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Chemical speciation of nucleotide 5′-monophosphates in the presence of biogenic amines

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ABSTRACT

The interaction of adenosine-, uridine-, inosine- and guanosine-5′-monophosphates with protonated ethylenediamine, putrescine, cadaverine, spermidine and spermine, was studied potentiometrically, at $t = 25°C$. The species ALH_2^0 are formed with diamines, ALH_2^0, ALH_3^+ with triamine, and, ALH_2^0, ALH_3^+ and ALH_4^{2+} with tetramine. The stability of these species is strongly dependent on the product charge, $\zeta = |z_{anion} \cdot z_{cation}|$: the ratio $\log K_r/\zeta$ is fairly constant, and, for the different systems, varies from 0.53 to 0.59. This behaviour is very similar to that shown by the analogous species of ATP ($\log K_r/\zeta = 0.52$), already studied in these laboratories. Two other factors are taken into account for the analysis of stability data, *i.e.*, the length of alkyl chain(s) in the amine, and the difference between total and protonated amino groups. These factors are related to steric arrangement and to the possibility of hydrogen bonding contribution. The relevance of these complexes in the chemical speciation of biological fluids is discussed.

Keywords: nucleotide 5′-monophosphates, biogenic polyamines, nucleotide 5′-monophosphate-polyammonium cation complexes, speciation

INTRODUCTION

Naturally occurring polyamines (such as putrescine, cadaverine, spermidine and spermine), also called biogenic amines, are a group of low molecular weight aliphatic polycations (at physiological pH values) containing amine functions separated by three or four methylene groups. They can be found in all living organisms, such as animal cells (total intracellular concentration range is 2–3 mmol L^{-1}). Biogenic amines play a fundamental role in many biological processes such as cell proliferation and differentiation, protein syntheses, aggregation, structural integrity, function of ribosomal subunits, DNA replication, membrane stabilization, and in the activity of several enzymes. It is believed that the polyamines mediate biological processes involving nucleic acid by association in a way that induces specific conformational changes. It is well known that they stabilize DNA against thermal and alkaline denaturation, enzymatic degradation, radiation damage and the intercalation of aromatic dyes. Many of the cellular effects of these polyamines (*e.g.*, spermine and spermidine) have been attributed to their ability to interact with the phosphate backbone of RNA and DNA and also with inositol-phosphatase. It has been suspected that polyamine molecules may interact with parts of the nucleic molecule other than the phosphate

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oxygen, as the π electrons of the bases and the lone pairs on nitrogen and oxygen. These interactions might stabilize more ordered conformations (Bolton and Kearns, 1977; Esposito *et al*., 1997; Heerschap, *et al*., 1985; Mernissi-Arifi *et al*., 1996; Tabor, 1962).

A great number of studies have been carried out on the formation of mixed complexes of AMP, ADP and ATP with other ligands (generally amines and aminoacids) and divalent metal ions (M = Cu^{2+}, Zn^{2+}) (Andrés *et al*., 1993; Antonelli *et al*., 1989; Bunce and Kong, 1978; Dietrich *et al*., 1981; Hosseini *et al.,* 1987; Kimura *et al*., 1982; Kimura *et al.*, 1990; Smith *et al.,* 1991). Polyammonium cations can replace the metal bound to ATP, and this kind of reaction might be of considerable interest in the speciation of biological fluids. However, often the possibility of interaction between the two ligands without the metal ion has been neglected. It has been reported that macrocyclic polyamines influence ATP dephosphorylation reactions and biogenic amines affect the coordination of Mg^{2+} to the nucleotides (Voige and Elliott, 1982).

Biogenic amines are largely protonated under physiological conditions and, on the other hand, nucleotides are polyanions at the same pH, with the phospate group largely ionised. In these conditions they interact electrostatically, in such a way that the polyanion is stabilised by positively charged polyamine. Labadi *et al*. have demonstrated the interaction between pyrophosphate ion and various polyamines using calorimetric and potentiometric techniques (Labadi *et al.*, 1991).

