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THE INTERACTIONS BETWEEN NUCLEIC ACIDS AND POLYAMINES. I. HIGH RESOLUTION CARBON-13 AND HYDROGEN-1 NUCLEAR MAGNETIC RESONANCE STUDIES OF SPERMIDINE AND 5′-AMP

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In 0.5 M solution at pH 7.6, interaction of spermidine and 5′-AMP is demonstrated by downfield proton NMR shifts. Shifts of ribose and adenine protons support a model in which triprotonated spermidine engages the phosphate anion with the C-3 diamine segment in a conformation to maximize interaction and the C-4 amino segment extended to interact with adenine N-7 (base *anti*, 2′endo, g′g′ and gg nucleoside conformation). Changes in carbon-13 chemical shifts for ribose C-5′ (downfield), C-2′ C-3′, and C-4′ (upfield) and for adenine C-6 and C-8 (upfield) support this model. In 0.006 M solution no significant changes in proton shifts and therefore no evidence for interaction was found. Spermidine and 5′-UMP (0.006 M) showed interaction at pH 10.5 (small upfield shifts in the proton nmr) interpreted as changing conformation by solvent interaction. In 0.006 M 3′-UMP at pH 10.5 small downfield proton shifts induced by spermidine are attributed to interactions with phosphate anion.

1. Introduction

Polyamines have a major role in protein synthesis which is not well understood [1]. They have been shown to affect DNA, RNA and protein synthesis [2, 3]; aggregation, structural integrity, and function of ribosomal subunits [2,4–6]; and function of *t*-RNA [7]. It is believed that the way in which polyamines mediate biological processes involving nucleic acids is by association in a way to induce specific conformational changes [8,9].

Polyamines bind strongly to nucleic acids; at high concentrations precipitation results [10,11]. Studies of the interaction of polyamines with nucleic acids in nonliving systems have centered on observed changes in physical properties, such as the temperature of melting [10,12] circular dichroism [13], and more recently, streaming birefringence [14]. Other techniques which have been of value in studies *in vitro* of the related role of Mg^{+2} in protein synthesis, such as Raman spectroscopy [15] and transition enthalpies and energies [16], and especially NMR spectroscopy, have been little used in probing the interactions of polyamines with nucleic acids.

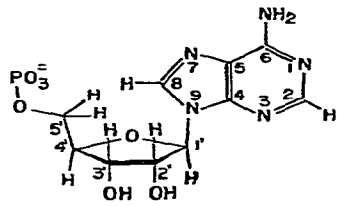
Polyamines are largely protonated at physiological pH. We have calculated, from ionization constants of spermidine of 8.34, 9.81 and 10.89 [17] that spermidine is 95.6% triprotonated and 4.3% diprotonated at pH 7.0, and 83.6% triprotonated, 15.2% diprotonated and 1.0% monoprotonated at pH 7.6. Polynucleotides are polyanions at this pH, with the phosphate group largely ionized, so that almost every nucleotide unit bears a full negative charge. The simplest interaction that has been proposed is that of a polyanion stabilized by the positively charged centers of polyamine molecules, in the same general way that nucleic acids are stabilized by magnesium ions. The stabilization of nucleic acids by Mg^{++} seems not to involve specific site binding, but rather stabilization by a surrounding mobile ion cloud [18]. Liquori et al. have proposed a model which does involve specific site binding: for one case of polyamine-nucleic acid interaction tetra-protonated spermine was suggested to fit into the small groove of the DNA double helix by binding to phosphate oxygens [19]. This model was not confirmed by the X-ray study by Suwalsky et al. [20].

It has been suspected that polyamine molecules may interact with parts of the nucleic acid molecule other than the phosphate oxygen. Electron-rich centers on the nucleic acid include the π electrons of the

bases and the lone pairs on nitrogen and oxygen [21]. These interactions might stabilize conformations of the kind that lead to more ordered systems. Elegant NMR studies of mononucleotides and dinucleoside phosphates during the past decade have investigated the 2'*endo*-3'*endo* conformational equilibrium of the sugar-ring, the sugar-base torsional angle and the phosphate-sugar bond angle [22–30]. The high resolution NMR studies of conformations of model nucleotides have also given indications of changes which accompany base stacking and the formation of more ordered nucleic acid structures [24,25,31–34].

We have examined the possibility of formation of a complex of Mg^{++} with polyamines (which change the interaction with nucleic acid) and could find no evidence for complex formation at physiological pH. In the course of that work, we found anomalous results when a divalent cation selective electrode was used to measure Mg^{++} concentration in the presence of a polyamine [35].

We are not aware of any unchallenged evidence to support binding of polyamines at specific sites, much less at sites other than phosphate oxygen; indeed evidence from studies of spermidine substitution for Mg^{++} in bacterial systems indicates that a 2:3 stoichiometric ratio is maintained, as would be expected from a charge neutralization model. We propose to study interactions in simpler systems and begin with the interaction of 5'-AMP (I) and spermidine (II) using high resolution NMR as a probe. Both continuous wave (CW) and Fourier transform (FT) techniques have been used in this study.



