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Crystal Structure of Propylguanidinium Diethyl Phosphate

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The crystal structure of the propylguanidinium salt of diethyl phosphate has been derived from three-dimensional X-ray data collected by counter methods. The space group is $P2_1/c$. The structure was refined by block diagonal least squares techniques to $R = 0.089$ for 1798 observed reflections. The terminal carbon atoms of the ethyl and propyl moieties vibrate strongly and one of them is possibly disordered. The conformation of the diethyl phosphate ion is *gauche* at both P–O bonds. The propylguanidinium ion is nearly planar. The two amino groups of the guanidinium residues are linked by two relatively strong hydrogen bonds to the *oxo* oxygen atoms in the same phosphate group.

Interactions between nucleic acids and proteins play a fundamental role in biology and it would appear to be of importance to establish the stereochemistry of these interactions on the atomic level. A general model of the structure of complexes between protamines and DNA has been proposed,[1,2] in which the polypeptide chain winds helically around the DNA molecule and over, or in, the small groove on it. Bonding occurs between the acid phosphate groups and the basic side chains of the protein. The detailed stereochemistry of this interaction is, however, not described and very little appears to be known about it. We have therefore started an investigation of the structures of some model complexes between secondary phosphates and derivatives simulating basic protein side chains. In this paper the crystal structure of the complex between diethylphosphoric acid and propylguanidin is described, the latter substance being analogous to the arginine side chain.

EXPERIMENTAL

The potassium salt of diethyl phosphate was prepared by treating an alcoholic solution of triethyl phosphate with potassium hydroxide.[3] The salt was suspended in ethyl acetate and the suspension acidified with hydrochloric acid. Removal of potassium chloride and evaporation yielded diethylphosphoric acid. It was mixed with an equivalent amount of propylguanidin [4] in methyl alcohol. The reaction was accompanied by considerable heat evolution. Crystals of the complex big enough for X-ray crystallographic analysis were obtained by letting ethyl acetate vapor slowly enter the solution in a closed system.

The crystals were very soft and easily deformed. They had the shape of plates with (010) predominant.

Weissenberg photographs showed the crystals to have space group $P2_1/c$. Cell dimensions were measured on a manual diffractometer and found to be $a = 8.292(1)$ Å, $b = 19.527(3)$ Å, $c = 12.231(2)$ Å, and $\beta = 131.97(1)°$. The uncertainties quoted are estimated standard deviations.

Table 1. Positional ($\times 10^5$) and thermal parameters ($\times 10^4$) with estimated standard deviations. The temperature factor is given by $\exp -(B_{11}h^2 + B_{22}k^2 + B_{33}l^2 + B_{12}hk + B_{13}hl + B_{23}kl)$.

Atom	x	y	z	B_{11}(B)	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
P	19676	15981	21649	244	52	82	24	144	14
	21	9	12	4	1	1	3	4	2
O1	32031	14751	16183	336	70	142	51	286	22
	55	21	35	1	2	5	6	13	5
O2	20660	9187	28677	666	54	261	−9	561	21
	73	24	45	19	2	74	10	22	6
O3	31573	20902	34077	281	72	101	−8	142	−23
	51	24	34	10	10	4	8	11	5
O4	2732	17904	8258	285	84	77	70	103	−10
	52	24	31	10	2	4	8	11	5
N1	72467	32491	27690	288	57	87	18	144	−10
	59	25	36	13	2	5	8	13	5
N2	74332	27530	11418	250	67	85	59	97	−5
	61	26	38	12	2	5	9	13	6
N3	4075	28061	35951	314	68	85	50	122	6
	67	27	39	14	3	5	10	14	6
C1	55131	13095	26224	374	83	264	64	403	56
	97	41	74	22	4	11	15	29	11
C2	6088	10317	17578	600	92	449	96	803	−25
	123	48	91	32	5	18	19	45	15
C3	9382	3069	19828	1037	115	458	97	943	−28
	181	58	114	59	7	23	30	67	20
C4	12437	−2574	29281	3012	48	1565	−4	3959	98
	294	50	213	140	4	72	39	196	28
C5	83159	29365	24989	298	40	109	3	226	−7
	75	28	47	15	2	6	10	17	6
C6	49041	33756	16850	332	88	144	106	228	−10
	90	39	58	18	4	8	15	21	9
C7	41350	37177	22896	510	129	287	168	466	−28
	116	50	84	29	6	13	21	36	15
C8	17372	38237	11903	487	180	354	156	473	−39
	130	64	103	31	8	17	26	41	21
H N1	7986	3466	3641	6.6					
	72	24	51	1.2					
H1N2	6483	2902	537	4.7					
	60	21	40	1.0					
H2N2	8412	2452	1023	6.4					
	70	23	47	1.2					
H1N3	1520	2548	3429	5.8					
	69	23	46	1.2					
H2N3	666	3085	4313	8.3					
	78	29	53	1.4					