In consideration of the great importance that amines have in biological processes, for many years our research group has been involved in a series of thermodynamic studies (by determining potentiometrically and calorimetrically ΔG^0, ΔH^0 and $T\Delta S^0$) on the interaction between open chain polyammonium cations with inorganic (Cl^-, SO_4^{2-}, HPO_4^{2-}, $P_2O_7^{4-}$, $P_3O_{10}^{5-}$) (Daniele *et al.*, 1997b; De Robertis *et al*., 1999) and organic ligands (acetate, malate, malonate, oxalate, tartrate, citrate, tricarballylate, butantetracarboxylate) (Daniele *et al.*, 2002; De Robertis *et al*., 2001) and *ATP* (De Stefano *et al*., 1996; De Stefano *et al*., 1998), as a contribution to speciation studies of biofluids. Some of these investigations have been specifically dedicated to the complexing ability of biogenic amines with some ligands of biological interest (De Robertis *et al*., 2001, and references therein) and one of our projects has examined the possibility of finding appreciable interactions in urine between these amines and oxalate as a further step in elucidating part of apparent urine supersaturation (De Stefano *et al.*, 1999). An interesting feature of all the polyammonium cation–polyanion systems is that the formation thermodynamic parameters are strictly dependent on charges, and simple ΔG^0 and $T\Delta S^0$-charge relationships can be written fairly independently of the type of amine or anion considered. The experience obtained in this field allows us to extend our studies to the interaction of amines with important nucleotide anions for potential applications as a model for natural processes involving phosphate groups.

This paper includes quantitative data relative to the amine (ethylenediamine, putrescine, cadaverine, spermidine and spermine) – nucleotide ligand systems (adenosine 5′-monophosphate, inosine 5′-monophosphate, guanosine 5′-monophosphate, uridine 5′-monophosphate) in aqueous solution, at t = 25°C, using the potentiometric method (H^+-glass electrode). Although ethylenediamine is not a biogenic amine, it has been also considered for comparison with the two biogenic diamines (putrescine and cadaverine).

MATERIALS AND METHODS

Reagents

Solutions of amines [ethylenediamine (en), 1,4-diaminobutane or putrescine (ptr), 1,5-diaminopentane or cadaverine (cdv), N-(3-aminopropyl)-1,4-diaminobutane or spermidine (spd) and N,N′-bis(3-aminopropyl)-1,4-diaminobutane or spermine (sper)] were prepared from corresponding Fluka products. The amines were purified by trasformation into the corresponding hydrochlorides. The purity of nucleotides investigated [adenosine 5′-monophosphate (AMP), inosine 5′-monophosphate (IMP), guanosine 5′-monophosphate (GMP), uridine 5′-monophosphate (UMP)], all in the form of disodium salt, by Acros Organic) ranged from 98 to 99%. The purity value of each nucleotide was taken into account in the calculations. Hydrochloric acid and sodium hydroxide solutions were standardised against sodium carbonate and potassium biphthalate, respectively. All the solutions were prepared using water of analytical grade (R = 18 MΩ cm^{-1}), and grade A glassware was always employed.

Apparatus and procedure

The measurements were carried out by means a potentiometric apparatus consisting of an automatic titrant dispenser Metrohm mod. 665 coupled with a potentiometer Crison mod. MicropH 2002 and by using a combination Orion-Ross 8172 glass-electrode. The estimated accuracy of this system was ± 0.15 mV and ± 0.003 cm^3 for e.m.f. and titrant volume reading, respectively. Pure nitrogen was bubbled in a titration cell in order to avoid O_2 and CO_2 inside, and the solutions were magnetically stirred. A volume of 20–25 mL of solution containing the amine hydrochloride (2–16 mmol L^{-1} for diamines, 3–10 mmol L^{-1} for triamine and tetramine), hydrochloric acid (3–17 mmol L^{-1}) and nucleotides (1–10 mmol L^{-1}) was titrated with sodium hydroxide solutions in the pH range 5–10. No background salt was added in these solutions, to avoid interferences of alkali metal cation with nucleotides, and of anion (Cl^-) with

amines. To determine the electrode formal potential (E°), independent titration of HCl solution (10 mmol L^{-1}), by using NaCl 0.01 mol L^{-1} as background salt, with NaOH standard solution as titrant was performed, in the same experimental conditions of temperature as the systems under study.

