

Molecular recognition of ADP over ATP in aqueous solution by a polyammonium receptor containing a pyrimidine residue

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Experimental procedures

Materials. Ligands HL was synthesised as previously described.¹

Potentiometric measurements

All pH-metric measurements ($\text{pH} = -\log [\text{H}^+]$) employed for the determination of equilibrium constants were carried out in 0.1 M NMe_4NO_3 solutions at 298.1 ± 0.1 K, by using the equipment and the methodology that has been already described.² The combined Hamilton glass electrode (LIQ-GLASS 238000/08) was calibrated as a hydrogen concentration probe by titrating known amounts of HCl with CO_2 -free NMe_4OH solutions and determining the equivalent point by Gran's method³ which allows one to determine the standard potential E° and the ionic product of water ($\text{pK}_w = 13.83(1)$ at $298.10.1$ K in 0.1 M NMe_4NO_3). At least three measurements were performed for each system in the pH ranges 2.5-10.5. In all experiments the ligand concentration $[\text{L}]$ was about 1×10^{-3} M while the anion concentration $[\text{A}]$ was varied in the range 5×10^{-4} - 1×10^{-3} M. The computer program HYPERQUAD⁴ was used to calculate the equilibrium constants from e.m.f. data.

¹H and ³¹P NMR measurements

¹H (400 MHz) and ³¹P (161 MHz) in D_2O solutions at different pH values were recorded at 298 K on a Bruker-Advance III 400 MHz spectrometer. Small amounts of 0.01 M NaOD and DCl were used to adjust the solutions pD. Complexation-induced ³¹P and ¹H NMR chemical shifts (CIS, ppm) were measured as $(\delta_{\text{OBS}} - \delta)100/\%_{\text{cplx}}$ where δ_{OBS} is the chemical shift of a signal measured in D_2O solutions containing receptor and substrate in a 1:1 molar ratio (both 5×10^{-3} M), δ is the chemical shift of the corresponding signal of the not complexed receptor or substrate and $\%_{\text{cplx}}$ is the percentage of complex species present in solution under the condition used to record the spectrum. The pH was calculated from the measured pD value by using the relationship $\text{pH} = \text{pD} - 0.40$.⁵

Modelling calculations

Simulated annealing procedures (10 ps equilibrating time, 10 ps running time and 10 ps cooling time, running temperature 600 K, starting and ending temperature 0K) were carried out to analyze the adducts formed by H_3L^{2+} with ADP^{3-} , HADP^{2-} and H_2ADP^- , or ATP^{4-} , HATP^{3-} and $\text{H}_2\text{ATP}^{2-}$. The AMBER forcefield, as implemented in the version 7.51 of HyperChem,⁶ was used for all calculations. Atomic charges were estimated at the semiempirical level (PM3 method)⁷ and an implicit simulation of aqueous environment (variable dielectric constant) was applied.

80 conformers per adduct were obtained and the conformers featuring similar conformations were grouped in families. Only the families within 2 kcal/mol from the minimum were then selected.