I

$H_2NCH_2CH_2CH_2NHCH_2CH_2CH_2CH_2NH_2$

1 2 3 4 5 6 7

c b c c a a c

II

2. Materials and methods

Adenosien-5'-monophosphate (5'-AMP) was purchased from Calbiochem, La Jolla, California. Uridine-3'-monophosphate (3'-UMP) and uridine-5'-monophosphate (5'-UMP) were obtained from Sigma Chemical Company, St. Louis, Missouri. These were used without further purification or drying.

Spermidine trihydrochloride was purchased from Calbiochem, La Jolla, California. It was recrystallized from an ethanol-water solution (80% ethanol).

Deuterium oxide 99.8% and 100.0% as well as sodium deuteroxide and dueterium chloride were purchased from Aldrich Chemical Company, Milwaukee, Wisconsin. NaOD and DCl were used for pD adjustment monitored by a Fisher Microprobe Combination Electrode (Cat. No. 13-639-92) (pD = 0.4 + pH meter reading [36,37]). Standard reference compounds used for the NMR work included, for PMR spectroscopy: tetramethylammonium chloride (TMA) from Aldrich Chemical Company; sodium 2,2-dimethyl-2-silapentane-5-sulfonate, $Si(CH_3)_3-CH_2-CH_2-CH_2SO_3^- Na^+$, (DSS) prepared by R. Kent in our laboratory using Tiers and Coon's method [38]; tetramethylsilane (TMS) from Aldrich Chemical Company; and terephthalic acid [TPA] from the Will Corporation, Rochester, New York; and, for carbon-13 spectroscopy: 1,4-dioxane from the Fisher Scientific Company, Fairlawn, New Jersey.

The paramagnetic-ion-trapping reagent disodium ethylenediaminetetraacetate (EDTA) was purchased from the Fisher Scientific Company, Fairlawn, Jew Jersey.

Two types of freeze-drying equipment have been used: a Virtis freeze-drying machine, and a high vacuum rack equipped with a mercury diffusion pump. Both have the capability of reducing the pressure to 10^{-5} torr.

CW hydrogen-1 nmr spectra were obtained with a Varian HA-100 spectrometer. FT hydrogen-1 and carbon-13 spectra were measured using a Bruker WP-60, a Varian XL-100-15, and a JEOL FX-60 spectrometer.

To prepare a sample for hydrogen-1 nmr spectroscopy, a calculated amount of mononucleotide and/or spermidine was lyophilized three times with 2–3 ml of 98.8% D_2O together with the standard reference compound and some EDTA (to trap paramagnetic ions such as Mn^{2+} or Cu^{2+}). The pD adjustment was

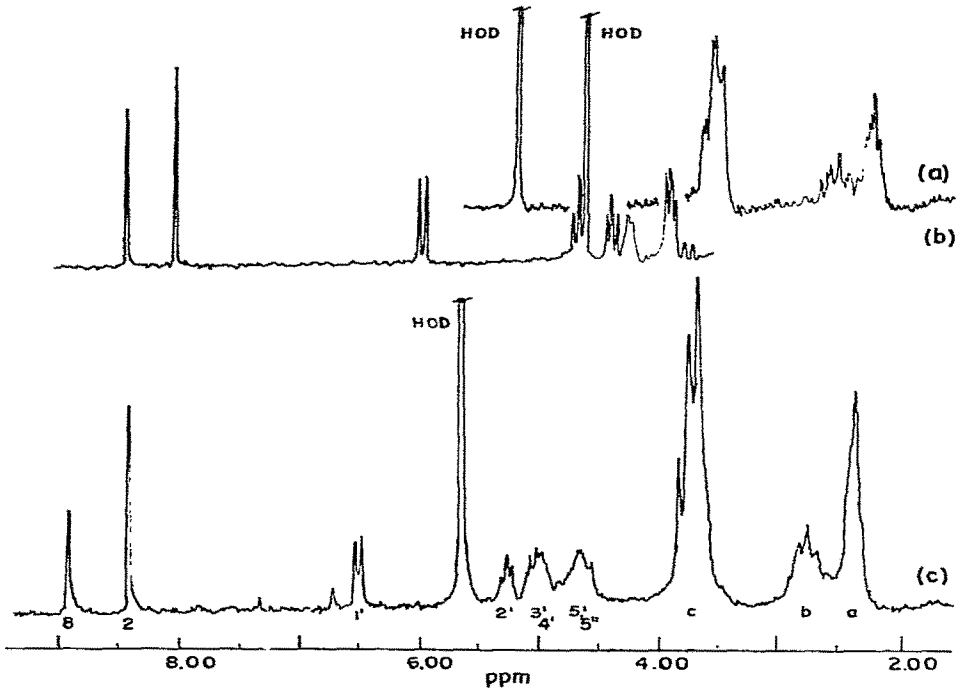


Fig. 1. Chemical shifts (δ) of the protons of (a) spermidine, (b) 5'-AMP, and (c) equimolar spermidine and 5'-AMP at pD = 8.0, 0.5 M in D_2O, 100 MHz, TMS reference in concentric NMR tube.

made before the first lyophilization. A suitable amount of 100.0% D_2O was added after the third lyophilization to give the desired concentration. The addition of D_2O to the lyophilized sample was done within a dry box containing P_2O_5 and nitrogen.