Calculations

All the parameters relative to electrode system (formal potential E^0, coefficient of junction potential j_a, $E_j = j_a [H^+]$) and to alkalimetric purity determination were refined using the nonlinear least squares computer program ESAB2M. Formation constants were refined using the nonlinear least squares computer programs STACO and BSTAC. Speciation profiles were obtained by the computer program ES4EC. Details on the calculation methods and programs have already been reported (De Stefano *et al*., 1997).

No background salt was added to the solutions under study in order to avoid interference. In fact, amines form weak complexes with anions (such as Cl^-, NO_3^- or ClO_4^-) (De Robertis *et al*., 2001) and phosphates interact significantly with alkali metal cations (Daniele *et al*., 1994). The interaction with small amounts of Cl^- and Na^+, coming from amine hydrochlorides and titrant NaOH, respectively, were taken into account in the calculations. The formation constants were corrected to zero ionic strength as already reported (Daniele *et al*., 1997a). Both computer programs BSTAC and STACO can deal with potentiometric data obtained in variable ionic strength conditions and perform corrections to $I = 0$ mol L^{-1}. All the concentration and complex formation data are given in the molar (mol L^{-1}) scale. Uncertainties in the different parameters are reported as ≥95% confidence interval.

RESULTS AND DISCUSSION

Stability of polyanion-polycation species – thermodynamic data

Equilibrium constants for the formation of nucleotide phosphate–polyammonium complexes refer to the reaction

$$H_rA^{r+} + L^{z-} = ALH_r^{(r-z)+} \qquad K_r$$

The values of nucleotide protonation constants were taken from the critical review of Smith *et al.* (1991): at $t = 25$°C and $I = 0$ mol L^{-1}, they report $\log K_1^H = 6.67$, 6.66, 6.66 and 6.63 for AMP, GMP, IMP and UMP, respectively. Moreover, monophosphates form weak complexes with Na^+, and the mean value is $\log K = 0.88$ ($t = 25$°C, $I = 0.1$ mol L^{-1} Me_4NCl) was used for all the phosphate ligands here considered. The error on these parameters (±0.02 for $\log K^H$, ±0.1 for the formation constant of Na^+ weak complexes) does not affect significantly the formation parameters of XMP-amine complexes. Protonation constants and weak Cl^- complex formation constants of amines were reported in a previous works (De Robertis *et al*., 2001).

The first macroscopic observation on the data in Table 1 relates to the effect of charge on the stability of XMP-polyammonium cation complexes: in fact the ratio $\log K_r/\zeta$ (where ζ is the charges product $|z_{cation} \cdot z_{anion}|$) is fairly constant:

$$\log K_r/\zeta = a \qquad (1)$$

$a = 0.55 \pm 0.04$ AMP
$a = 0.53 \pm 0.06$ UMP
$a = 0.59 \pm 0.07$ IMP
$a = 0.54 \pm 0.05$ GMP

Table 1 Formation constants[a] for the AMP-, UMP-, IMP-, GMP-amine complexes at $t = 25$°C and I = 0 mol L^{-1}

	r	$\log K_r \pm \epsilon$[b]	$\log K_r/\zeta$
AMP			
en	2	2.61 ± 0.03	0.7
cdv	2	2.22 ± 0.03	0.6
ptr	2	2.24 ± 0.03	0.6
spd	2	2.52 ± 0.08	0.6
	3	3.04 ± 0.05	0.5
sper	2	2.63 ± 0.04	0.7
	3	3.44 ± 0.03	0.6
	4	4.09 ± 0.03	0.5
UMP			
en	2	2.64 ± 0.03	0.7
cdv	2	2.04 ± 0.10	0.5
ptr	2	2.10 ± 0.03	0.5
spd	2	3.13 ± 0.08	0.8
	3	3.17 ± 0.05	0.5
sper	2	2.67 ± 0.05	0.7
	3	3.19 ± 0.08	0.5
	4	3.76 ± 0.04	0.5
IMP			
en	2	2.73 ± 0.03	0.7
cdv	2	2.31 ± 0.05	0.6
ptr	2	2.30 ± 0.05	0.6
spd	2	3.54 ± 0.05	0.9
	3	3.51 ± 0.03	0.6
sper	2	3.19 ± 0.10	0.8
	3	3.21 ± 0.12	0.5
	4	4.09 ± 0.08	0.5
GMP			
en	2	2.65 ± 0.03	0.7
cdv	2	1.95 ± 0.03	0.5
ptr	2	1.61 ± 0.12	0.4
spd	2	3.09 ± 0.12	0.8
	3	3.24 ± 0.03	0.5
sper	2	2.70 ± 0.03	0.7
	3	3.01 ± 0.05	0.5
	4	4.04 ± 0.04	0.5

