Nuclear Magnetic Resonance Studies of Catecholamines. Ternary Complexes with Adenosine 5′-Triphosphate and Divalent Metal Ions in Aqueous Solution

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Abstract: ^{1}H NMR investigations of the association between fully protonated catecholamines (dopamine and norepinephrine) and ATP-divalent metal ion (Mg, Co, Ni, Mn) chelates have been carried out in order to elucidate the stoichiometry and the structure of their ternary complexes. Analysis of the induced chemical shifts indicates that catecholamine-ATP-M^{2+} complexes are formed with practically only a 1:1:1 stoichiometry. It is confirmed that the binding of the catecholamine molecule in the ternary complexes is via its association with ATP alone, with no direct interaction with the metal ion. The stability of these complexes is found to be independent of the type of the ATP-chelated divalent metal ion, the average formation constants being 11 and 13 M^{-1} for the complexes of dopamine and norepinephrine, respectively. The results, however, indicate that the chelation of divalent metal ion in the ternary complex reduces the ability of ATP to bind catecholamines. Spin-lattice relaxation data for the protons of catecholamines bound in the ternary complexes were used to determine metal-proton distances. The results are consistent with outer-sphere coordination of the catecholamines. Further, direct evidence for this outer-sphere coordination is provided by monitoring the effect of ATP and catecholamines on the EPR spectrum of Mn^{2+}, and the effect of divalent metal ions and catecholamines on the ^{31}P spectrum of ATP. A model for the ternary complex is proposed on the basis of the present results, coupled with a previously suggested model for the binary catecholamine-ATP complex.

Divalent metal ions, usually associated with nucleotides, are known to play a role in the mechanisms involved in the release and reuptake of catecholamines in biogenic organelles. Numerous data reported provide considerable evidence for metal or metal-ATP dependence of these transport processes. The finding[1] that, in addition to adenine nucleotides, divalent metal ions occur also in storage sites of catecholamines, led to the suggestion that metal coordination may be important in the binding mechanism of the amines. Models for catecholamine-nucleotide-divalent metal ion complexes interacting with adrenergic receptors have been suggested[2] on the basis of tentative, or indirect, rather than firm experimental grounds.

In a previous study[3] it has been shown that the interactions between catecholamines and divalent metal ions, or 1:1 metal-ATP chelates (MATP), depend considerably on the ionization state of the amines. At acidic or neutral pH, the catecholamines, being in the cationic state[4] (i.e., un-ionized ring hydroxyls and protonated ammonium group), do not interact with divalent metal ions. At pH >7, however, as the phenolic hydroxyl of the catecholamines starts to ionize, a strong metal association is found to take place, involving this site. In the presence of ATP, ternary complexes of two types are formed. Both exhibit strong metal-ATP chelation, but different pH-dependent catecholamine binding modes. It was suggested that at pH $\lesssim$7 the catecholamine binding takes place only through association with ATP, with no direct interaction with the metal ion. At higher pH the catecholamine would bind directly to the metal ion via the ionized ring hydroxyl, apparently with no association with the ATP molecule. The association involved in the two types of ternary complexes can be schematically represented as "catecholamine-ATP-M^{2+}" and "catecholamine-M^{2+}-ATP", respectively.

Ternary complexes of the second type were investigated in some detail in a series of studies by Rajan et al.,[5] and possible correlations between the experimental results and the biological activity of the amine were sugested. It is to be noted, however, that physiological pH, the major portion of the catecholamines (ca. 97%[4]) is in the cationic form, i.e., fully protonated. Even though metal ions may somewhat increase the acidity of the amine ring hydroxyls,[3] nevertheless ternary complexes of the first type would greatly predominate.

In the present work a quantitative ^{1}H NMR study of ternary catecholamine-MATP complexes of the first type is provided. The stoichiometry, the formation constants, and the structure of these complexes have been determined through appropriate analysis of paramagnetic shift and relaxation data. The experiments were carried out at pD ~6.4[6] in order to eliminate possible formation of the second type of ternary complexes. (Ionization of the first phenolic group of catecholamines, in metal-free, deuterium oxide solutions, occurs with pK_as in the region 9.4-9.6.[4])

Experimental Section

Materials. The amines,[7] as hydrochlorides, and ATP disodium salt of the highest purity, were obtained from Sigma. The metal salts $Co(NO_3)_2 \cdot 6H_2O$ and $MgCl_2 \cdot 6H_2O$ were obtained from Merck; $NiCl_2 \cdot 6H_2O$ and $MnCl_2 \cdot 4H_2O$ were from BDH Chemicals. The metal salts (except that of manganese) were dried in vacuo with slight heating. Experimental solutions were made up by dissolving the materials in D_2O (99.7%).

