed.[56] Examples of such plots are given in Figure 1 for adenosine and in Figure 2 for ATP. These and the results listed in Table II show that the influence of pH on K_{St} is much more significant than the presence of the triphosphate moiety. In fact, the stabilities of (Trp)(Ade) and (Trp)(ATP) are identical within experimental error at a given pH. For both adducts, the stability increases somewhat with increasing pH. This probably results from an electrostatic repulsion between the adenine moiety protonated at N(1) in the lower pH range and the positively charged ammonium group of tryptophan. The corresponding observation was made for the self- and hetero-association of purine and pyrimidine derivatives.[57,58]

Overall, the stability of these adducts is in the order found for similar systems.[9,10,25,59] Morita[11] determined for (ATP)(Trp) at 25°C and pH 8 in 2×10^{-2} *M* phosphate buffer log K_{St} = 1.3. This value differs by about 0.7 log unit from the present result; this is probably due to the different experimental conditions, especially the higher ionic strength (0.5, $NaClO_4$) used in this study.

The data of Table II also show that the electronic inter-

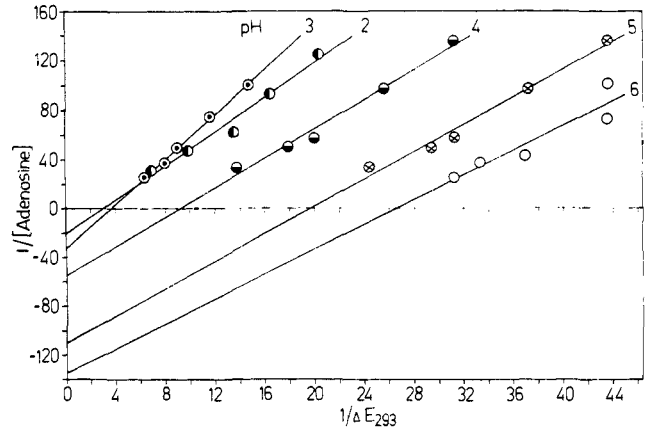


Figure 1. Determination of K_{St} (25°C; I = 0.5, $NaClO_4$) for the stacking adduct between adenosine and Trp (10^{-3} *M*). Intercepts with the *y* axis, i.e., $-K_{St}$: −21.0 (pH 2.00, ◐), −33.0 (3.00, ⊙), −55.1 (4.00, ◒), −109.9 (5.00, ⊗), and −135.0 (6.00, ○); with the *x* axis, i.e., $1/\Delta E_{max}$: 3.02 (pH 2.00), 3.62 (3.00), 9.20 (4.00), 19.7 (5.00), and 26.6 (6.00).

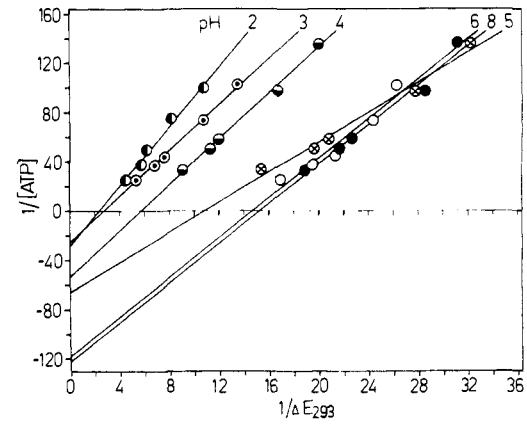


Figure 2. Determination of K_{St} (25°C, I = 0.5, $NaClO_4$) for the stacking adduct between ATP and Trp (10^{-3} *M*). Intercepts with the *y* axis, i.e., $-K_{St}$: −28.7 (pH 2.00, ◐), −26.1 (3.00, ⊙), −53.7 (4.00, ◒), −65.6 (pH 5.00, ⊗), −119.0 (6.00, ○), and −122.3 (8.00, ●); with the *x* axis, i.e., $1/\Delta E_{max}$: 2.25 (pH 2.00), 2.79 (3.00), 5.89 (4.00), 10.83 (5.00), 14.74 (6.00), and 15.30 (8.00).

Table II. Logarithms of the Apparent Stability Constants, Log K_{St} (eq 8), and Molar Absorptivities, ϵ (M^{-1} cm^{-1}),[a] as a Function of pH for the Stacking Adducts between the Purine Moiety of Adenosine or ATP and the Indole Group of DL-Tryptophan as Determined in Aqueous Solution (25°C; I = 0.5, $NaClO_4$) by Uv-Difference Spectra[b,52]

	Adenosine		ATP	
pH	Log K_{St}	ϵ_{293}	Log K_{St}	ϵ_{293}
1.00	1.65 ± 0.20	249 ± 98	1.62 ± 0.12	326 ± 48
2.00	1.50 ± 0.32	332 ± 119	1.52 ± 0.15	393 ± 125
3.00	1.50 ± 0.28	288 ± 96	1.45 ± 0.20	376 ± 103
4.00	1.73 ± 0.29	131 ± 35	1.68 ± 0.27	189 ± 51
5.00	1.97 ± 0.21	49 ± 10	1.80 ± 0.17	82 ± 15
6.00	2.19 ± 0.23	39 ± 8	1.96 ± 0.25	66 ± 8
8.00[c]			2.07 ± 0.28	63 ± 13

[a] Measured at 293 nm. Owing to stray light problems, the extinction coefficients are only relative: the absolute values may be somewhat higher. [b] The results are the average of at least six, in general nine, independent series of measurements. [c] At values of pH >6, the solubility of adenosine is too low for meaningful measurements.

