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Structural interactions of polyamines

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Abstract

Polyamines are ubiquitous biological cations. We have determined the X-ray crystal structures of a number of polyamine salts and complexes of diamines with acidic amino acids in order to obtain structural information on polyamine interactions. The conformations of diamines in the complexes display variations reflecting the flexibility in their backbone geometries. In all the structures, the amino group protons are involved in hydrogen bonds without large torsional strain on the terminal C–N bond. Evaluation of the relative size of electrostatic, van der Waals and hydrogen-bonding interactions suggests that all the terms might be of significance in the formation of polyamine complexes.

Keywords: Polyamines, X-ray diffraction, structure, interaction.

1. Introduction

Since the discovery of spermine by Anthony von Leeuwenhoek in human semen over two hundred years ago, there have been periodic bursts of activity reporting the role of polyamines in a variety of cellular functions. Polyamines are the major representatives of cellular organic cations, the other important cations being inorganic ions. This has led, until recently, to the conviction that polyamines are essential mainly for the maintenance of the charged state of the living cell.

Recent biochemical research has dispelled much of these convictions. The biosynthesis and degradation of polyamines in a variety of tissues have been extensively investigated. Polyamines are now implicated in an almost bewildering mace of cellular functions[1–6].

Apart from participating in cellular functions, polyamines also appear to be essential for the integrity of the native structures of several biological macromolecules and their distinct topological assemblies. Polyamines stabilize the double helical structure of nucleic acids[7]. Distinct polyamine-binding sites have been localized in the three-dimensional structure of *t*RNA[8]. Several viral genomes contain tightly bound polyamines[7]. They are essential for the neutralization of the negative charges of phosphates. They are also probably required for compacting nucleic acid and its encapsidation in the protein coat[9]. The function of polyamines is not easily replaced by other inorganic cations in the stability of virions of belladonna mottle virus, suggesting a degree of specificity to polyamine interactions[10].

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In contrast to the extensive biochemical literature on polyamines, studies on their structure and interactions with other biomolecules are relatively rare. These studies are limited to the inorganic salts of polyamines[11] and polyamines complexed with nucleic acids, where polyamines are an inevitable part of the crystallization cocktail[12]. To obtain quantitative information on different interactions responsible for polyamine specificity, we have determined a number of structures containing polyamines in different chemical contexts. Brief reports of these structures have been published[13–18]. Here we report an analysis of these structures.

2. Crystallization of polyamine salts

The structures of two inorganic salts of polyamines and six complexes of three diamines with two acidic amino acids are presented and analysed in this paper.

2.1. Cadaverine dihydrochloride monohydrate

Structures of the chloride salts of diamines of the type $H_2N(CH_2)_nNH_2$ for $n = 3$, 4 and 6 have earlier been determined by X-ray diffraction methods[19–21]. However, attempts to determine the structure for the amine when $n = 5$ (1,5-diaminopentane) were not successful[11]. 1,5-diaminopentane or cadaverine is found as an intrinsic component of the plant virus turnip yellow mosaic virus[22]. Cadaverine dihydrochloride purchased from Sigma was passed through a Dowex1 (OH^- form) column which retains chloride ions. The free amine eluted was neutralized to pH 7.0 by the addition of 1 N HCl. The resulting solution was concentrated by lyophilization. This was layered with propanol and left undisturbed in a sealed test tube. Very hygroscopic, needle-shaped crystals appeared in a few days. It was observed that these crystals could be used for diffraction studies if mounted in a sealed glass capillary tube.

2.2. Sym-homospermidine triphosphate monohydrate

Sym-homospermidine ($NH_2(CH_2)_4NH(CH_2)_4NH_2$) is a rare polyamine found in relatively large concentrations in sandal leaves and is a homologue of the more common spermidine. It was purified from sandal leaves following published procedures[23]. *Sym*-homospermidine chloride obtained from this procedure was passed through Dowex1 resin. The eluted free amine was titrated with 2 N phosphoric acid till the pH reduced to 3 and the sample was dried in vacuum. The phosphate salt of the amine crystallized from an aqueous solution by slow diffusion of propanol. The needle-shaped crystals obtained after a few days were stable when exposed to air. In contrast, the chloride salt prepared by a similar procedure was highly hygroscopic and unsuitable for diffraction experiments. Hygroscopicity is a common property shared by many other chloride salts of polyamines. This is related to the large hydration energy of chloride ions.

