Interaction of Hexaazaalkanes with Phosphate Type Anions. Thermodynamic, Kinetic, and Electrochemical Considerations

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The interaction of adenosine 5′-triphosphate, pyrophosphate, and hexacyanoferrate(II) anions with the cyclic polyamines 1,4,7,10,13,16-hexaazacyclooctadecane ([18]aneN$_6$) and 1,4,7,13-tetramethyl-1,4,7,10,13,16-hexaazacyclooctadecane (Me$_4$[18]aneN$_6$) and with the open-chain polyamine 1,14-bis(methylamino)-3,6,9,12-tetraazatetradecane (Me$_2$pentaen) has been studied in the pH range 3–10 by potentiometry and cyclic voltammetry at 298.1 K in 0.15 mol dm^{-3} NaClO$_4$ as well as by ^{31}P NMR spectroscopy. Me$_4$[18]aneN$_6$ in its protonated forms is the receptor displaying the largest stepwise stability constants for the addition of any one of the three fully deprotonated substrates. Despite this fact, ternary distribution diagrams, ^{31}P NMR, and competitive cyclic voltammetry with [Fe(CN)$_6$]$^{4-}$ show that Me$_2$pentaen is the best receptor for the considered substrates. Relative selectivities of the three receptors are determined using potentiometric and electrochemical methods. The kinetics of ATP hydrolysis induced by the three receptors have been followed by monitoring the loss of ATP by ^{31}P NMR spectroscopy. Me$_4$[18]aneN$_6$ is the receptor inducing at pH 3 the largest rate accelerations. The activation energies for the three processes have been determined. Considerations regarding the nucleophilicity and topologies of the receptors are invoked to explain the observed trend.

Introduction

In a recent paper[1] it was reported that ATP and related phosphate type anions strongly interact with "large" polyazacycloalkanes of the [3*k*]aneN$_k$ series from [21]aneN$_7$ to [36]aneN$_{12}$. Such interactions could provide in some instances a model for enzymes promoting ATP hydrolysis.[1-7] It has been suggested that ATP dephosphorylation reactions induced by cyclic polyammonium receptors proceed, at least at not very acidic pH values, via formation of a covalent phosphoramidate intermediate. This intermediate would be formed by means of nucleophilic attack on the γ-phosphorus of ATP by a nonprotonated nitrogen of the receptor. The nucleophilic character of these nitrogens is a function of their nature as well as of the overall charge present in the complexed species.[1] On the other hand, good topological complementarity between the receptor and ATP can favor cleavage of the latter.[1,6,7] Therefore, studying ATP interactions with polyammonium receptors displaying different topological features as well as different nucleophilicities in their nitrogens is of interest to obtain further insight into the factors governing ATP catalytic dephosphorylation.

With this purpose, we have studied the interaction of the 18-membered polyazacycloalkanes 1,4,7,10,13,16-hexaazacyclooctadecane ([18]aneN$_6$) and 1,4,7,13-tetramethyl-1,4,7,10,13,16-hexaazacyclooctadecane (Me$_4$[18]aneN$_6$) as well as of the open-chain polyamine 1,14-bis(methylamino)-3,6,9,12-tetraazatetradecane (Me$_2$pentaen) (see Chart I) with ATP and pyrophosphate anions by using potentiometric and ^{31}P NMR methods. The kinetics of ATP hydrolysis has been also followed by ^{31}P NMR. In order to use competitive cyclic voltammetry to better characterize these systems, we have extended these studies to the interaction of the polyammonium receptors herein considered with the complex anion hexacyanoferrate(II).

Experimental Section

Materials. Me$_4$[18]aneN$_6$ and Me$_2$pentaen were synthesized as described in refs 8 and 9. [18]aneN$_6$ was purchased from Aldrich or Fluka and used as its hexahydrochloride salt. The sodium salt of ATP was obtained from Boehringer-Mannheim. NaClO$_4$ used as a supporting electrolyte was purified according to a published procedure.[10] All other chemicals were Merck reagent grade and were used without further purification.

Electromotive Force Measurements. The potentiometric titrations were carried out in 0.15 mol dm^{-3} NaClO$_4$ at 298.1 K, using equipment previously described.[9] The reference electrode was an Ag–AgCl electrode in saturated KCl solution. The glass electrode was calibrated as a hydrogen-concentration probe by titrating well-known amounts of HCl with CO$_2$-free NaOH solutions and determining the equivalent point by Gran's method,[12] which yields the standard potential E° and the ionic product of water (pK_w = 13.73(1) in 0.15 mol dm^{-3} NaClO$_4$ at 298.1 K).

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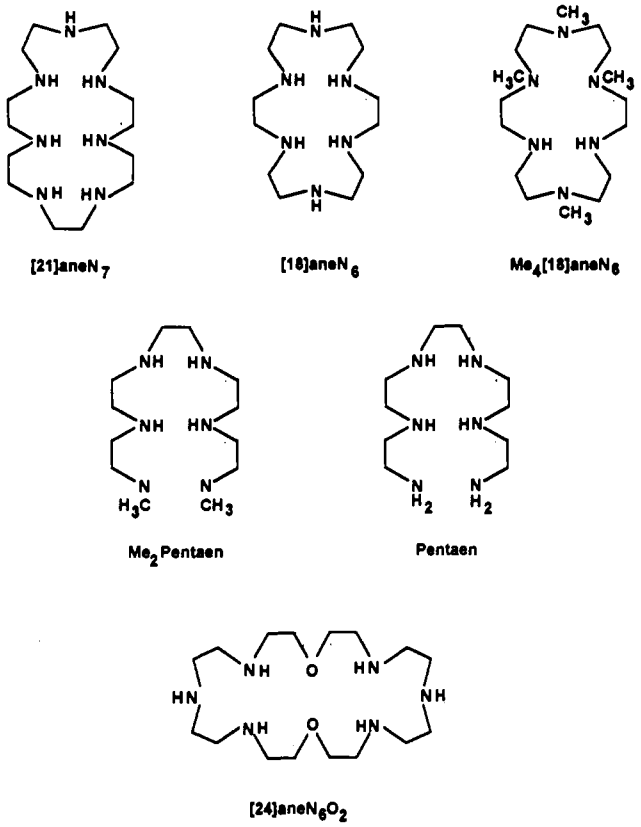
Chart I