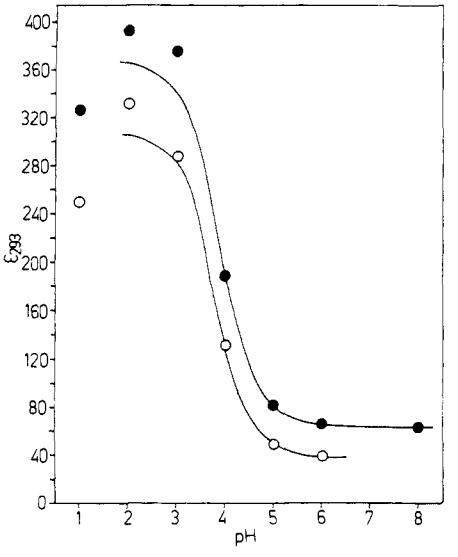


Figure 3. Dependence of the molar absorptivities, ϵ_{293} (M^{-1} cm^{-1}), of the adenosine-Trp (○) and ATP-Trp (●) adducts on pH; cf. Table II.

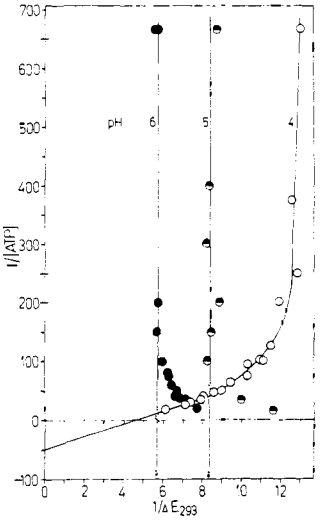


Figure 4. Attempted determination (see text) of the apparent stability constants at pH 4.00, 5.00, and 6.00 (25°C; I = 0.5, $NaClO_4$) for the stacked adduct between ATP and Trp in the presence of Cu^{2+} ([$Cu(ClO_4)_2$] = [Trp] = [Leu] = 10^{-3} M; cf. Experimental Section).

action within the stacked adduct depends considerably on the pH for both systems. This is even more evident from Figure 3 where the molar absorptivities are plotted vs. pH. Both curves, the one corresponds to (Trp)(Ade) (empty circles) and the other to (Trp)(ATP) (full circles), show inflection points which correspond within experimental error with the known acidity constants for the deprotonation of N(1) in adenosine and ATP: $pK^{H}_{H(Ade)}$ = 3.55 (cf. ref 60) and $pK^{H}_{H2(ATP)}$ = 4.06.[24,25] Assuming that the measured absorbance results from a charge transfer within the stacked adducts, then an increasing ϵ with decreasing pH indicates that the adenine moiety functions as an acceptor. This means protonation of the adenine residue should enhance its acceptor capacity, and this should lead[61] to an increasing absorbance; however, other explanations appear also possible.[29-32]

Finally, the addition of Mn^{2+}, Cu^{2+}, or Zn^{2+} does not alter K_{St} or ϵ of the adenosine-tryptophan system. In all cases, the experimental values are the same as the values of Table II. However, owing to precipitation, experiments could only be carried out in the pH range below 5, and there only Cu^{2+} complexes appreciably with tryptophan. The coordination tendency of adenosine toward Mn^{2+}, Cu^{2+}, or Zn^{2+} is very low;[49] hence the formation of metal ion bridged stacking adducts could not be expected under these conditions.

Spectrophotometric Evidence for Metal Ion Bridged Stacked Adducts. In the mixed-ligand systems, the metal ion will coordinate to the phosphate chain of ATP and to the amino acetate part of tryptophan. In such a ternary complex, an intramolecular stacking between the purine moiety of ATP and the indole residue of tryptophan is possible. Indeed, uv-difference spectra correspond in position and shape to those observed in the absence of metal ions.

In Figure 4 is shown an attempt to determine the stability constant of eq 3 by spectrophotometric measurements, i.e., by plotting at several values of constant pH 1/[ATP] vs. $1/\Delta E_{293}$. The decrease of the intercepts between the dotted lines (experiments at pH 5 and 6) and the x axis with increasing pH is in accord with an increasing concentration of $Cu(Trp)^{+}$; the latter leads to an increasing concentration of the ternary adduct and, hence, to an increasing absorbance, ΔE_{293}. Furthermore, in the concentration range of ATP from 1.5×10^{-3} M to 6.67×10^{-3} M ([Trp] = [Cu^{2+}] = 10^{-3} M), the experimental data at pH 5 and 6 fit straight lines which are parallel to the y axis within experimental error, indicating a high but indeterminate stability of the ternary complex. An estimation[25] of the lower limit of the stability constant gives log $K^{Cu(Trp)}_{Cu(Trp)(ATP)} > 4$, which agrees with the result of Table I. The deviation of the data from the straight lines at [ATP] $> 1.5 \times 10^{-2}$ M, due to a decrease of the absorbance, ΔE_{293}, indicates displacement of tryptophan from the coordination sphere of Cu^{2+} by the high concentrations of ATP.