To prepare a sample for carbon-13 nmr spectroscopy, a weighed amount of the solute was dissolved in 98.8% D_2O and then the internal reference was added. The pD was then adjusted by addition of NaOD and DCl.

LAOCOON III computer simulation was used in interpretation of the hydrogen-1 NMR spectra [39]. Computer simulation work was performed in State University of New York, Albany, using a UNIVAC 1108 computer, and a program developed by Sarma and Lee [28].

3. Results

3.1. CW Hydrogen-1 NMR spectra of 0.5 M 5'-AMP-spermidine

CW ^1H NMR spectra of 0.5 M 5'-AMP, spermidine and an equimolar mixture of the two were measured at 100 MHz (fig. 1), using TMS in concentric NMR tubes as reference. Since the samples were prepared in D_2O, protons of the hydroxyl groups on the ribose and the amino groups of adenine and spermidine exchanged rapidly with deuterium and we did not observe these resonances. Instead, an HOD peak was always present in the spectra.

At pD 8.0, the 5'-AMP spectrum was well-defined: the resonance peaks due to the two non-exchangeable base protons appeared furthest downfield whereas the resonance peaks due to the non-exchangeable ribosyl

able 1
roton chemical shifts of 5'-AMP, spen · idine, and 1:1 mixture in 0.5 M solution, pD 8.0

	5'-AMP								Spermidine		
	H-8	H-2	H·i'	H-2'	H-3'	H-4'	H-5'	H-5"	H-c	H-b	H-a
riginal Chemical shifts, δ (ppm) [a]	8.46	8.05	5.97	4.67	4.40	4.24	3.91	3.91	3.55	2.51	2.22
oupling constants, *J* (herz) [a]	$J_{1'2'}$ 5.4	$J_{2'3'}$ 5.0	$J_{3'4'}$ 5.2	$J_{4'5'}$ 3.8	$J_{4'5''}$ 3.8	$J_{4'P}$ 2.0	$J_{5'5''}$ −12.6	$J_{5'P}$ 4.3	$J_{5''P}$ 4.3		
hemical shifts under interaction, δ' (ppm)	8.93	8.40	6.52	5.28	5.08	4.98	4.66	4.66	3.76	2.77	2.38
δ = δ' − δ	+0.47	+0.35	+0.55	+0.61	+0.68	+0.74	+0.75	+0.75	+0.21	+0.26	+0.16

[a] δ values of H-8 and H-2 were measured directly from the experimental spectrum whereas all the other δ values due to the ribosyl protons and the J_{ij} values were confirmed by computer simulation.

The peak assignments were made with reference to he work by Fujiwara and Uetsuki [40] as well as that y Kan and Ts'o [41]. The spectra observed were anal-zed by computer simulation and the chemical shifts nd coupling constants are listed in table 1.

The spermidine spectrum at room temperature and H 8.0 appeared to be composed of three sets of mul-iplets having their resonances at ca. 3.5, 2.5 and 2.2 pm. Peak assignments were made on the assumption hat the resonances due to the methylene prtons *alpha* o the amino groups should appear farther downfield ompared to those due to the *beta* methylene protons. he three multiplets have been assigned as *a, b, c* and he ratio of protons was confirmed to be 4:2:8 by ntegration.

It was possible to do a comparative study on the pectra of the mixture of 5'-AMP and spermidine be-ause the resonance peaks of the respective compunds id not overlap. There were significant changes in the pectrum as 5'-AMP was allowed to interact with sper-nidine (fig. 1). Much perturbation was detected in-luding changes in the line shape and also the chemical hifts. All resonance peaks shifted downfield (see able 1). The base protons of adenine H-8 and H-2 vere shifted 0.47 and 0.35 ppm downfield respective-y. In the ribose moiety of the 5'-AMP we observed an nteresting trend, namely, the deshielding effect in-reases from 1' to 5' and 5" protons. The amount of leshielding, Δδ, for protons 1', 2', 3', 4' and (5', 5") was 0.55, 0.61, 0.68, 0.74 and 0.75 ppm respectively (positive sign indicating a deshielding effect).

3.2. Proton-decoupled carbon-13 FTNMR study of 0.5 M 5'AMP-spermidine

Since the NMR spectra indicated interactions between 0.5 M 5'-AMP and spermidine using CW proton spectra, we were interested to confirm this result using natural abundance carbon-13 NMR spectroscopy.

The 15 MHz spectra of 0.3 M 5'-AMP, 0.3 M spermidine and a mixture of 0.3 M 5'-AMP and 0.3 M spermidine at pD 8.0 were measured (fig. 2). Chemical shifts were measured relative to dioxane, 67.39 ppm.

Chemical shift assignments of 5'-AMP were made with reference to the reported proton-decoupled carbon-13 NMR studies of nucleotides and nucleosides [42–47].

No carbon-13 NMR spectrum for spermidine has been reported to data. Chemical shift assignments given in table 2 were made with reference to the work of Brown [48] and Lippmaa and Pehk [49] on α,ω-polymethylenediamines, and also to that of Weser et al. [47] on spermine.

