Interaction of polyamines, their protonated salts and metal complexes with nucleic acid fragments

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Abstract - The paper describes our attempt to prove earlier conclusions on the
conformation and inter-ionic interactions of protonated Put, Spd and Spm
cations, based initially on the X-ray and IR data of only two types of salts:
hydrochlorides and hydrated monohydrogen phosphates. These two sets of salts
composed at the beginning only of native polyamines (Put^4, Spd$^{3.4}$ and Spm$^{3.4.3}$)
were later on extended by their homologs (Put^2, Spd$^{3.3}$ and Spm$^{3.3.3}$), and
further also by nitrates of Put and Spm. The number of water molecules within
homologous sets of crystalline monohydrogen phosphate salts, meets our pre-
dictions and depends on geometry and distribution of acceptor centers within
dianions of monohydrogen phosphates. Underway are microcalorimetric measure-
ments which should help us to understand the role of water molecules within
specific networks of hydrogen bonds in single crystals of hydrated phosphates.

Nitrate anions having planar structure and being stabilized by charge delocaliza-
tion, interact in a completely different manner with protonated Put and Spm
cations, than the tetrahydrally oriented acceptor centers of chloride and
phosphate anions. As a consequence, interesting similarities observed in
proton-donor activities of N$^+$H$_3$ group within pairs of hydrochlorides and
phosphates do not exist any more in nitrate within the same PA cations.

The last mentioned differences could be observed by measurements of melting
midpoint temperature (Tm) of standard 5S rRNA solution after addition of
equimilimolar amounts of hydrochloride or nitrate salt of Spm. The latter
observation seems to indicate that counteranions could modulate within
quite a wide range and in a special manner the interaction of protonated PA
cations with different kinds of nucleic acids. The comparisons of the inter-
actions of protonated and fully N-methylated sperminium cations (Spm4H^{4+} and
Me$_{10}$Spm^{4+}) with 5S rRNA seems to indicate that purely electrostatic attrac-
tion which is very often assumed as the main binding force between PAs and
nucleic acids, in fact plays a much less significant role than to that of
the networks of specific hydrogen bonds.

I. INTRODUCTORY REMARKS ON NATURALLY OCCURRING POLYAMINES AND NATURAL PRODUCT CHEMISTRY

Today, it is generally accepted that aliphatic biogenic amines, especially putrescine
(Put), spermine (Spm) and spermidine (Spd) count among the most important natural products
present in all living organisms. Here one may ask why this class of compounds has not so far
been the subject of a plenary lecture at our symposia on natural product chemistry organized
every second year since 1960. It is difficult to answer this simple question and although I
am the one who has posed it, I am not sure I can answer it in a convincing way. Not giving
the matter much thought, one could say not without reason, that there are probably other
classes of natural products which deserve this distinction more. But is this the whole truth?
Certainly not. I think that a majority of organic and bioorganic chemists, as well as bio-
chemists, ignore or underestimate this class of compounds, like I ignored them untill
recently, because of their exceptionally simple molecular structure consisting of nitrogen-
carbon chains without chiral centers or branches, and likewise simple chemistry leading to
easy and efficient total syntheses. At first sight, it seems that synthetic and physical
organic chemists have already studied all the interesting and important aspects of biogenic
polyamines. In fact, the accumulation of knowledge on the chemistry and structure of poly-
amines, especially rapid in the last 20 years, has provided incentive for the biochemical,

physiological and genetic studies of their biological role and their applications in medicine, agriculture and numerous branches of biotechnology.

At this point let us recall some statements from the recently published reviews on that subject by Tabor and Tabor (ref. 1) and Goldstone *et al.* (ref. 2). Interest has been increasing during the last twenty years in the naturally occurring polyamines (PA). Their specific function is still obscure, but their ubiquitous distribution, their high concentration in cells, and the increase in their concentrations found in rapidly growing tissues have stimulated many investigations on these compounds. The biosynthetic pathways for Put, Spd and Spm are well established. The enzymes involved in the specific steps have been purified and characterized from a variety of sources. Three of these enzymes are of particular interest, namely: ornithine and arginine decarboxylase and adenosyl-methionine-decarboxylase. The short turnover times of these enzymes presumably reflect the physiological importance of PAs and indicate the cells' need for rapid responses in PAs levels to a variety of stimuli (ref. 1). The most important recent developments in the PA area are the preparations of a number of mutants that have defects in the biosynthetic pathway for PA, as well as the availability of specific irreversible mechanism-based inhibitors of ornithine and arginine decarboxylases. Both the mutants and the inhibitor treated cells are being used to study the effects of PA depletion *in vivo*, demonstrating that PA are required for growth and their lack stimulates the entry of the cell to a senescent state culminating in lysis (ref. 1). PAs are thought to function in the regulation of nucleic acid structure at several levels of organization. Spm-DNA complexes have a regular conformation which stabilizes the nucleic acid molecule against thermal denaturation *in vitro*. Depletion of intracellular PAs likewise promotes alteration of DNA conformation, which could be important in controlling nucleosome assembly and gene expression (ref. 2). Sakai and Cohen (ref. 3) have summarized data which suggest that PAs modify the conformation and reactivity of bacterial and yeast tRNAs and Loftfield *et al.* (ref. 4) have recently demonstrated that both the role and precision of aminoacylation of tRNA molecules is enhanced by mM concentrations of Spm. PA bound to plant tRNA and rRNA are assumed to have equivalent functions *in vivo* (ref. 2).