[a]referred to the reaction $H_rA^{r+} + L^{2-} = ALH_r^{(r-2)+}$
[b]ϵ = 95% CI

indicating a strong contribution of electrostatic interaction to the stability of these complexes. Values of $\log K_r/\zeta$ can be compared with those obtained for the analogous complexes of ATP (De Stefano *et al*., 1996) (reported in Table 2)

$$a = 0.52 \pm 0.03$$

Both differences among monophosphates and between monophosphates and ATP are not statistically significant. By considering monophosphates and ATP altogether we have

$$a = 0.54 \pm 0.03$$

(standard deviation on the fit, $\sigma = 0.37$) and, using the crude approximation $\zeta = 2\underline{n}$ ($\underline{n}$ = number of possible salt bridges):

$$-\Delta G^0 = 6.2 \pm 0.2 \text{ kJ mol}^{-1}\ \underline{n}^{-1} \qquad (1a)$$

that represents an average free energy contribution per bond.

However, the fairly large deviations on the fit (σ_{fit} = 0.28, 0.47, 0.59, 0.48 and 0.44, for AMP, UMP, IMP, GMP and ATP, respectively), clearly indicate that other factors influencing the stability must be taken into account. A more detailed inspection of formation constants in Tables 1 and 2 leads to the following observations.

(a) The stability of XMP and ATP–polyammonium cation complexes is a function of the length of alkyl chain(s).
(b) The stability of $A(XMP)H_r^{(r-2)}$ species is also a function of the number of amino groups in the amine.

The first point can be related to steric factors, as pointed out in other investigations dealing with similar system (De Robertis *et al*., 1996). The second factor may account for the possibility of hydrogen bonding between unprotonated amino groups and phosphate group, $-NH \cdots {}^{-}O-$. If we consider the ratio $R_{N/C}$ = (number of *N* atoms) / (number of *C* atoms) in the amine (en = 1; ptr = 0.5; cdv =0.4; spd = 0.43; sper = 0.4) and the difference (*d*) between total and protonated amino groups, we found (after some trials) the new linear empirical equation

$$\log K_r = a_0\zeta + a_1 R_{N/C} + a_2\, d \qquad (2)$$

whose parameters, calculated by fitting all the $\log K_r$ data, are

$$a_0 = 0.47 \pm 0.02$$
$$a_1 = 0.82 \pm 0.16$$
$$a_2 = 0.23 \pm 0.07$$

The standard deviation on the fit $\sigma = 0.25$ is significantly lower than that obtained by fitting equation (1). Parameters of equation (2) can be interpreted as follows. The first one, a_0, has the same meaning as the term a in equation (1), *i.e.*

$$-\Delta G^0_{el} = 5.4 \pm 0.2 \text{ kJ mol}^{-1}\ \underline{n}^{-1} \qquad (2a)$$

represents the average free energy contribution for a single salt bridge, when amino groups are fully protonated and are separated by very long alkyl chains ($R_{N/C} \rightarrow \infty$). The parameter a_1 represents the contribution due to the formation of stable rings, with a maximum reached for $R_{N/C} = 1$. The last contribution to the stability, represented by a_2, can be related to the formation of hydrogen bonds, with an average free energy

$$-\Delta G^0_{hb} = 1.3 \pm 0.4 \text{ kJ mol}^{-1}\ d^{-1} \qquad (2b)$$

Though the above separated contributions are affected by large uncertainties, they are very representative of

Table 2 Formation constants for the reaction for the ATP-amine complexes at t = 25°C and I = 0 mol L^{-1}

	r	$\log K_r$	$\log K_r/\zeta$
en	2	5.0	0.6
cdv	2	3.9	0.5
ptr	2	4.3	0.5
sprd	2	3.8	0.5
	3	5.7	0.5
sper	2	4.3	0.5
	3	6.2	0.5
	4	8.1	0.5