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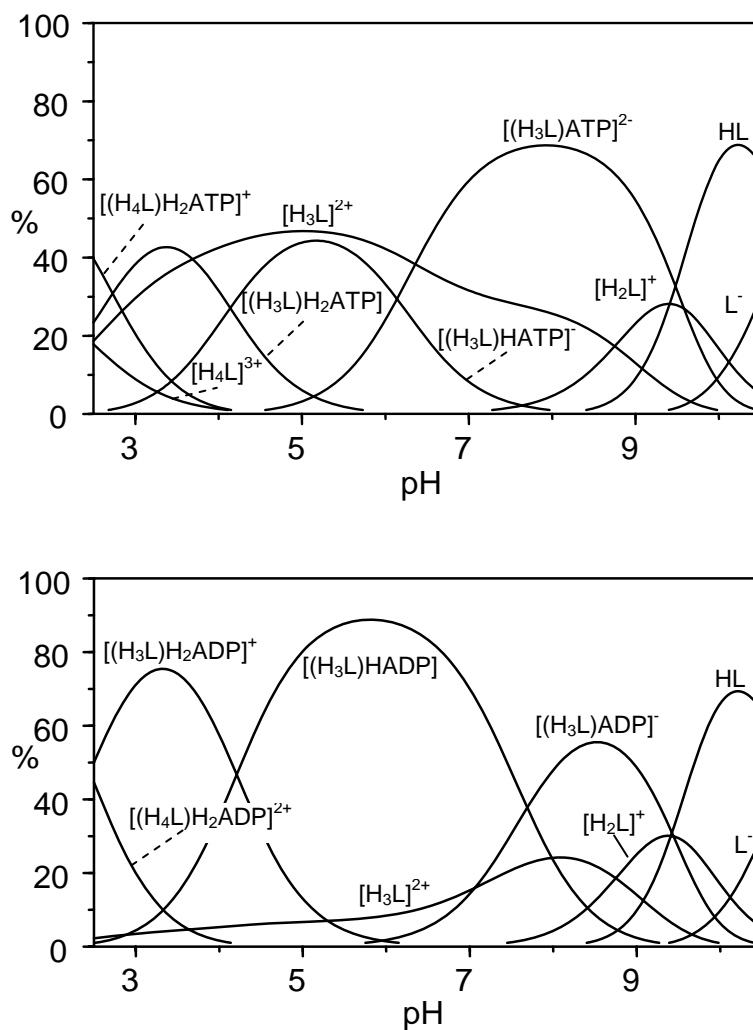


Figure S1. Distribution diagrams of the complexes formed by HL with ATP, ADP. 0.1 M NMe_4Cl at 298.1 ± 0.1 K.