The emf data were monitored by means of the computer program PASAT.[13] The computer program SUPERQUAD[14] was used to calculate the stability constants. The ligand protonation constants as well as those of ATP and $P_2O_7^{4-}$ employed in the calculations have been reported elsewhere.[1,8,9,15,16] Protonation of $[Fe(CN)_6]^{4-}$ occurs at pH <4; thus the protonation constant of this anion has also been taken into account.[17] The titration curves were treated either as a single set or as separate entities without significant variations in the values of the stability constants. Calculation of the distribution diagrams was performed by means of the computer program DISPO.[18]

Electrochemical Measurements. Cyclic voltammetric and chronoamperometric measurements were carried out with a battery-powered potentiostat and a triangular-wave generator (Newtronic 200P). Cleaned and activated[19] platinum, glassy-carbon, and gold electrodes were employed as working electrodes; a platinum-wire auxiliary electrode and the saturated calomel reference electrode (SCE) completed the three-electrode standard cell. The sweep rate varied from 50 to 500 mV s^{-1}. All experiments were carried out under argon atmosphere in a cell thermostated at 298.1 K. All samples were 0.15 mol dm^{-3} in $NaClO_4$ to maintain constant the ionic strength. The pH was adjusted to the desired value by adding appropriate amounts of $HClO_4$ or NaOH.

To compare potentiometric and cyclic voltammetric data by evaluation of the molar fractions of complexed hexacyanoferrate(II), α_M, computer-assisted simulated cyclic voltammograms were obtained by using the formal potentials and diffusion coefficients for free and complexed $[Fe(CN)_6]^{4-}$. As previously reported,[20] a simplified program calculates the i–E curves for a given set of parameters which define the electrochemical experiment (initial and switching potentials, sweep rate), providing the concentration, formal potential, and diffusion coefficients are known. In several simulation procedures[21] it is assumed that all species diffuse at the same rate; however, this is an oversimplification in a number of systems. In particular, drastic changes in the peak current occur in the systems studied here as a function of pH and of the ligand/$[Fe(CN)_6]^{4-}$ ratio.

To improve the reliability of electrochemical data, chronoamperometry has been used to determine the α_M values from experimental mean diffusion coefficients of the electroactive species as in polarographic techniques.[22] Then, α_M can be calculated from the relationship $\alpha_M=(D-D_M)/(D_{ML}-D_M)$, where D_M is the diffusion coefficient for $[Fe(CN)_6]^{4-}$, D_{ML} is the limiting diffusion coefficient for the system $[Fe(CN)_6]^{4-}$/ligand, and D is the actually determined mean diffusion coefficient.

Potentiostatic i–t curves were obtained at a potential sufficiently large to ensure diffusion-limited current for all the species. Since it has recently been stated that chronoamperometric data obtained at short times (<1 s) are often unreliable and seem to imply that such data cannot be used to accurately determine diffusion coefficients,[23] conventional long-time experiments were used. After current background correction, linear relationships i vs $t^{-1/2}$ were observed for times up to 5 s after the potential step.

Diffusion coefficients were calculated from the experimental i–t curves (i) from the least-squares slope of the $t^{-1/2}$ plot (Cottrell equation, $i = nFAc(\pi D)^{-1/2}t^{-1/2}$), (ii) from the least-squares intercept of the $it^{1/2}$ vs $t^{1/2}$ plot, and (iii) by linear extrapolation of i at infinite time. A good agreement for the D values calculated by the different methods was obtained.

NMR Measurements. The ^{31}P NMR spectra were recorded at 121.42 MHz on a Varian Unity 300-MHz instrument. Chemical shifts are relative to an external reference of 85% H_3PO_4. Probe temperature was regulated by a variable-temperature accessory. Adjustments to the desired pH were made using drops of HCl or NaOH solutions. Kinetic studies were performed by following at different pH values the time-dependent change in the integrals of the resolved ^{31}P NMR signals of P_α, P_β, and P_γ of ATP and peaks for inorganic phosphate and ADP. The kinetic parameters for the conversion of ATP into ADP were calculated by following the disappearance with time of the P_β NMR signal in samples with molar ratio ATP/macrocycle ≤ 1 or the signal corresponding to formation of inorganic phosphate in samples with an excess of ATP. Initial concentrations of ATP and polyamines varied in the range 10^{-2}–3 $\times 10^{-2}$ mol dm^{-3}. Plots of log [ATP] vs time were linear for several half-lives. For this purpose, an automated array of spectra was used. Calibration curves were employed when the integral ratios were not equal because of variations in the ^{31}P relaxation times.

Results and Discussion

Electromotive Force Data. In Tables I–III[24] are presented the stability constants for the interaction of ATP, $P_2O_7^{4-}$, and $[Fe(CN)_6]^{4-}$ with the hexaamines Me2pentaen, $Me_4[18]aneN_6$, and $[18]aneN_6$. Several features regarding the thermodynamic aspects of the interactions deserve to be discussed. First of all, the stoichiometry found in solution for the anion complexes formed is always 1/1. Such a stoichiometry is derived from the electromotive force data analysis using the computer program SUPERQUAD, as well as, in the case of the phosphate anions,

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(24) The stability constants reported in Tables I–III are conditional constants for 298.1 K and 0.15 mol dm^{-3} $NaClO_4$. Owing to complex formation between Na^+ and phosphate type anions, they are valid for solutions in which Na^+ concentration is about 0.15 mol dm^{-3}. As Na^+ is a standard state for physiological solutions, we have preferred to present the uncorrected stability constants. However, an easy correction can be carried out as reported by: Sigel, H.; Tribolet, R.; Malini-Balakrishnan, R.; Martin, R. B. *Inorg. Chem.* **1987**, *26*, 2149.