The observations at pH 4 (Figure 4) correspond at low concentrations of ATP to the ones made at pH 5 and 6, while for [ATP] $> 8 \times 10^{-3}$ M a new situation occurs. The concentrations of metal ion complexes are rather low, and thus at high concentrations of ATP, the binary interaction between ATP and tryptophan dominates the whole system; indeed, the data with [ATP] $> 2 \times 10^{-2}$ M fit a straight line with an intercept on the y axis of $-K_{St} = -50$; i.e., log K_{St} = 1.7. This value is in excellent accord with the apparent stability constant of the stacking adduct, (Trp)(ATP), at pH 4 (cf. Table II); the same holds for the molar absorptivity (cf. Figure 2).

Obviously, the experimental alternative to the spectrophotometric determination at 293 nm of the equilibrium constant of the ternary complex would be the determination of the one due to eq 2. This would mean doing experiments analogous to those in Figure 4 but using increasing concentrations of tryptophan. Unfortunately, this is not possible as the self-absorption of tryptophan is too high. Hence, the only remaining possibility was to measure the change in absorbance at 293 nm as a function of pH for solutions containing M^{2+}, ATP, and Trp in the ratio 1:1:1, and compare the result with the distribution of complex species as calculated with the equilibrium constants determined by potentiometric titrations.

In the upper part of Figure 5, ΔE_{293} is plotted vs. pH for the Cu^{2+}-ATP-Trp system; for comparison the absorbance of the corresponding metal ion-free system is also shown. It is obvious that the presence of Cu^{2+} favors the indole-purine interaction. This is further evidence for an increased stability of the stacking adduct resulting from the formation of a metal ion bridge. However, an estimation (assuming complete complex formation) of the molar absorptivity of the metal ion bridged adduct gives $\epsilon_{293} \geq 180$ M^{-1} cm^{-1} at pH 6 to 8. As it is difficult to see why the metal ion bridge should alter the molar absorptivity, this value ap-

Table III. Logarithms of the Equilibrium Constants Used, besides Those of Table I, for the Calculation of the Concentration of the Species Shown in Figures 6 and 8 (I = 0.1; 25°C)

Equilibrium	Mn^{2+}	Cu^{2+}	Zn^{2+}	Ref
$M^{2+} + HATP^{3-} \rightleftharpoons M(HATP)^{-}$	2.39	3.12	2.67	45
$M(ATP)(H_2O)^{2-} \rightleftharpoons M(ATP)(OH)^{3-} + H^{+}$	−10.7	−8.17[a]	−8.87	46
$M(Trp)^{+} + HATP^{3-} \rightleftharpoons M(Trp)(HATP)^{2-}$	1.4	1.8	2.5	b,c
$M(Trp)^{+} + Trp^{-} \rightleftharpoons M(Trp)_2$	1.7[d]	7.07[e]	3.8[d]	b

[a] Reference 37. [b] Estimated values; the corresponding equilibria play practically no role (cf. Figures 6 and 8). [c] Example: $\log K^{Mn(Trp)}_{Mn(Trp)(ATP)} - \log K^{Mn}_{Mn(ATP)} = 3.82 - 4.78 = -0.96 = \Delta \log K_{Mn}$; hence, one obtains for $\log K^{Mn(Trp)}_{Mn(Trp)(HATP)} = \log K^{Mn}_{Mn(HATP)} + \Delta \log K_{Mn} = 2.39 - 0.96 \cong 1.4$. [d] For Mn^{2+} and Zn^{2+} complexes of amino acids holds, $\log K^{Mn}_{MnL} - \log K^{MnL}_{MnL_2} \cong 0.8$ and $\log K^{Zn}_{ZnL} - \log K^{ZnL}_{ZnL_2} \cong 0.9$, respectively;[36] hence, $\log K^{Mn(Trp)}_{Mn(Trp)_2} = \log K^{Mn}_{Mn(Trp)} - 0.8 = 2.50 - 0.8 \cong 1.7$ and $\log K^{Zn(Trp)}_{Zn(Trp)_2} = \log K^{Zn}_{Zn(Trp)} - 0.9 = 4.69 - 0.9 \cong 3.8$. [e] Cf. footnote e of Table I.