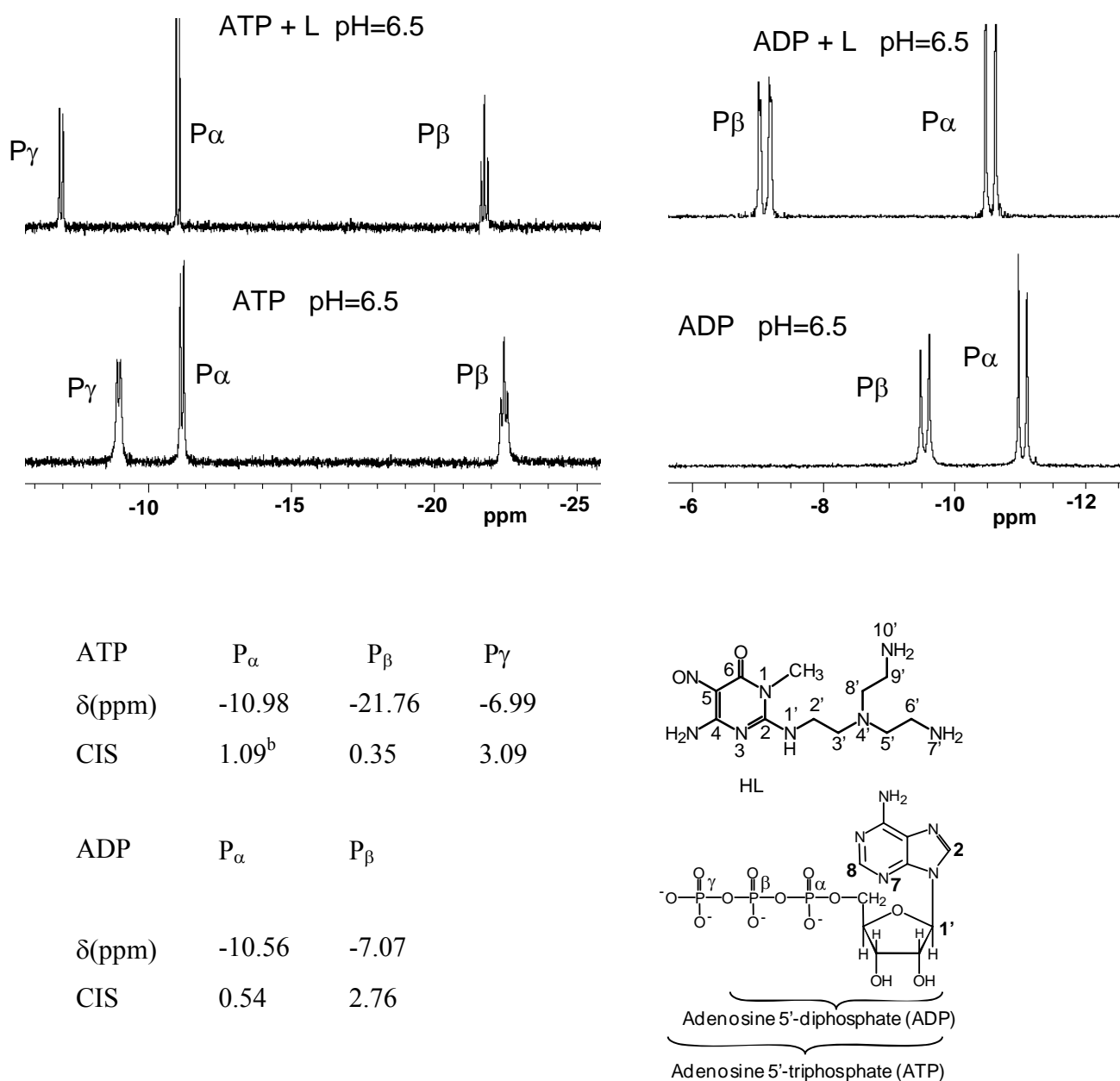


Figure S2. ^{31}P NMR spectra of ATP and ADP in the absence and in the presence (1:1 molar ratio) of HL, and complexation-induced chemical shifts (CIS, ppm) measured in D_2O solution at pH 6.5, 298.1 K. CIS are calculated for 100% complexation.

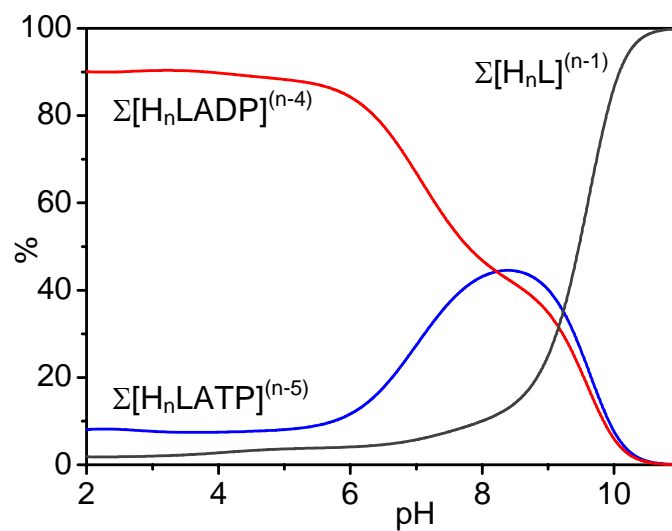


Figure S3. Overall percentage of ADP and ATP bound to HL, as a function of pH, in a system containing HL, ADP and ATP in 1×10^{-3} M concentration. As can be seen from this figure, below pH 6, HL is able to bind 90% of ADP even in the presence of equimolar amounts of ATP.