Table I. Stability Constants for the Interaction of the Polyamines Me_2pentaen, [18]aneN$_6$, and Me_4[18]aneN$_6$ with ATP (A) at 298.1 K in 0.15 mol dm^{-3} $NaClO_4$

	log K		
reaction	Me_2pentaen	[18]aneN$_6$	Me_4[18]aneN$_6$
L + 3H + A = H_3LA[a]	31.53(3)[b]	30.99[c]	29.66(2)
L + 4H + A = H_4LA	39.95(2)	38.70	36.41(2)
L + 5H + A = H_5LA	46.46(2)	43.92	40.93(2)
L + 6H + A = H_6LA	51.41(2)		44.18(3)
L + 7H + A = H_7LA	55.19(2)		
L + 8H + A = H_8LA	57.75(3)		
H_3L + A = H_3LA	2.89	2.47	3.30
H_4L + A = H_4LA	4.77	5.91	7.48
H_5L + A = H_5LA	7.47	8.92	
H_6L + A = H_6LA	9.86		
H_4L + HA = H_5LA			5.76
H_4L + H_2A = H_6LA			5.01

[a] Charges have been omitted for clarity. [b] Values in parentheses are standard deviations in the last significant figure. [c] Taken from ref 1.

Table II. Stability Constants for the Interaction of the Polyamines Me_2pentaen, [18]aneN$_6$, and Me_4[18]aneN$_6$ with $P_2O_7^{4-}$ (A) at 298.1 K in 0.15 mol dm^{-3} $NaClO_4$

	log K		
reaction	Me_2pentaen	[18]aneN$_6$	Me_4[18]aneN$_6$
L + 3H + A = H_3LA[a]	31.11(5)[b]	31.79(2)	30.24(2)
L + 4H + A = H_4LA	40.51(2)	40.01(3)	37.90(2)
L + 5H + A = H_5LA	47.73(1)	46.60(4)	44.23(2)
L + 6HA + A = H_6LA	53.51(1)	50.83(6)	49.8(1)
L + 7H + A = H_7LA	57.67(1)		
L + 8H + A = H_8LA	59.83(3)		
H_3L + A = H_3LA	2.47	3.27	3.88
H_4L + A = H_4LA	5.33	7.22	7.48
H_5L + A = H_5LA	8.74	11.60	
H_6L + A = H_6LA	12.01	14.83	
H_4L + HA = H_5LA			7.16
H_4L + H_2A = H_6LA			4.87

[a] Charges have been omitted for clarity. [b] Values in parentheses are standard deviations in the last significant figure.

Table III. Stability Constants for the Interaction of the Polyamines Me_2pentaen, [18]aneN$_6$, and Me_4[18]aneN$_6$ with $[Fe(CN)_6]^{4-}$ (A) at 298.1 K in 0.15 mol dm^{-3} $NaClO_4$

	log K		
reaction	Me_2pentaen	[18]aneN$_6$	Me_4[18]aneN$_6$
L + 3H + A = H_3LA[a]	31.21(4)[b]	31.64(2)	31.02(2)
L + 4H + A = H_4LA	39.21(2)	39.07(3)	35.34(2)
L + 5H + A = H_5LA	44.25(2)	42.28(4)	37.92(2)
L + 6H + A = H_6LA	47.37(2)		
H_3L + A = H_3LA	2.57	3.12	4.67
H_4L + A = H_4LA	4.03	6.28	6.41
H_5L + A = H_5LA	5.26	7.28	
H_6L + A = H_6LA	5.87		

[a] Charges have been omitted for clarity. [b] Values in parentheses are standard deviations in the last significant figure.

by the variatons in the ^{31}P chemical shifts as a function of the receptor/substrate molar ratio. These curves, which are illustrated in Figure 1 for the interaction at pH 3 of ATP with Me_2pentaen and Me_4[18]aneN$_6$, present sharp breaks close to a value of 1. It is to be noted that Me_2pentaen produces larger variations in the ^{31}P chemical shift than Me_4[18]aneN$_6$, in accord with a greater interaction at this pH (*vide infra*). As will be discussed later, the cycle voltammetric measurements are also in agreement with this stoichiometry. In Figure 2 the stepwise stability constants for the interaction of the tetraanions with the protonated forms of the receptors ($A^{4-} + H_nL^{n+} = A(H_nL)^{(n-4)+}$), are plotted as a function of the protonation degree, n, of the supramolecular species. In accord with similar studies,[1,3,20,25,26] for a given ligand

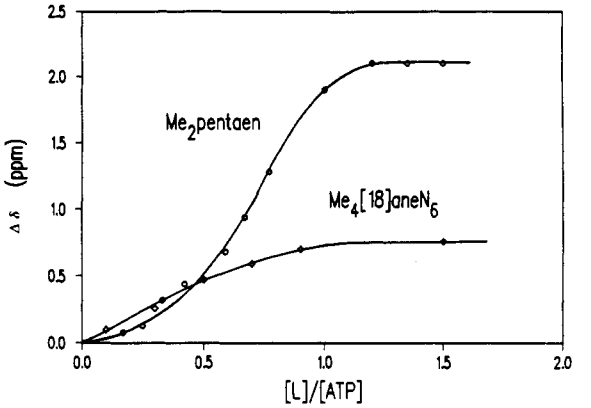

Figure 1. Variation of the ^{31}P chemical shifts of P_γ of ATP as a function of the receptor/ATP molar ratio at pH 3: (a) Me_2pentaen; (b) Me_4-[18]aneN$_6$.