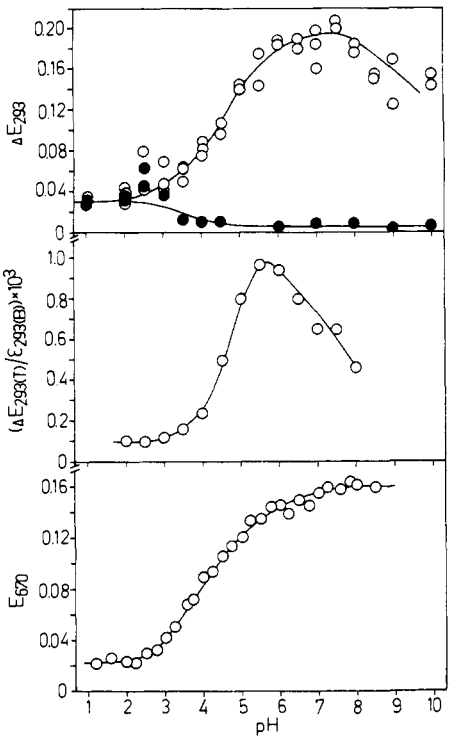


Figure 5. Spectrophotometric measurements showing the formation of $Cu(ATP)(Trp)^{3-}$ as a function of pH ($[Cu^{2+}] = [ATP] = [Trp] = 10^{-3}$ M; 25°C; I = 0.5, $NaClO_4$). (Upper) Uv-difference absorbance, ΔE_{293}, measured in the presence of leucine (○) (10^{-3} M; cf. Experimental Section); the same system but without Cu^{2+} gives ●. (Middle) The uv-difference absorbance of the ternary system, $\Delta E_{293(T)}$, was measured in the presence of leucine and triphosphate (each 10^{-3} M; cf. Experimental Section); the average of five independent measurements was used and divided by the molar absorptivities, $\epsilon_{293(B)}$ (cf. Table II), of the Trp-ATP adducts, i.e., $\Delta E_{293(T)}/\epsilon_{293(B)}$ was plotted (see text). (Lower) Optical absorbance at 670 nm of the ternary system (measured in 4-cm cells; the reference was H_2O).

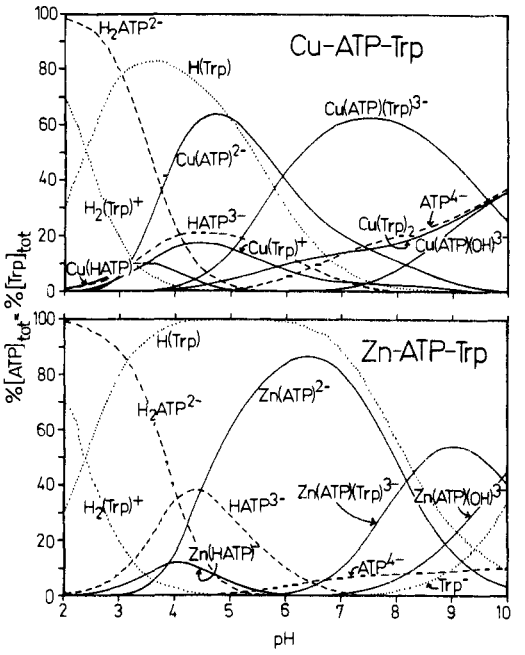


Figure 6. Effect of pH on the concentrations of the species present in an aqueous solution (I = 0.1; 25°) of M^{2+}, ATP, and Trp, given as the percentage of the total ATP or Trp (or M^{2+}) present; computed with the constants of Tables I and III for concentrations of 10^{-3} M for each reactant. Complexes with a partially protonated phosphate chain were not considered in the calculations because the corresponding constants are not known. However, if such species occur they would exist only below pH 3. (Upper) Cu^{2+}, ATP, and Trp (concentrations of $Cu(HATP)(Trp)^{2-}$ and Trp^{-} are <0.3 and ≤0.5%, respectively). (Lower) Zn^{2+}, ATP, and Trp (concentrations of $Zn(HATP)(Trp)^{2-}$, $Zn(Trp)^{+}$, and $Zn(Trp)_2$ are <0.1, ≤5.2, and ≤7.1%, respectively).

pears to be too high by a factor of at least 2 (cf. Table II). Hence, this suggests the presence of an additional absorbance, probably arising from a Cu^{2+}–tryptophan interaction because this would not be cancelled by a corresponding interaction in the reference beam. This could not be investigated directly as in a binary Cu^{2+}–Trp system precipitation occurs in this pH region. Therefore, in addition to the measurements described above, a reference solution containing Cu^{2+}, TP, and Trp (cf. Experimental Section) was used, using hydrogen triphosphate to mimic ATP. The absorbances of the ternary system, $\Delta E_{293(T)}$, measured in this way are of the expected order; however, below pH 4 they are also of the same order as observed for the metal ion free systems. Therefore, to obtain a direct measure for the extent of formation of the Cu^{2+} bridged stacking adduct, $\Delta E_{293(T)}$ was divided by $\epsilon_{293(B)}$ of the Cu^{2+}-free systems (Table II and Figure 3). The results are plotted in the middle part of Figure 5; they parallel the former results (upper part) reasonably well, at least up to pH about 5.5 where a sharp break and decrease in the absorbance occurs. This arises from a change in the reference solution, as $Cu(HTP)^{2-}$ is deprotonated ($pK^{H}_{Cu(HTP)} = 5.6$).[62]

The upper and middle part of Figure 5 show clearly that the stability of the stacking adduct depends on the metal ion bridge, i.e., on the coordination of Cu^{2+} to ATP and Trp. This change in the coordination sphere of Cu^{2+} should be reflected in the visible region of the absorbance spectrum. Indeed, this is observed, as is shown in the lower part of Figure 5 where E_{670} is plotted vs. pH;[63] the similarities between the parts of Figure 5 are obvious.