NMR Spectra. ^{1}H NMR chemical shifts were measured on a Bruker HFX-10 spectrometer operating at 90 MHz. The deuterium signal of the solvent was used for locking. A trace of dioxane in the experimental solutions served as an internal reference. Upfield shifts (expressed in hertz) are denoted by a positive sign. Each measurement was repeated three-four times in order to reduce random errors. The experimental uncertainty in the diamagnetic shifts is estimated as ±0.5 Hz and in the paramagnetic shifts as 3-7%, depending on the line widths.

Proton spin-lattice relaxation times were determined using a 180°-τ-90° sequence on a Bruker WH-270 spectrometer, equipped with a Nicolet Model 1180 32K computer, operating at 270 MHz. Homodecoupling was used to suppress the HDO signal to minimize its interference. The experimental error in the relaxation times measurements is estimated as 10-15%.

All the measurements were performed at an ambient probe temperature of 27 °C. The notation of the proton resonances of the studied amines is as given in structure **1**.

Results and Discussion

(A) Stoichiometry, Formation Constants, and Intrinsic Shifts. The proton chemical shifts ($\Delta\delta$) induced by ATP chelates with divalent metal ions (Co^{2+}, Ni^{2+}, and Mg^{2+}) in the series of phenolic amines (PEA, TA, DA, and NE)[7] were investigated by two types of titrations: (a) titration of amine (at constant

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Table I. Formation Constants (K_1) and Bound-State Shifts (δ_1) for the Amines in Their 1:1 Complexes with M^{2+}-ATP Chelates

Complex	K_1, M^{-1}	δ_1, Hz		
		Ring protons[a]	Side-chain protons	
			β	α
PEA-CoATP	10.5 ± 0.9	232 ± 9 (×2); 158 ± 11 (×3)	331 ± 11	315 ± 19
TA-CoATP	11.0 ± 1.2	241 ± 10 (×4)	397 ± 16	357 ± 12
DA-CoATP	11.3 ± 1.0	311 ± 22 (H_6); 228 ± 20 (H_5); 235 ± 20 (H_2)	447 ± 13	377 ± 10
NE-CoATP	13.5 ± 1.4	296 ± 24 (H_6); 207 ± 23 (H_5); 208 ± 23 (H_2)	409 ± 15	393 ± 8; 539 ± 14
DA-NiATP	11.6 ± 2.0	34 ± 4 (×3)	21 ± 3	11 ± 2
NE-NiATP	12.6 ± 2.0	27 ± 3 (×3)	*b*	8 ± 2
DA-MgATP	12.1 ± 1.3	36 ± 2 (H_6); 34 ± 2 (H_5); 31 ± 2 (H_2)	21 ± 2	11 ± 1
NE-MgATP	13.4 ± 2.0	25 ± 2 (H_6); 27 ± 2 (H_5); 17 ± 2 (H_2)	*b*	7 ± 1
DA-ATP[c]	16.3 ± 1.8	35 ± 2 (H_6); 31 ± 2 (H_5); 30 ± 2 (H_2)	22 ± 2	12 ± 2
NE-ATP[c]	17.1 ± 2.0	23 ± 2 (H_6); 25 ± 2 (H_5); 14 ± 2 (H_2)	*b*	6 ± 1

[a] The assignment of the ring protons is given in parentheses. In cases of ambiguous identification, due to overlapping resonances, the relative integrated intensities are given. [b] Obscured by the HDO signal. [c] From ref 9.

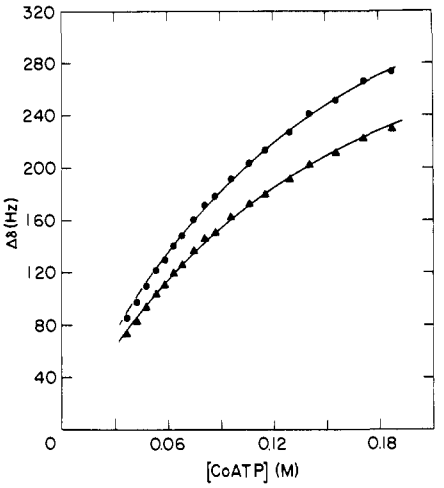


Figure 1. Paramagnetic shifts induced by CoATP in the H_α (triangles) and H_β (circles) protons of DA (70 mM).

