NMR STUDIES OF CATECHOLAMINES. INTERACTIONS WITH ADENINE NUCLEOTIDES BY ^{31}P MAGNETIC RESONANCE

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1. Introduction

Elucidation, on the molecular level, of the interactions between catecholamines and adenine nucleotides, particularly ATP, can provide a basis for understanding the biochemical mechanisms involved in the processes of storage, release, uptake and neural action of catecholamines. ^{1}H-NMR spectroscopy studies [1] have demonstrated that catecholamines and adenine nucleotides form in aqueous solution binary complexes primarily through stacking interaction between the catechol and the adenine rings. In addition, an electrostatic interaction between the positively charged ammonium group of the catecholamines and the negative phosphate moiety of the nucleotides was found to play an important role in stabilizing these complexes. It is the purpose of the present study to further investigate the involvement of the phosphate groups of adenine nucleotides in the association with catecholamines, by means of ^{31}P-NMR. Chemical shifts, spin couplings and spin-lattice relaxation times were measured for the phosphorus resonances of the nucleotides. The effects of catecholamines were found to be rather small, apparently due to the relatively weak, through-space, interactions involved. However, due to the high sensitivity of phosphate groups to their chemical environment, the results were of sufficient significance to allow gaining a better insight into the phosphate–amine interactions.

2. Materials and methods

Dopamine (DA) and L-norepinephrine (NE) as hydrochlorides, and adenine nucleotides (AMP, ADP and ATP) and ribose 5′-phosphate as sodium salts were obtained from Sigma Chemical Co. Experimental solutions were made up by dissolving the materials in deuteriumoxide (99.7%). The concentrations used were 30–60 mM for the phosphates and 5–200 mM for the amines. The pD values were adjusted by addition of concentrated DCl or NaOD, and are reported as the meter readings corrected for deuterium isotope effect. All samples were treated with Chelex-100 (sodium form; Bio-Rad Laboratories) to remove traces of paramagnetic impurities. In addition, 1 mM EDTA were dissolved in the experimental solutions.

^{31}P-NMR spectra were recorded on a FT-Bruker WH-270 spectrometer operating at 109.3 MHz, equipped with a Nicolet model 1180 32K computer. The deuterium signal of the solvent was used for a field-frequency locking. Phosphorus chemical shifts and POP spin coupling constants were obtained during broad-band proton decoupling. Relaxation times and P–H couplings were obtained from proton undecoupled spectra. The chemical shifts were measured relative to H_3PO_4 external reference present in a 2 mm coaxial tube (10 mm NMR tubes were used for the ^{31}P-NMR measurements). Spin-lattice relaxation times (T_1) were determined using a $180°-\tau-90°$ sequence. The T_1 values were obtained from a plot of $\ln(I_\infty - I_\tau)$ versus τ, where I denotes the intensity of the phosphorus resonances. The uncertainty in the chemical shifts and the coupling constants is ±0.1 Hz, and in the spin-lattice relaxation times ~10%. All spectra were recorded at ambient probe temp. 27°C.

3. Results and discussion

3.1. *Chemical shifts*

Phosphorus chemical shifts depend strongly on the

pH in aqueous solutions. In order to avoid acid–base reactions (the pK_a values associated with secondary phosphate ionizations of adenine nucleotides are ~7 [2]), the measurements were carried out at pD 3.5 and pD 5.4. Varying concentrations of DA and NE were added to solutions containing adenine nucleotides. All the phosphorus signals were found to shift progressively upon addition of catecholamines, either down- or up-field, reflecting the participation of the phosphate groups in the association between the nucleotides and catecholamines. Typical shift data are given in table 1.

Phosphorus chemical shifts may be dominated by several factors, e.g., the total occupation of the d_π orbitals of the phosphorus, the effective electronegativity of the neighbouring oxygen [3], the O–P–O bond angles [3,4] and the phosphate torsional angles [5]. The effect of catecholamine association on the phosphorus chemical shifts of adenine nucleotides may be interpreted in terms of two principal mechanisms:

1. Ionization of the terminal phosphate due to competitive effect of the positive ammonium group of the catecholamines (mechanism I). Proton dissociation would actually affect all the factors above [4–7], resulting in down-field shifts.