The change in concentration in dependence on pH for the several species present in a Cu^{2+}–ATP–Trp, 1:1:1, system under the conditions of Figure 5 is shown in the upper part of Figure 6 (Tables I and III).[64] By comparing the curve E_{670} vs. pH with the concentration of the several Cu^{2+} complexes, one realizes that this curve is a superposition of

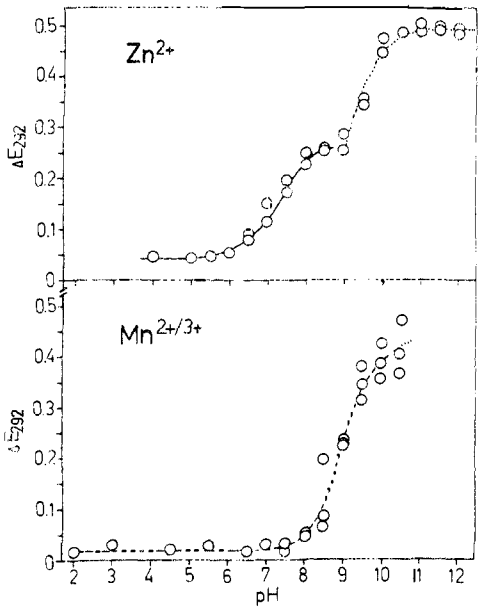


Figure 7. Uv-difference absorbance, ΔE_{292}, indicating the formation of M(ATP)(Trp) in dependence on pH (cf. Experimental Section; $[M^{2+}]$ = [ATP] = [Trp] = [Leu] = 10^{-3} *M*; 25°C; *I* = 0.5, $NaClO_4$). The dotted line parts indicate regions of precipitation. (Upper) Zn^{2+}-ATP-Trp system. (Lower) $Mn^{2+/3+}$-ATP-Trp system; Mn^{2+} was to a remarkable extent oxidized to Mn^{3+} (see text).

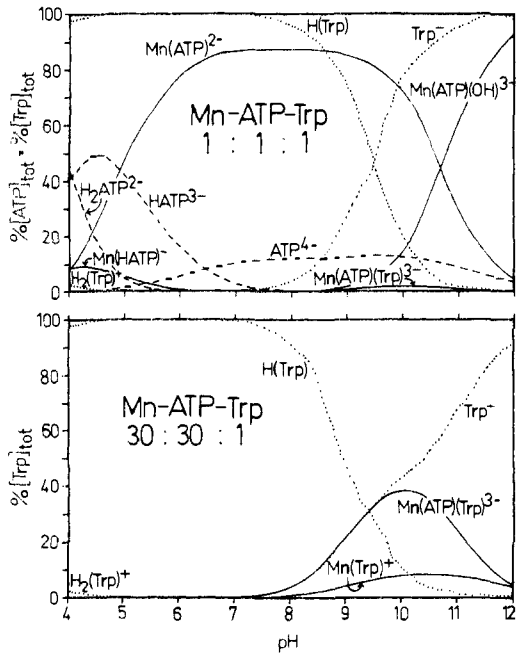


Figure 8. Influence of pH on the concentrations of the several species present in an aqueous solution (*I* = 0.1; 25°) of Mn^{2+}, ATP, and Trp; for constants, cf. Tables I and III. Complexes with a partially protonated phosphate chain were not considered because the corresponding constants are not known. However, if such species occur, they would exist only below pH 3. (Upper) $[Mn^{2+}]$ = [ATP] = [Trp] = 10^{-3} *M*; the concentrations of the several species are given as the percentage of the total ATP or Trp (or Mn^{2+}) present (concentrations of $Mn(HATP)(Trp)^{2-}$, $Mn(Trp)^{+}$, and $Mn(Trp)_2$ are <0.01, ≤2.4, and ≤0.1%, respectively). (Lower) $[Mn^{2+}]$ = [ATP] = 3×10^{-2} *M* and [Trp] = 10^{-3} *M*; concentrations of the several species containing Trp are given as the percentage of the total Trp present (concentration of $Mn(HATP)(Trp)^{2-}$ is <0.01%).

the absorbance of all the complexes present, whereas the absorbances measured at 293 nm, due to the indole–purine stacking, resemble very closely the concentration of $Cu(ATP)(Trp)^{3-}$ in dependence on pH; in both curves of the upper parts of Figures 5 and 6, the maximum is located at pH 7.5. This is a conclusive proof of the intramolecular stacking in $Cu(ATP)(Trp)^{3-}$.