The most pressing questions in the PA field are the following: what is the physiological role of the PA and what is the molecular mechanism of their action? The most obvious specific characteristic of the PAs is their polybasic character, which gives them a much higher affinity for acidic constituents than that exhibited by Na^+, K^+, Mg^{2+} or Ca^{2+} (ref. 1). Many of the biological functions of PAs appear to be attributable to the cationic nature of these basic molecules, and as a consequence of the fact that they are strongly protonated at physiological pH their electrostatic interactions with polyanionic nucleic acids and negatively charged functional groups of membranes and enzymatic or structural proteins in the cell (ref. 2). It is generally assumed that these interactions are responsible for the regulation of various cellular processes but still in any given experiment, especially in studies *in vitro*, it is very difficult to know whether the effects observed are of physiological relevance, or whether they are artifacts resulting from non-specific polyamine-polyacid interactions (ref. 1). In biochemical and physiological considerations of the biological role and function of PAs, very often the terms "bind" and "binding" are used, which in my opinion are meaningless from the structural and mechanistic point of view. Mostly it is assumed that the nature of the binding of PA to the negatively charged groups in nucleic acid, membrane phospholipids and some proteins is exclusively electrostatic, but if so, there is no reasonable explanation for the high specifity of some of these interactions.

It appears that to solve the above problems we should use a new approach, a new methodology in natural product chemistry and molecular biology. We should find a method for combining simple and even trivial structures of some ubiquitous biomolecules possessing a great variety of their specific interactions with different and important biopolymers.

Till now, in natural product chemistry we have concentrated our efforts, new experimental techniques, our inventivity and imagination on the elucidation of each sophisiticated structures of important biomolecules and then relate the solved structure to its mostly unique biological function. The studies on the structure and synthesis of chlorophyll, vitamin B12, cytochrome, insuline, bacteriorhodopsin, neurotoxin, tRNA molecules are only a few examples illustrating this approach which is still most valuable and fundamental, and gives new information on the structure and function of unknown links in different metabolic pathways of living organisms.

The best known example of a new approach which slowly emerges from the present investigations is just in the field of biogenic amines. We should try to look at the molecular structures of these simple compounds through their different kinds of intermolecular interactions, and since at physiological pH they are almost completely protonated, we should first of all learn about the preferred conformations of the fully protonated cations of Put ($Put2H^{2+}$), Spd ($Spd3H^{3+}$) and Spm ($Spm3H^{4+}$), and about the degree and manner in which they can be changed by specific interactions with different counter anions. In other words, if we want to understand the molecular mechanism of varieties of specific interactions of simple protonated PAs, we should try to find their specific intrinsic properties through their interactions with different counter anions and ubiquitous water molecules. The simplest way to do this is in

my opinion to solve the molecular and crystal structures of a number of PA salts with the same fully protonated cations and different counter anions.

In the case of biomolecules with exotic, very complex and partly rigid architecture, it is mostly sufficient to solve by X-ray the structure of one crystalline form of given compounds, and very often not caring whether it is a pure compound or its salt or even a derivative. In the case of PA we should build our structural knowledge on the structure of many single crystals of the same PA in which special attention is paid to the degree of protonation and to the information which can be gathered not only from the molecular but also crystal structure, since in the latter data all types of intermolecular interactions which express the personality of a given PA are concealed.

II. OUR OWN INTEREST IN POLYAMINES FIELD

My entry into the field of PA was not accidental. Having been deeply engaged for some time in the study of the structure-function relation of tRNA, it was obvious to me that I ought to make my own observations on the influence of organic and metallic cations on the dynamic conformation of tRNA molecules. It was also obvious to me that the term "organic cations" should mainly denote protonated PA, but I did not intend to start our own systematic structural study of PA at the level of their simple salts with simple inorganic acids. However, this has not been and will not be our main stream of research. Consequently, progress in the study has been rather slow, but nevertheless, we are satisfied that our own collection of crystalline PA salts continues to grow, and on the basis of this we have been able to solve the structure of two more Put and one more Spm salt, prepared a huge set of IR spectra, and what is most important, we are able to carry on current comparative structural study of PAs taking into account all the published and our own X-ray data.