Table 1
Chemical shift data[a]

	Δδ (Hz)			
	pD	P_α	P_β	P_γ
ATP+DA	3.5	+ 8.1	− 3.0	− 0.5
ADP+DA	3.5	+ 5.5	− 1.2	
AMP+DA	3.5	+ 4.0		
ATP+NE	3.5	+ 1.8	− 9.5	− 3.6
ADP+NE	3.5	+ 4.8	− 5.6	
AMP+NE	3.5	+ 5.5		
ATP+DA	5.4	− 4.4	−17.2	−24.9
ADP+DA	5.4	− 4.9	−24.5	
AMP+DA	5.4	−16.6		
ATP+NE	5.4	− 5.0	−19.6	−29.3
ADP+NE	5.4	− 2.5	−24.2	
AMP+NE	5.4	−23.4		

[a] The chemical shifts are given relative to the respective shift values in the uncomplexed state. The nucleotide and catecholamine concentrations are 30 mM and 150 mM, respectively

2. The direct effect of the protonated ammonium group (mechanisms II). This can be rationalized as producing distortion in the electronic charges of the phosphate group, accompanied by alteration in the diamagnetic shielding experienced by the phosphorus nuclei, the effect of which has an opposite direction to that of proton dissociation, hence resulting in up-field shifts.

While the second mechanism is expected to be much weaker than mechanism I, due to larger separation between the interacting groups, the contribution of the latter at low pH values, far from the pK_a, would be small, as only a minute fraction of the nucleotides would undergo secondary ionization. The observed chemical shifts are the weighted sum of the two contributions. Evidently, (Cf. table 1) at pD 5.4 mechanism I is the dominant. The decrease of this contribution as the pD is lowered to 3.5 would cause mechanism II to have a higher relative effect. This is compatible with the observation that the α-phosphate signals of all the nucleotides were shifted up-field. Furthermore, at this pD both the β- and γ-phosphate of ATP were shifted down-field, but with P_β being more shifted than P_γ. Since under the action of mechanism I P_γ should be the one which is shifted more (e.g., complete secondary deprotonation is found to cause ~5.5 ppm and 1.5 ppm down-field shifting of the P_γ and P_β resonances, respectively) the results indicate a considerable contribution of mechanism II to the shifts of the γ-phosphate as well. It can thus be concluded that the α- and γ-phosphates are preferentially involved in the association of ATP and catecholamines.

The contribution of mechanism II is not expected to differ appreciably at pD 5.4 relative to pD 3.5. However additional labelization of the terminal protons upon increasing the pD brings about larger contribution of mechanism I which in fact overcomes that of mechanism II. The induced down-field shifts at pD 5.4 follow the sequence

$|\delta(P_\gamma)| > |\delta(P_\beta)| > |\delta(P_\alpha)|$ for ATP

and $|\delta(P_\beta)| > |\delta(P_\alpha)|$ for ADP.

These are actually proper trends for chemical shifts dominated by mechanism I.

The phosphorus chemical shifts induced by NE follow in general similar trends as those of DA, but are somewhat more negative (particularly at pD 3.5), implying larger contribution of mechanism I. This

may be attributed to participation of the β-hydroxyl group of NE in the association with nucleotides. Possible formation of hydrogen bonding between this group and the phosphate moiety [8] would affect the phosphorus shifts predominately by shifting the pK_a [4], i.e., through mechanism I, in agreement with the experimental results.

3.2. *Spin-lattice relaxation times*

Phosphorus spin-lattice relaxation times for adenine nucleotides were measured in the absence and presence of catecholamines, at pD 7.0. At the region of this pD, changes in the proton concentration were found to have little or no effect on the T_1 values [9]. Computed T_1 values are given in table 2.

The principal interactions contributing to the relaxation rate of phosphorus nuclei are the dipole–dipole (DD), the chemical shift anisotropy (CSA) and the spin rotation (SR). Under the experimental conditions (i.e., deuterium oxide solutions and applied magnetic field of 63.4 kG) the phosphorus relaxation of adenine nucleotide was found to be dominated by CSA [9]. Intermolecular interactions due to association of nucleotides with catecholamines are not expected to affect significantly the mechanisms for phosphorus relaxation. The observed shortening of the T_1 values can thus be best interpreted in terms of changes in the correlation time for the relaxation. Under conditions of motional narrowing and isotropic tumbling, that usually characterize the motion of small molecules, the relaxation rate arising from CSA is directly proportional to the reorientational correlation time [10]. Shortening of the tumbling rate due to molecular association can thus account for the observed increase in the relaxation rates. Such effect should however affect relaxation rates of all the phosphorus nuclei of a certain nucleotide to the same extent. Selective changes, as found for ADP and ATP (cf. table 2) may indicate restriction of the internal rotation of the phosphate groups. This implies, in accordance with the chemical shift results, the preferred involvement of the α- and γ-phosphates of ATP in the complexation with catecholamines. Note also the more pronounced changes in T_1 values produced by NE relative to DA, being attributable to the effect of the β-hydroxyl group.