The uv-difference absorbance, ΔE_{292}, for the Zn^{2+}-ATP-tryptophan system is plotted vs. pH in the upper part of Figure 7. The distribution of the species as a function of pH for the same system and under the same conditions is shown in the lower part of Figure 6. A comparison shows the close relation between the absorbance at 292 nm and $[Zn(ATP)(Trp)^{3-}]$. Although a precipitate forms at pH >9, the formation of a plateau (ΔE_{292}) at this pH where the maximal concentration of $Zn(ATP)(Trp)^{3-}$ occurs is unequivocal. A comparison of ΔE_{max} for the Cu^{2+} and Zn^{2+} systems (upper parts of Figures 5 and 7) reveals that this is about 30% higher for the latter system; this could be an indication of a larger extent of intramolecular stacking in $Zn(ATP)(Trp)^{3-}$ compared with that in $Cu(ATP)(Trp)^{3-}$.[65]

The attempt to study the Mn^{2+}-ATP-tryptophan system led to unexpected results. The uv-difference absorbance, ΔE_{292}, is plotted vs. pH in the lower part of Figure 7; a comparison with the distribution of the species under the same conditions, as shown in the upper part of Figure 8, reveals indeed the occurrence of an absorption in the pH region where $Mn(ATP)(Trp)^{3-}$ is formed. However, this ternary complex exists only in very low concentrations (2%), and, as the metal ion is relatively far away from the region where the indole–purine interaction occurs, a significant change of the molar absorptivity cannot be expected. Hence, this suggests that actually the absorbance of the stacking interaction of another species is measured. Repetition of the experiments in the absence of Mn^{2+}, but at constant ionic strength (*I* = 0.5, $NaClO_4$), demonstrated unequivocally the dependence of ΔE_{292} on the presence of this metal ion, and that Na^+ is not able to link the aromatic moieties together significantly. The only conclusive explanation is that we were actually measuring the absorbance due to stacking in a Mn^{III} species, i.e., of $Mn(ATP)(Trp)^{2-}$. In accord with this are (1) the observation that the solutions turned slightly yellowish in the pH range of 9 to 10, and (2) the known stabilization of Mn^{III} by phosphates,[66] e.g., HPO_4^{2-} and $P_2O_7^{4-}$. When Mn^{2+} and ATP are in a 30-fold excess compared with Trp, it is possible to obtain $Mn(ATP)(Trp)^{3-}$ in considerable concentrations (cf. the lower part of Figure 8).[67]

^{1}H NMR Studies of the Tryptophan-Zn^{2+}-ATP System. ^{1}H NMR measurements were carried out to detect the intramolecular stacking in the mixed-ligand complexes containing ATP and tryptophan by another independent method. When a stacking adduct is formed by two aromatic systems, the ^{1}H NMR resonances of those protons lying above or below a π system are shifted to higher field compared with the signals of the free molecule.[9b,10] As paramagnetic metal ions, like Cu^{2+} and Mn^{2+},[24,26,41] broaden the proton resonances, the only suitable metal ion of those used in this study is the diamagnetic Zn^{2+}.[68]

The ^{1}H NMR signals of ATP and tryptophan were assigned according to Cohn and Hughes[69] and Bak et al.,[70] respectively. Figure 9 shows the influence of increasing amounts of tryptophan on the chemical shift of H(2) and H(8) of ATP, and of H(2), H(4), H(5,6), and H(7) of tryptophan (cf. Scheme I) at equimolar concentrations of Zn^{2+} and ATP^{4-} in D_2O at pD 9.4[71] and 10.4.[72] The signals due to the protons of ATP are shifted to higher field, as are the resonances of tryptophan when compared with those of the free ligand.[73] The relatively constant size of Δ shift for the tryptophan protons is in accord with the high formation degree of the mixed-ligand Zn^{2+} complex under these condi-

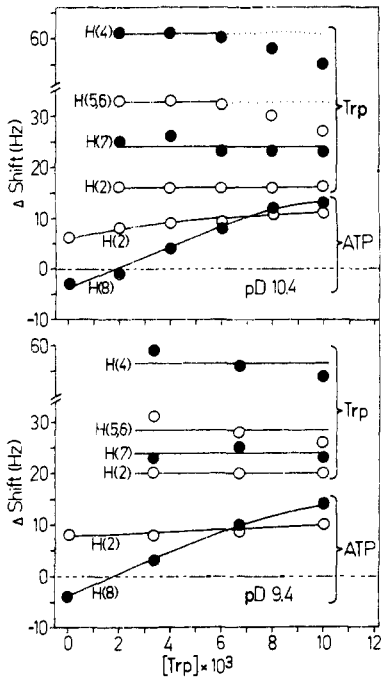


Figure 9. Change in chemical shift, Δ shift, of the ^1H NMR resonances for H(2) and H(8) of ATP and for H(2), H(4), H(5,6), and H(7) of Trp (cf. Scheme I) in D_2O at pD 9.4 (lower part) and 10.4 (upper part) in the presence of Zn^{2+} in dependence on [Trp]; [ATP] = $[Zn(ClO_4)_2] = 10^{-2}$ *M*; 25°C. The chemical shifts of the free ligands at pD 9.4 (first value) and pD 10.4 (second value) are for ATP: H(8) 769, 768; H(2) 742, 742 Hz, and for Trp: H(2) 665, 656; H(4) 680, 672; H(5,6) 652, 644; H(7) 699, 693 Hz. In the case of multiplets, the data refer to the midpoint of the pattern.

tions.[74] Overall, the positive shift values for H(2) and H(8) of ATP evidence clearly the existence of an intramolecular stacking within $Zn(ATP)(Trp)^{3-}$. In the binary complex, $Zn(ATP)^{2-}$, the signal of H(8) is shifted in the opposite direction.[68]