III. OUR EARLIER OBSERVATIONS

Three papers which we published under the same running title (ref. 5, 6, 7): "Comparative structural analysis of selected salts of PAs in the aspects of their interactions with nucleic acids" were based on X-ray data of PA salts, mostly solved by other laboratories (ref. 8a-f) and on our IR spectral data of the same crystalline preparations which were used for X-ray analysis. For a better characterization of each PA salt we have used additionally the IR spectra of O, N deuterated preparations, since besides the conformation of protonated PA cations we are also interested in the type of intermolecular interactions, which are well visible and could be mutually compared in the IR spectra of deuterated PA salts.

We base our comparative structural analysis on a quantitative evaluation of the networks of hydrogen bonds (HBs) existing in single crystals of a given PA salt, using the ΔHB parameters, and the enthalpy difference values $-\Delta H°$. The parameters: ΔHB and $-\Delta H°$, were determined according to (ref. 5, 9) in the following way:

$\Delta HB = rDo + rAc - da \times 10^3$

where: rDo and rAc = van der Waals radii of proton-donor and acceptor atoms; $rO = 1.45$, $rN = 1.55$, $rCl = 1.86$ Å; da = Do......Ac distance in Å (from X-ray data)

$-\Delta H°$ is derived from ΔHB. Assuming that the enthalpy differences $(-\Delta H°)$ between hydrogen bonded and unbonded Do and Ac atoms, oscillate between 0-25 Kcal/mol, a tentative correlation between ΔHB values (0-500 units) and $-\Delta H°_{HB}$ values (0-25.5 Kcal/mol) has been attempted (ref. 5, 9 and Table below).

ΔHB	00	50	100	150	200	250	300	350	400	Å 10^{-3}
$-\Delta H°_{HB}$	0.0	0.75	1.5	2.25	3.0	4.5	6.0	7.5	9.0	Kcal mol^{-1}

ΔHB	450	460	470	480	490	500	Å 10^{-3}
$-\Delta H°_{HB}$	10.5	13.5	16.5	19.5	22.5	25.5	Kcal mol^{-1}

This approach enables us to characterize (in Kcal/mol units) the activity of each proton-donor (Do) and proton-acceptor (Ac) group engaged in one particular HB and/or in a set of HBs. In the first paper (ref. 5) we gave detailed characteristics of Do and Ac groups present in single crystals of the four following salts of Spd and Spm:

$Spm4H^{4+}.4C\bar{l}$ $Spm4H^{4+}.2(HPO_4^{2-}).6H_2O$

$Spd3H^{3+}.3C\bar{l}$ $2(Spd3H^{3+}).3(HPO_4^{2-}).6H_2O$
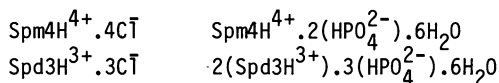
In our next paper (ref. 7) we enlarged the above set by two analog salts of Put:

$$Put2H^{2+}.2C\bar{l} \qquad Put2H^{2+}.HPO_4^{2-}.2H_2O$$

and having in hand a full set of anhydrous hydrochlorides and hydrated phosphates of Put, Spd and Spm we were able to compare among them the following items:

1. conformation of fully protonated $Put2H^{2+}$, $Spd3H^{3+}$ and $Spm4H^{4+}$ cations in both types of salts,
2. proton-donor activities of $-N^+H_3$ (within a single cation and a whole set) and N^+H_2 groups (within $Spd3H^{3+}$ and $Spm4H^{4+}$ cations in both salts),
3. proton-acceptor activities of chloride ($C\bar{l}$) and hydrogen phosphate (HPO_4^{2-}) anions within the same and different counter cations,
4. Do and Ac activities of water molecules,
5. summaric - H° values for a whole set of HBs operating within given single PA crystals.

A very detailed discussion of the collected data is given in published papers (ref. 5-7). Now, I will try to indicate only the most important observations most of which presumably have a general significance.

