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The formation of Proton and Alkali-Metal Complexes with Ligands of Biological Interest in Aqueous Solution. Potentiometric and Calorimetric Investigation of H^+, Na^+ and K^+ Complexes with Adenosine-5'-triphosphate

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ATP (adenosine-5'-triphosphate) takes part in many fundamental biochemical reactions. Besides, it is known that ATP in biological fluids is complexed with many metal ions. Therefore it is important to know the formation constants of these complexes. Sodium and potassium (the former in particular) are among the higher ionic constituents of all biological fluids [1], so it is necessary to know: i) if alkali-metal complexes of ATP are formed in aqueous solution and, if this occurs, ii) the values of formation constants as accurately as possible. Moreover, in order to have a full picture of the thermodynamic parameters for the above mentioned systems, it is advisable to determine the formation enthalpy values. The knowledge of the thermodynamic parameters for the protonation of ATP is preliminary to these studies.

Several values of ATP protonation constants are reported in the literature [2, 3], at different ionic strengths and temperatures, in different ionic media. It is also generally accepted that ATP does form complexes with alkali-metal ions. Till now no datum relative to formation enthalpy of ATP-Na^+ or K^+ complexes has been reported.

Experimental

Reagents and apparatus (for potentiometric and calorimetric measurements) were as previously reported [4, 5].

ATP, disodium salt, was from Merck; its inorganic phosphate content was 2% and the H_2O content about 6%. This was taken into account in the calculations.

Apparatus

The potentiometric measurements were carried out by means of two potentiometers (Radiometer PHM 52 and Orion 801 A) using glass electrodes (Ingold and Metrohm). The calorimetric measurements were carried out at 25 ± 0.001 °C employing a LKB precision calorimeter (model 8700) and a 100 cm^3 titration vessel (model 8726-1). The reproducibility of potentiometric apparatus was ±0.005 pH units. The Q-value reproducibility was ±0.015 cal.

Potentiometric titrations

20–25 cm^3 of solution containing ATP disodium salt 3–6 mmol dm^{-3} and sodium perchlorate, potassium nitrate and tetraethylammonium bromide ($C_{Na} + C_K + C_{Et_4N} = 0.1$ mol dm^{-3}), were titrated with standard CO_2-free KOH. The electrode system was standardized before and after each run by titrating a solution of HNO_3 (at the same ionic strength as the solution under study) with standard KOH.

Calorimetric titrations

80–90 cm^3 of solution containing ATP disodium salt and Na^+, K^+ and Et_4N^+ salts at the total ionic strength 0.1 mol dm^{-3} were titrated with standard KOH until complete deprotonation. Furthermore, solutions containing ATP^{4-}, at the same ionic strength as above, were titrated with standard $HClO_4$ until complete protonation.

Calculations

All the calculations were performed by using least squares computer programs: 1) ACBA (for protonation constants) (6); 2) MINIQUAD 76A (for stability constants in general) (7); 3) DOEC (for formation enthalpy) (8); 4) REGRE, a general regression analysis program (9).

Results and Discussion

Potentiometric data were analyzed firstly by using ACBA without allowing for alkali-metal–ATP interactions. The protonation constant values follow the order $Et_4N^+ \gg K^+ > Na^+$. Assuming that i) the tetraethylammonium cation does not form complexes with ATP and ii) the activity coefficients are the same* in all mixtures of $NaClO_4$, KNO_3 and Et_4NBr;

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*For footnote please see overleaf.

TABLE I. H^+, Na^+, and K^+–ATP Formation Constants at I = 0.1 mol dm^{-3} and t = 25 °C.

$\log K_1^H$		$\log K_2^H$	$\log K_{Na(ATP)}$	$\log K_{K(ATP)}$	
7.10(5)^a	$C_{Et_4N} = 0.1$	–	1.54(9)	1.41(9)	
6.45(4)	$C_{Na} = 0.1$				equations [1] and [2]
6.54(4)	$C_K = 0.1$				
–		4.01(5)	–	–	mean value from ACBA calculations
7.105(25)		4.05(4)	1.57(6)	1.42(6)	MINIQUAD 76A

$^a3\sigma$ in parenthesis.

TABLE II. Thermodynamic Parameters for ATP Protonation and Complexation with Sodium and Potassium at I = 0.1 mol dm^{-3} and t = 25 °C.