3. Preparation of the complexes of diamines with acidic amino acids

The complexes of diamines 1,3-diaminopropane, 1,4-diaminobutane and 1,6-diaminohexane with acidic amino acids aspartic and glutamic acid were used in the present analysis. All the diamines and amino acids were purchased from Sigma. Chloride-free amines were

Table I

List of structures reported in this paper

No. Title	*Chemical formula*	*Nomenclature*
1. Cadaverine dihydrochloride monohydrate	$NH_3^+(CH_2)_5NH_3^+ \cdot 2Cl^- \cdot H_2O$	1,5-diaminopentane dihydrochloride monohydrate
2. *Sym*-homospermidine triphosphate monohydrate	$NH_3^+(CH_2)_4NH_2^+(CH_2)_4NH_3^+ \cdot (H_2PO_4)_3^- \cdot H_2O$	1,9-diamino-5-azanonane triphosphate monohydrate
3. Putrescine L-glutamic acid complex	$(NH_3^+(CH_2)_4NH_3^+) \cdot 2\left(^-O_2C(CH_2)_2CH(NH_3^+)CO_2^-\right)$	1,4-diaminobutane 2(glutamic acid)
4. Putrescine DL-glutamic acid complex	$(NH_3^+(CH_2)_4NH_3^+) \cdot 2\left(^-O_2C(CH_2)_2CH(NH_3^+)CO_2^-\right)$	1,4-diaminobutane 2(glutamic acid)
5. Putrescine L-aspartic acid complex	$(NH_3^+(CH_2)_4NH_3^+) \cdot 2\left(^-O_2C(CH_2)CH(NH_3^+)CO_2^-\right)$	1,4-diaminobutane 2(aspartic acid)
6. Propanediamine L-glutamic acid complex	$(NH_3^+(CH_2)_3NH_3^+) \cdot 2\left(^-O_2C(CH_2)_2CH(NH_3^+)CO_2^-\right)$	1,3-diaminopropane 2(glutamic acid)
7. Propanediamine DL-glutamic acid complex	$(NH_3^+(CH_2)_3NH_3^+) \cdot 2\left(^-O_2C(CH_2)_2CH(NH_3^+)CO_2^-\right)$	1,3-diaminopropane 2(glutamic acid)
8. Hexanediamine L-glutamic acid complex	$(NH_3^+(CH_2)_6NH_3^+) \cdot 2\left(^-O_2C(CH_2)_2CH(NH_3^+)CO_2^-\right) \cdot 2H_2O$	1,3-diaminohexane 2(glutamic acid) dihydrate

prepared by passing through a DOWEX1 (OH^-) column. The free amines so obtained were titrated against free acidic amino acids till the pH reached 7.0. All the complexes were crystallized by the addition of propanol to the aqueous solutions of the complexes. Table I lists the complexes examined by X-ray diffraction. Similar procedures for preparing complexes of tri- and tetra-amines with amino acids have not been successful.

Table II

Crystallographic parameters of the structures reported in this paper

Parameter	*Structure*							
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Chemical formula	$C_{10}H_{32}N_4Cl_4O_2$	$C_8H_{32}O_{13}N_5P_3$	$C_{14}H_{30}O_8N_4$	$C_7H_{15}O_4N_2$	$C_{13}H_{30}O_8N_4$	$C_{13}H_{28}O_8N_4$	$C_{13}H_{58}O_8N_4$	$C_{14}O_{10}H_{18}N_4$
Formula wt	382.2	469.3	382.4	191.2	358.4	368.3	368.3	446.5
D_c	1.22	1.59	1.37	1.36	1.39	1.39	1.39	1.32
Wavelength (Å)	1.542	0.711	0.711	0.711	1.542	0.711	0.711	0.711
μ (cm^{-1})	53.4	3.63	1.05	1.04	9.50	1.07	1.07	1.01
Cell[1] a	11.814	8.754	5.175	5.231	4.779	5.199	15.218	5.171
b	4.517	15.534	22.884	22.815	8.350	16.832	5.169	22.044
c	20.370	8.246	7.840	7.857	11.015	20.076	22.457	10.181
α	90.00	91.60	90.00	90.00	97.45	90.00	90.00	90.00
β	106.56	114.60	93.47	93.34	102.54	90.00	90.00	104.51
γ	90.00	74.11	90.00	90.00	89.98	90.00	90.00	90.00
Space group	P2	$P\bar{1}$	$P2_1$	$P2_1/n$	P1(C2)	$P2_12_12_1$	$Pna2_1$	$P2_1$
Z	4	2	2	4	1	4	4	2
No. of observed reflections	1480	2644	1490	1174	1385	1411	1945	2058
Sigma cut-off limit on Fs for parameters	5	5	5	5	5	5	5	5
No. of parameters refined (non-hydrogen atom)	175	239	234	118	212	226	225	270
No. of reflections above cut-off	1383	2221	917	863	1300	1276	993	1010
R-factor[2]	0.101	0.057	0.044	0.079	0.082	0.040	0.058	0.054
Goodness of fit[3]	1.32	1.21	1.22	1.42	0.88	0.61	1.56	1.26
$\{\Delta/\sigma\}^4_{max}$	0.219	0.052	0.024	0.036	0.031	0.067	0.143	0.015
ρ^5_{max}	0.59	0.60	0.23	0.77	0.43	0.21	0.28	0.27
ρ^6_{min}	−0.64	−0.46	−0.24	−0.25	−0.42	−0.18	−0.32	−0.23

[1]Distances are in Å; angles are in degrees.

