Multiple Molecular Recognition and Catalysis. Nucleotide Binding and ATP Hydrolysis by a Receptor Molecule Bearing an Anion Binding Site, an Intercalator Group, and a Catalytic Site

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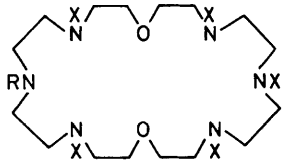
The polytopic receptor molecule (**1**), containing a macrocyclic polyamine as anion receptor subunit and an acridine group for stacking interaction, strongly binds nucleotides in aqueous solution by multiple site binding and catalyses ATP hydrolysis with increased selectivity.

The design of receptor molecules displaying high molecular recognition and selective reactivity requires the incorporation of sites for multiple interaction with the substrate species. This may be achieved with polytopic receptors containing subunits complementary to the different functional groups present in the substrate(s).[1]

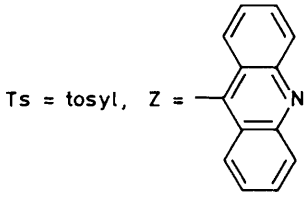
We now report the binding properties and hydrolytic reactivity towards nucleotide substrates of the polyfunctional receptor molecule (**1**) that combines three functional subunits: a macrocyclic polyamine moiety (**2**) as anion binding site, an acridine side-arm for stacking interaction, and catalytic amino groups on the macrocycle (**2**) for facilitating hydrolytic reactions.

Indeed, the protonated macrocyclic hexamine (**2**), in common with other macrocyclic polyamines, strongly binds nucleoside polyphosphates[2,3] by electrostatic interactions and catalyses the hydrolysis of ATP.[2] On the other hand, acridine derivatives[4] associate with the nucleic bases of nucleotides and nucleic acids by stacking[5] and by intercalation.[6]

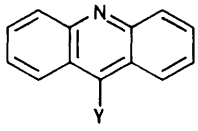
Treatment of (**3**)[7] with acrylonitrile in tetrahydrofuran (THF) gave (**4**) (94% yield) which was reduced by B_2H_6/THF to (**5**) (61% yield). The reaction of (**5**) with 9-chloroacridine[8] in phenol gave (**6**) (44% yield) which, after careful purification by chromatography, was deprotected by treatment with HBr–AcOH in the presence of phenol affording the salt (**1**).8HBr. The hydrochloride salt of (**1**) was prepared by addition of HCl to the free base obtained by chromatography on anion exchange resin (90% yield). The model compound (**8**) was obtained by treatment of 9-chloroacridine with 3-dimethylamino-1-propylamine in phenol.†



(**1**) X = H, R = $(CH_2)_3NHZ$
(**2**) X = R = H
(**3**) X = Ts, R = H
(**4**) X = Ts, R = $(CH_2)_2CN$
(**5**) X = Ts, R = $(CH_2)_3NH_2$
(**6**) X = Ts, R = $(CH_2)_3NHZ$



Ts = tosyl, Z =



(**7**) Y = NH_2
(**8**) Y = $NH(CH_2)_3N(Me)_2$

Compound (**1**) absorbs light in the visible domain (λ_{max} 408 nm, ε_{max} 10 000, aqueous solution, pH 4 or 7) and shows strong fluorescence (λ_{max} 450 nm) (Table 1). Binding of nucleotides by protonated (**1**) was demonstrated at different pH values by ^{31}P n.m.r., fluorescence (Table 1), and ^{1}H n.m.r. spectroscopy (Table 2).

In the presence of (**1**), the ^{31}P n.m.r. signals of ATP and ADP are significantly altered. The P(α) signal of ATP is unchanged whereas P(β), and particularly the terminal phosphate P(γ) signals, are shifted considerably downfield [1.32 p.p.m. for P(β) and 2.22 p.p.m. for P(γ)] demonstrating the binding of the polyphosphate chain of ATP and ADP by (**1**)–nH$^+$.