The spectrum of the mixture appeared to be well separated into three discrete regions, thus allowing unambiguous spectral interpretation. We found that in the system of interacting molecules, there were significant upfield shifts for carbons 6 and 8 on adenine,

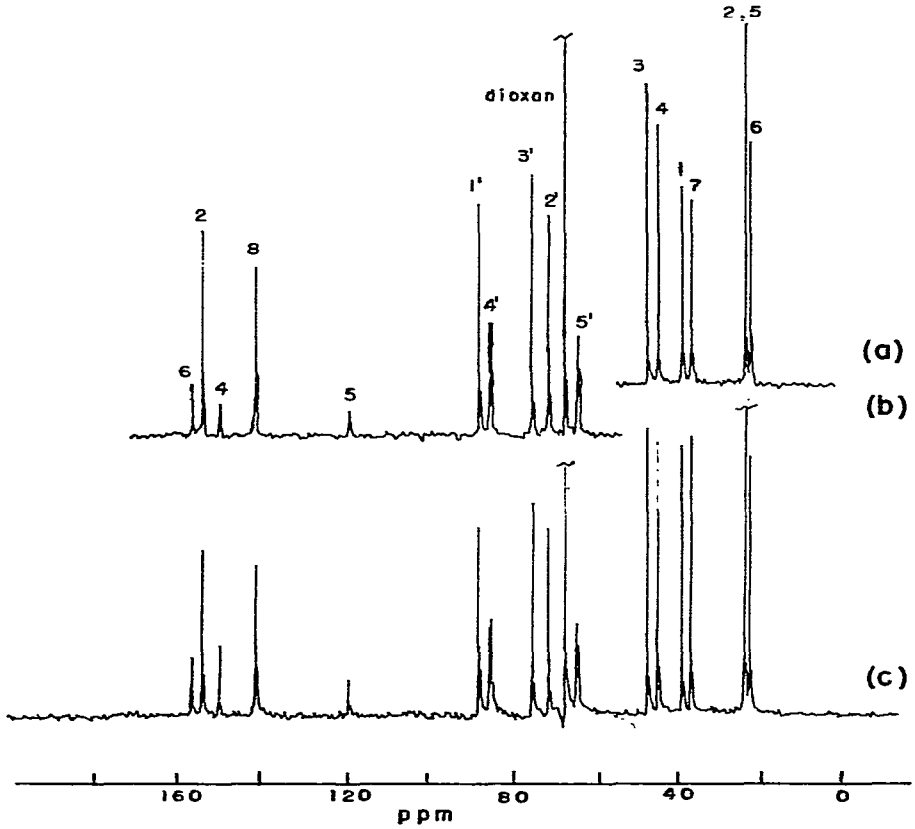


Fig. 2. Chemical shifts (δ) of the carbon atoms of (a) spermidine, (b) 5′-AMP, and (c) equimolar spermidine and 5′-AMP at pD = 8.0, 0.3 M in D_2O, proton decoupled, 15 MHz, dioxane reference.

for ribosyl carbons 2′,3′ and 4′, and for the two carbons (3 and 4) adjacent to the secondary amino group on spermidine (see table 2). Since the reproducibility of the data was in the order of ± 0.05 ppm, we regarded only chemical shifts more than 0.05 ppm as significant.

3.3. FT hydrogen-1 NMR study of 0.006 M 5′-AMP-spermidine

It is known that even simpler units of polynucleotides undergo base stacking in solutions of high concentration [50–53]; such interaction is reported to affect the conformation of dinucleoside phosphates [24,25,31–34]. It was therefore of interest to determine if the chemical shifts observed in the hydrogen-1 and carbon-13 NMR spectra in mixtures of spermidine and 5′-AMP could be observed at the concentration range of physiological conditions.

Spectra were measured at pD 7.4 using terephthalate (7.87 ppm) as reference. The concentration of 5′-AMP was fixed at 0.006 M and that of spermidine was varied; nucleotide-spermidine ratios were 1:1, 3:2, 3:1 and 6:1. Spectra of 5′-AMP, spermidine, and a 1:1 mixture are given in fig. 3.

There were no detectable changes in the chemical shifts and coupling constants on mixing at any of these molar ratios in the dilute solution. Some changes in the line shapes were detected in the spermidine resonances, but the fact that these are multiplets made analysis difficult.

Table 2
Carbon-13 chemical shifts of 5'-AMP, spermidine and 1:1 mixture in 0.3 M solution, pD 8.0

	5'-AMP										Spermidine				
	6	2	4	8	5	1'	4' [a]	3'	2'	5' [a]	3	4	1	7	2+5	6
δ	155.74	153.21	149.29	140.73	118.83	87.89	85.22 J 0.59	75.37	71.41	64.49 J 0.06	47.89	45.41	39.70	37.49	24.69	23.59
$\delta'_{mixture}$	155.60	153.28	149.24	140.67	118.83	87.91	85.05 J 0.51	75.17	71.28	64.52 J 0.26	47.76	45.29	39.63	37.49	24.75	23.58
$\Delta\delta$	−0.14	+0.07	−0.05	−0.06	−0.00	+0.02	−0.17	−0.20	−0.13	+0.03	−0.13	−0.12	−0.07	0.00	+0.06	−0.01

a) 4' and 5' resonance peaks are split into doublets due to coupling with the phosphorus nucleus.