The stabilizing influence of Zn^{2+} on the stacked adduct becomes even more evident when increasing concentrations of Zn^{2+} are added to solutions containing equimolar amounts of ATP and Trp (cf. Figure 10). In the absence of Zn^{2+}, a small positive Δ shift is observed, confirming stacking between the aromatic moieties of the two ligands. However, as soon as both ligands are linked together, Δ shift increases tremendously, indicating considerably increased stacking. As in Figure 9, the Δ shifts in Figure 10, due to H(8) of ATP, and H(4) and H(5,6) of Trp, are especially significant. This may imply that the imidazole part of the adenine moiety is located over the benzene ring of the indole residue within $Zn(ATP)(Trp)^{3-}$

Discussion

The first hint of intramolecular stacking in the mixed-ligand complexes, $M(ATP)(Trp)^{3-}$, was obtained from the stability data measured by potentiometric titrations. Studies of the uv-difference absorbances and the shifts in the ^1H NMR spectra gave conclusive evidence for the formation of metal ion bridged stacked adducts. Therefore, the question arises of the position of the intramolecular equilibrium 9 between an open and a stacked form.

$$\text{ATP-M}^{2+}\text{-Trp} \overset{K'}{\rightleftharpoons} \begin{matrix}\text{ATP}\\ \diagdown\\ \text{M}^{2+}\\ \diagup\\ \text{Trp}\end{matrix} \qquad (9)$$

One may try to make a rough guess about the order of the dimensionless constant K' based on the relative magnitudes of the molar absorptivities of the stacking adducts.

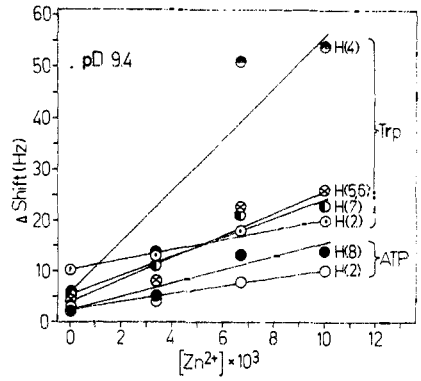


Figure 10. Change in Δ shift of the ^1H NMR resonances for H(2) and H(8) of ATP and for H(2), H(4), H(5,6), and H(7) of Trp in D_2O at pD 9.4 in dependence on $[Zn^{2+}]$; [ATP] = [Trp] = 10^{-2} *M*; 25°C; cf. Figure 9.

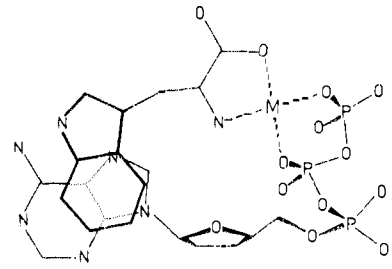


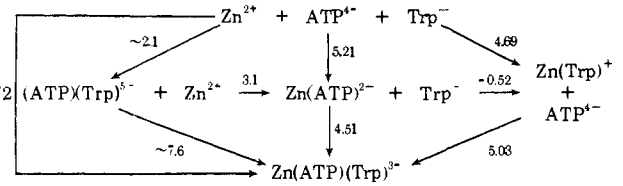
Figure 11. Tentative and simplified structure of M(ATP)(Trp).

The values of ϵ, which are assembled in Table II for the metal ion-free systems, suggest for the metal ion linked adducts a lower limit for the molar absorptivity of about 60 M^{-1} cm^{-1} and an upper limit of about 400 M^{-1} cm^{-1}. As the latter value corresponds to a species with a protonated adenine moiety, which does not occur in the ternary complexes, one expects a value closer to the lower limit, i.e., to 60 M^{-1} cm^{-1}. Based on Figure 5, one estimates[75] for $Cu(ATP)(Trp)^{3-}$: $\epsilon_{293(Cu,ATP,Trp)} \simeq 150$ M^{-1} cm^{-1}. Hence, this value might well correspond to conditions where $Cu(ATP)(Trp)^{3-}$ exists practically completely in the stacked form; this would be also in accord with Δ log K_{Cu}, which is larger for the ternary complex containing tryptophan compared to the one for the complex with alanine (cf. Table 1). However, to be on the safe side, we conclude only $K'_{Cu(ATP)(Trp)} \geq 1$. The same holds probably for $Mn(ATP)(Trp)^{3-}$, as may be surmised from the values due to Δ log K_{Mn} of the tryptophan and alanine systems.

A comparison of the upper parts of Figures 5 and 7, by taking also into account the results of Figure 6, suggests that the intramolecular stacking in $Zn(ATP)(Trp)^{3-}$ is somewhat more pronounced than in $Cu(ATP)(Trp)^{3-}$. This is in accord with the relatively large value of Δ log K_{Zn} for the Zn^{2+}-ATP-Trp system. Hence, there is some evidence that the degree of the intramolecular stacking in these ternary complexes depends also on the kind of metal ion involved, i.e., on the steric conditions imposed by the geometry of its coordination sphere. A tentative and simplified structure of these metal ion bridged stacking adducts is depicted in Figure 11; it is based on the ^1H NMR results of the Zn^{2+}-ATP-Trp system. Also, the metal ion is shown as being coordinated only to the β- and γ-phosphate group of ATP, which is true for Cu^{2+} and Zn^{2+}, while Mn^{2+} coordinates to all three phosphate groups;[69] however, such a terdentate coordination is in the stacking adduct, sterically also well possible.