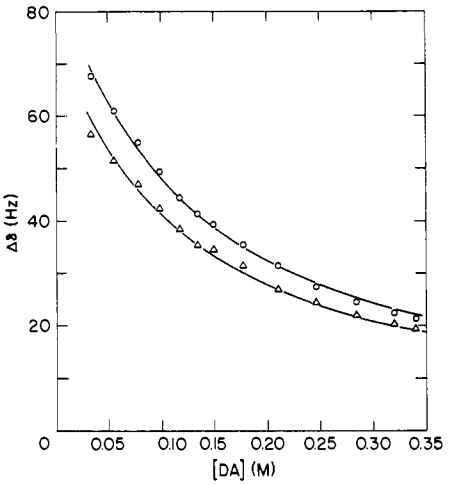
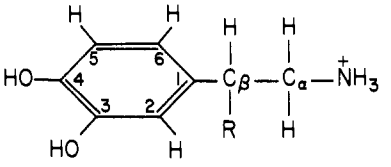


Figure 2. Paramagnetic shifts induced by CoATP (22 mM) in the H_α (triangles) and H_β (circles) protons of DA, as a function of its concentration.



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concentration of 40-70 mM) by 1:1 M^{2+}-ATP (MATP, 0.01-0.2 M); (b) titration of MATP (at constant concentration of ca. 20 mM) by amine (0.02-0.35 M). Under the experimental conditions the metal ions and ATP can be regarded as being completely complexed in a 1:1 chelate.[8] Typical titration curves are shown for the DA-CoATP system in Figures 1 and 2. The experimental data of both titrations have been simultaneously analyzed assuming a possible binding of either one or two amine molecules by the M^{2+}-ATP chelate, i.e., that both 1:1 and 2:1 amine-MATP complexes are formed, with stepwise formation constants K_1 and K_2. The method of analysis has been described elsewhere.[9] The analysis yielded $K_1 \simeq 10\ M^{-1}$ and $K_2 \simeq 0$ for the systems under investigation. It follows that within the experimental error the formation of a MATP·(amine)$_2$ complex can be regarded as negligible. The data were then subjected to a least-squares analysis assuming the formation of a 1:1 complex only. The calculated formation constants (K_1) and intrinsic shifts (δ_1) are given in Table I.

In order to establish the formation of a 1:1 catecholamine-MATP complex throughout the whole relevant concentration range, use was made of the Scatchard equation:[10,11]

$$\nu/L_f = K(n - \nu) \qquad (1)$$

In the present case, bearing in mind that $L_b/L_0 = \Delta\delta/\delta_1$, we have:

$$\nu = L_b/A_0 = \Delta\delta L_0/(\delta_1 A_0)$$

$$L_f = L_0 - L_b = (1 - \Delta\delta/\delta_1)L_0$$

where L_0, L_b, and L_f denote, respectively, total, bound, and free amine concentrations, and A_0 is total MATP concentration. n is the number of amine molecules bound (equivalently and independently) to a monomeric unit of the MATP chelate and K is the overall formation constant of the complex. According to the above equation a plot of ν/L_f vs. ν should yield a linear line with intercepts of n and nK with the abscissa and the ordinate, respectively. The representation of data by means of a Scatchard plot is very sensitive to the model employed. Even small deviations (e.g., alteration in stoichiometry) which may not have been detected in regular data representation (e.g., titration curves) would be prominently displayed by this plot. A Scatchard plot for the data of Figures 1 and 2 is shown in Figure 3. The straight line is the theoretical one for $n = 1$ and $K = 11.3\ M^{-1}$. The good correlation between the theoretical line and the experimental data clearly demonstrates the formation of catecholamine-MATP complexes with practically only 1:1 stoichiometry.

The formation of catecholamine-MATP complexes of such

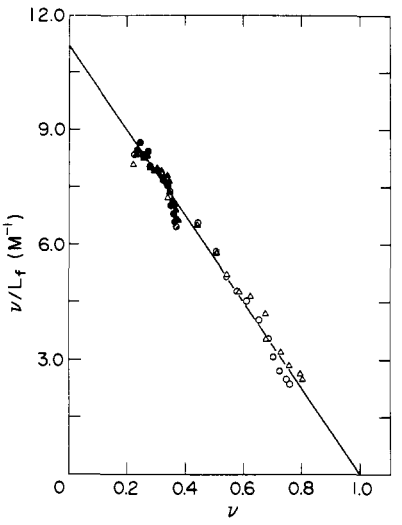

Figure 3. Scatchard plot for the data in Figures 1 and 2. The straight line was calculated by means of eq 1, taking $n = 1$ and $K = 11.3\ M^{-1}$.

stoichiometry only can be rationalized by using a space-filling model for the M^{2+}-ATP complex. Such a model, constructed on the basis of previous results,[8,12-16] under the assumption that association with catecholamines does not significantly alter its conformation (see below), reveals that the metal ion coordinated to N_7 of the adenine ring (either directly or via a water bridge) together with the phosphate chain, which chelates the ion and folds around it, form a bulky group on one side of the adenine ring. This group would considerably interfere with the association of amine on this side. Moreover, the protonated ammonium group would be in close proximity to the positively charged metal ion, resulting in electrostatic repulsion. It thus appears that, in contrast with metal-free ATP, in metal-associated ATP only one side of the adenine ring is available for association with a catecholamine molecule.