3.4. FT hydrogen-1 study of interactions between uridine nucleotides and spermidine

The absence of any evidence for interaction between 5'-AMP and spermidine at millimolar concentrations led us to examine if interactions could be observed at higher pH between 5'-UMP and spermidine at these low concentrations. For comparison purposes, 3'-UMP-spermidine mixtures were also studied. 100 MHz FT proton NMR spectra were obtained for spermidine 5'-UMP and a 1:1 molar mixture (fig. 4) and for spermidine, 3'-UMP, and a 1:1 molar mixture (fig. 5) all at pD 10.9 and 0.006 M, using terephthalate as reference. The resonances due to the pyrimidine base were well separated and no complications arose from peak overlaps; the results are presented in table 3.

Chemical shift assignments for 5'-UMP and 3'-UMP were made with reference to previously published work [54–56]. Evidence for some interactions between spermidine and 5'-UMP under these conditions was noted; all of the peaks were shifted upfield from −0.05 to −0.16 ppm.

In the 3'-UMP and spermidine mixture, there was a downfield shift of most of the 3'-UMP peaks except that of the 2'-proton whereas the resonances due to spermidine remained unchanged.

4. Discussion

4.1. 5'-AMP-spermidine in 0.5 M solution

Significant changes in the chemical shifts of 5'-AMP and spermidine were observed on interaction in relatively concentrated solution. The largest downfield shifts, approximately 0.75 ppm, were noted for the ribose protons on carbons nearest to the phosphate group, and somewhat smaller chemical shift changes (0.70 to 0.55 ppm) for the more distant ribose protons bonded to carbon. The downfield shift of the 5'-protons is interpreted as arising from approach of an ammonium cation to the phosphate O^-. The regular trend of deshielding which was observed must be due only in part to inductive effects of this interaction along the ribose chain, and in part to the proximity of positive centers of protonated spermidine.

Somewhat smaller downfield chamical shift changes were observed for the adenine protons. A similar de-

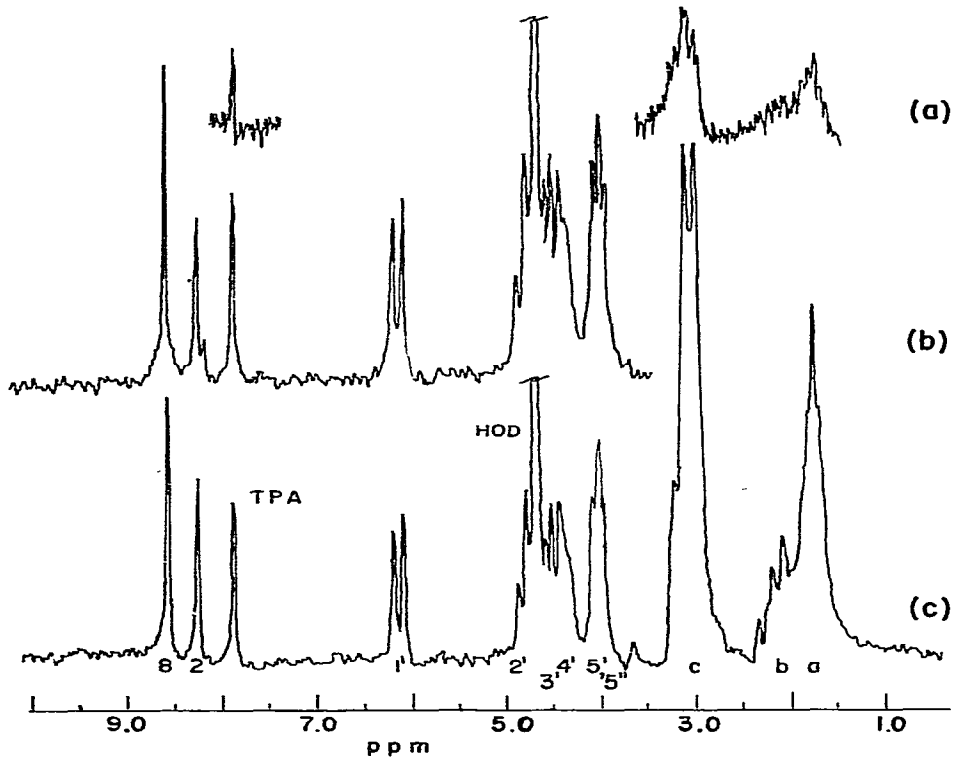


Fig. 3. Fourier transformed proton NMR spectra of (a) spermidine; (b) 5'-AMP, and (c) equimolar spermidine and 5'-AMP at pD = 7.4, 0.006 M in D_2O, 60 MHz, terephthalate ion reference.