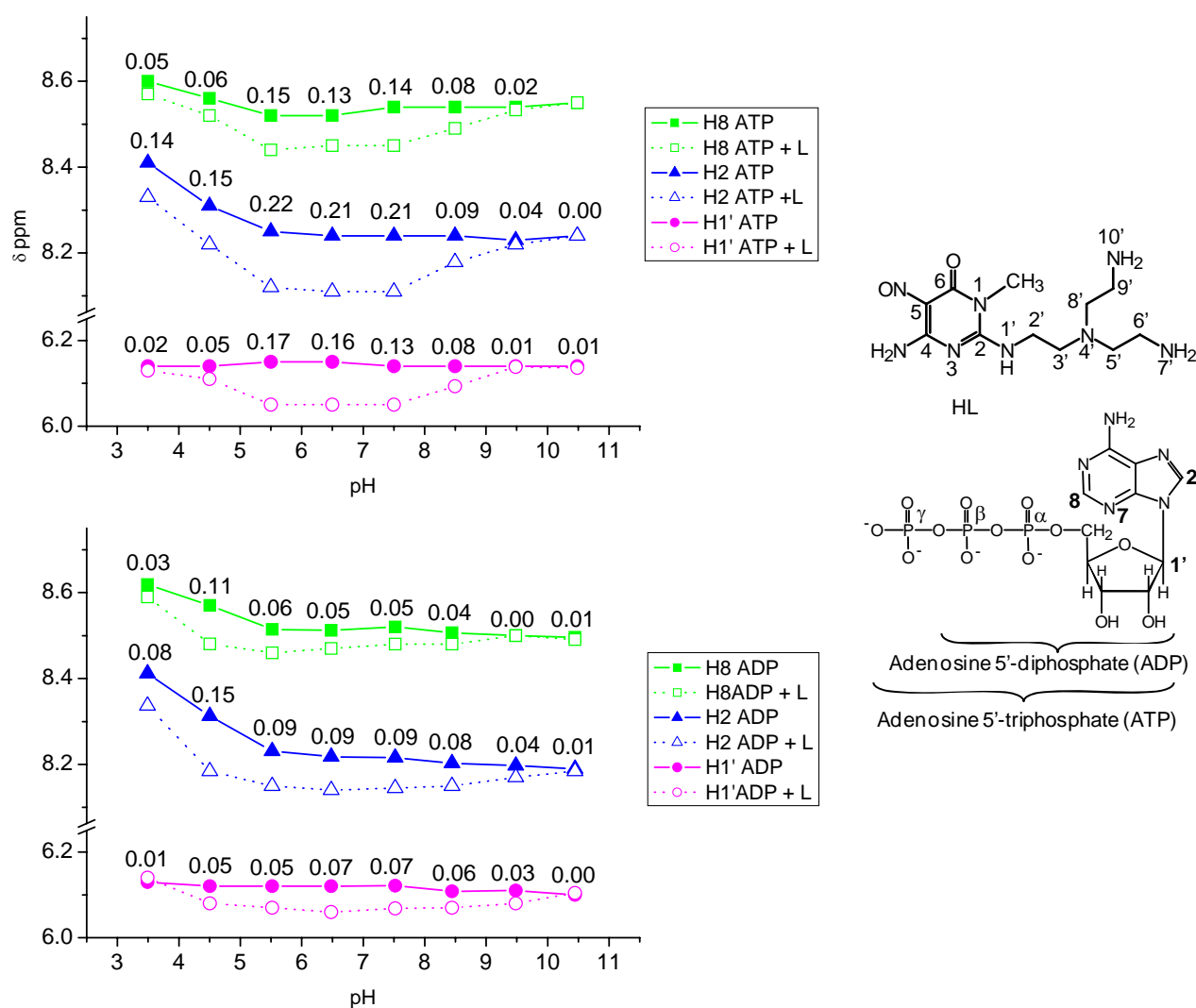


Figure S4. pH dependence of aromatic (H2, H8) and anomeric (H1') ^1H NMR signals and complexation-induced shifts (CIS) for ATP, ADP and AMP solutions in the absence and in the presence (1:1 molar ratio) of HL. CIS are calculated for 100% complexation.

Table S1 Logarithms of the stability constants for the formation of ATP and ADP complexes with HL. 0.1 M NMe₄Cl at 298.1±0.1 K. A = anion.

	Log <i>K</i>	
	ADP ³⁻	ATP ⁴⁻
H ₃ L + A = [(H ₃ L)A] ^a	3.85(3)	3.97(3)
H ₃ L + HA = [(H ₃ L)HA]	5.30(6)	3.34(5)
H ₃ L + H ₂ A = [(H ₃ L)H ₂ A]	5.72(8)	3.59(6)
H ₄ L + H ₂ A = [(H ₄ L)H ₂ A]	5.70(8)	3.84(7)

^a Charges omitted for simplicity