Inspection of Table I shows a constant trend in the magnitudes of the intrinsic shifts along the series of the amine-MATP complexes, i.e., PEA < TA < DA < NE. This trend, which is similar to that found in the binary amine-ATP complexes,[9] actually reflects the association of the amines with ATP in the ternary complexes. Moreover, the similarities between the values of the formation constants for the amine-MATP and the corresponding amine-ATP[9] complexes, and between the values of the shifts of catecholamines in their complexes with ATP or MgATP (cf. Table I), indicate that introducing divalent metal ion to form the ternary complex does not significantly affect the interactions between the amine and the ATP molecules. The results thus imply, in agreement with a former suggestion,[3] that in the catecholamine-MATP complexes the catecholamine molecule associates only with ATP and not with the metal ion. The somewhat lower formation constants in the ternary, relative to the respective binary complexes,[9] may be attributed to weakening of the electrostatic interaction between the side chain of the amine and the phosphate moiety of ATP in the presence of the M^{2+} ion. It has indeed been shown[3] that charge neutralization of AMP^{2-} by its binding to Co^{2+} considerably decreases its ability to bind catecholamines. Inspection of atomic models reveals that additional decrease of the electrostatic interaction is due to a larger separation between the phosphate and the ammonium groups in the ternary complex, ca. 6 Å, compared to ca. 3 Å in the binary complex.

The observed paramagnetic shifts may arise from two contributions: the contact (scalar) and the pseudocontact (dipolar) hyperfine interactions. Examination of the shifts of catecholamines in the complexes with NiATP in comparison with those in the MgATP complexes (cf. Table I) reveals that there is actually no net contact contribution to the induced shifts in the ternary complexes. This implies that the paramagnetic shifts induced by CoATP are of through-space pseudocontact origin only, as is expected for outer-sphere coordination of the amines in the ternary complexes. Considering the spatial dependence of the pseudocontact shifts, the increase of the intrinsic shifts along the series of the amine-CoATP complexes, upon substitution of amine ring hydroxyl groups, is compatible with the amine approaching nearer to the ATP molecule, and subsequently to the metal ion, as has been similarly observed in the corresponding binary complexes.

It is interesting to note the splitting of the $H_{\alpha\alpha'}$ signal of NE into two distinct, well-separated lines (cf. Table I), brought about by its complexation to CoATP; this is not observed with the other amine complexes. Due to the asymmetric β-carbon of NE the α-hydrogens become nonequivalent. However, because of small differences in the diamagnetic shielding experienced by these nuclei in the various rotamers of NE, either free in solution or complexed with ATP or MgATP, the signals of the α-hydrogens become accidently isochronous. The large paramagnetic shifts induced by Co^{2+} serve to augment the nonequivalency of H_α and $H_{\alpha'}$, in this case arising mainly from different internuclear orientations relative to the metal ion, in each rotamer of NE bound to CoATP. The possibility of resolving these resonances implies,[17] in support of the previous suggestion,[9] that the association of NE with ATP involves considerable stabilization of a preferred conformation, apparently through hydrogen bond formation between the β-hydroxyl and the phosphate moiety.[18]

(B) Spin-Lattice Relaxation. Spin-lattice relaxation times (T_1) were measured for the protons of DA (62 mM) and NE (57 mM) as a function of the concentration of added MgATP (6-47 mM), CoATP (2-30 mM), NiATP (0.3-6 mM), and MnATP (0.2-3 mM). The intrinsic relaxation rates due to the complexation of catecholamines with the MATP chelates were obtained by fitting the experimental data to the relation:

$$\frac{1}{T_1} = \frac{1}{T_{1,0}} + \frac{L_1}{L_0}\left(\frac{1}{T_{1,C} + \tau_M} - \frac{1}{T_{1,0}}\right) \qquad (2)$$

where $T_{1,0}$ and $T_{1,C}$ refer to relaxation times of catecholamines in the unbound state and in the 1:1 complex with MATP, respectively; L_0 is the total concentration of the catecholamine and L_1 is the concentration of the complex calculated[9] with the appropriate K_1 values from Table I. Since it follows from Table I that the formation constants for the catecholamine-MATP complexes are, within experimental uncertainty, independent of the type of divalent metal ion, the average values 11.3 and 13.2 M^{-1} were used for the DA-MnATP and NE-MnATP complexes, respectively. τ_M in eq 2 is the mean residence time of the metal ion in the complex. Values for τ_M obtained for the MATP complexes[15] were employed in the calculations. Actually it is found that for the ternary complexes $\tau_M \ll T_{1,C}$ and this term is practically negligible in eq 2. The calculated intrinsic relaxation rates are given in Table II.