and a given receptor the stability grows with the overall charge present in the supramolecular species. Among the three studied substrates, for a given protonation degree of the supramolecular species, Me_4[18]aneN$_6$ is the one interacting more strongly with all three anions. However, to analyze which are the prevailing species in solution throughout the pH range studied, the basicities of both substrates and receptors have also to be considered (see footnote 27). If selectivity is understood as the preferential coordination of a substrate over another one under equivalent experimental conditions (analytical concentrations of substrate and receptor, pH, etc.), the actual protonation degrees of the reactants should be known to evaluate such a parameter. Nevertheless, this evaluation is rather difficult due to the complexity of these systems. We have recently suggested[28] that an appropriate way to overcome this difficulty is to calculate the distribution diagrams for the ternary systems (substrate **A**)–(substrate **B**)–receptor and represent the overall percentages of free and complexed receptor as a function of pH. Such plots can be extended to compare the relative affinities of two receptors for one substrate. This method presents the additional advantage of not requiring any assumption regarding the location of the protons in the host and guest species. Thus, calculating distribution diagrams for the ternary system (receptor **A**)/(receptor **B**)/substrate and representing the overall amounts of complexed receptor as a function of pH result in a clear establishment of selectivity patterns in these systems. In Figure 3a such a diagram is presented for the system ATP–Me_4[18]aneN$_6$–Me_2pentaen. Calculations have been made, in all cases, with respect to ATP and for 10^{-3} mol dm^{-3} concentrations in all the reactants. Me_4-[18]aneN$_6$–ATP-complexed species never prevail in solution because the concentrations of Me_2pentaen–ATP-complexed species are higher throughout the entire pH range. This can be attributed to the fact that Me_2pentaen, presenting larger protonation constants than Me_4[18]aneN$_6$,[27] takes up protons at higher pH values, favoring the interaction with the anionic forms of ATP. Similar conclusions can be drawn for the system ATP–[18]aneN$_6$–Me_2pentaen, also with ATP–Me_2pentaen-complexed species being the main ones in solution. However, when comparing ATP–Me_4[18]aneN$_6$ and ATP–[18]aneN$_6$ systems, one observes an intermediate situation (Figure 3b). ATP–Me_4[18]aneN$_6$-

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(27) Values taken from refs 1, 8, 9, 15, and 16. Basicity constants for ATP, $P_2O_7^{4-}$, Me_2pentaen, [18]aneN$_6$, and Me_4[18]aneN$_6$ taken from refs 1, 6, 7, 12: ATP, log β_1 = 6.26, log β_2 = 10.24, log β_3 = 12.01; $P_2O_7^{4-}$, log β_1 = 8.14, log β_2 = 14.06, log β_3 = 16.00; Me_2pentaen, log β_1 = 10.279, log β_2 = 19.799, log β_3 = 28.640, log β_4 = 35.182, log β_5 = 38.99, log β_6 = 41.50; Me_4[18]aneN$_6$, log β_1 = 9.75, log β_2 = 18.87, log β_3 = 26.40, log β_4 = 28.99; [18]aneN$_6$, log β_1 = 10.15, log β_2 = 19.63, log β_3 = 28.52, log β_4 = 32.76, log β_5 = 35.00, log β_6 = 36.00.

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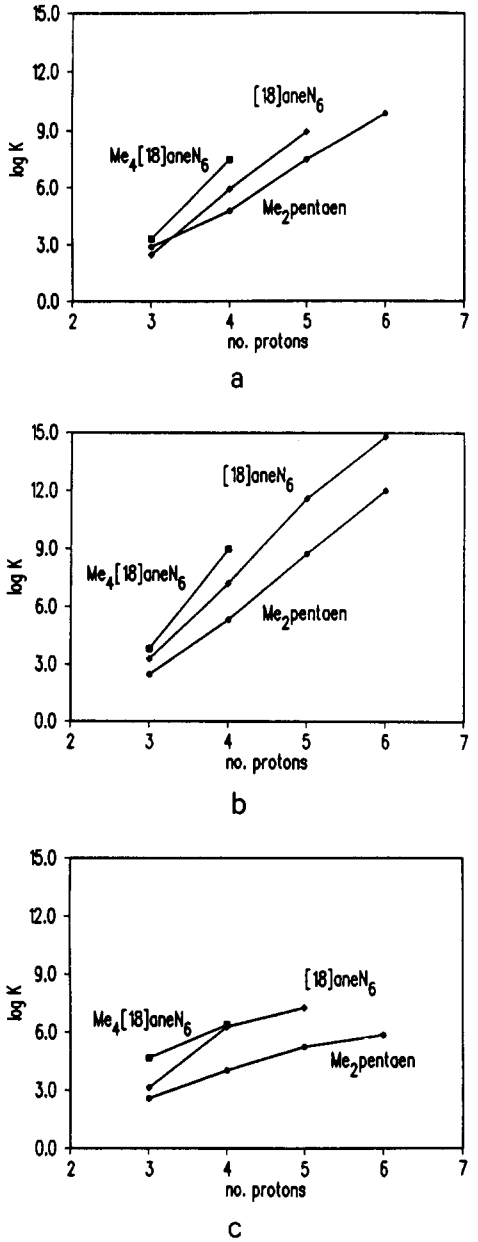

Figure 2. Plot of the logarithms of the stepwise stability constants as a function of the number of protons, n, for the interaction of $Me_4[18]aneN_6$, $[18]aneN_6$, and Me_2pentaen with (a) ATP, (b) $P_2O_7^{4-}$, and (c) $[Fe(CN)_6]^{4-}$.

complexed species slightly predominate throughout the pH range 6.5–8.2, while for lower and higher values the reverse situation occurs. pH values 6.5 and 8.2 correspond to points at which both receptors display the same affinity for ATP. By analogy with the terminology adopted in other representations, these points may be defined as *isoselectivity points*. On the other hand, these representations show that selectivity can be modulated by several factors, one of them being the pH.

Figure 4 shows that in the system ATP–$P_2O_7^{4-}$–$[18]aneN_6$, ATP-complexed species predominate for pH lower than *ca.* 6.2 and, in the system ATP–$P_2O_7^{4-}$–Me_2pentaen, for pH lower than 7.3. So, 6.2 and 7.3 would represent isoselectivity pH values under the experimental conditions used to determine these diagrams. For ATP–$P_2O_7^{4-}$–$Me_4[18]aneN_6$, pyrophosphate species are the main ones throughout the pH range, the differences in relative concentrations being higher for pH <5.5.

These results highlight the critical importance of considering the different basicities in establishing selectivity patterns.

Electrochemistry. Electroanalytical techniques have long found wide application in the characterization of complex ions in solution.