1. All "trans" (maximum stretched) is the preferred conformation of fully protonated PA cations, since only one cation ($Spm4H^{4+}$) adopts $tttg^+tttg^-ttt$ conformation (9xt + 2xg) in the presence of $C\bar{l}$ counter anions (ref. 5-7).
2. All N^+-H groups (6 - in $Put2H^{2+}$, 8 - in $Spd3H^{3+}$, 10 - in $Spm4H^{4+}$ cations) are involved in hydrogen bonding system with the accepting center of counter anions.
3. Summaric Do activities of N^+H_3 within salt-pairs of Put, Spd and Spm (see Table 3 in ref. 7) are very similar, indicating that these values are related to intrinsic properties of given cations (presumably with the pKa I value), hence they are weakly sensitive to different interactions with chloride and monohydrogen phosphate anions.
4. Do activities of N^+H_2 groups depend on counter anions, and they are always more active in the presence of $C\bar{l}$ than HPO_4^{2-} (see Table 3 in ref. 7).
5. Acceptor activities of $C\bar{l}$ anions depend on the donor activities of PA cations and are in some way related to their pKa I values (Table 3 in ref. 7).
6. Acceptor activities of HPO_4^{2-} anions depend very little on the donor activities of PA cations, presumably due to the buffering action of crystalline water molecules the accepting activities of which are lowest in $Spm2H_3PO_4.6H_2=$ and the highest in $Put2H_3PO_4.2H_2O$.
7. The twice bent conformation of $Spm4H^{4+}$ cation in its hydrochloride salt is presumably derived from the Ac properties of $C\bar{l}$ anions which prefer to act as triple acceptor centers and since in this salt the stoichiometric ratio: N^+-H/Cl equals 2.5, the $C\bar{l}$ anion forces the bent conformation which fulfills the steric requirements to form two bifurcated HBs, changing as a consequence the N^+-H/Cl ratio to the favorable value of 3.

IV. OUR FURTHER STUDIES IN POLYAMINES FIELD

A comparative analysis of the number and distribution of Ac centers in HPO_4^{2-} anions with the number of water molecules in crystalline monohydrogen phosphate of Put, Spd and Spm seems to indicate that the HPO_4^{2-} anions prefer to act as seven- to nine-fold acceptors. To fulfill this intrinsic desire the Put, Spd and Spm monohydrogen phosphates should crystallize respectively with 1-3, 5-9 and 4-8 water molecules. To check the validity of these very simple calculations we prepared three additional monohydrogen phosphates, starting with the homologs of native Put^4, $Spd^{3.4}$ and $Spm^{3.4.3}$, which in all cases have one methylene group less (Put^3, $Spd^{3.3}$, $Spm^{3.3.3}$). According to our expectation all homologous phosphates crystallize easily with crystalline water molecules whose number fits our predictions, which is seen in the Table below.

Monohydrogen phosphates (HPO_4^{2-}) of:

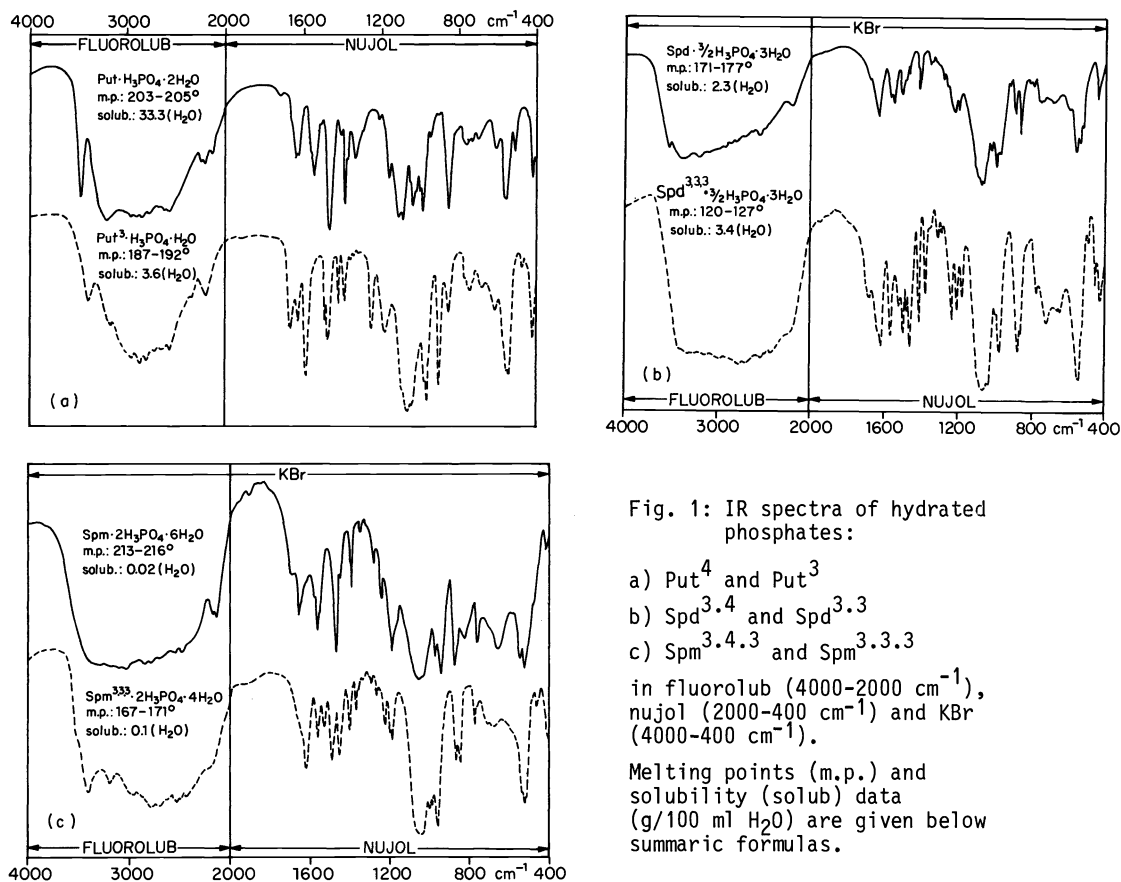| Native PA		Put^4	$Spd^{3.4}$	$Spm^{3.4.3}$
	present	2	6	6
Number of H_2O per formula	predicted	1-3	5-9	4-8
	present	1	6	4
Homolog of PA		Put^3	$Spd^{3.3}$	$Spm^{3.3.3}$