The different reactions which lead to the formation of such a mixed-ligand complex, M(ATP)(Trp), may be sum-

Scheme II



marized as is shown for Zn^{2+} in Scheme II. The logarithms of the equilibrium constants are given for each step; these data were either taken or calculated from the results given in Tables I and II. The data for the systems with Cu^{2+} or Mn^{2+} may be obtained analogously.

Two further features should be considered. One follows from Figure 8, where the lower part demonstrates that excess of Mn^{2+}-ATP^{4-} leads to a significant formation of $Mn(ATP)(Trp)^{3-}$ and therefore to an increased stacking interaction. This situation resembles natural systems where the concentration of substrate dominates that of the enzyme: even a low coordination tendency may then lead to a satisfactory formation degree of complexes. The other follows from the attempt to study the uv-difference absorbance of the Mn^{2+}-ATP-tryptophan system which was not very satisfactory from an experimental point of view, but it evidences that the change in the oxidation state of the metal ion from +II to +III leads not only to a higher coordination tendency of the metal ion but also to an increased stacking between the indole and purine moieties. Certainly, the corresponding reduction from +III to +II will induce the reverse process. Hence, assuming (1) a metal ion is located at a specific site, and (2) a given ligand group will only coordinate if the metal ion is in a particular oxidation state, then a kind of a "trigger" is created by inducing stacking through coordination, and a change in conformation may be induced at a site relatively remote from the location of the metal ion. Again, such a situation appears possible in natural systems.

General Conclusions

Today it is clear that specific interactions between aromatic moieties play an important role in biological systems. In many cases, aromatic amino acid residues are in the active center of enzymes. Furthermore, it was suggested, e.g., for microsomal (Na^+ and K^+) ATPase,[76] that the adenine moiety of ATP is necessary for binding to the enzyme. Similarly, based on studies with 1,N^6-etheno derivatives of such coenzymes, it was concluded that the adenine residue is not used to activate the coenzyme but rather to bind it to the enzyme by stacking.[20]

Such stacking adducts are usually rather weak if they are not additionally stabilized by polar interactions, e.g., proton or ionic bridges. For example, in the adduct between glucose oxidase and FAD, a tryptophanyl group stacks with one of the aromatic moieties of FAD (either the adenine or the isoalloxazine part), while the phosphate and ribose residues of the coenzyme are connected to the enzyme by polar interactions.[77] The high stability of the stacking adduct between Trp(62) in the active center of lysozyme and nicotinylium moieties is explained in a similar way.[78]

More directly related to the present results is the observation that ATP will only be hydrolyzed by heavy meromyosin in the presence of Ca^{2+} or Mg^{2+}. Moreover, a new absorbance in the uv-difference spectrum of the enzyme and ATP in the 290-nm region is only observable in the presence of Ca^{2+} or Mg^{2+}.[7] This is interpreted as an effect of the stacking between the purine residue of ATP and a tryptophanyl indole group in the active center of the enzyme.[7,11] Indeed, the shape and position of this difference spectrum are in accord with those obtained now for the ternary M^{2+}-ATP-Trp systems. Similarly, pyruvate kinase binds Mn^{2+} tightly only in the presence of nucleotides.[79] A study of the binding tendency between arginine kinase and Mn^{2+}, ADP, or ATP revealed that $Mn(ADP)^-$ or $Mn(ATP)^{2-}$ are more tightly bound to the enzyme than Mn^{2+} or the nucleotides alone;[80] this may also be interpreted in terms of an increased stability of ternary complexes[50,54,55] and a stacking interaction. Furthermore, a study of the helical interactions of poly(N^6-[Δ^2-isopentenyl]adenylic acid) showed an increased stacking and base pairing in the presence of Mg^{2+}.[81]

Finally, it must be emphasized that similar results to those outlined here for the model system containing M^{2+}, ATP, and tryptophan must be expected for corresponding systems containing other purine or pyrimidine nucleotides, metal ions, and aromatic amino acid residues. For example, the tyrosinyl[82] or the histidinyl[83] moieties are known to form stacking adducts with nucleotides, while salicylate fits into the adenosine-binding pocket of liver alcohol dehydrogenase.[84]

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(74) The signals due to H(4) and H(5,6) are somewhat overlapping leading to an increased error in the evaluation of Δ shift for these protons.
(75) From the middle part of Figure 5 one obtains at pH 6 $\Delta E_{293(T)}/\epsilon_{293(B)} \simeq 0.95 \times 10^{-3}$ and from Table II $\epsilon_{293(B)} = 66$; hence $\Delta E_{293(T)} = 0.95 \times 10^{-3} \times 66 \simeq 0.063$. $[Cu(ATP)(Tp)] = 4.3 \times 10^{-4}$ *M* (cf. upper part of Figure 6); therefore, $\epsilon_{293(Cu,ATP,Trp)} = 0.063/4.3 \times 10^{-4} \simeq 150\ M^{-1}\ cm^{-1}$.
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