The variations in the $T_{1,C}$ values for the ring and the side-chain protons of the catecholamines upon complexation with MgATP reflect the involvement of both groups in the association. However, while these values are similar for the ring protons of DA and NE, a more pronounced relaxation is observed for the H_α protons of NE as compared to those of DA. This indicates a higher degree of stabilization of the side chain of the complexed NE, in agreement with the chemical-shift results discussed above.

The relaxation rates in the paramagnetic MATP complexes of catecholamines follow the sequence $H_\beta > H_\alpha > H_6 > H_2 > H_5$. A similar trend is observed also with the CoATP-induced paramagnetic shifts (cf. Table I). These trends, having a direct implication on the relative metal-proton distances, are

Table II. Spin-Lattice Relaxation Rates ($T_{1,C}^{-1}$, s^{-1}) for Dopamine and Norepinephrine in Their 1:1 Complexes with M^{2+}-ATP Chelates

	Proton				
Complex	6	5	2	β	α
DA	0.42	0.25	0.25	0.92	0.97
DA-MgATP	3.2	3.0	2.5	5.6	4.4
DA-CoATP	24	20	21	35	32
DA-NiATP	180	138	145	202	167
DA-MnATP	2085	1710	1820	3260	3000
NE	0.29	0.23	0.42	*a*	1.23
NE-MgATP	3.1	2.9	3.5	*a*	7.3
NE-CoATP	25	25	23	62	47; 74
NE-NiATP	175	149	155	*a*	305
NE-MnATP	2080	1880	1890	*a*	4000

[a] Obscured by the HDO signal.

in good agreement with the model for the binary catecholamine-ATP complex proposed on the basis of a ring-current shift analysis.[9]

(C) Structure of the Ternary Complex. Intrinsic relaxation rates can be used to calculate intermolecular distances (R). In the absence of contact interaction the net paramagnetic spin-lattice relaxation is given by:[19]

$$\frac{1}{T_{1,M}} = \frac{1}{T_{1,C}}(\text{MATP}) - \frac{1}{T_{1,C}}(\text{MgATP}) = CR^{-6} \quad (3)$$

$$C = \frac{2}{15} g^2\beta^2 S(S+1)\gamma_I^2 \left[\frac{3\tau_{1,C}}{1+\omega_I^2\tau_{1,C}^2} + \frac{7\tau_{2,C}^2}{1+\omega_S^2\tau_{2,C}^2}\right] \quad (4)$$

where the intrinsic relaxation is corrected for the diamagnetic contribution as measured in the ternary complex with MgATP. In eq 4 ω_I and ω_S are the precession frequencies of the nucleus and the electron, respectively; γ_I is the magnetogyric ratio of the nucleus; $\tau_{1,C}$ and $\tau_{2,C}$ are the correlation times for the magnetic dipole interaction, and are given by:

$$\tau_{i,C}^{-1} = \tau_r^{-1} + \tau_M^{-1} + \tau_{i,S}^{-1} \qquad (i = 1 \text{ or } 2) \quad (5)$$

Here, τ_r is the rotational correlation time; $\tau_{1,S}$ and $\tau_{2,S}$ are, respectively, the longitudinal and the transverse relaxation times of the electronic spin.

The crucial factor in the calculation of distances by means of eq 3 is the determination of the correlation times. Unfortunately, these can only rarely be properly evaluated. By using a scaling model it is sometimes possible to by-pass the difficulties encountered in this problem. This purpose can be served by a nucleus which fulfills two conditions: (a) its relaxation by the paramagnetic metal ion is governed by processes with correlation times similar to those of the nuclei under investigation; (b) its distance from the metal ion is known or can be independently calculated. In the present study, the protons of the solvent molecules, which are known to be coordinated in the MATP complexes,[8] appear to be suitable scaling nuclei. It can be reasonably assumed that the relaxations of the protons of both the water and the catecholamine molecules are dominated by similar correlation times, e.g., the electronic spin relaxation for the Co^{2+} and Ni^{2+} complexes and the reorientational correlation time for the Mn^{2+} complexes. Taking the distance between the metal ion and the water proton as 2.8 Å,[20,21] the distances for the catecholamine protons can be directly calculated with the relation:

$$R = 2.8[T_{1,M}/T_1(\text{HDO})]^{1/6} \quad (6)$$

where T_1(HDO) is the proton relaxation time of the coordinated water in the MATP complex.