Fig. 1: IR spectra of hydrated phosphates:

a) Put[4] and Put[3]

b) Spd[3.4] and Spd[3.3]

c) Spm[3.4.3] and Spm[3.3.3]

in fluorolub (4000-2000 cm^{-1}), nujol (2000-400 cm^{-1}) and KBr (4000-400 cm^{-1}).

Melting points (m.p.) and solubility (solub) data (g/100 ml H_2O) are given below summaric formulas.

Our seemingly correct prediction encouraged us to make further speculations on the conformation of protonated cationic homologs of Put[3], Spd[3.3] and Spm[3.3.3] as their hydrated phosphates in the light of their IR spectra, melting points, and solubility data (see Fig. 1a-1c).

In all the cases the IR spectra show more similarities than differences within homologous pairs, which could indicate that the loss of one methylene group does not change the maximum stretched conformation in three homologous cations of PAs. This hypothetic conclusion is also based on our previous statement that crystalline water molecules buffer the activities of counter anions and prevent their action against the preferred conformation of PA cations. Only in the case of a homologous pair of Spd the loss of methylene groups does not change the degree of hydration and as a consequence the solubility of both Spd phosphates is almost the same (see Fig. 1). In this respect the behaviour of the pair of Put and Spm phosphates is significantly different, since in both cases the degree of hydration is decreased (by 50% for Put[3] and by 33% for Spm[3.4.3]). As a result, in both pairs the solubilities in water are drastically changed, however not in the same direction. The lower hydration of Put[3] phosphate diminishes its water solubility 10 times, but a similar processfor Spm[3.3.3] phosphate increases its water solubility. Our tentative explanation of the last two cases is as follows. In the case of Put[3] phosphate the lower degree of hydration improves the total organization of the HBs networks, hence there is a decrease of water solubility of that salt, and in the case of Spm[3.3.3] phosphate the HBs networks are poorly organized than in the phosphate of native Spm.

Even if the above explanations are right, only a full X-ray analysis of Spm[3.3.3].2H3PO4.4H2O and Put[3].H3PO4.H2O could answer the knawing question why the decrease of the number of crystalline water molecules in one case improves and in other worsens the organization of HBs networks? Hence, we decided to solve by X-ray analyses the crystal and molecular structures of all the three hydrated homologous phosphates.

Very unique sets of hydrated monohydrogen phosphates of native and homologous PA encouraged us to enrich the method which we had so far been using in our structural studies by applying thermodynamic measurements, and especially by: *i*) thermogravimetric analysis, *ii*) the determination of the first heat of solvation, and *iii*) the plots of differential scanning calorimetry. Reconnaissance measurements performed in the Institute of Physical Chemistry, Polish Academy of Sciences - were very promising, hence we are trying to develop the thermodynamic measurements rapidly and quite extensively, believing that they will provide us with a lot of
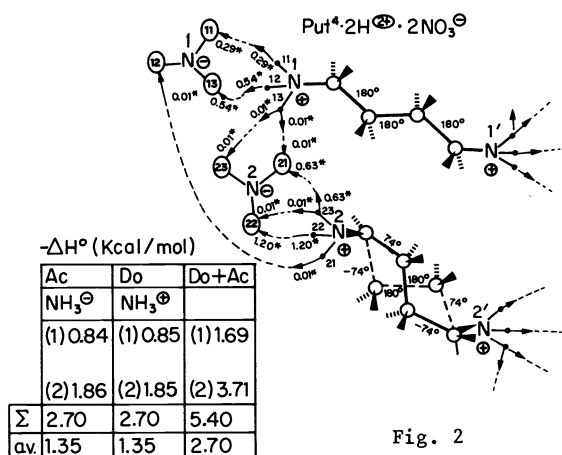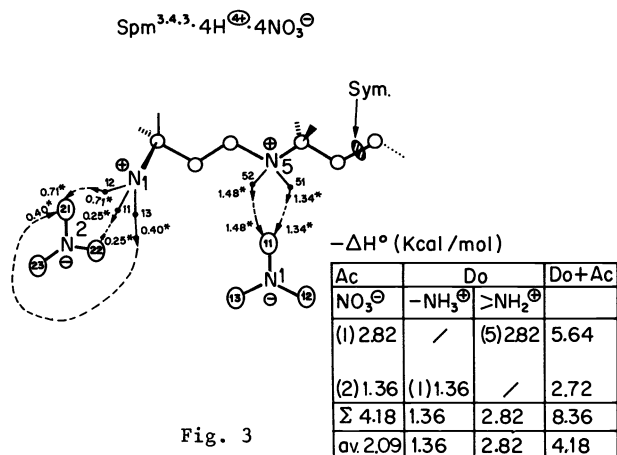
Fig. 2 and 3. Quantitative characterization of each HB as well as Do and Ac activities of its components expressed by $-\Delta H°$ parameter (Kcal/mol) in the crystal structure of the nitrate of Put (Fig. 2) and Spm (Fig. 3, ref. 11). $-\Delta H°$ values with an asteric concern particular HBs, and those in the table - Do and Ac activities of given atom groups.