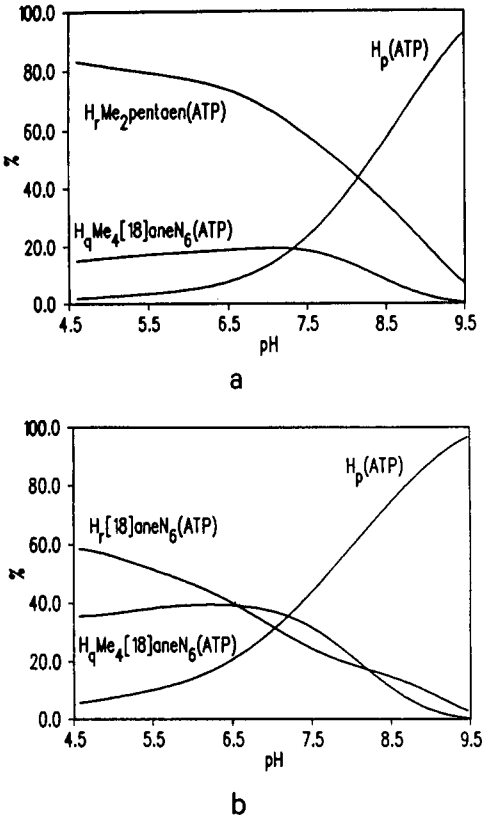

Figure 3. Calculated distribution diagrams for the ternary systems (a) Me_2pentaen–$Me_4[18]aneN_6$–ATP and (b) $[18]aneN_6$–$Me_4[18]aneN_6$–ATP. The sums of the percentages of complexed species and ATP are presented as a function of pH. Concentrations in all reagents are 1×10^{-3} mol dm^{-3}. Percentages are calculated with respect to ATP.

Although usual treatments are limited to electroactive species, cyclic voltammetry and chronoamperometry can be applied to characterizing nonelectroactive species such as is the case for the interaction between ATP and polyazaalkanes.[28,29] This method makes use of the phenomenon of the competitive formation of electroactive complexed species between the $[Fe(CN)_6]^{4-}$ anion and the same ligands.

Accordingly, the interaction between $[Fe(CN)_6]^{4-}$ and Me_2-pentaen, $[18]aneN_6$, and $Me_4[18]aneN_6$ was studied by cyclic voltammetry and chronoamperometry. Both the hexacyanoferrate(II) ion and its complexes with these ligands exhibit a one-electron reversible (E_{pc}-E_{pa} = 60 mV) and diffusion-controlled (i_p proportional to the square root of sweep rate) redox couple which can be represented as

$$M^{II}LH_q \leftrightarrow M^{III}LH_q + e \qquad (1)$$

As expected, the electrochemical parameters are strongly dependent on pH and the molar ratio ligand/$[Fe(CN)_6]^{4-}$ (L/M). Thus addition of increasing amounts of ligand to a hexacyanoferrate(II) solution at a given pH leads to a decrease of the peak current and to a shift of the peaks toward more positive potentials.

In the cases where the ligand is present in less than stoichiometric amounts, the CV's are rather broad and look like the overlap of two couples, but for L/M ratios close to unity only one couple appears. Cyclic voltammograms remain almost unaltered for L/M ratios >1, confirming the stoichiometry already inferred from the potentiometric studies. Molar ratio curves obtained by changing the concentration of ligand and keeping constant the

(29) Peter, F.; Gross, M.; Hosseini, M. W.; Lehn, J.-M.; Sessions, R. B. *J. Chem. Soc., Chem. Commun.* **1981**, 1067. The authors suggested the possibility of using competitive cyclic voltammetry between electroactive and nonelectroactive substrates to evaluate interaction strengths.

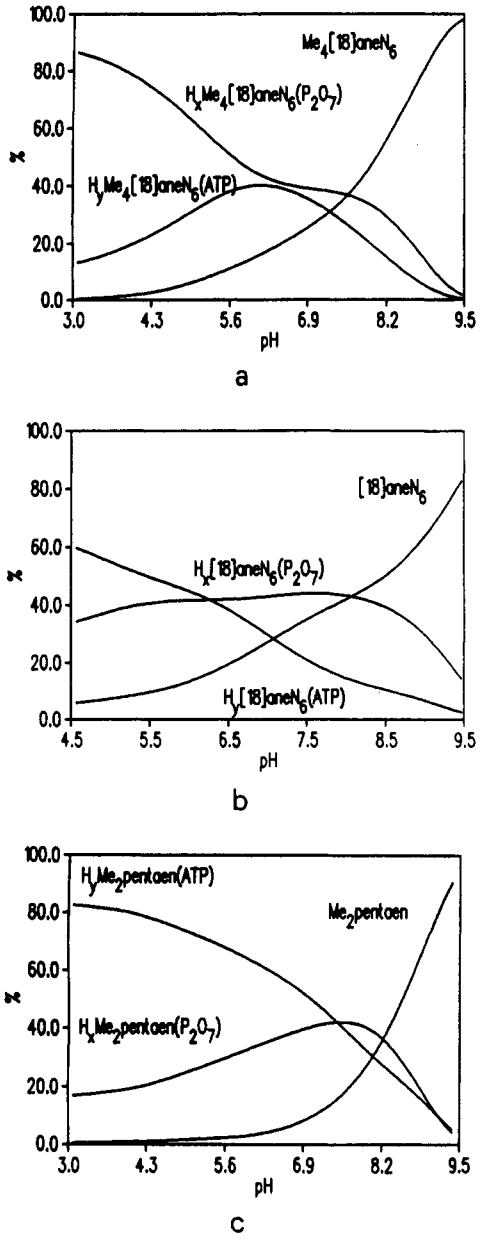

Figure 4. Calculated distribution diagrams for the ternary systems (a) $Me_4[18]aneN_6$–$P_2O_7^{4-}$–ATP, (b) $[18]aneN_6$–$P_2O_7^{4-}$–ATP, and (c) Me_2pentaen–$P_2O_7^{4-}$–ATP. The sums of percentages of complexed species and ATP are plotted as a function of pH. Concentrations in all reagents are 1×10^{-3} mol dm^{-3}. Percentages are calculated with respect to the ligands.

$[Fe(CN)_6]^{4-}$ concentration (see Figure 5) clearly indicate 1/1 anion–receptor complexed species.

Since ATP solutions were found to be nonelectroactive and no influence on the cyclic voltammetric response of $[Fe(CN)_6]^{4-}$ solutions was detected (for molar ratios ATP/M from 0.0 to 2.5), it can be expected that addition of increasing amounts of ATP to ligand–$[Fe(CN)_6]^{4-}$ solutions causes a measurable alteration of their electrochemical response. As shown in Figure 5, experimental results agree well with these expectations: voltammetric peaks are shifted toward less positive potentials while peak current increases, denoting the increase in concentration of uncomplexed $[Fe(CN)_6]^{4-}$ at the expense of the complexed species.