Table 2. Distances (Å) and angles (°). E.s.d. in parenthesis.

	Distances Uncorrected	Corrected for "riding motion"		Angles
P–O1	1.572(4)	1.596	O1–P–O2	107.3(2)
P–O2	1.552(5)	1.564	O1–P–O3	111.0(2)
P–O3	1.483(3)	1.510	O1–P–O4	105.1(2)
P–O4	1.486(3)	1.521	O2–P–O3	103.5(2)
O1–C1	1.462(7)	1.478	O2–P–O4	112.9(3)
O2–C3	1.455(12)	1.465	O3–P–O4	116.8(2)
C1–C2	1.471(10)	1.526	P–O1–C1	122.5(3)
C3–C4	1.491(17)	1.540	O1–C1–C2	108.5(5)
N1–C5	1.289(7)	1.302	P–O2–C3	121.3(5)
N2–C5	1.340(6)	1.373	O2–C3–C4	109.7(9)
N3–C5	1.327(6)	1.376	N2–C5–N3	117.6(4)
N1–C6	1.466(7)	1.489	N2–C5–N1	123.0(4)
C6–C7	1.425(9)	1.485	N3–C5–N1	119.4(4)
C7–C8	1.494(10)	1.539	C5–N1–C6	124.9(4)
N1–HN1	0.91(5)		N1–C6–C7	113.1(5)
N2–H1N2	0.69(4)		C6–C7–C8	112.8(7)
N2–H2N2	1.09(4)			
N3–H1N3	1.18(4)			
N3–H2N3	0.93(5)			
N1–O4′	2.816			
N2–O4′	2.878			
N2–O3′	2.828			
N3–O3′	2.813			
N3–O1′	3.090			

By flotation in mixtures of *m*-xylene and bromobenzene the density of the crystals was found to be 1.15 g/cm³, corresponding to four (calc. 4.00) formula units $(C_2H_5O)_2PO(OH).CH_3(CH_2)_2.NH.C(NH)NH_2$ in the unit cell.

A roughly cube-shaped crystal fragment of dimensions $0.48 \times 0.35 \times 0.27$ mm was used for the intensity measurements, which were carried out on a Picker automatic diffractometer using graphite monochromated Mo$K\alpha$ radiation ($\lambda = 0.71069$ Å) and $\omega/2\theta$ scan technique. All 3217 reflections with $2\theta < 55°$ were measured. Of these, 1798 had intensities above a 2.5σ cut-off limit and were used for the structure analysis. The intensities of three standard reflections decreased slightly (by about 5 %) during the experiment and the measurements were corrected accordingly. The standard deviations of the intensities were based on counter statistics and an assumed 2 % instrumental instability. Corrections for absorption and secondary extinction were applied. The atomic scattering factors were those of Hanson *et al.*[5] for non-hydrogen atoms and of Stewart *et al.*[6] for hydrogen atoms. All programs used are described in Ref. 7.

STRUCTURE ANALYSIS

The Patterson map showed the position of the phosphorus atom and the orientation of the phosphate group, and a weighted Fourier synthesis revealed the positions of all non-hydrogen atoms. The structure was refined by block diagonal least squares techniques, applying anisotropic temperature factors for the non-hydrogen atoms. The positions of the hydrogen atoms were postulated and an isotropic temperature factor assigned to them. The param-

Table 3. Observed and calculated structure factors (× 10).