[2]$R = \sum (||F(o)| - |F(c)||) / \sum |F(o)|$

[3]$GOF = (\sum w(|F(o)| - |F(c)|)^2/(m-n))^{1/2}$ where m is the number of reflections and n the number of parameters refined.

[4]Maximum shifted/esd in final cycle of refinement.

[5, 6]Maximum and minimum values of electron density in difference Fourier at the end of refinement.

D_c: Density calculated.

μ: Linear absorption coefficient.

4. X-ray examination and structure solution

All crystals were initially examined using X-rays from a sealed tube generator equipped with a Cu anode. The cell dimensions and crystal symmetry of the compounds investigated are recorded in Table II. After the determination of unit cell dimensions and space group photographically, the crystals were transferred to an Enraf Nonius 4-circle diffractometer. In the case of putrescine aspartic acid complex, visual examination of the Weissenberg photographs suggested the crystals to be monoclinic C2. Generally, X-ray

diffraction data were collected to a resolution of 0.85 Å, using $w/2\theta$ scan. In most cases, only one asymmetric unit of data was recorded. The reflection intensities were corrected for radiation damage (which was always insignificant), and Lorentz and polarization factors. The structures were determined using MULTAN or SHELEX86, the direct methods programs. In most of the complexes all the non-hydrogen atoms could be located in the initial E-map. However, in cadaverine dihydrochloride monohydrate and *sym*-homospermidine the water molecules could be located only after some initial refinement of the rest of the structure. During refinement (full matrix using a modified version of the SHELX76 program), most of the non-hydrogen atoms were associated with anisotropic temperature factors. One of the phosphate groups in *sym*-homospermidine, water oxygens in cadaverine and the two central carbon atoms of putrescine in the putrescine–aspartic acid complex, which were partially disordered, were associated with isotropic temperature factors. Most of the hydrogen atoms could be located in difference Fourier maps. Only these hydrogen positions have been used in the present analysis. Table II lists some parameters reflecting the quality of structure determination.

Putrescine–aspartic acid complex was initially refined in the space group C2. In this group, two aspartic acid molecules are related by a two-fold symmetry. However, statistically significant intensity differences exist in reflections related by monoclinic symmetry (R - mrg = 14.8%). Hence, the structure was also refined in triclinic P1. There was a significant improvement in the goodness of fit on reducing the space group symmetry. Hence, this complex might belong to triclinic P1 with a high degree of pseudosymmetry. The conformations of the two independently refined aspartic acid molecules are nearly identical. The side chain has a *gauche* configuration, which corresponds to the most frequently observed structure for aspartic acid. In this conformation, the charged main- and side-chain carboxyls are most separated.

Although similar efforts were expended on the preparation of aspartic acid and glutamic acid complexes, it was only with the latter that success was consistently achieved. At neutral pH, the diamines carry two positive charges while the acidic amino acids have one negative charge. Hence, the stoichiometry of the complexes is 2 amino acids : 1 diamine. None of the shorter diamine complexes has water of crystallization. In contrast, the structure of polyamine salts as well as hexanediamine glutamic acid contains water molecules.

5. Conformation of glutamic and aspartic acid molecules

As most complexes involve two independent glutamic acid molecules, it is of some interest to observe the extent of variability of glutamic acid conformation in these complexes. The mean lengths and bond angles are in good agreement with the average values determined from the 36 glutamic acid molecules for which coordinates are available in the Cambridge Structural Database (CSD)[24]. Hence, the major differences in the conformation of the glutamic acid molecules are due to differences in conformational angles (Table III).

Table III

Torsion angles in the glutamic acid molecules

Angle	*Structures*								
	3(1)	*3(2)*	*4*	*6(1)*	*6(2)*	*7(1)*	*7(2)*	*8(1)*	*8(2)*
O1–C′–Cα–N1	−11.5 (0.9)	−24.6 (0.9)	−21.9 (0.8)	−10.3 (0.6)	−5.0 (0.6)	−28.4 (1.9)	−30.6 (1.8)	−45.8 (1.6)	−13.8 (1.6)
O2–C′–Cα–N1	168.4 (0.5)	157.6 (0.6)	160.9 (0.5)	172.2 (0.4)	175.1 (0.4)	157.2 (1.2)	151.6 (1.3)	134.7 (1.2)	165.7 (1.2)
O2–C′–Cα–Cβ	−67.4 (0.8)	−76.9 (0.8)	106.8 (0.7)	−64.1 (0.5)	−59.4 (0.5)	−83.4 (1.5)	−83.0 (1.6)	−109.9 (1.3)	−70.2 (1.5)
O1–C′–Cα–Cβ	112.5 (0.8)	100.7 (0.7)	−70.3 (0.7)	113.3 (0.5)	120.2 (0.5)	90.8 (1.7)	94.6 (1.6)	69.4 (1.6)	110.2 (1.4)
C′–Cα–Cβ–C	−57.9 (0.80)	−61.3 (0.8)	−59.8 (0.8)	−55.6 (0.5)	−63.5 (0.5)	−63.4 (1.5)	−70.9 (1.5)	62.4 (1.4)	−49.5 (1.6)
N1–Cα–Cβ–C	64.9 (0.8)	64.5 (0.8)	66.0 (0.8)	66.2 (0.5)	60.9 (0.5)	55.5 (1.6)	53.8 (1.6)	178.1 (1.0)	73.0 (1.5)
Cα–Cβ–C–Cδ	−178.9 (0.6)	173.7 (0.6)	169.2 (0.6)	172.0 (0.4)	−175.3 (0.4)	158.6 (1.2)	171.1 (1.1)	74.1 (1.4)	−74.2 (1.7)
Cβ–C–Cδ–O_6	−176.8 (0.7)	126.5 (0.7)	−169.6 (0.7)	177.6 (0.4)	173.4 (0.4)	−142.9 (1.4)	85.2 (1.7)	−171.4 (1.1)	−173.6 (1.2)
Cβ–C–Cδ–O_7	7.2 (1.1)	−52.0 (0.9)	16.6 (1.1)	−4.1 (0.7)	−5.3 (0.7)	39.7 (1.9)	−94.8 (1.6)	8.1 (1.6)	6.4 (1.1)