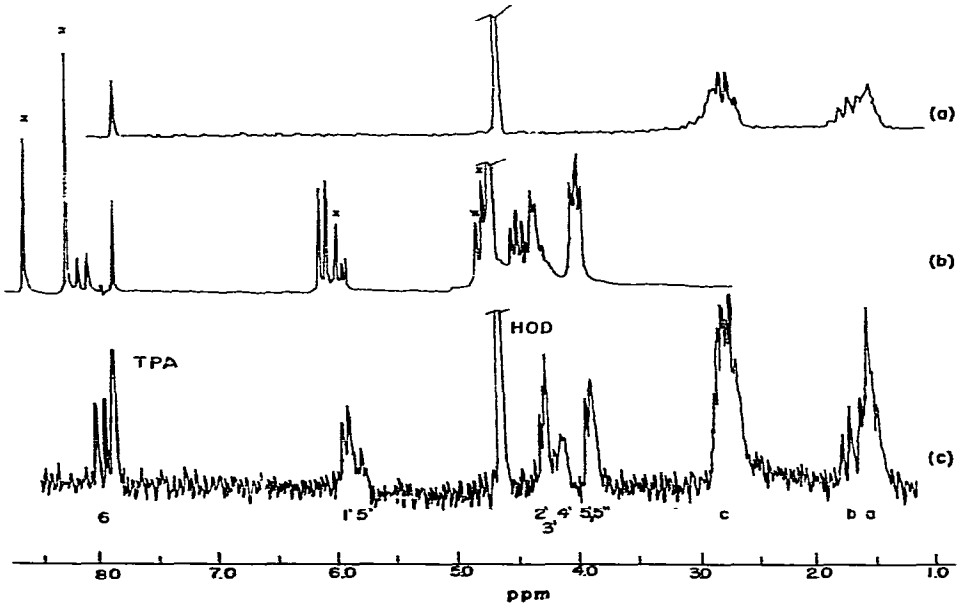


Fig. 4. Fourier transformed proton NMR spectra of (a) spermidine, (b) 5'-UMP, and (c) equimolar spermidine and 5'-UMP at pD = 10.9, 0.006 M in D_2O, 100 M Hz, terephthalate ion reference. X indicates an extraneous peak of unknown origin.

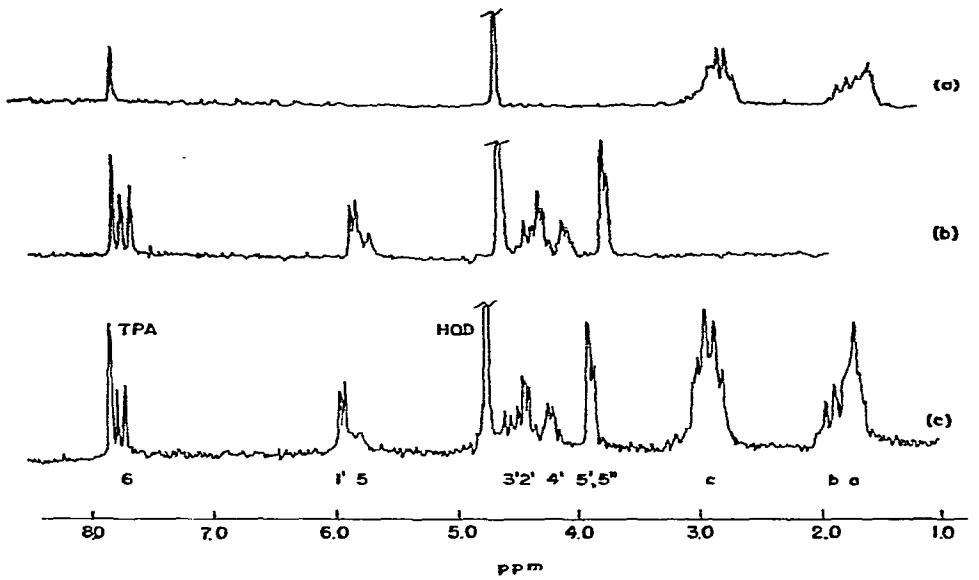


Fig. 5. Fourier transformed proton NMR spectra of (a) spermidine, (b) 3'-UMP, and (c) equimolar spermidine and 3'-UMP at pD = 10.9, 0.006 M in D_2O, 100 MHz, terephthalate ion reference.

Table 3
Proton chemical shifts of 5'-UMP, 3'UMP spermidine, and 1:1 mixtures in 0.006 M solution, pD 10.9

	5'-UMP								Spermidine		
	6	1'	5	2'	3'	4'	5'	5"	c	b	a
Original chemical shifts, δ (ppm)	8.11	6.12	5.95	4.50	4.39	4.34	4.02	4.01	2.85	1.85	1.67
Chemical shifts, δ', under interaction	7.99	5.97	5.85	4.36	4.30	4.18	3.93	3.92	2.77	1.78	1.62
Δδ = δ'−δ	−0.12	−0.15	−0.10	−0.14	−0.09	−0.16	−0.09	−0.09	−0.08	−0.07	−0.05
	3'-UMP										
	6	1'	5	3'	2'	4'	5'	5"	c	b	a
Original chemical shifts, δ (ppm)	7.76	5.90	5.77	4.36	4.45	4.17	3.85	3.83	2.85	1.85	1.67
Chemical shifts, δ', under interaction	7.78	5.92	5.80	4.47	4.39	4.21	3.89	3.86	2.85	1.85	1.68
Δδ = δ'−δ	+0.02	+0.02	+0.03	+0.11	−0.06	+0.04	+0.04	+0.03	0.00	0.00	+0.01