This competitive effect can be clearly seen in molar ratio curves and in plots of the formal potential vs pH. For instance, Figure 6 shows the pH dependence of the formal potential ($E^{\circ\prime} = (E_{pa} + E_{pc})/2$) for a solution of $K_4[Fe(CN)_6]$ and $Me_4[18]aneN_6$ (L/M = 0.5 and ATP/M = 0.90) and those corresponding to a solution of only $[Fe(CN)_6]^{4-}$ and ligand (L/M = 1.2). The presence of ATP causes a shift of the formal potential to values

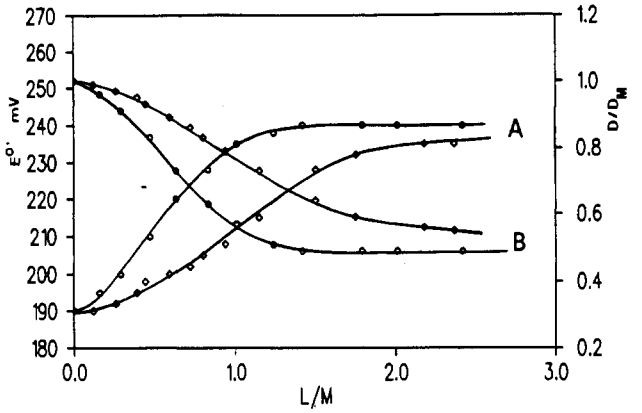

Figure 5. Effect on the formal potential (A) and diffusion coefficients (B) of the ligand/$[Fe(CN)_6]^{4-}$ ratio at pH 6.15 in the system $[Fe(CN)_6]^{4-}$–Me_2pentaen–ATP: circles, $c_M = 2.14 \times 10^{-3}$ mol dm^{-3}; squares, $c_M = 2.14 \times 10^{-3}$ mol dm^{-3}, $c_A = 2.14 \times 10^{-3}$ mol dm^{-3}.

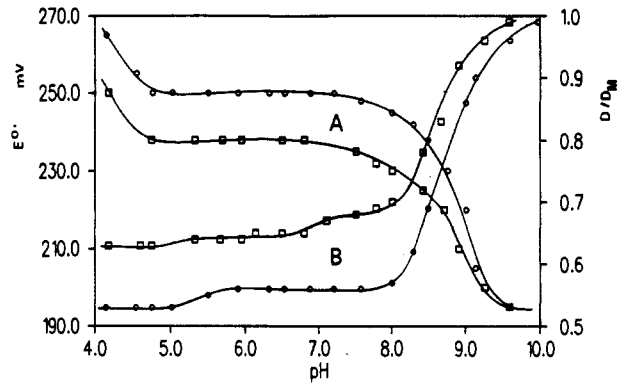

Figure 6. pH dependence of the formal potential (A) and diffusion coefficients (B): circles, $[Fe(CN)_6]^{4-}$–$Me_4[18]aneN_6$; squares, $[Fe(CN)_6]^{4-}$–ATP–$Me_4[18]aneN_6$. $c_M = 1.36 \times 10^{-3}$ mol dm^{-3}; $c_L = 1.44 \times 10^{-3}$ mol dm^{-3}; $c_A = 1.25 \times 10^{-3}$ mol dm^{-3}.

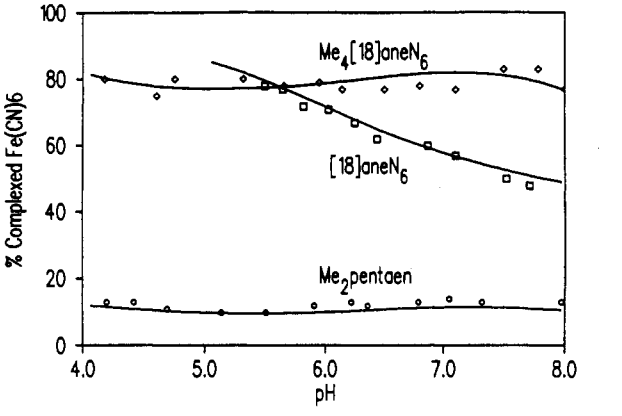

Figure 7. Plot of the percentages of complexed $[Fe(CN)_6]^{4-}$ as a function of pH for the ternary systems $[Fe(CN)_6]^{4-}$–ATP–$Me_4[18]aneN_6$, $[Fe(CN)_6]^{4-}$–ATP–$[18]aneN_6$, and $[Fe(CN)_6]^{4-}$–ATP–Me_2pentaen. Points represent the electrochemical values, and continuous lines, those calculated from the potentiometric constants with the program DISPO.

close to that of the $[Fe(CN)_6]^{4-}$ couple, indicating a strong ATP–$Me_4[18]aneN_6$ interaction.

If compared with the case of $[Fe(CN)_6]^{4-}$, a weaker interaction is observed between ATP and the macrocyclic receptors $[18]aneN_6$ and $Me_4[18]aneN_6$ than with Me_2pentaen. Comparisons between experimental cyclic voltammograms and working curves were used to obtain estimates for the molar fraction of complexed hexacyanoferrate(II), α_M, in molar ratio experiments. Alternatively, diffusion chronoamperometric coefficients were used. In Figure 7, the points represent the molar fractions of complexed $[Fe(CN)_6]^{4-}$ obtained from the electrochemical data and the continuous lines those calculated by means of the potentiometric

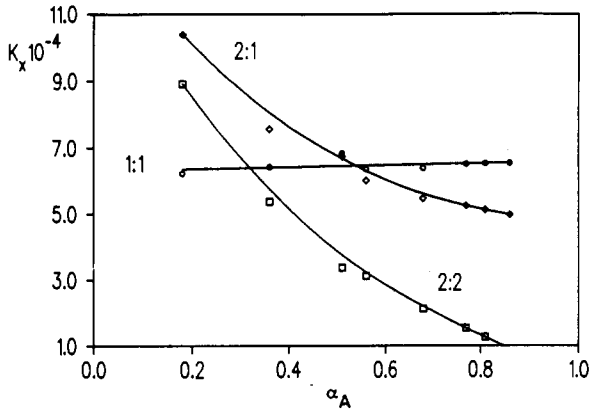

Figure 8. Application of the molar ratio method to the system $[Fe(CN)_6]^{4-}$–Me_2pentaen–ATP at pH 5.8: plots of K_A vs α_A for different stoichiometries.

stability constants. A reasonable agreement between both techniques is observed.