(Angles are in degrees. ESDs are in parenthesis)

The conformation of the glutamic acid side-chain is characterized by the two dihedral angles X^1 and X^2. In nearly half (16) of the earlier 36 structures, a *gauche–trans* configuration is observed for these angles. Seven of the nine glutamic acids in the present analysis also possess this configuration. A recent analysis of glutamic acid side-chain conformations in proteins also shows a similar distribution[25]. All the three different conformations of the side-chain (*gauche–gauche*, *gauche–trans*, *trans–gauche*) observed in the present case have also been observed earlier. The overall conformation of one of the glutamic acid molecules in its complex with hexanediamine is different from the other eight structures. This glutamic acid has an internal hydrogen bond between the main-chain amino and side-chain carboxyl groups. A major source of difference in the glutamic acid molecules is in the orientation of the terminal carboxyl groups (Table III).

6. Conformation of diamines

In contrast to the nearly invariant features of amino acids, the conformation of diamines displays interesting variations. Putrescine and propanediamine are present in three and two complexes, respectively. Cadaverine, hexanediamine and *sym*-homospermidine are represented in only one structure. Most of the bond lengths and angles of the diamines agree, within the limits of experimental error, with the corresponding values observed in the structures of their inorganic salts.

The only hexanediamine molecule present in these complexes is in its most favourable all *trans* conformation. All the diamine structures reported earlier also have all *trans* conformation in the structures of their chloride salts.

Propanediamine conformation is different in its complex with L- and DL-glutamic acid molecules. In the achiral environment provided by DL-glutamic acid, propanediamine is in its most favourable *trans–trans* conformation. In contrast, it assumes a *trans–gauche* conformation during interaction with L-glutamic acid. The main feature of packing in the propanediamine–L-glutamic acid complex is the arrangement of the diaminopropane around dimers of antiparallel, juxtaposed L-glutamic acid molecules. The diaminopropane in the DL-complex is sandwiched between the two antiparallel glutamic acid molecules of the same chirality.

In two of the three complexes of putrescine, it is present in its most favourable all *trans* conformation. It assumes a *gauche–trans–gauche* conformation in its complex with aspartic acid. Both the structures have earlier been observed for putrescine in its chloride[20] and phosphate[26] salts, respectively.

Cadaverine adopts an all *trans* conformation in its chloride salt. However, the crystal structure has a high degree of pseudosymmetry. The water molecules present in the structure are partially disordered, and probably account for the earlier difficulties in its structure determination. The molecules, despite adopting an all *trans* conformation, display slight curvature. The only triamine, *sym*-homospermidine, included in the present analysis exists in its most favourable *trans* conformation.

The variations in the conformation of diamines within the limited range of chemical environment provided by inorganic ions and acidic amino acids in these crystal structures reflect the inherent conformational flexibility of these diamines. This is undoubtedly one of the reasons for their being excellent candidates for maximizing stabilizing interactions with other biomolecules. Table IV gives a summary of diamine conformations observed in different structures.

7. Hydrogen bonding in complexes

Diamines used in the present studies have six protons which can participate as donors in hydrogen bonds. Each acidic amino acid has three proton donors and four carboxyl

Table IV
Summary of conformations of diamines*

Diamine	*Observed conformations*
1,3-Diaminopropane	*trans–planar & trans–gauche*
1,4-Diaminobutane	*trans–planar & trans–gauche*
1,5-Diaminopentane	*trans–planar*
1,6-Diaminohexane	*trans–planar*

*The table also includes conformations observed in inorganic salts of diamines[11,19–21].