In the presence of ATP, a slight bathochromic shift (about 2 nm) is observed for both absorption and fluorescence of (**1**). The absorption of (**1**) shows a weak hypochromic effect whereas its fluorescence is significantly enhanced in the presence of ATP (Table 1). In contrast, addition of triphosphate (TP) has no effect on the absorption of (**1**) and produces a slight quenching of its fluorescence. Titration of (**1**) with ATP followed by fluorescence gives a 1 : 1 stoicheiometry for the complexes formed between ATP and (**1**)–nH$^+$. On addition of ATP to the reference compounds (**7**) and (**8**), no appreciable changes are detected for (**7**), whereas (**8**) shows a slight fluorescence enhancement.

Table 1. Absorption and fluorescence spectroscopy studies of nucleotides and polyphosphate binding by (**1**)[a].

	Absorption		Fluorescence	
Substrate	$\lambda_{max.}$/nm	$\varepsilon_{max.} \times 10^{-3}$	$\lambda_{max.}$[b]	I_{rel}[c]
None	408	10	450	75
ATP	410	9.5	452	100
ADP	408	10	452	78
AMP[d]	408	10	450	80
TP	408	10	450	65
PP	408	10	450	65

[a] Measurements performed on a 3 ml aqueous solution containing [(**1**).8HCl] = [Substrate] = 10^{-5} M, at pH 7.6 (tris-buffer 0.1 M), 25 °C. [b] Excitation at 408 nm. [c] Relative emission intensity. [d] [AMP] = 10^{-3} M.

† All new compounds have n.m.r. spectra and microanalytical data in agreement with their structure.

Table 2. ^{1}H n.m.r. spectroscopy studies of nucleotide binding by (**1**)[a].

	^{1}H n.m.r. δ						
	Acridine moiety[b]				Aromatic and anomeric protons of nucleotides[c]		
Substrate	a	b	c	d	H-2	H-8	H′-1
None	8.47	8.02	7.87	7.62			
ATP[d]	8.25	7.88	7.58	7.47	8.21 (8.73)	7.90 (8.46)	5.72 (6.24)
ADP	8.27	7.98	7.75	7.57	8.13 (8.68)	7.77 (8.43)	5.75 (6.23)
AMP	8.26	7.98	7.81	7.55	8.35 (8.70)	8.08 (8.39)	6.02 (6.22)
TP	8.36	7.47	7.74	7.60			
AP_2A	8.19	7.93	7.69	7.51	8.05 (8.34)	7.87 (8.22)	5.76 (6.06)

[a] ^{1}H N.m.r. spectra were measured at 200 MHz, in a D_2O solution at 25 °C containing [(**1**).8HCl] = [nucleotide] = [TP] = 10^{-3} M each, adjusted to pH 4 by addition of 5 M HCl or NaOH. Bu^{t}OH was used as internal standard. [b] a and c doublets, b and c triplets; signal assignment as shown in structure (**7**) based on literature results for 9-aminoacridine derivatives.[11] [c] Values in parentheses are those observed for the free substrates. [d] In the presence of (**2**) at pH 4, δ 8.59, 8.38, and 6.22 for H-2, H-8 and H′-1 of ATP, respectively.

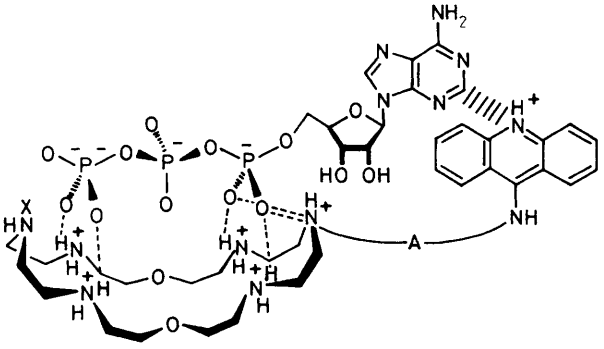


Figure 1. Schematic representation of the preferred *syn* orientation of the two partners in the complex formed by (**1**)–nH$^+$ with ATP, allowing simultaneous electrostatic (– – –) and stacking (|||||) interactions. The exact shape of the species and positioning of the two components are not known [A = $(CH_2)_3$]. Depending on the hydrolysis mechanism X represents either a lone pair or a proton (see ref. 2). Phosphate groups are labelled α, β, γ from the ribose moiety; protons H′-1, H-2, and H-8 are respectively the anomeric proton and the imidazole and pyrimidine protons of the adenine group.