Proton spin-lattice relaxation times of the water in solutions containing ATP were determined as a function of divalent metal ion concentration (keeping $[M^{2+}] \ll [\text{ATP}]$ to eliminate coordination of water to free metal ions). The relaxation rates for the bound water were calculated assuming three coordinated water molecules,[8,14,22] and were corrected for exchange contribution, using the reported values for water exchange rates in the aqueous complexes of Co^{2+},[23] Ni^{2+},[21] and Mn^{2+}.[24] Actually, it was found that only in the case of the nickelous comples ($\tau_M^{-1} = 3 \times 10^4\ s^{-1}$) the exchange term contributes significantly to the relaxation of the water protons. The results are given in Table III. Calculated metal-proton distances for the catecholamine complexes are given in Table IV. Included also are distances measured from an atomic model for the ternary complex based on geometries suggested for the binary DA-ATP[9] and M^{2+}-ATP[8,13-16] complexes.

Table III. Spin-Lattice Relaxation Rates for Water Molecules Coordinated in the M^{2+}-ATP Chelates[a]

	M^{2+}			
	Mg^{2+}	Co^{2+}	Ni^{2+}	Mn^{2+}
$1/T_1$(HDO), s^{-1}	0.2	1.6×10^3	2.0×10^4	2.8×10^5

[a] It is assumed that three water molecules are inner-sphere coordinated in the complex.

Table IV. Metal-Proton Distances (in Å) for Dopamine and Norepinephrine in Their Ternary Complexes with MATP

	Proton				
	6	5	2	β	α
DA-CoATP	5.9	6.1	6.0	5.5	5.6
DA-NiATP	6.2	6.4	6.4	6.1	6.2
DA-MnATP	6.2	6.5	6.4	5.8	5.9
Ne-CoATP	5.8	5.8	5.9	4.9	4.8; 5.2
NE-NiATP	6.2	6.3	6.3		5.7
NE-MnATP	6.2	6.3	6.3		5.7
Model for DA-MATP	6.2	6.7	6.7	5.2	7.4

In evaluating the distances from eq 6, the following assumptions have been made: (a) the correlation time for the catecholamine protons is comparable to that of the water coordinated to the metal ion in the MATP complex; (b) the distances between the metal ion and the coordinated water molecules are unchanged upon association of the catecholamine; (c) the number of water molecules in the first hydration sphere is three. Evidence for outer-sphere coordination of the catecholamine in the ternary complex provided in the present work evidently supports the first two assumptions. The third assumption involves a certain degree of uncertainty since coordination of two[12,16] or three kinetically nonequivalent water molecules[13] in the MATP complex has also been suggested. Combining all sources of uncertainty (including those in $T_{1,M}$, τ_M, etc.) the error in the ratio $T_{1,M}/T_1$(HDO) can be reasonably estimated not to exceed a factor of two. Due to the one-sixth power dependence, this corresponds to ca. 10% uncertainty in the metal-proton distances.

The metal-proton distances, found to be in good agreement with the values obtained from the atomic model (except for H_α, see below), are consistent with outer-sphere coordination of the catecholamines. Furthermore the relative magnitudes of the ring and of the side-chain distances, i.e., $R(H_6) < R(H_5) \simeq R(H_2)$ and $R(H_\beta) < R(H_\alpha)$, are in accordance with the proposed structure for the binary catecholamine-ATP complex.[9] Thus, it is further confirmed that the association of catecholamines with ATP in the ternary complex is very similar to that in their binary complex.

Examination of Table IV reveals that the NE molecule, particularly its side-chain protons, is closer to the metal ion than is the case for DA. This result substantiates a previous