Equilibrium	$-\Delta G$ (Kcal mol^{-1})	ΔH (Kcal mol^{-1})	ΔS (cal mol^{-1} deg^{-1})
$H^+ + ATP^{4-} \rightleftharpoons [H(ATP)]^{3-}$	9.69(3)^a	−0.2(1)	31.8(4)
$2H^+ + ATP^{4-} \rightleftharpoons [H_2(ATP)]^{2-}$	15.25(5)	−3.8(2)	38(1)
$Na^+ + ATP^{4-} \rightleftharpoons [Na(ATP)]^{3-}$	2.14(8)	−0.2(4)	7(1)
$K^+ + ATP^{4-} \rightleftharpoons [K(ATP)]^{3-}$	1.94(8)	0.3(5)	8(2)

$^a3\sigma$ in parenthesis.

the noticeable difference in the $\log K_1^H$ values is ascribable to the formation of $[M(ATP)]^{3+}$ complexes. Being quite constant in all ionic media, the value of log K_2^H seem to indicate no formation of $[MH(ATP)]^{2-}$ complexes.

All the potentiometric data were also analyzed simultaneously a) using a linear regression program for the equation

$$\log K_1^H = 10 \cdot a_1 \cdot C_{Na} + 10 \cdot a_2 \cdot C_K + 10 \cdot a_3 \cdot C_{Et_4N} \qquad (1)$$

from whic it has been possible to obtain the coefficients a_1 = log K_1^H (C_{Na} = 0.1), a_2 = $\log K_1^H$ (C_K = 0.1) and a_3 = $\log K_1^H$ (C_{Et_4N} = 0.1); b) using the computer program MINIQUAD 76A for the simultaneous calculation of protonation constants and alkali-metal–ATP formation constants.

Approximate constants of the complexes $[M(ATP)]^{3-}$ have been obtained from the equation

$$K_{M(ATP)} = (10^{\delta \log K} - 1)/C_M \qquad (2)$$

(δlogK = difference between the protonation constants in Et_4N^+ and M^+) and have been used as input values for the calculation with MIQUAD 76A.

The results of this analysis are summarized in Table I.

The calculation of the calorimetric data were performed by DOEC program, obtaining simultaneously H^+ and M^+–ATP formation enthalpy. The thermodynamic parameters for ATP protonation and complexation with alkali-metal ions are reported in Table II.

Formation constants of the complexes $[M(ATP)]^{3-}$ show a high stability (in general alkali-metal complexes have stability constants lower than 10 mol^{-1} dm^3); for example at C_K = 0.1 mol dm^{-3}, C_{ATP} = 5 mmol dm^{-3} and pH = 7, 70% of ATP is present as $[K(ATP)]^{3-}$. Therefore, the importance of accounting for the formation of these complexes is confirmed**.

The values of formation constants for $[M(ATP)]^{3-}$ complexes agree with those previously reported, making allowance for the different experimental conditions [2, 3]. The presence of species $[MH(ATP)]^{2-}$ and $[M_2(ATP)]^{2-}$ has been hypothesized [2, 3, 13], but, at least up to C_M = 0.1 mol dm^{-3}, this hypothesis is to be excluded on the ground of our experimental data.

Alkali-metal complexes with ATP have low formation ΔH values and are stabilized mainly entropically,

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*It is known [10–12] that, when taking into account weak interactions, for I ≤ 0.1 mol dm^{-3} the activity coefficients in various media are not very different, while they are significantly different at I > 1 mol dm^{-3}.

**In blood plasma conditions (C_{Na} = 0.15 mol dm^{-3}) δ log K = 0.7. This difference, if not taken into account, can seriously alter the picture of ATP–(multi-metal) models.

as pointed out previously for similar complexes [4, 5]

The low ΔH value for the first protonation step of ATP and for the formation of alkali-metal complexes was confirmed by potentiometric measurements at $t \neq 25$ °C (for example, at 10 °C the formation constants for $[H(ATP)]^{3-}$ and $[M(ATP)]^{3-}$ differ from those obtained at 25 °C by less than 0.1 log units)

From calorimetric and potentiometric measurements, including those at $t \neq 25$ °C, it was possible to obtain the general equation for the first protonation step of ATP

$$\log K_1^H = 7.10 - 0.15\frac{\Delta T}{T} + 0.033\frac{(\Delta T)^2}{T} - \log(1 + 37\,C_{Na}) - \log(1 + 26\,C_K)$$

where $\Delta T = T - 298.16$ (T, K), in the range $10 \leqslant t \leqslant 40$ °C, at $I = 0.1$ mol dm^{-3}

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