Binding of nucleotides by (**1**)–nH$^+$ is unambiguously demonstrated by ^{1}H n.m.r. spectroscopy (Table 2). On addition of 1 equiv. of ATP, ADP, or AMP to a solution of (**1**) at pH 4 or 7, the proton n.m.r. signals corresponding to the CH_2 groups of the macrocyclic moiety of (**1**) are shifted downfield, whereas the aromatic signals of the acridine part of (**1**) undergo significant upfield shifts. On the other hand, the aromatic protons (H-2 and H-8) and the anomeric proton (H′-1) signals of ATP, ADP, and AMP are also shifted strongly upfield. The shifts of H-2, H-8, and H′-1 (*ca.* 0.5 p.p.m.) are consistent with a stacking interaction between the acridine group of (**1**)–nH$^+$ and the adenine unit in ATP, ADP, or AMP.‡

Competition experiments between (**1**) and (**2**) (followed by ^{1}H n.m.r. and fluorescence spectroscopy) show that (**1**)–nH$^+$ binds ATP about twice as strongly as (**2**)–nH$^+$. Similarly, competition experiments between ATP and TP for binding to (**1**)–nH$^+$ indicate that ATP is bound more strongly than TP by a factor of *ca.* 2.

Assuming that the (poly)phosphate group of the nucleotides binds to the positively charged macrocyclic moiety of the receptor (**1**), ATP, ADP, and AMP may form two types of complexes in which the relative orientation of the two partners is such as to allow (*syn*) or not to allow (*anti*) interaction between the acridine part of (**1**) and the adenine group of the nucleotide. ^{1}H n.m.r. and fluorescence spectra of (**1**) + ATP solutions are unchanged in the temperature range 5—25 °C, thus indicating that the complexes present are also unaffected. Binding studies of the symmetrical substrate AP_2A by ^{1}H n.m.r. spectroscopy show that the shifts observed for the aromatic proton signals of (**1**) are similar to those obtained for ATP, but the shifts for H-2, H-8 and H′-1 protons signals of AP_2A are about half of those observed for ATP. On the basis of these observations and the fact that (**1**)–nH$^+$ forms stronger complexes with ATP than (**2**)–nH$^+$, it seems reasonable to propose that complexes in which both the polyphosphate chain and the nucleic base of the nucleotide interact simultaneously with (**1**)–nH$^+$ (Figure 1) are the predominant species in solution.

Compound (**1**) catalyses ATP (k_{obs} = 0.0126 min^{-1}) and ADP (k_{obs} = 0.0015 min^{-1}) hydrolysis at pH 7 and 84 °C. As for (**2**)[2] the reaction proceeds, at least in part, through a covalent phosphoramidate intermediate. The ratio of the observed first order rate constants k_{obs} for ATP over ADP is 8.4 for (**1**) and 2.7 for (**2**). Although less effective than (**2**) in both hydrolytic reactions, (**1**) shows greater selectivity between ATP and ADP than (**2**).

Compound (**1**) was found to bind strongly to the supercoiled circular double stranded DNA plasmid pBR322 at pH 7.6 (tris-buffer) probably *via* a double type of interaction, involving both intercalation and electrostatic interaction with the phosphate groups at 10^{-6} M concentration, as analysed by band displacement in gel electrophoresis. Mono- and poly-intercalators built on biogenic polyamines (spermine, spermidine) have been shown to bind strongly to DNA.[10]

In conclusion, the receptor (**1**) recognises nucleotides, in particular, ATP, by simultaneous interactions with both their polyphosphate chain and their nucleic base group and, furthermore, it catalyses their hydrolysis with greater selectivity than the parent compound (**2**). These results illustrate how molecular engineering of binding and reactive sites in polytopic receptors enhances structural and catalytic selectivities in supramolecular species.

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‡ Ethenoadenosine triphosphate (ATP), a fluorescent analogue of ATP,[9] is also complexed by (**1**)–nH$^+$ and shows the same type of behaviour, as shown by ^{1}H n.m.r. and fluorescence observations on both components.

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