[a]referred to the reaction $H_rA^{r+} + L^{4-} = ALH_r^{(r-4)+}$

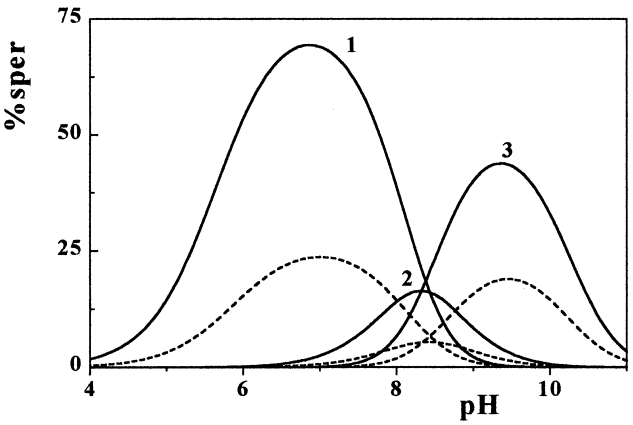

Figure 1 *Distribution of the species vs. pH in the system H^+-sper-UMP. C^0_{sper} = 0.005 mol L^{-1}; C^0_{UMP} = 0.01 mol L^{-1}. solid line: I = 0 in NaCl; dashed line I= 0.15 mol L^{-1} in NaCl. 1 = Sper(UMP)H_4^{2+}; 2 = Sper(UMP)H_3^+; 3 = Sper(UMP)H_2^0.*

Table 3 Formation percentages of the species UMP-, AMP-, GMP-, IMP-amine-H^+ complexes at different pH values and t = 25°C

			pH = 6		pH = 7.5		pH = 9	
L	A	r	I = 0	I = 0.15	I = 0	I = 0.15	I = 0	I = 0.15
UMP	en	2	27.2	13.4	23.1	10.0	1.3	0.5
	ptr	2	9.9	4.1	23.2	8.0	17.4	6.2
	cdv	2	11.2	4.6	25.8	9.2	24.0	8.5
	spd	2	0.1	0.1	5.2	1.9	36.0	15.1
		3	34.3	12.1	52.3	19.8	11.4	5.0
Σ %[a]			34.4	12.2	57.5	21.7	47.4	20.1
	sper	2	0	0	1.1	0.3	40.2	15.9
		3	0.2	0.1	7.0	2.0	8.4	3.5
		4	53.7	15.4	60.5	21.5	2.2	1.2
Σ % [a]			53.9	15.5	68.6	23.8	50.8	20.6
AMP	en	2	24.8	12.1	21.9	9.4	1.2	0.5
	ptr	2	13.9	5.9	31.7	11.9	24.6	9.4
	cdv	2	13.4	5.7	31.0	11.6	29.1	10.9
	spd	2	0.1	0	5.2	1.9	36.0	0.4
		3	32.9	11.6	52.2	19.8	11.4	13.1
Σ % [a]			33.0	11.6	57.4	21.7	47.4	13.5
	sper	2	0	0	0.5	0.2	25.7	11.9
		3	0.7	0.2	20.7	8.3	34.9	16.8
		4	69.8	25.9	63.5	32.2	3.3	2.0
Σ % [a]			70.5	26.1	84.7	40.7	63.9	30.7
IMP	en	2	30.3	15.3	26.6	11.9	1.6	0.6
	ptr	2	16.1	7.0	35.1	13.6	27.6	10.8
	cdv	2	15.8	6.8	34.9	13.5	32.8	12.7
	spd	2	0.7	0.3	21.7	11.8	80.2	57.1
		3	58.3	26.5	61.3	34.6	7.2	5.3
Σ % [a]			59.0	26.8	83.0	46.4	87.4	62.4
	sper	2	0	0	1.9	0.7	60.7	33.4
		3	0.4	0.1	13.1	5.1	13.3	7.6
		4	70.4	26.1	68.4	33.4	2.1	1.6
Σ % [a]			70.8	26.2	83.4	39.2	76.1	42.6
GMP	en	2	26.8	13.2	23.4	10.2	1.3	0.5
	ptr	2	18.9	8.4	39.7	16.1	31.6	12.8
	cdv	2	12.1	5.1	28.3	10.3	26.5	9.6
	spd	2	0.3	0.1	13.3	5.8	64.1	36.3
		3	43.7	17.0	57.1	25.9	8.7	5.1
Σ % [a]			44.0	17.1	70.4	31.7	72.8	41.4
	sper	2	0	0	0.7	0.2	37.1	15.7
		3	0.3	0.1	9.5	3.5	15.9	7.0
		4	68.0	24.2	70.4	32.1	3.7	2.0
Σ % [a]			68.3	24.3	80.6	35.8	56.7	24.7