From the diffusion coefficients, a generalization of the molar ratio method[30] was applied to confirm the stoichiometry of the complexed species from well-known pH-constant molar ratio curves when the concentration of ligand was varied as the concentration of $[Fe(CN)_6]^{4-}$ was kept constant. Assuming a unique complex is formed at a given pH

$$mM + nL + qH \leftrightarrow M_mL_nH_q \quad (2)$$

only substitution of the correct m and n values must satisfy the equation

$$K_M = \frac{\alpha_M^{1/m}}{m^{1/n}(1-\alpha_M)^{m/n}(c_L - \alpha_M c_M)} \quad (3)$$

where c_M and c_L are the total concentrations of M and L. K_M is related to β_q as $\beta_q = K_M^n(1 + \sum\beta_j[H]^j)[H]^{-q}$, β_j being the cumulative protonation constants for the ligand determined potentiometrically.

In order to confirm the stoichiometry of the ATP–L species and evaluate the formation constants from electrochemical measurements, a second series of α_M values were obtained after successive additions of ligand to a solution containing $[Fe(CN)_6]^{4-}$ and ATP. Therefore, the molar ratio method can be extended to competitive equilibria, providing only one ATP–L complex is present in solution at the same pH value

$$r\text{ATP} + sL + wH = (\text{ATP})_rL_sH_w \quad (4)$$

Providing the values for m, n, and K_M are known, the molar fraction of ATP complex, α_A, can be calculated from the α_M values by means of the relationship

$$\alpha_A = \frac{c_L - (n/m)\alpha_M - \alpha_M/(1-\alpha_M)^{m/n}K_M m^{1/n}}{(s/r)c_A} \quad (5)$$

c_A being the total concentration of ATP. Therefore, the correct stoichiometric coefficients for the ATP–L complex can be verified:

$$K_A = \frac{m^{1/n}\alpha_A^{1/s}(1-\alpha_M)^{m/n}K_M m^{1/n}}{r^{1/s}(1-\alpha_A)^{r/s}\alpha_M^{1/n}} \quad (6)$$

and $\beta_w = K_A(1 + \sum\beta_j[H]^j)[H]^{-w}$. For a set of favorable cases, electrochemical measurements allow direct estimates of K_M and K_A which are in good agreement with the potentiometric data. For instance, Figure 8 shows a plot of K_A vs α_A for different stoichiometries corresponding to the ATP–Me_2pentaen–$[Fe(CN)_6]^{4-}$ system at pH 6.1. A constant K_A value is only obtained for 1/1 ligand/hexacyanoferrate(II) stoichiometry. From the electrochemical data a value of $K_A = 6.5 \times 10^4$ ($\log \beta_4 = 40.1$) in excellent agreement with the potentiometric value, $\log \beta_4 = 39.95$, is obtained.

(30) Beltrán, A.; Beltrán, D.; Cervilla, A.; Ramírez, J. A. *Talanta* **1983**, *30*, 124.

Table IV. Rate Constants (±10%) and Activation Parameters for the Hydrolysis of 0.01 mol dm^{-3} ATP at pH 3 in the Presence of Polyammonium Receptors Me_4[18]aneN$_6$, [18]aneN$_6$, and Me_2pentaen (0.01 mol dm^{-3})

	T (°C)	10^3k^a	$E_a{}^b$	ΔH^*	ΔG^*	ΔS^*
Me_4[18]aneN$_6$	25	0.40				
	50	4.5				
	55	5.9				
	60	8.0				
	65	15				
	80	43	74 ± 2	71 ± 2	108.2 ± 0.3	−105 ± 7
	85	63				
[18]aneN$_6$	40	0.10				
	60	1.5				
	65	2.8				
	70	5.3				
	75	11				
	80	13	114 ± 4	111 ± 4	111.7 ± 0.3	−2 ± 12
Me_2pentaen	45	1.9				
	50	2.2				
	55	2.9				
	70	10				
	80	24	73 ± 4	70 ± 4	109.9 ±0.3	−113 ± 12
	85	34				

[a] Rate constants are in min^{-1}. [b] E_a, ΔH^*, and ΔG^* are in kJ mol^{-1}, and ΔS^* is in J mol^{-1} K^{-1}.

Kinetic Studies. It is widely known that several polyammonium receptors catalyze ATP cleavage to larger or lesser extents. Among those studied up to now, 1,4,7,10,13,16,19-heptaazacycloheni-cosane ([21]aneN$_7$) is the one inducing the largest rate enhancement with respect to free ATP.[1] The effect of smaller and larger macrocycles of this [3k]aneN$_k$ series is much reduced. Also the ditopic macrocycle bisdien, [24]aneN$_6$O$_2$,[3,6] displays good properties in this respect, presenting similar rate enhancements at neutral and acidic pH values. The ability of the polyammonium receptors to induce rate accelerations in the ATP hydrolysis has been suggested to depend on factors such as (i) formation of stable anion complexes between host and guest, (ii) presence of nucleophilic nitrogens in the host species, (iii) overall charge in the host and in the substrate:receptor assembly, (iv) pH, etc.

We have examined the ATP cleavage promoted by the polyammonium receptors Me_2pentaen and Me_4[18]aneN$_6$ monitoring the ATP loss by means of ^{31}P NMR spectroscopy. Measurements were performed at different temperatures and pH values. Although, data relevant to the system ATP–[18]-aneN$_6$ can be found in the literature,[3,6] we have performed new experiments under our experimental conditions in order to have strictly comparable data. First-order reactions with respect to the concentration of the ATP–macrocycle complexes were found, in all three systems, for the catalytic conversion of ATP into ADP and inorganic phosphate. Experiments performed with excess of either ATP or macrocycle do not show significant changes in the rate constants with respect to those with ATP/L = 1 molar ratio. Therefore, the dominant species in solution, in the temperature range used in the kinetic measurements (see Table IV), should be macrocycle–ATP complexes of 1/1 stoichiometry.