oxygens with potential for hydrogen bonding. Hence, in each structure, eight acceptor centres exist for 12 protons. Since each acceptor can participate in more than one hydrogen bond, the number of acceptor centres suffices to form all possible hydrogen bonds with protons. However, not all possible hydrogen bonds with carboxyl oxygens can be formed with the protons available. It is found that all the protons in the complexes participate in hydrogen bonding interactions. These hydrogen bonds are mainly with carboxyl oxygens, with the exception of hexanediamine glutamic acid complex. The amino group protons in hexanediamine glutamic acid complex hydrogen-bond with water oxygens also. Due to the large differences in the end-to-end distance of terminal amino groups of hexanediamine in its most favourable all *trans* geometry and in the distance between the carboxyl groups of glutamic acid in any conformation, it is not possible to form all hydrogen bonds between the amino groups and carboxylate ions directly. Hence, inclusion of water of crystallization in hexanediamine glutamic acid complex is probably due to the hydrogen-bonding properties of the diamine. None of the other complexes contain water of crystallization.

It has earlier been suggested that the inclusion of water molecules in phosphate salts of polyamines is determined by the hydrogen-bonding requirements of monohydrogen phosphate groups[27]. Each $(HPO_4)^{2-}$ anion prefers to act as a seven to nine acceptor centre. Considering the number of proton donors associated with different polyamines and the number of protons required for hydrogen bonding with neutralizing phosphate groups, Bratek-Weiwiorowska *et al.*[27] suggested a method of predicting the number of water molecules required for hydrogen bonding. However, the addition of a water molecule not only provides two protons but also introduces two additional acceptor centres. Hence, it is difficult to predict the number of included water molecules solely on the basis of hydrogen-bonding requirements of phosphate groups. In contrast to phosphate salts of spermidine and spermine, which contain three[28] and six[29] water molecules, respectively, the structure of *sym*-homospermidine phosphate contains only one water molecule per formula unit.

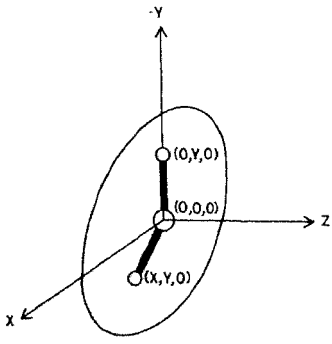


Fig. 1. Definition of the orthogonal coordinate system used for describing hydrogen bonding at the acceptor (or donor). In this system the hydrogen bonding group is on the *Y* axis. The atom bonded to this group is at the origin, while the atom bonded to the atom at the origin is in the *XY* plane.

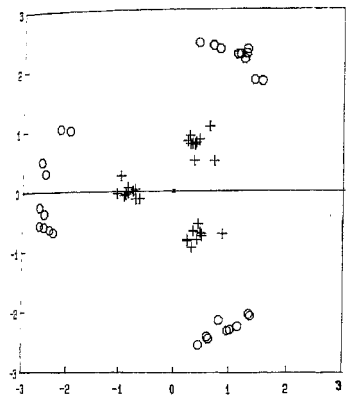


Fig. 2(a)

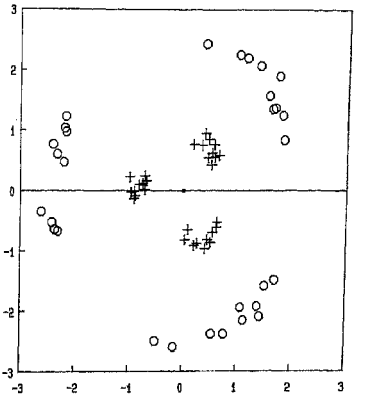


Fig. 2(b)

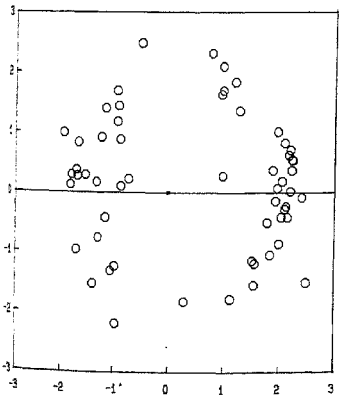


Fig. 2(c)

FIG. 2. XZ projection of the acceptor positions (○) and protons (+) bonded to the donors at the amino group of (a) diamines, (b) amino acids, and (c) XZ projection of the donor positions of hydrogen bonds at the carboxylates of amino acids.

The geometry of hydrogen-bonding interactions in polyamine complexes was analysed as follows. The molecules were transformed to an orthogonal coordinate system such that the acceptor (or donor) group is on the *Y* axis and the atom bonded to this group is at the origin of the system. The other atom bonded to the atom at the origin was made to lie on the *XY* plane (Fig. 1). The position of the groups hydrogen-bonded to the acceptor (or donor) centres was determined in this coordinate system. Figure 2a shows the *XZ* projection of the acceptor centres involved in hydrogen bonding with primary amino groups ofpolyamines along with the proton positions. In this projection, three acceptor groups approximately separated by 120° are anticipated for each amino group included in the diagram. Three clusters of acceptor centres are observed which are staggered with respect to the bond in the *XY* plane. The position of protons is also staggered with respect to this bond. This suggests that acceptor centres are clustered so as to make hydrogen bonds without torsional strain on the bond along the *Y*-axis. Figure 2b illustrates the bonding at the amino group of amino acids. The features are very similar to those of Fig. 2a except for slightly larger scatter in the positions of acceptors. These results illustrate the importance of hydrogen bonding in polyamine interactions. The smaller scatter of acceptor positions hydrogen bonded to polyamines is probable due to the flexibility of the polyamine backbone.