[a] For *spd*: % *spd*(XMP)H_2^0 + % *spd*(XMP)H_3^+; For *sper*: % *sper*(XMP)H_2^0 + % *sper*(XMP)H_3^+ + % *sper*(XMP)H_4^{2+}.

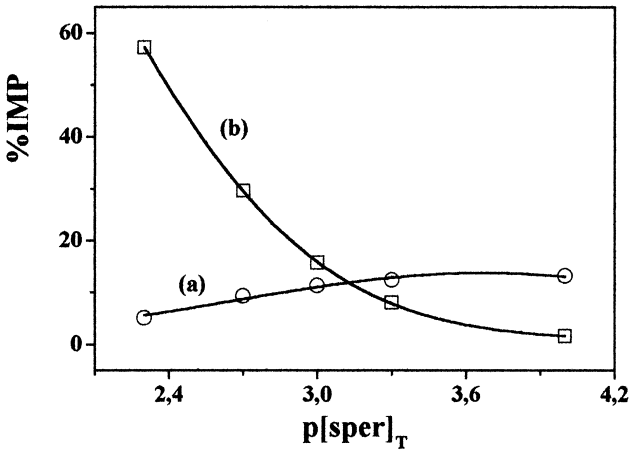

Figure 2 *Competition between Mg^{2+}-IMP(a) and spermine cation -IMP-polyammonium (b) species, at t = 25°C. C_{IMP} = 5 mmol L^{-1}; C_{Mg} = 5 mmol L^{-1}.*

the stability picture of nucleotide–polyammonium cation complexes.

Speciation of nucleotide–biogenic amines aqueous systems

In all the systems studied in this work the formation of polyammonium cation–XMP polyanion is quite significant since high yields are observed in the pH range of interest for biochemical reactions. In Figure 1 we report the speciation profile for the system H^+-spermine–UMP: in pure water solution we have formation percentages >60, whilst in 0.15 (NaCl) mol L^{-1} aqueous solution the maximum formation percentage is ≈20%. This lowering is due to the ion pair formation Na^+–UMP^{2-} (Smith *et al.*, 1991) and, mainly, to the formation of different chloride (*sper*)$H_rCl^{(r-1)+}$ species (De Robertis *et al.*, 2001). The same trend is followed by all the other systems. To give a complete picture of these systems we report in Table 3 for comparison the formation percentages at I = 0 and 0.15 mol L^{-1} (NaCl) and at pH = 6, 7.5 and 9.

It has been suggested that polyamines can be competitive with respect metal ions in the complex formation with nucleotide mono, di and triphosphates. For example when considering Mg^{2+}, we have the formation of a $Mg(XMP)^0$ complex species whose formation constant is $\log K_r$ = 2.00 ± 0.05 for all the monophosphates (Smith *et al.* , 1991). In the presence of polyamines, Mg^{2+} is substituted in the complex species by the polyammonium cation. As an example, in Figure 2 we plotted %IMP complexed (a) with magnesium(II) and (b) with polyammonium cation(s), vs $-\log C_{cation} = p[sper]_T$. Owing to the higher formation constants, polyammonium cations are strongly competitive in the binding to the nucleotide phosphates; in the reported example the competition is in favour of protonated spermine for quite low amine concentration, being the intersection of complex percentage curves at $C_{sper} = 10^{-3.2}$ mol L^{-1}. Stability data reported in this work can be used to obtain speciation profiles for several polyammonium cation–nucleotide systems, and the predictive relationships (1) and, in particular, (2) allow to extend our knowledge to several other similar systems.

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