At pH 3, ATP hydrolysis promoted by Me_4[18]aneN$_6$, [18]aneN$_6$, and Me_2pentaen yields initially the formation of ADP and inorganic phosphate; when the concentration of ADP starts to be significant, a new signal very close to that of inorganic phosphate attributable to AMP appears in the ^{31}P NMR spectra. As the pH is increased, the protonation degree of the ATP–polyamine complexes decreases and therefore, as expected, the

rates of the ATP hydrolyses induced by these receptors change with pH. For instance, for $Me_4[18]aneN_6$ the k (min^{-1}) values obtained are as follows: pH 4, $k = (40 \pm 2) \times 10^{-3}$; pH 5.1, $k = (12 \pm 1) \times 10^{-3}$; pH 6.5, $k = (8.4 \pm 0.5) \times 10^{-3}$; pH 7, $k = (2.2 \pm 0.1) \times 10^{-3}$. At pH 7, the separation between the resonances of inorganic phosphate and AMP is much larger, and so, by integration of the different resonances, it is possible to estimate the evolution of the amounts of the different species with time. Although, as suggested in the literature,[1-7] phosphoramidate could be present as an intermediate species, we have not detected any characteristic signal at lower field than the AMP resonance.

The data in Table IV show that $Me_4[18]aneN_6$ is, by far, the receptor inducing the largest rate acceleration for the ATP dephosphorylation. While at pH 3 and 80 °C, $Me_4[18]aneN_6$ produces a 20-fold rate enhancement with respect to free ATP, Me_2pentaen and $[18]aneN_6$ yield 10-fold and 5-fold rate enhancements, respectively. The fact that a partial methylation of $[18]aneN_6$ to give $Me_4[18]aneN_6$ results in a better catalyst of ATP hydrolysis is, at first glance, a striking result. Some points may be advanced to try to justify this result. First of all, methylation yields a clear drop in basicity;[8,27] while at pH 3, in the absence of ATP, $Me_4[18]aneN_6$ exists in solution mainly in its triprotonated form, $H_3(Me_4[18]aneN_6)^{3+}$, $[18]aneN_6$ is in its tetraprotonated form $H_4([18]aneN_6)^{4+}$. As indicated by the potentiometric results (see Table I), this would produce a weaker ATP–macrocycle complex in the case of $Me_4[18]aneN_6$. However, it has been pointed out that, although formation of stable complexes is required as a first step in the catalytic pathway, their relative stabilities are not necessarily related to the rate enhancement produced and it seems that a compromise between stability and electronic and structural features of the guest is of greater relevance in this respect. The introduction of electron-donor methyl groups should produce an increase in the nucleophilic character of the molecule with respect to $[18]aneN_6$. This feature is further accentuated by its lower overall charge at this pH. Therefore, apart from possible topological considerations we cannot still present, nucleophilicity should be at the origin of the higher rates found for $Me_4[18]aneN_6$. Probably, the effects of partial methylation are also reflected in the higher rates we have found for Me_2pentaen compared to those reported in the literature for its nonmethylated linear analog pentaethylenehexamine (see Chart I). It has been reported that $Me_6[24]aneN_6O_2$,[6] the fully methylated derivative of $[24]aneN_6O_2$, produces lower catalytic rates than its precursor but, probably in this case, the decrease in stability and/or the distortions in optimal topology are not further balanced by the increase in nucleophilicity produced by the methyl groups.

The fact that some kind of compromise between stability and nucleophilicity is required to significantly hydrolyze ATP is manifested by our data at pH 7. At this pH value the rate constant differences between $Me_4[18]aneN_6$ and $[18]aneN_6$ are much less ($k = (2.2 \pm 0.1) \times 10^{-3}$ min^{-1} and $k = (1.3 \pm 0.1) \times 10^{-3}$ min^{-1}, respectively), indicating perhaps that the anchorage of ATP produced by $Me_4[18]aneN_6$ is not sufficiently large to effectively cleave ATP.

Plots of ln k vs $1/T$, for the ATP to ADP conversion, allow us to estimate the activation energy for all three systems. From these parameters the thermodynamic activation quantities ΔG^*, ΔH^*, and ΔS^* can be obtained (Table IV).[31] While Me_2pentaen and $Me_4[18]aneN_6$ present similar unfavorable values of ΔS^*, the value found for $[18]aneN_6$ is close to zero.

The main pattern of a generally accepted mechanism[32,33] for the acidic hydrolysis of ATP implicates a first nucleophilic addition of solvent to the γ-phosphate group of ATP followed by elimination to produce ADP. We can assume that a similar addition–elimination mechanism is also operating in the presence of the polyammonium catalyst.[6] In this case, elimination of phosphate takes place from the ATP complex. The negative entropies of activation obtained in the presence of $Me_4[18]aneN_6$ and Me_2-pentaen could be ascribed to the fact that the rate-limiting step in the catalyzed dephosphorylation of ATP is a bimolecular reaction involving an addition process. On the other hand, the less unfavorable ΔS^* value obtained in presence of the unmethylated $[18]aneN_6$ could suggest a monomolecular elimination reaction as the rate-determining step. We are currently extending these studies to related receptors to gain further insight into these reactions.

Acknowledgment. We are indebted to the DGICYT (Grant PB90-0857) and IVEI for financial support as well as the Italian Ministero della Università e della Ricerca Scientifica e Tecnologica (quota 60%). We thank one of the reviewers for helpful comments on the kinetic discussion.

(31) Kinetic thermodynamic parameters have been calculated by means of the equations $E_a = \Delta H^* + RT$, $\Delta G^* = -RT \ln kh/Tk_b$, and $\Delta G^* = \Delta H^* - T\delta S^*$, k_b being the Boltzmann constant, h the Planck constant, and k the rate constant in s^{-1}. See for instance: Jencks, W. P. *Catalysis in Chemistry and Enzymology*; McGraw-Hill: New York, 1969.

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