Table 2
Spin-lattice relaxation data[a]

	T_1 (s)			ΔR_1 (s^{-1})[b]		
	P_α	P_β	P_γ	P_α	P_β	P_γ
ATP	3.7	4.9	8.3			
ATP+DA	3.0	4.8	6.8	0.06	0.01	0.03
ATP+NE	2.6	4.4	5.2	0.11	0.02	0.07
ADP	4.6	8.1				
ADP+DA	2.9	5.7		0.12	0.06	
ADP+NE	2.6	5.6		0.16	0.06	
AMP	8.2					
AMP+DA	6.1			0.04		
AMP+NE	5.5			0.06		

[a] Nucleotide and catecholamine concentrations are 60 mM and 200 mM, respectively

[b] $R_1 \equiv T_1^{-1}$. ΔR^1 is the increase in the relaxation rate due to catecholamine association

3.3. *Coupling constants*

P–O–P couplings ($J_{\alpha\gamma}$, $J_{\beta\gamma}$) and P–H couplings for adenine nucleotides in the absence and presence of catecholamines are given in table 3. The variation in the coupling constants are found to be small, however the observed trends in these quantities appear to be of significance. Recent ^1H and ^{31}P studies [11–16] carried out on 5′-nucleotides have shown that their preferred conformation in solution is *gauche–gauche* (*gg*) about the $C_{4'}$–$C_{5'}$ bond, and *gauche–gauche* (*g′g′*) about the $C_{5'}$–$O_{5'}$ bond. The fractional populations of the *gg* and *g′g′* conformations are directly

Table 3
Spin-coupling data[a]

	$J_{\alpha\beta}$	$J_{\beta\gamma}$	$J_{H_{5'},P_\alpha} + J_{H_{5''},P_\alpha}$	$J_{H_{5'},P_\alpha}$
ATP	19.2	19.2	10.8	2.1
ATP+DA	19.0	19.0	11.0	2.0
ATP+NE	18.9	18.9	11.0	2.0
ADP	21.5		10.4	1.8
ADP+DA	21.5		10.6	1.7
ADP+NE	21.4		10.8	1.6
AMP			9.2	1.5
AMP+DA			9.4	1.2
AMP+NE			9.4	1.2

[a] Nucleotide and catecholamine concentrations are 60 mM and 200 mM, respectively. Coupling constants are in Hz

related to the magnitudes of the P–H spin couplings [14]. Inspection of table 3 indicates a reduction in $^4J_{H_{4'}P_\alpha}$ accompanied by increase in the sum $^3J_{H_{5'}P_\alpha} + {}^3J_{H_{5''}P_\alpha}$ upon association of the adenine nucleotides with the catecholamines. These changes are consistent with the interrelations between the spin coupling constants proposed [14], and imply destabilization to some extent of the *gg–g′g′* conformation. Using space-filling models, based on the structure proposed for the binary catecholamine–ATP complex [17], it is indeed found that closer approach of the α-phosphate toward the ammonium group, under the effect of attractive electrostatic interaction, would necessitate rotations about the $C_{4'}$–$C_{5'}$ and $C_{5'}$–$O_{5'}$ bonds, which subsequently would disturb the *gg′–gg′* conformation.

3.4. *Amine–phosphate interactions in the absence of rings association*

The effect of catechol–adenine rings association on the amine–phosphate interaction was examined by using ribose 5′-phosphate as a base-lacking nucleotide derivative. Upon titration with catecholamines, at pD 3.5, small up-field shifts (~2 Hz for molar ratio 1:4) were detected. These shifts are significantly smaller than those obtained for AMP (cf. table 1). The present finding thus support the conclusion, based on ^{1}H-NMR studies [1], that in the absence of rings association the amine–phosphate interaction is considerably reduced.

4. Conclusions

The study of the effects of catecholamines on the phosphorus resonances of adenine nucleotides led to the following conclusions:

1. It is confirmed that the phosphate moiety of the nucleotides is involved in the association with catecholamines, most likely through electrostatic interaction with the protonated ammonium group. This would be true also at physiological pH since ammonium ionization of catecholamines occurs with $pK > 9.7$.

2. The amine–phosphate interaction is augmented by association between the catechol and the adenine rings and also by participation of substituted β-hydroxyl group in the binding (via hydrogen bond formation).

3. The amine–phosphate interaction involves predominantly the α-phosphate and to a lesser extent the terminal phosphate, i.e., P_β of ADP and P_γ of ATP. In view of the flexibility of the phosphate moiety and the finding that 1:1 or 2:1 catecholamine–nucleotide complexes are mainly formed [1,17], this conclusion is compatible with a model in which the first catecholamine molecule bound to the nucleotide interacts mainly with the α-phosphate, whereas the second molecule, associated on the other side of the nucleotide interacts with the terminal phosphate.

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