The Stereochemical Structure of Disodium DL-Glycerol 3-Phosphate Hexahydrate, the D Isomer of which is an Inhibitor of Triose Phosphate Isomerase

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(Received 10 May 1972)

The stereochemistry of DL-glycerol 3-phosphate was studied by X-ray-crystallographic techniques. All the bond lengths and angles are within normally accepted limits except the ester bond, which is one of the largest yet noted, being 0.1637 nm. The conformation of the molecule is such that an intramolecular hydrogen bond is formed between the hydroxyl group on the β -carbon atom and the phosphate group. The crystal, which was grown by alcohol diffusion into an aqueous solution, is held together by sodium co-ordination and a complex system of hydrogen bonds. A table of the observed and calculated structure factors, $|F_{\text{obs}}|$ and F_{calc} , has been deposited as Supplementary Publication 50010 at the National Lending Library for Science and Technology, Boston Spa, Yorks. LS23 7BQ, U.K., from whom copies can be obtained on the terms indicated in *Biochem. J.* (1972) 126, 5.

D-Glycerol 3-phosphate is closely related to the substrates of triose phosphate isomerase (EC 5.3.1.1), dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate (Fig. 1), and it is a competitive inhibitor of the enzyme activity (Johnson & Wolfenden, 1970). The structural relationship is shown in the Fischer diagrams of Fig. 1, which also shows the course of the enzyme-catalysed reaction, as suggested by Rose (1962), and the structures of the proposed intermediate transition states.

The three-dimensional structure of D-glycerol 3-phosphate has been studied by X-ray crystallography as part of a programme to study analogues of these substrates and reaction intermediates, and the substrates themselves, when these can be crystallized, with a view to providing precise stereochemical data for use in elucidating details of the catalytic mechanism. When current crystallographic studies of the enzyme (Banner *et al.*, 1971) are more advanced the structures of these individual molecules will be compared in detail with those seen in complexes involving the enzyme.

Results and Discussion

A molecule of D-glycerol 3-phosphate is shown in Fig. 2, and a list of the bond lengths and angles for this structure is given in Table 1. Throughout this paper the use of ' $\pm$ ' indicates one estimated standard deviation.

Since 1960 there has been considerable interest in organic phosphate structures; the phosphate groups

of some of these are compared in Table 2. In glycerol 3-phosphate the bond lengths from the phosphorus to the terminal oxygen atoms are in good agreement with the previous structures, whereas the ester bond is one of the longest yet noted, being 0.1637 nm. In common with other structures one of the terminal oxygen atoms makes an O-P-O angle with the ester bond oxygen of significantly less than the tetrahedral angle.

Several structures have also been published which contain glycerol moieties. These are compared with the present structure in Table 3. The oxygen-carbon bond lengths on the glycerol section are all equal to the accepted value of 0.143 nm (Kennard, 1962) to within one estimated standard deviation and O-1 forms the normally high bond angle between P and C-3, in this case 121.9°. In the preliminary publication of this work (Fenn & Marshall, 1971) the C-C bonds were quoted as 0.152 nm, but further refinement has decreased these to 0.1495 ± 0.0015 nm and 0.1510 ± 0.0015 nm. This may seem short compared with the normally accepted value of 0.1541 nm (Kennard, 1962) but Lide (1962) has suggested that the C-C bond when both carbon atoms have sp^3 -type bonding is 0.1526 ± 0.0002 nm. This seems to be confirmed by the structures listed in Table 3 and so, since the present bonds are within two estimated standard deviations of this value, they are not considered to be significantly short.

With the nomenclature of Bastiansen (1949) the basic configuration of the glycerol moiety can be described as $\alpha\gamma$, which is the second lowest energy state as shown in Table 4 (Koningsveld, 1968), but the

Fig. 1. Fischer diagrams of the proposed transition between glyceraldehyde 3-phosphate and dihydroxyacetone phosphate and also the inhibitor glycerol 3-phosphate

stagger along the present C-2-C-3 bond is not perfect (Fig. 3), probably because of the intramolecular hydrogen bond between O-4 and O-5. This bond is possible because of the abnormal configuration of the bonds C-2-C-3...O-1-P as shown in Fig. 3 and because of the orientation of the phosphate oxygen atoms bringing O-4 to 0.266 nm from O-5. The existence of this hydrogen bond is further supported by the indication of a hydrogen atom in the final difference electron-density map. Although this map showed considerable 'noise', a probable hydrogen position was found at 0.103 nm from O-5 and forming an O-H...O angle of $171 \pm 16^\circ$. The result of this hydrogen bond is to form a puckered, seven-membered ring holding C-2, C-3 and the phosphate group in a stable configuration. This bond is of considerable

interest, since it can be seen in Fig. 1 that the hydroxyl group that we have found to be bonded to the phosphate group in the inhibitor plays an important role in Rose's (1962) suggested transformation via the two intermediate states in the enzymic action.

Of the seven hydrogen atoms associated with the molecule indications of the positions of six were found in the final difference map and it was possible to refine five of these positions. All six hydrogen atoms are listed in the tables, but no standard deviation is given for the postulated atom H-6.

Six water molecules are associated with each molecule of glycerol 3-phosphate in the crystal structure, and they are involved in close approaches to other oxygen atoms, forming an intricate hydrogen-bonding scheme which holds the crystal together as shown in

Fig. 2. Projection of D-glycerol 3-phosphate molecule showing the numbering convention

Fig. 4. The most probable bonding scheme has been selected and presented in Table 5. It was determined by considering the bond lengths and angles between neighbouring oxygen atoms. It may be noted that all

oxygen atoms, except O-1, are involved in four relations with other atoms, i.e. hydrogen bonds, sodium co-ordination or covalent bonds. The exception is only involved in three relations. Apart from the hydrogen atoms associated with the molecule only one peak was found in the final difference map which could represent a hydrogen atom along any of the probable hydrogen bonds. It was considered that no significance could be attached to this peak, which was one of many found in the difference electron-density map with a height barely above background.