valuable data due to a completely new approach. This will certainly broaden our knowledge of the fascinating field of PA, and of the role of water molecules in biological systems. Calorimetric data will also introduce some important corrections into our tentative correlation between ΔHB and $-\Delta H°$ parameters, which thus far have played a very important role in our structural considerations.

Recently we noticed in the course of our independent studies on the structure of protonated nucleosides (ref. 10), that nitrate anions due to their planar geometry which is stabilized by charge delocalization, can drastically change the conformation of protonated cytidine (CydH[+]) relatively to that present in cytidine hydrochloride (CydH[+].Cl^{−}). To prove the scope of our conclusion concerning the intrinsic properties of fully protonated PA cations (see point 3 on page 4 of this paper) based only on the structural determination of their hydrochloride and phosphate salts, one of us recently solved the structure of Put[4] and Spm[3.4.3] nitrates (Put[4]2H[2+].2NO$_3^-$, Spm[3.4.3]4H[4+].4NO$_3^-$ (ref. 11). According to our predictions in both salts the nitrate anions, thanks to their specific interaction with Put[4].2H[2+] and Spm[3.4.3]4H[4+] cations (see Fig. 2 and 3) change their all "trans" to partly "bent" conformations (see Fig. 4). However, completely unpredictable for us were the drastic changes in the Do activities of N[+]H$_3$ groups within Put[4]2H[2+] and Spm[3.4.3]4H cations as nitrate salts - relative to the corresponding hydrochloride and phosphate salts (see Table 1).

So far we have assumed that the very similar $-\Delta H°$ values of $-N^+H_3$ groups in hydrochlorides and phosphates of Put[4] (3.72 and 3.88 Kcal/mol) and in analogous salts of Spm[3.4.3] (6.29 and 6.82 Kcal/mol) - indicate a negligible influence of counter anions on these important features of PAs, responsible for their current involvement in networks of intermolecular HBs. The mean Do activity of $-N^+H_3$ groups in both nitrates (Put[4] and Spm[3.4.3]) are not only much lower than calculated for Put hydrochloride and phosphate but are almost identical to that for Spm (1.35 and 1.36 Kcal/mol, see Table 1). These values are roughly 3 times and 5 times lower than that previously estimated, respectively, for Put and Spm (see Table 1). It means that Do activities of Put and Spm cations in nitrate salts are mainly determined by the acceptor activities of nitrate anions.

TABLE 1

The protonodonor activities (Kcal/mole) of $-\overset{+}{N}H_3$ and $=\overset{+}{N}H_2$ groups in Put[4] and Spm[34.3] hydrochlorides,phosphates and nitrates. In parantheses, acceptor activities of counteranion (Kcal/mole)

PA cation	$-\overset{+}{N}H_3$			$=\overset{+}{N}H_2$		
	Cl^{−}	HPO$_4^{2-}$	NO$_3^-$	Cl^{−}	HPO$_4^{2-}$	NO$_3^-$
Put 2H[2+]	3.72	3.88	1.35			
	(3.72)	(12.70)	(1.35)			
Spm 4H[4+]	6.89	6.82	1.36	4.45	2.25	2.82
	(5.67)	(13.57)	(2.09)			
references		8 a-g				

TABLE 2
CHARACTERIZATION OF THE PROTON ACCEPTOR PROPERTIES OF THE NITRATE ANION.