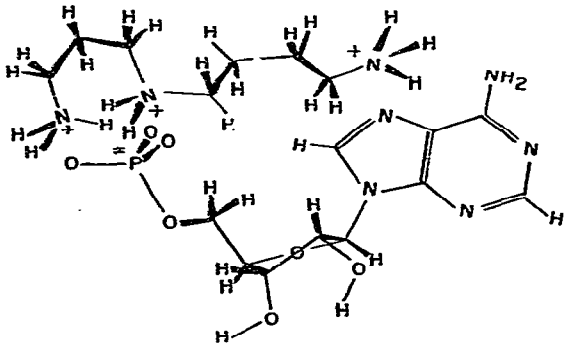


Fig. 6. Model for the interaction of spermidine and 5'-AMP at physiological pH, 0.5 M.

shielding effect, arising from the third cationic site of spermidine (two are believed to be engaged with the phosphate dianion), is more pronounced for adenine H-8 then for H-2. For 5'-AMP as for most 5'-mononucleotides, the sugar-base preferred conformation is *anti* [51,57,58], and H-8 would then be closer to the spermidine cationic site.

Specific site binding of the protonated amino group of spermidine on the N-7 of adenine would intensify the differing effect of spermidine on H-2 and H-8. N-7 is two atoms away from H-8 but five atoms away from H-2. Fig. 6 shows a model for such interaction. This interpretation is strengthened by the fact that N-7 on adenine has been reported to be quite susceptible to binding by positive charges [8,47,59].

These changes in chemical shifts are very much larger than those reported to result from interaction of Mg^{+2} with 5'-UMP at pD 5.4 in 0.1 M solution [50].

The observed changes in the chemical shifts of the spermidine protons are also in accord with this interpretation of the interaction. It was found that the changes in chemical shifts for protons *c*, *b*, *a* were 0.21, 0.26 and 0.16 ppm respectively. Since the changes were most significant on protons *b* and *c* and much less for *a*, we concluded that the segment $D_3\overset{+}{N}CH_2CH_2CH_2\overset{+}{N}D_2-$ was more affected by interaction than the segment $-\overset{+}{N}D_2CH_2CH_2CH_2CH_2\overset{+}{N}D_3$.

The interaction was first thought to cause upfield shifts in spermidine protons (contrary to the observations) because of proximity to a center bearing nearly two full negative changes. There is, however, good reason to anticipate an increase in the equilibrium proton tion of nitrogen (which should cause a downfield shift of protons on nearby carbon) when it is situated so that (linear) hydrogen bonds to a negative center can be formed by the protonated species. Yamdagni and Kebarle [60] have shown that gas phase proton affinities of α,ω-diamines $H_2N(CH_2)_n NH_2$, where $n=3, 5,$ or 7, are 15–20 kcal mol^{-1} larger than for the corresponding aliphatic monoamines. The increased stabilization of the monoprotonated species is a measure of the hydrogen bond energy in the cyclic structure $-\overset{+}{N}H_2-H\text{----}NH_2-$. In the case of spermidine – 5'-AMP interactions in relatively concentrated solution, two of the spermidine nitrogens (those separated by the three carbon chain) can interact with the phosphate oxygen negative centers in this way, and the resulting increase in protonation might lead to a slight downfield shift. The magnitude of such a shift is open to speculation. Sudmeier and Reilley [61] have shown downfield shifts on protonation of nitrogen in putrescine of 0.43 ppm for protons on the α carbons, and 0.32 ppm for those on the β carbons. Experiments in this laboratory with putrescine [62] have confirmed the magnitude of these shifts and established that the change in chemical shift for a change from pD 7.6 to pD 5.6 was only 0.03 ppm for α protons and 0.02 ppm for β protons.

These small effects might be superimposed on a general small downfield shift caused by aromatic ring currents; our model suggests that most of the spermidine protons would fall within the deshielding zone of the adenine [53].

It could be argued that the chemical shift changes of all the resonances in the 5'-AMP and spermidine mixture may be due to a so-called "base-unstacking phenomenon" with spermidine acting as the unstacking agent. Evans and Sarma [53] found that the resonances of base protons 2, 8, as well as those of ribosyl protons 1', 2', did go downfield on dilution (leading to unstacking), however, the ribosyl 3' proton resonance remained almost constant and the ribosyl 4' and 5' protons were shifted upfield. Since we found a general downfield shift of all resonances, this unstacking argument can be ruled out.

We thus tentatively conclude that the following model of interaction between 5'-AMP and spermidine at high concentration is likely: – The segment $D_3\overset{+}{N}(CH_2)_3\overset{+}{N}D_2-$ (with two positively charged amino

groups) binds to the phosphate moiety whereas the segment $-(CH_2)_4\overset{+}{N}D_3$ stretches out and binds to the N-7 atom on adenine. We postulate that the conformation of 5′-AMP in 0.5 M solution, roughly equal 2′ endo ←→ 3′ endo ribose conformation, predominant g′g′ C(5′)–O(5′) and gg C(4′)–C(5′), and predominant *anti* sugar base torsional angle (29) need be little changed in the association; we have illustrated what may be a preferred association of the 2′endo ribose conformer.