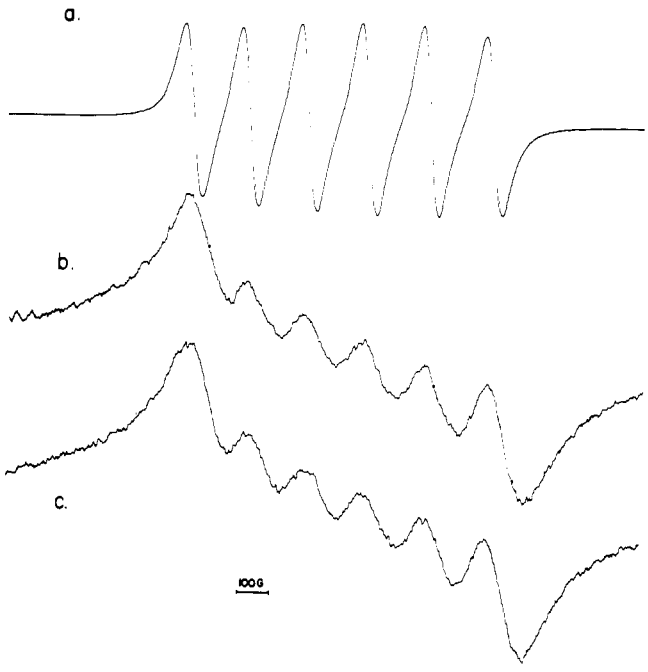

Figure 4. The EPR spectra, at the x-band, of (a) aqueous solution of Mn^{2+} (4.8 mM); (b) solution a + 6.5 mM ATP; (c) solution b + 16 mM DA.

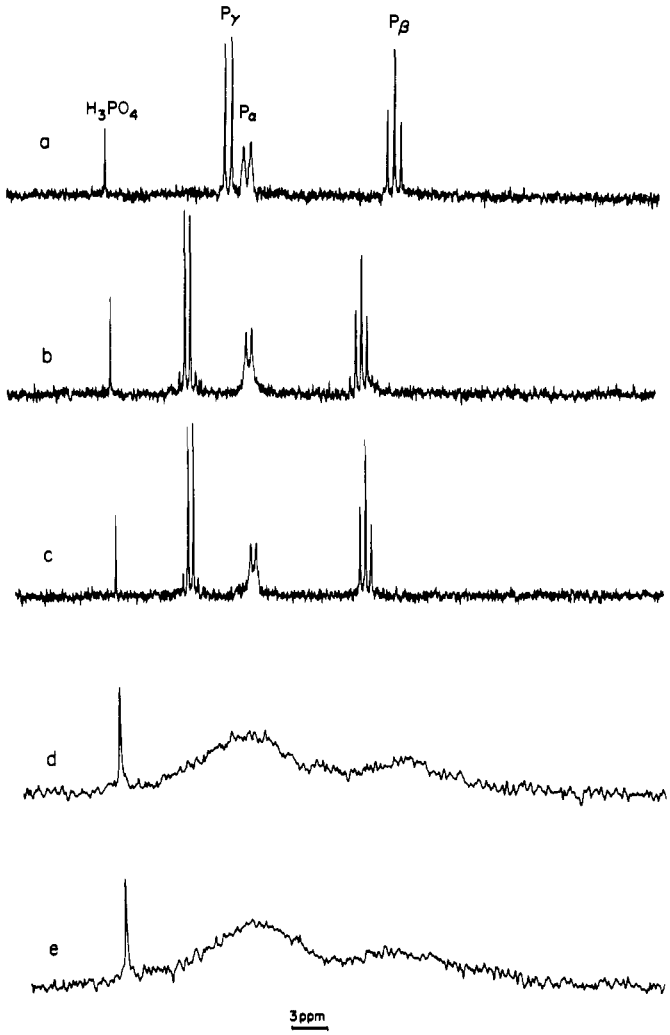

Figure 5. ^{31}P spectra of (a) aqueous solution of ATP (71 mM); (b) solution a + 76 mM Mg^{2+}; (c) solution b + 92 mM DA; (d) solution a + 8 mM Co^{2+}; (e) solution d + 0.19 M DA.

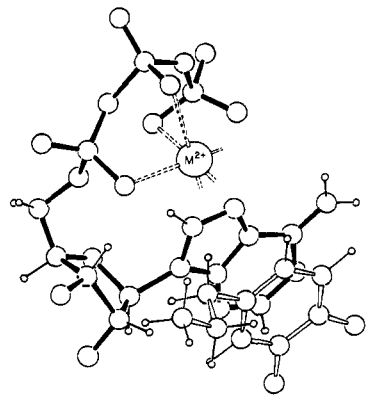

Figure 6. The structure of the ternary DA-ATP-M^{2+} (M = Co, Ni, Mn) complex implied by the experimental results. The rings of DA and ATP are stacked at a vertical separation of 3.4 Å. The metal ion chelated by the phosphate moiety of ATP associates also with N_7 of the adenine ring, either directly or via a water bridge.

suggestion,[9] based on ring-current shift studies, that due to hydrogen bonding involving the β-hydroxyl group, the NE molecule would be pulled toward the phosphate chain of ATP Consequently NE would be closer to the metal ion which is chelated by the phosphates. From the atomic model it appears that steric interference of the ribose moiety of ATP can account for hindered rotation of the side chain of NE as implied by the relaxation data.