SALT	DO group	Ac. activities Kcal/mole	THE SUM AND DISTRIBUTION OF ACTIVE AC. CENTERS WITHIN NO$_3$			
			Σ	O[1]	O[2]	O[3]
Put·2HNO$_3$	$\overset{+}{N}H_3^2$	1.90	5	2	2	1
	$\overset{+}{N}H_3^1$	0.84	3	1	1	1
Spm·4HNO$_3$	$\overset{+}{N}H_3$	1.36	3	2	1	—
	$\overset{+}{N}H_2$	2.82	2	2	—	—

Since two cations of Put.2H^{2+} in different conformations are present in an elementary Put.2HNO$_3$ cell (see Fig. 2 and ref. 11), it comprises also two non-equivalent -N$^+$H$_3$ groups associated with two nitrate anions characterized by the following Ac actiyities: 0.84 and 1.90 Kcal/mol (mean value: 1.35, see Table 1). On the other hand in Spm4H^{4+}.4(NO$_3^-$), the Spm cations occur in one symmetrically twice bent conformation (g$^+$tttttttttg$^-$), hence two N$^+$H$_3$ groups possess identical Do activities, which conform with the Ac activities of NO$_3$ anions associated with them and equal 1.36 Kcal/mol (Table 1). In a molecule of Spm.4HNO$_3$, an N$^+$H$_2$ group and a nitrate anion associated with it in a specific manner, are additionally present. The donor activity of the N$^+$H$_2$ group equals 2.82 Kcal/mol, so it is two times greater than that of -N$^+$H$_3$, occurring in the same salt. This is another surprise and another significant deviation from our observations on hydrochlorides and phosphates of Put and Spm (see Table 1). In these salts the donor activities of the N$^+$H$_2$ group are respectively 1.5 and 3 times lower than the donor activity of the -N$^+$H$_3$ group. A relatively high proton-donor value of the N$^+$H$_2$ group in Spm.4HNO$_3$ is in agreement with the activity of the nitrate anion associated with it, and equals 2.82 Kcal/mol. The two discussed Put and Spm nitrates contain four nitrate anions differing in the mode of association and summaric proton-donor activity, as depicted in Table 2.

Table 2 shows that the multitude of association forms that a nitrate anion can easily adapt. The problem requires further studies, but even at present we can state that nitrate anions interact with organic counter cations in a variety of ways both by evoking conformational changes as well as by distinct modulation of proton-donor activities of these cations.

In the case of Put.2HNO$_3$, X-ray analysis (ref. 11) showed the presence of two Put.2H^{2+} cations; one adopts a fully stretched conformation, and the other a double bent gauche$^+$-trans-gauche$^-$ conformation which occurs in two isomeric forms with unchanged location of end N-C bonds (see Fig. 2).

IR temperature spectra performed on a crystalline film of Put.2HNO$_3$ seem to confirm the above observation, and may allow the determination of the temperature of phase transition of both conformers in a crystal. These studies are under way, also with calorimetric data of the peculiar behaviour of Put and Spm nitrates.
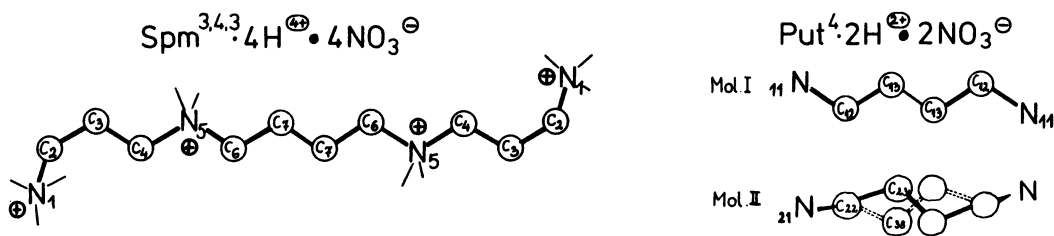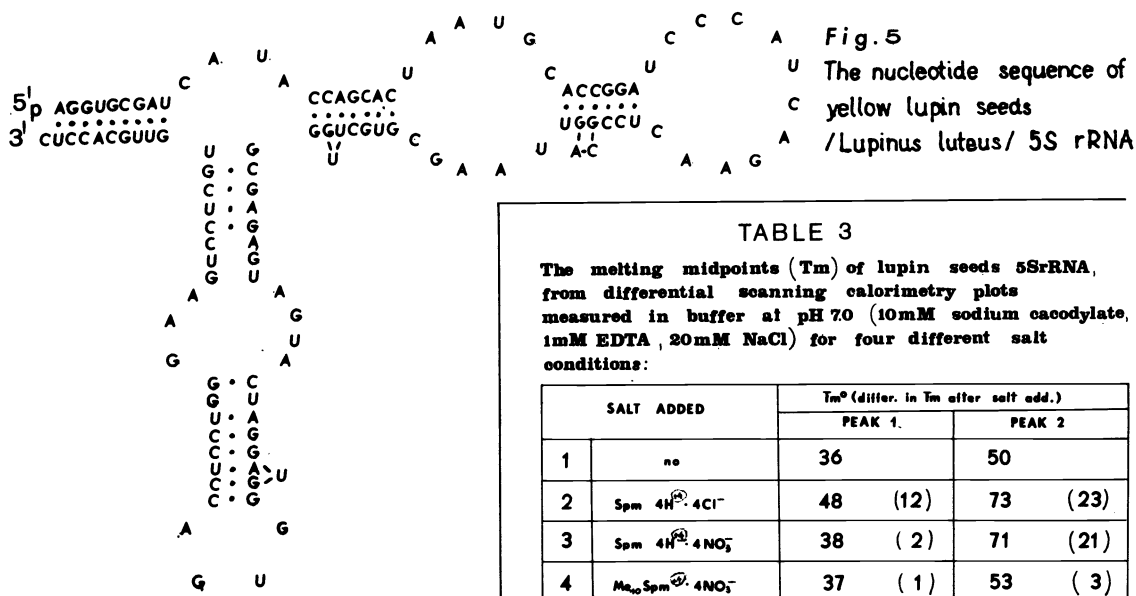