Figure 2c shows the donor centres around carboxylate acceptors of complexes. The donor centres are clustered close to the *XY* plane and very few occur close to the *YZ* plane, suggesting that the lone pair electrons are in the carboxylate plane. Also, the donor centres eclipsed to the other carboxylate oxygen (*syn* position) are clustered to a greater extent than the donor centres in *trans* position (*anti* position). The RMS deviations of the donor centres from their mean positions in the *syn* and *anti* positions are 1.19 and 1.38 Å, respectively.

8. Electrostatic interactions

Inorganic ions in crystal structures of polyamine salts primarily provide strong electrostatic interaction between the oppositely charged groups with some additional stabilization through hydrogen bonding. Hence, many of the proposed models of polyamine interactions with nucleic acids were based on the cationic nature of polyamines. It is, therefore, of interest to examine the extent of clustering of oppositely charged groups in polyamine complexes, which is likely to be a more realistic representation of the electrostatic component of their interaction.

In the following analysis it is assumed that each primary amino group carries a net positive charge and each carboxyl group carries a net negative charge. The positions of these groups were represented by the coordinates of the nitrogen and carboxyl oxygen atoms. The average number of charged groups occurring around amino groups of all polyamines in concentric shells of equal volume (50 $Å^3$) are shown in Fig. 3a. Figure 3b depicts the same distribution around the amino acid amino groups. The figure also includes the distance in Å of each shell from the charge centre under consideration. There is a clear clustering of negatively charged groups around the amino groups. This negatively charged shell extends to approximately 3.5 Å around the cationic group. This shell is surrounded by a shell of small but significant positive charge. The distribution of the positive and negative charges becomes nearly random after about

5 Å. Figures 3c and 3d depict the distribution of charges around the main-chain and side-chain carboxylates of glutamic and aspartic acids. In these distributions, the occurrence of the partial charge on the other carboxylate oxygen has been omitted. The pattern of charge clustering around the carboxylates is complementary to that around the amino groups.

9. Van der Waals interactions and crystal packing

An important aspect of the structures of these complexes is the effect of clustering of nonpolar atoms contributing significantly to stability through van der Waals interactions. All the complexes with glutamic acid have one of their cell dimensions close to 5.2 Å. This

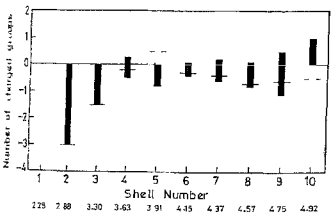


Fig. 3(a)

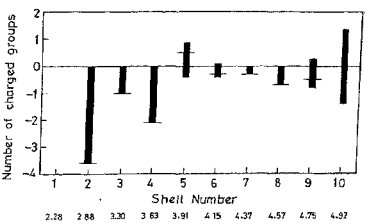


Fig. 3(b)

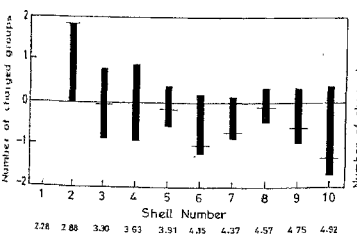


Fig. 3(c)

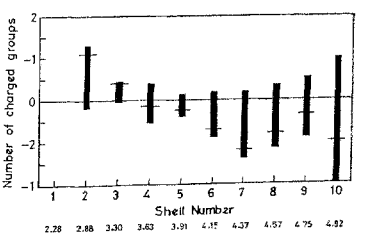


Fig. 3(d)

Fig. 3. Average number of charged groups around (a) amino groups of diamines, (b) amino groups of amino acids, (c) main-chain carboxylates of amino acids, and (d) side-chain carboxylates of amino acids, shown as a histogram corresponding to shells of 50 Å^3. The distance from the charge group under consideration to the edge of the shell is also indicated. The horizontal bar shows the average residual charge.

