Complexation of Nucleotides *via* Multiple π-Stacking and Photoionisation of the Resultant Complex

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A water-soluble, highly-fluorescent complexing reagent, consisting of two diazapyrenium subunits linked by a *meta*-xylene spacer, binds nucleotides as nonfluorescent, but photolabile, 1 : 1 complexes in which the substrate is sandwiched between the diazapyrenium subunits.

There is much current interest in the design of polytopic molecular receptors which reversibly bind substrates in water such that the resultant complex can be *selectively activated* by light or an electrode.[1] The specificity of binding may increase for receptors whose conformation can adapt to enfold the substrate (Scheme 1). Such artificial tropism enhances complex stability by maximising hydrophobic, polar, hydrogen-bonding and electrostatic interactions between substrate and receptor.[2,3] We now describe a system in which 2,2′-(1,3-xylyl)-bis(7-methyl-2,7-diazapyrenium) tetrachloride (*m*-bis-

Scheme 1

Table 1 Parameters for the 1:1 complexes formed between *m*-bisDAP^{4+} and nucleotides in neutral aqueous solution containing KCl (10^{-2} mol dm^{-3})

Substrate	$E°$/V (vs. NSE)[g]	K^i/ dm^3 mol^{-1}	n^i	$\tau_{CR}{}^i$/ps
Adenine	0.95[h]	325	1.1	140
AMP[a]	0.95	740	1.0	125
ADP[b]	0.95	1005	0.9	105
ATP[c]	0.95	1220	0.9	120
CMP[d]	1.20	55	1.0	260
GMP[e]	0.85	945	0.9	50
TMP[f]	1.05	210	1.0	220

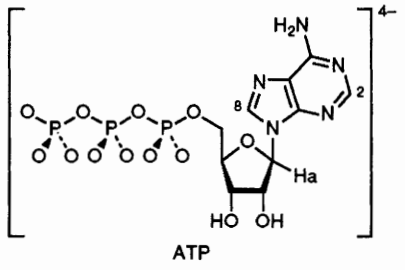
[a] 2′-Deoxyadenosine-5′-monophosphate. [b] Adenosine-5′-diphosphate. [c] Adenosine-5′-triphosphate. [d] 2′-Deoxycytidine-5′-monophosphate. [e] 2′-Deoxyguanosine-5′-monophosphate. [f] Thymidine-5′-monophosphate. [g] NSE = normal sulfate electrode. [h] V *vs.* 0.5 mol dm^{-3} sulfate electrode taken from ref. 8 and refers only to the nucleic acid base. [i] ±10%. [j] ±15 ps.

DAP^{4+}) binds nucleotides in water in the form of photoactive complexes.

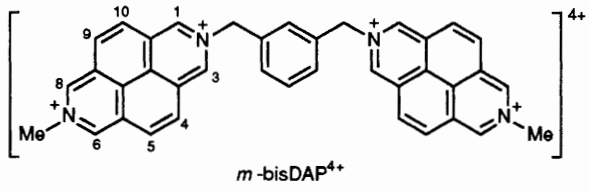
The receptor was synthesized by heating 2,7-diazapyrene[4] with two equivalents of α,α′-dibromo-1,3-xylene in refluxing acetonitrile followed by methylation with an excess of iodomethane,[5] and ion exchange.† Relative to *N,N*′-dimethyl-2,7-diazapyrenium dichloride (DAP^{2+}),[4] there were no shifts in the ^{1}H NMR signals while UV–VIS spectra displayed small (*ca.* 4 nm) red shifts, slight broadening, and decreased (*ca.* 15%) molar absorption coefficients. These results indicate that the two diazapyrenium subunits interact only very weakly and are probably held in an *anti* configuration. Fluorescence spectroscopy, however, indicated modest quenching for *m*-bisDAP^{4+} since the fluorescence quantum yield ($\phi_f = 0.42$) and lifetime ($\tau_f = 6.8$ ns) were reduced relative to DAP^{2+} ($\phi_f = 0.63$; $\tau_f = 9.0$ ns).[6]