Fig. 4. Schematic representation of the conformation of fully protonated cations of Spm and Put on the basis of X-ray data (ref. 11).

Fig.5
The nucleotide sequence of yellow lupin seeds /Lupinus luteus/ 5S rRNA

TABLE 3

The melting midpoints (Tm) of lupin seeds 5SrRNA, from differential scanning calorimetry plots measured in buffer at pH 7.0 (10mM sodium cacodylate, 1mM EDTA , 20mM NaCl) for four different salt conditions:

SALT ADDED		Tm° (differ. in Tm after salt add.)			
		PEAK 1.		PEAK 2	
1	no	36		50	
2	Spm 4H$^{(+)}$. 4Cl$^-$	48	(12)	73	(23)
3	Spm 4H$^{(+)}$. 4NO$_3^-$	38	(2)	71	(21)
4	Me$_{10}$Spm$^{(+)}$. 4NO$_3^-$	37	(1)	53	(3)

Unexpected interactions of nitrate counter anions with protonated Put and Spm cations
encouraged us to start with our own observations on the influence of PA salts on the dynamic
conformation of tRNA and 5S rRNA molecules. At the first stage of these studies we took under
consideration two biopolymers which we chose for our models: 1) tRNA^{Phe} from different
organisms and 2) 5S rRNA from lupin seeds the primary structure of which was recently solved
by one of us (ref. 12) (see Fig. 5).

In turn, PAs were represented by the hydrochlorides and nitrates of Spm$^{3.4.3}$ and its homolog
Spm$^{3.3.3}$ as well as fully methylated tetrafold quaternary sperminium nitrate: Me$_{10}$Spm^{4+}.4NO$_3^-$.
The latter fully methylated Spm cation cannot form normal HBs since it has no N$^+$-H groups,
but being similarly four-fold charged as fully protonated Spm cation, it can "bind" to all
kinds of nucleic acids by purely electrostatic forces. We assumed that addition of equivalent
amounts of Spm4H^{4+}.4Cl$^-$, Spm4H^{4+}.4NO$_3^-$ and Me$_{10}$Spm^{4+}.4NO$_3^-$ to the same RNA preparation in the
same and proper buffer, should give us, if we were lucky, valuable information on the
similarities and differences in the type of interaction of a given PA with the same bio-
polymers. Of course, the results of such experiments depend on many factors, among which
the methodology and accuracy of analytical tools is most important. Our studies are at the
introductory state, and much effort must be still spent to transform our present qualitative
measurements into fully quantitative ones with high reproducibility and reversibility.

Nevertheless, we have decided to present very briefly some of our latest observations since
in our opinion they are very interesting, and we believe that in the near future we will be
able to supply all the necessary experimental details.

One of the methods which we have used to follow the influence of PA on the dynamic conforma-
tion of 5S rRNA, is the determination of melting midpoints (Tm) from differential scanning
calorimetry plots. In Table 3 two temperatures are given which characterize the phase transi-
tion of our 5S rRNA preparation in standard conditions, these are 36° and 50°C. After the
addition of mM amounts of Spm4H^{4+}.Cl$^-$ preparation both temperatures rose (by 12° and 23°C).
The addition of the same amounts of Spm4H^{4+}.4NO$_3$ shifted distinctly only the second peak,
whereas the addition of fully methylated sperminium cations had no distinct influence on
both peaks. What does this mean? No doubt that purely electrostatic attraction which very
often is assumed to be the main binding force between PA and nucleic acid in fact plays a
role with much less significance to that of the network of HBs which can be modulated and
tuned by the geometry and acceptor activities of counter anions.

Although our last statements introduce new complications to the already very complex image
of interactions of PA with nucleic acids, I believe that they are most important for proper
understanding, and if possible also for distinguishing between specific-high physiological
relevance and non-specific, unexpected and often undesired polyanions ══ polycations inter-
action. Slowly progressing but promising are also our own studies on the competitive inter-
actions of protonated PA cations and metal cations with model RNA molecules.

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