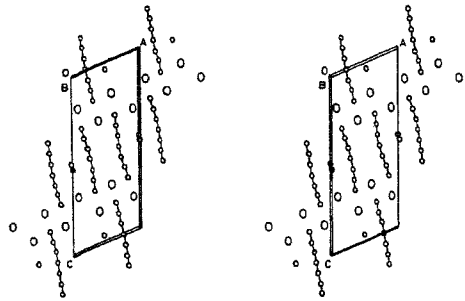


Fig. 4(a)

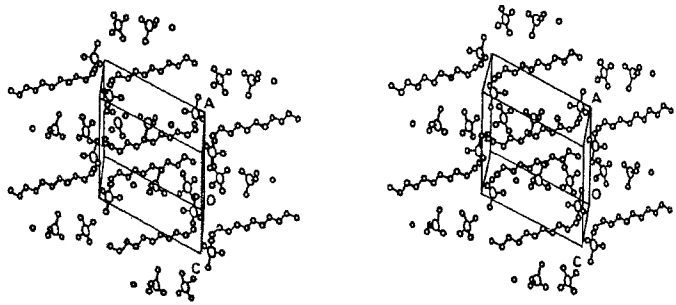
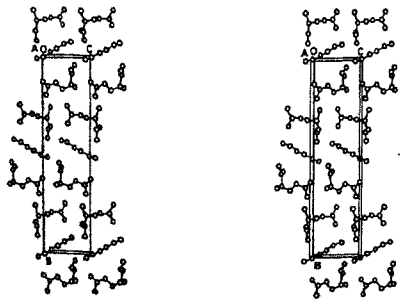


Fig. 4(b)



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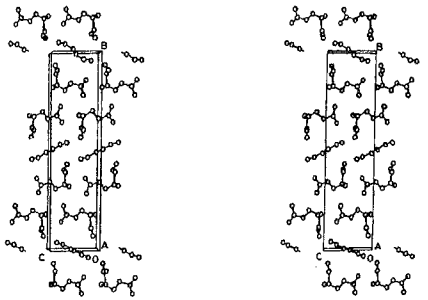


Fig 4(d)

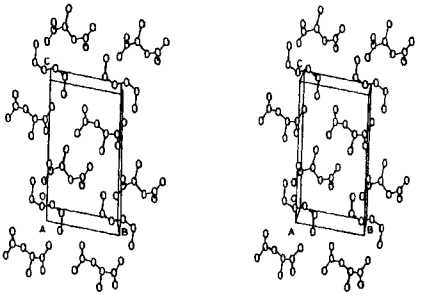


Fig 4(e)

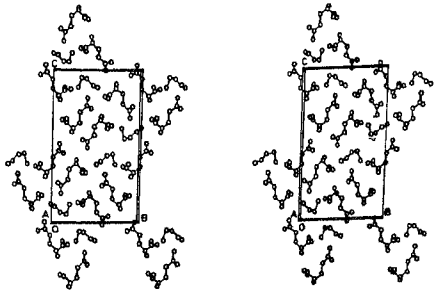
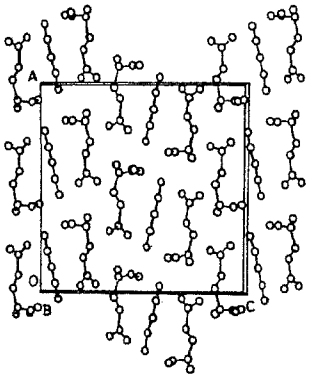


Fig. 4(f)



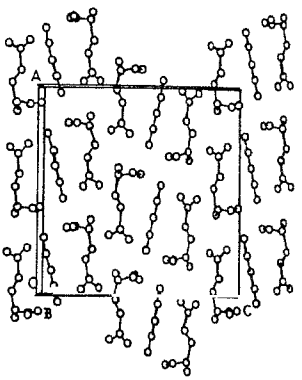
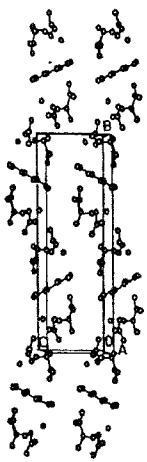


Fig. 4(g)



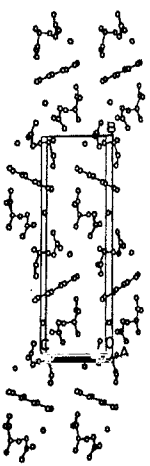


Fig. 4(h)

FIG. 4. Stereo packing diagrams of the structures reported in this paper. (a) Cadaverine dihydrochloride monohydrate; (b) *sym*-homospermidine triphosphate monohydrate; (c) putrescine L-glutamic acid complex; (d) putrescine DL-glutamic acid complex; (e) putrescine L-aspartic acid complex; (f) propanediamine L-glutamic acid complex; (g) propanediamine DL-glutamic acid complex; (h) hexanediamine L-glutamic acid complex.