Table 3. Continued.

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eters were refined only for the hydrogen atoms of the guanidinium group. Those of the other hydrogen atoms (C–H 1.05 Å, $B=18$ Å^2) were kept constant due to the large thermal vibrations of the carbon atoms. This holds especially for the terminal carbon C4 atom of one of the ethyl groups, for which the maximum temperature factor is 56 Å^2, (r.m.s. amplitude 0.85 Å). This direction is roughly normal to the terminal C–C bond, and a strong torsional vibration about the O2–C3 bond probably takes place in the crystal. Alternatively, the structure may be described in terms of disorder of one or

Table 4. Parameters of thermal ellipsoids ($10^3 \times$ Å).

Atom	R.m.s. ampli-tude	Components along x	y	z	Atom	R.m.s. ampli-tude	Components along x	y	z
P	317	058	314	038	C1	414	124	377	225
	251	−218	023	045		338	043	−125	341
	183	185	019	243		258	337	−045	182
O1	372	104	363	063	C2	460	078	335	363
	256	−227	039	036		421	376	239	456
	241	234	035	320		251	249	−097	027
O2	366	411	112	108	C3	511	264	411	−056
	341	249	204	369		443	509	−055	563
	301	037	223	224		387	−181	226	163
O3	375	008	369	−060	C4	845	987	039	1077
	291	−226	044	082		438	−268	205	153
	197	216	107	257		253	066	223	−064
O4	419	131	390	−030	C5	281	044	273	−028
	280	−219	102	057		250	279	−052	059
	176	185	−001	234		212	154	024	279
N1	339	088	319	036	C6	437	157	396	−038
	277	−239	093	032		266	−047	099	213
	189	189	009	252		232	298	−047	250
N2	376	133	431	−035	C7	537	174	481	−084
	273	−168	114	102		348	236	074	449
	178	204	−010	230		272	303	−106	094
N3	378	150	337	−030	C8	613	125	575	−109
	293	−225	133	051		405	142	109	471
	185	191	−001	247		286	366	−063	187

more of the ethyl carbon atoms, but refinements based on such a model did not significantly improve the agreement.

The final value of R is 0.089 ($R_w = 0.107$) for the observed reflections. The positional and thermal parameters of the atoms are given in Table 1, and in Table 3 observed and calculated structure factors are compared.

RESULTS AND DISCUSSION

The bond lengths and angles are given in Fig. 1 and Table 2, in which also the values obtained by "riding motion" correction are included. Estimated

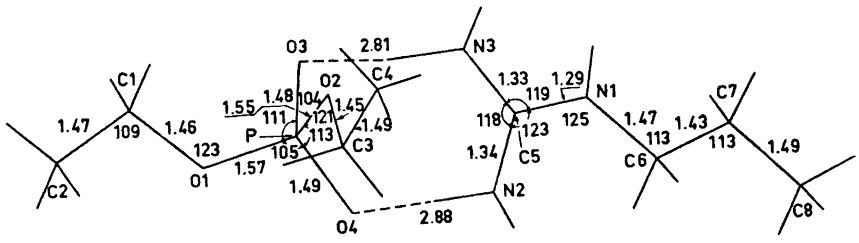

Fig. 1. Bond lengths (Å) and angles (°) (uncorrected values).

standard deviations in bond lengths are 0.003 – 0.004 Å for P – O bonds, 0.005 – 0.006 Å for C – N bonds and 0.01 – 0.02 Å for C – C bonds, but the errors may be considerably greater due to the large thermal effects. The parameters of the thermal ellipsoids are given in Table 4.