Finally, the somewhat larger deviations between the experimental and the model distances for the α protons of the catecholamines can be rationalized bearing in mind that the experimental results are the average over intramolecular rotations about single bonds, which are fast on the NMR time scale. The model distances given in Table IV were measured for the preferred conformation of DA bound to ATP, i.e., the trans conformation.[9] However, allowing rotation of the side chain would bring the α-hydrogens considerably closer to the metal ion (and would somewhat further remove the β-hydrogens). The average metal-proton separations, when the side chain of DA assumes a gauche conformation, are found (from the model) to be ca. 3.5 and 6.0 Å for H_α and H_β, respectively. Rapid interconversion of the rotamers would thus account for the discrepancy between the model and experimental values.

(D) Direct Evidence for Outer-Sphere Coordination of Catecholamines. The effect of association with catecholamines on the M^{2+}-ATP adduct has been studied by monitoring the EPR spectrum of Mn^{2+} [25] and the ^{31}P spectrum of ATP.[26] The results, shown for DA in Figures 4 and 5, clearly indicate that while a marked effect on the magnetic resonance spectra is brought about by formation of the M^{2+}-ATP chelate, almost no effect is observed upon addition of catecholamines. Note that under the experimental conditions (see legends of Figures 4 and 5) ~16% of the Mn^{2+} would be present in the MnATP-DA complex, and ca. 42 and 8% of the ATP would be present in the MgATP-DA and CoATP-DA complexes, respectively. The changes in the respective spectra upon association with DA are significantly less than these percentages, thus confirming the conclusion that catecholamines are outer-sphere coordinated in the ternary complex. Furthermore, these results imply that the catecholamine molecule bound in the ternary complex does not alter either the intermolecular geometry or the interactions between M^{2+} and ATP.

Conclusions

Catecholamines in aqueous solution at pH ~7 form ternary complexes with M^{2+}-ATP chelates. In these complexes the catecholamines are outer-sphere coordinated via their association with ATP. While ATP is strongly bound to the divalent

metal ion (with stability constants of ca. 10^4-10^5 M^{-1}), the association of the catecholamine molecule in the ternary complex is considerably weaker (ca. 11-13 M^{-1}). This association is similar to that observed in the binary catecholamine-ATP complexes in the interactions involved (i.e., ring stacking, hydrogen bonds, and electrostatic attraction), in the overall stability, and in the structural features. On the other hand, there appears to be practically no direct interaction between the catecholamine and the metal ion in the ternary complexes, and the stability of these complexes with regard to the catecholamine binding is actually independent of the type of the divalent metal ion chelated by ATP.

A model for the ternary catecholamine-ATP-M^{2+} complexes (M = Co, Ni, Mn), compatible with the experimental results, is depicted in Figure 6. It is important to note that, as implied by the present results, the geometry of the MATP adduct is actually unaffected by association with catecholamines. Thus, while the paramagnetic metal ions would bind simultaneously to the three phosphates of ATP,[15] the diamagnetic ions would bind to the β- and γ-phosphates[27] or the β-phosphates alone.[28] Furthermore, the metal ions may interact with the adenine ring, either directly,[12] or via a water molecule.[13,14] It should, however, be stressed that these possible different modes of binding do not significantly alter the intermolecular geometry of the ATP-catecholamine adduct in the ternary complex.

The present and previous results[9] imply that in aqueous solutions containing catecholamines and ATP, at pH ~7, in the presence or absence of divalent metal ions, the catecholamines would form mainly 1:1 and 2:1 complexes with ATP and 1:1:1 complexes with M^{2+}-ATP. In view of the lower stability of the ternary complexes, coupled with the ability of the MATP chelate to bind only one catecholamine molecule, it appears that metal-free rather than ternary complexes would be preferably formed. It has been previously suggested[2,9] that ATP by itself cannot be responsible for the storage of catecholamines in biological tissues. It now appears that this is even more true of the MATP chelates, Moreover, in agreement with our previous observation,[2] the present results indicate that addition of dialent metal ions would disrupt to some extent the binary catecholamine-ATP complex.

The secondary ionization of ATP occurs[21] with $pK_a \simeq 7$. Hence, at physiological pH ATP^{3-} and ATP^{4-} would be present at approximately equal concentrations in aqueous solution. It thus appears that the binary catecholamine-ATP complexes have 3− or 2− ionic charges, whereas the ternary complexes have 1− or 0 charges, compared with a charge of 1+ for the free catecholamine molecule. These changes in the overall molecular charge, apart from binary or ternary complex formation per se, may be of importance in the active processes involving catecholamines.

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References and Notes

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