allows side-by-side stacking of glutamic acid molecules, which leads to van der Waals stabilization as well as formation of hydrogen bonds between main-chain carboxyl and main-chain amino groups. The latter feature observed in nine of the eleven independent amino acid molecules has been proposed to be important for the prebiotic polymerization of amino acids[30]. Figures 4a–h gives the stereo packing diagrams of the structures discussed in this paper. The linear dimensions of putrescine and glutamic acid are similar in putrescine glutamic acid complex. This factor is utilized in the complex where putrescine is sandwiched between two *anti*-parallel glutamic acid molecules providing additional van der Waals interactions. In its interaction with the shorter aspartic acid, putrescine assumes a *gauche–trans–gauche* conformation, which is again suitable for maximizing van der Waals interaction with the complementary surface of aspartic acid. In contrast, the shorter diamine, propanediamine, exists in different conformations when complexed to L and DL-glutamic acid molecules. Only with DL-glutamic acid, the van der Waals interaction between the apolar groups of glutamic acid and propane diamine (Fig. 4g) contributes to stability. In the complex with L-glutamic acid, major share of van der Waals interaction occurs between the dimers of glutamic acid molecules. The propane diamine clusters around glutamic acid dimers, providing charge neutralization and hydrogen bonding (Fig. 4f). The linear dimension of the hexanediamine is longer than glutamic acid. In its complex with glutamic acid, van der Waals interaction between apolar groups is retained, which precludes the formation of all possible hydrogen bonds involving amino group protons (Fig. 4h). The hydrogen-bonding requirements are met by the inclusion of additional water molecules.

In *sym*-homospermidine, each formula unit of polyamine binds to three dihydrogen phosphate groups. Spermidine phosphate trihydrate crystal[28] structure contains 1.5 monohydrogen phosphate molecules per formula unit. In contrast to the single water molecule per amine in the present case, there are three water molecules in spermidine phosphate. Hence, hydrogen-bonding details of the two structures are different. However, in both the structures the primary and secondary amino groups participate in three and two hydrogen bonds, respectively. Although the details of organization of polar and apolar groups differ, both structures contain sheets of amine in which the zigzag backbone are arranged parallel to each other at a distance of about 4.5 Å and thus contribute to stability through van der Waals interactions.

An interesting heterogeneity is observed in the structure of cadaverine dihydrochloride monohydrate. This is the first example of a linear diamine with two molecules in the crystal asymmetric unit. Both the cadaverine molecules have an all *trans* conformation. However, due to small departures from ideal dihedral angle of 180°, the backbone exhibits a characteristic curvature in the two molecules (Fig. 4a). The molecular curvature allows reasonable van der Waals packing between neighbouring molecules with their convex surfaces towards each other. However, between neighbouring molecules with

facing concave surfaces, a cavity large enough to accommodate water channels is formed. Interestingly, these water molecules, in close proximity to apolar carbon atoms, are disordered. Although hydrophobic environment is anticipated to impose considerable ordering of water molecules[31], no fixed positions and hence regular order for water molecules in apolar regions are anticipated due to lack of hydrogen bonding.

The contribution of electrostatic, hydrogen bonding and van der Waals interactions in these structures was quantitated. Different energy terms were computed following the procedures suggested by Scheraga[32]. A dielectric constant of 4 was used for computing the electrostatic component. Table V shows the energy values of these terms for structures 3, 6 and 7. It was found that the contributions to crystal stabilization by hydrogen bonding, electrostatic and van der Waals terms were remarkably similar in different complexes. In the vicinity of polarizable groups, the electrostatic component might be anticipated to be lower.

Table V

Type of energy	*Energy values Energy (kcal/mol) structures*		
	3	*6*	*7*
H-bond	−13.7	−17.9	−10.5
Electrostatic	−186.3	−145.8	−165.9
van der Waals	−32.0	−57.2	−40.0
Total	−232.0	−220.9	−216.4

10. Conclusions

Earlier studies on polyamines were mainly concerned with the structures of their inorganic salts. The mode of interaction of polyamines with oligonucleotides was observed in certain studies as a consequence of using polyamines in the crystallization medium. The structures presented and analysed in this paper concentrate on the polyamines and illustrate different types of interactions that are important for understanding the role of polyamines.

It is clear from the foregoing analysis that hydrogen bonding also plays an important role in polyamine interactions. In every chemical context examined, hydrogen-bonding requirements of amino group protons were always satisfied. Additional stabilization by van der Waals interactions of apolar backbone atoms of polyamines also is significant. Most earlier models proposed for interaction of polyamines with other molecules were primarily concerned with the electrostatic component[33]. Although Feuerstein *et al.*[34] had considered all the interactions in their modelling, such studies are further complicated by the possibility of water-mediated hydrogen bonding. In any modelling study it is extremely difficult to take into consideration all these factors. Hence, most of the earlier studies have yielded poor agreement with experimental data[12,34].

These studies also have implications on the specificity of polyamines. In the course of these investigations, numerous attempts were made to crystallize complexes of tri- and

tetra-amines. However, the efforts were not successful, probably due to the somewhat lower specificity of diamines in comparison to tri- and tetra-amines. The higher specificity of the more complex polyamines probably does not permit the formation of stable complexes with any other arbitrarily chosen anionic molecules. A similar situation was encountered in attempts of crystallization of diamines with aspartic and glutamic acid molecules. Glutamic acid, with greater flexibility of its side-chain, led to more frequent success in obtaining crystals.

The results presented in this paper illustrate the inherent ability of polyamines to participate in a variety of complexes through hydrogen bonding, electrostatic and van der Waals interactions. The structures presented illustrate the flexibility in the backbone geometry of polyamines and the specificity of their bonding patterns. These properties of polyamines make them ideal candidates as biological cations.

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