The diethyl phosphate ion. The structure of this ion may be described in terms of two approximately planar zig-zag chains of atoms, O4 – P – O1 – C1 – C2 and O3 – P – O2 – C3 – C4, whose planes are nearly normal to each other (91°). The conformation is thus *gauche* at both P – O ester bonds. The same conformation was found for the barium salt of diethyl phosphate [8] and appears to be the preferred one for dialkyl phosphates.[9] The bond lengths and angles at the phosphorus atom are normal, the angle O4 – P – O3 being greater (116.8°) than the others. As mentioned above the thermal motion of the ethyl groups is exceptionally large and the positions of the terminal carbon atoms poorly defined. As usual in such cases the corresponding bond lengths are found too short. Similar effects were observed in crystals of barium diethyl phosphate.[8]

The propyl guanidinium ion. All non-hydrogen atoms in this ion lie in the same plane to within 0.06 Å. Atoms N1 – N2 – N3 – C5 are coplanar within the limits of error. The same holds for N1 – C6 – C7 – C8, and the angle between these planes is 6.3°. The C – N bonds to the primary amino groups are found to be 1.340 Å and 1.327 Å, considerably longer than that of 1.289 Å to the secondary amino group. The difference is, however, hardly significant, and in the most accurate analysis of a compound of this type, that of arginine hydrochloride monohydrate,[10] the two types of C – N bonds differ in length by only 0.007 Å. The C – C bonds in the propyl residue are found too short due to the large thermal vibrations also in this part of the structure.

The crystal structure. The crystal is built up of layers of propylguanidinium and diethyl phosphate ion parallel to the (x,z)-plane. Within the layers the ions are linked together by hydrogen bonds. There are no such bonds between the layers, the contact being mainly between the hydrocarbon "tails" which point out from the hydrogen-bonded core of the layers. This explains the

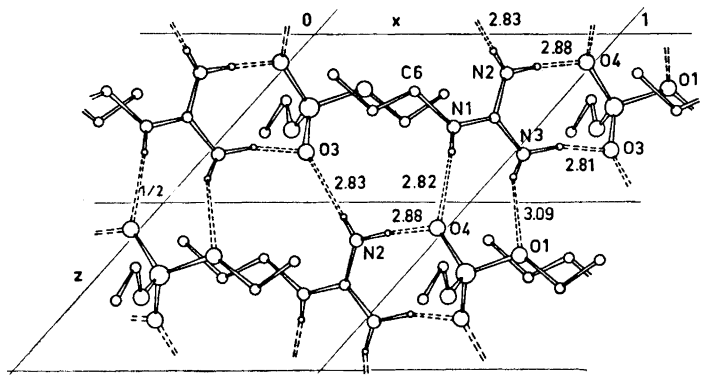

Fig. 2. The structure projected along b.

mechanical properties and the low density of the crystals, as well as the exceptionally large amplitudes of vibration of the atoms in the hydrocarbon parts of the ions.

Each propylguanidinium ion is connected by four relatively strong N – H···O hydrogen bonds (lengths 2.81 – 2.88 Å) to three neighbouring phosphate groups. The fifth hydrogen atom of the guanidinium group, H2N3, is probably forming a weak hydrogen bond between N3 and the ether oxygen atom O1, which are at a distance of 3.09 Å (Fig. 2).

Two of the strong hydrogen bonds, N3 – H···O3 and N2 – H···O4, connect the propylguanidinium ion to the two *oxo* oxygen atoms in the same diethyl phosphate ion. Apparently the bonding between these two ions is stronger than the bonding to their neighbours, and the crystal may be considered to be built up of propylguanidinium diethyl phosphate "molecules". The relative orientation of the two ions in the molecule is presumably governed mainly by the two *intra*-molecular hydrogen bonds. The guanidinium plane is roughly parallell to the O4PO1C1C2 plane at a distance of about 1 Å, but slightly twisted about the molecular axis so as to make the two hydrogen bonds fairly linear. The angle at the hydrogen atom is 170° in one of the bonds (N3···O3), 167° in the other. The atoms O3 and O4 are at distances of 0.28 Å and 0.75 Å, respectively, from the guanidinium plane and on the same side of it.

The geometry and bonding described above for the propylguanidinium diethyl phosphate complex may have important bearings on the structure of complexes between nucleic acids and basic proteins, such as protamines and histones, as the charged arginine side chains may be bonded in the same manner as the propylguanidinium ion.

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