The changes in the C-13 chemical shifts lend support to this model of the interaction of spermidine with 5′-AMP in relatively concentrated solution. The upfield shifts of the 6 and 8 carbons indicate interaction of some type; this must involve the electropositive ammonium group. Ribose carbons 2′, 3′ and 4′ are also shifted upfield by 0.13 to 0.2 ppm; these carbons are also in positions to be influenced by the cationic centers in the same way as the protons on these carbons. The *direction* and magnitude of the change of chemical shift in these cases is difficult to predict; it is the result of a balance of opposing charge effects operating through space and by induction [63]. There is a significant downfield shift of the ribose 5′ carbon which is believed to result from an inductive effect when cationic centers of spermidine interact with phosphate oxygens.

The chemical shift of spermidine carbons 1, 3, 4 and 7 was also significantly changed; upfield shifts of 0.1 to 0.15 ppm were noted. Upfield shifts of carbons α and β to amine functions on protonation of amino acids and amines were found by Horsley and Sternlicht [63]. In our case we have a trifunctional amine in which a small fraction of the amine at pH 7.0 is unprotonated, and hydrogen bonding in a cyclic structure [60] may also have an effect. Thus increased protonated of spermidine as it interacts with phosphate may be a partial explanation; a spatial effect of negative charge adjacent to two nitrogen cationic centers may also contribute.

4.2. 5′-AMP-spermidine in 0.006 M solution

Comparison of the chemical shifts of the protons of 5′-AMP at 0.5 M at 0.006 M concentrations indicated extensive association in the former case. Upfield changes in the shifts of H-8 (–0.16 ppm), H-2 (–0.28 ppm) and H-1′ (–0.19 ppm) were noted by Schweizer et al. [50] as the concentration of 5′-AMP was changed from infinite dilution to 0.3 M and were interpreted as a consequence of association of the mononucleotide. Chemical shifts of slightly different magnitude were reported by Evans and Sarma [53] for H-8, H-2, and also for H-2′, and also by Raszka and Kaplan [5]. Our solutions were at slightly different acidities (pD = 8.0 and 7.4), but the changes were roughly comparable ($\Delta\delta$H-8 = 0.11, $\Delta\delta$H-2 = 0.20, $\Delta\delta$H-1′ = 0.14 and $\Delta\delta$H-2′ = 0.11), downfield on dilution.

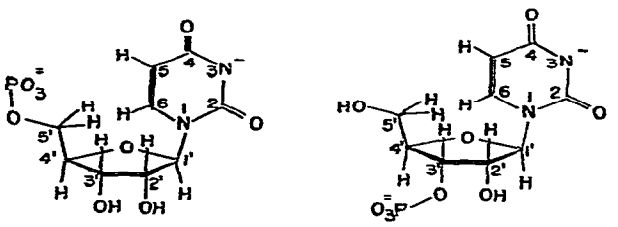
The changes in chemical shifts of the protons of 5-AMP and spermidine when they were mixed in various ratios in 0.006 M solution were too small to be significant, although there were some changes in line shapes. The lack of specific interaction must be a result of extensive solvation of both species; they may be insulated from each other in solvent cages.

4.3. Uridine nucleotides and spermidine in 0.006 M solution

The phosphate group of 5′-UMP is closer to the base moiety than is the case in 3′-UMP (fig. 7). It was thus our expectation that 5′-UMP might have more interaction with spermidine than 3′-UMP.

It was known that the pKa of the N-3 proton of the uracil moiety of 3′-UMP was about 9.43 and that of 5′-UMP was about 9.50 [64]. At pH 10.5, one expects a trianionic UMP to be obtained, and more interactions, thus, might be expected on the base moiety. pH 10.5 is outside pH range for physiological systems, but if there were sufficient triprotonated amine of if the increased energy of hydrogen bonding could induce an appreciable fraction of triprotonated amine, interactions might be evident from nmr spectral changes. In the 5′-UMP-spermidine system, it was calculated that there should be about 91% trianionic and 9% dianionic 5′-UMP and also 0.1% triprotonated, 12.6% diprotonated, 62.2% monoprotonated, and 25.2% non-protonated spermidine.

Prestegard and Chan [65] reported an upfield shift of the uracil H-6 resonance when salts like $Mg(ClO_4)_2$ and $NaClO_4$ interacted with 5′-UMP. These salts have a structurebreaking effect on the solvent and are responsible for the upfield shift of the H-6 resonance due to reorientation of the uracil base about the glycosidic bond. J 1′2′ changes due to changes in the amount of ring puckering were also reported. We de-



(a) (b)

Fig. 7. Conformation of (a) 5'-UMP and (b) 3'-UMP at pH 11.

tected a general phenomenon of upfield shift of resonances and also changes in $J\,1'2'$. These served as an indication that at pD 10.9, spermidine served as a structure-breaking solute to a 5'-UMP solution.

In the 3'-UMP-spermidine system, we calculated that there should be about 92.2% trianionic and 7.8% dianionic 3'-UMP.

The lack of changes in the spermidine resonances and a general downfield shift of the 3'-UMP resonances (most significant for the 3'-ribose proton) have suggested a slight electrostatic interaction between the monoprotonated spermidine and the phosphate moiety of 3'-UMP but little or no interaction between the uracil moiety and the spermidine.

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