Addition of a nucleotide to a neutral aqueous solution of *m*-bisDAP^{4+} containing KCl (10^{-2} mol dm^{-3}) at 25 °C caused a slight red shift and broadening of the diazapyrenium absorption bands, indicating complexation between the reagents.[6] Fluorescence from the diazapyrenium subunits was quenched upon addition of the nucleotide, the resultant complex being non-fluorescent ($\phi_f < 10^{-4}$). For each nucleotide, the fluorescence titration data gave excellent fits to the Hill equation;[7] the derived binding constants (K) and stoichiometries (n) are collected in Table 1. For adenine, the magnitude of K increases with increasing number of phosphate groups, although complexation is pronounced for adenine itself. For the monophosphates, K increases with increasing ease of oxidation of the base, as measured by the one-electron oxidation potential ($E°$).[8] Binding between receptor and substrate, therefore, includes both electrostatic and charge-transfer interactions.

Complexation of nucleotides was confirmed by ^{1}H NMR spectroscopic titration of the ligand with adenosine-5′-triphosphate (ATP) in D_2O at 25 °C. Complexation between DAP^{2+} and ATP caused a slight shielding of protons on both receptor and substrate; the purine and anomeric protons (2-H, 7-H, H^a) of ATP and the internal protons (4-H, 5-H, 9-H, 10-H) of DAP^{2+} were shifted upfield by 0.06 ppm. These modest shifts are indicative of relatively long-range stacking between the aromatic nuclei.[9] In contrast, complexation between *m*-bisDAP^{4+} and ATP caused significantly larger shifts; the anomeric proton, for example, was shifted upfield by 0.25 ppm, and the signals were subject to fluctuational broadening.[10] The two pairs of α-protons on the diazapyrenium subunits were readily distinguished in the complex, 1-H and 3-H being shifted upfield by only 0.07 ppm whilst 6-H and 8-H were shifted upfield by 0.15 ppm. The two diazapyrenium subunits appeared equivalent in the NMR studies but NOE experiments showed the complexes to be in dynamic equilibrium on these time scales.



ATP



m-bisDAP^{4+}

Relative to DAP^{2+}, the complexes formed between nucleotides and *m*-bisDAP^{4+} are about six times more stable.[6] The 1:1 stoichiometry indicates well defined structures while the linear Hill plots show that the two diazapyrenium subunits exhibit positive cooperativity. Time-resolved fluorescence studies, with 20 ps time resolution, could not distinguish the two diazapyrenium subunits in *m*-bisDAP^{4+}, such that both must be involved in complexation. These findings suggest that *m*-bisDAP^{4+} adopts a *syn* configuration upon binding to a nucleotide and that the substrate is sandwiched between the two diazapyrenium subunits. The ^{1}H NMR data further indicate that the purine base is only partially encompassed by the diazapyrenium subunits, presumably because of steric restrictions. The substrate, however, appears to be in van der Waals contact with both diazapyrenium subunits, forming a 3-membered π-stack.

Excitation of the 1:1 complexes with a 30 ps laser pulse resulted in immediate formation of the diazapyrenium radical cation, as identified by its characteristic differential absorption spectrum.[6] This species arises from rapid photoinduced electron transfer from the base to a diazapyrenium subunit. In each case, the diazapyrenium radical cation decayed by first-order kinetics, independent of concentration of base or monitoring wavelength, and the lifetimes (τ_{CR}) are collected in Table 1. The complexes do not dissociate upon excitation, since no long-lived species were observed, and the decay process is attributed to charge recombination within the complex. The rate of charge recombination ($k_{CR} = 1/\tau_{CR}$) decreases with increasing $E°$ (Table 1), as expected if reaction falls within the Marcus 'inverted' region.[11]

We have shown that *m*-bisDAP^{4+} forms photoactive, 3-membered π-stacks with nucleotides in water. The conformation of the receptor changes dramatically upon complexation, since the noncomplexed form attempts to minimise electrostatic repulsion between the diazapyrenium subunits whilst the complex tries to align the polycycles. This artifical tropism is a key feature in the design of efficient multipurpose receptors. It is also considered that *m*-bisDAP^{4+} may function as a novel bis-intercalator since the two diazapyrenium subunits could be separated only by a single base pair.

† All compounds gave satisfactory ^{1}H NMR and elemental analyses.

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