The sodium co-ordination also helps to bind the crystal together and is shown in Fig. 5 and listed in Table 5. All three sodium atoms are six co-ordinated, but the approximation to octahedral is better for Na-1 and Na-2, which are situated on symmetry elements.

From a study of other inhibitors of triose phosphate isomerase it may be possible to ascertain if these have any similar stereochemical features, especially in the region of C-2, and also to find if the hydroxyl group associated with C-2 has any constraints on its position as in the present material.

Table 1. Intramolecular bond lengths and angles in glycerol 3-phosphate

One estimated standard deviation is also given.

Intramolecular bond lengths (nm)

P-O-1	0.1637 ± 0.0007	H-1-O-6	0.094 ± 0.016
P-O-2	0.1516 ± 0.0007	H-2-C-1	0.106 ± 0.017
P-O-3	0.1510 ± 0.0007	H-3-C-1	0.099 ± 0.018
P-O-4	0.1493 ± 0.0008	H-4-O-5	0.103 ± 0.017
		H-5-C-2	0.094 ± 0.016
O-1-C-3	0.1436 ± 0.0011	H-6-C-3	0.107^*
C-3-C-2	0.1495 ± 0.0015		
C-2-C-1	0.1510 ± 0.0012		
C-1-O-6	0.1428 ± 0.0013		
C-2-O-5	0.1441 ± 0.0011		

Intramolecular bond angles ($^{\circ}$)

O-1-P-O-2	107.8 ± 0.4	C-1-O-6-H-1	105 ± 11
O-1-P-O-3	101.2 ± 0.4	O-6-C-1-H-2	102 ± 10
O-1-P-O-4	106.0 ± 0.4	O-6-C-1-H-3	107 ± 11
O-2-P-O-3	114.2 ± 0.4	C-2-C-1-H-2	109 ± 9
O-2-P-O-4	112.3 ± 0.4	C-2-C-1-H-3	112 ± 10
O-3-P-O-4	114.1 ± 0.4	H-2-C-1-H-3	115 ± 14
		C-1-C-2-H-5	103 ± 10
P-O-1-C-3	121.9 ± 0.6	C-3-C-2-H-5	101 ± 11
O-1-C-3-C-2	113.0 ± 0.7	C-2-C-3-H-6	92^*
C-3-C-2-C-1	111.0 ± 0.8	O-1-C-3-H-6	128^*
C-2-C-1-O-6	111.0 ± 0.8	H-5-C-2-O-5	117 ± 11
C-3-C-2-O-5	112.7 ± 0.7	C-2-O-5-H-4	106 ± 10
C-1-C-2-O-5	111.0 ± 0.8	O-5-H-4-O-4	171 ± 16

* Suggested position of H-6, see the text.

Table 2. Comparison of P-O bond lengths (nm) and angles (°) and their average estimated standard deviations for some phosphate groups

The numbering scheme used here is not necessarily that used in the original paper.

Material	Reference	Ester bond O-1	Av. estimated s.d.				Av. estimated s.d.						
			O-2	O-3	O-4	O-1-P-O-2	O-1-P-O-3	O-1-P-O-4	O-2-P-O-3	O-2-P-O-4	O-3-P-O-4		
DL-Glycerol 3-phosphate	Present work	0.1637	0.1516	0.1510	0.1493	0.0007	107.8	101.2	106.0	114.2	112.3	114.1	0.4
Glycerol 2-phosphate	Ul-Haque & Caughlan (1966)	0.1618	0.1527	0.1516	0.1457	0.0012	108.3	109.7	100.8	113.3	110.4	113.5	0.4
DL-O-Serine phosphate	Putkey & Sundaralingam (1970)	0.1590	0.1530	0.1522	0.1499		108.2	106.2	103.7	108.6	114.3	115.1	
Thymidylate	Trueblood <i>et al.</i> (1961)	0.1587	0.1514	0.1486	0.1474	0.0011	107.6	107.6	102.1	106.0	114.4	118.4	0.7
Glucose 1-phosphate	Bevers & Maconochie (1965)	0.159	0.153	0.152	0.148	0.003							
2-Aminoethanol phosphate	Kraut (1961)	0.1591	0.1557	0.1503	0.1493	0.0005	106.2	109.6	103.9	109.3	109.8	117.4	0.2
Diethyl phosphate	Kyogoku & Iitaka (1966)	0.162	0.159*	0.152	0.152	0.002	103.5	104.6	111.9	110.5	103.4	121.6	0.8
Uridine 5'-phosphate	Shefter & Trueblood (1965)	0.164	0.155	0.152	0.151		103	108	118	108	110		
1-Naphthyl phosphate	{ Chi-Tang Li & Caughlan (1965)	0.1592	0.1529	0.1505	0.1494	0.0008	104.4	103.6	110.8	108.3	111.9	116.8	0.4
		0.1591	0.1573	0.1475	0.1472	0.0008	105.2	109.7	102.9	111.5	109.7	116.6	0.4
Pyridoxal phosphate oxime	Barrett & Palmer (1969)	0.159	0.156	0.148	0.146	0.001	107.1	109.0	103.1	108.7	110.7	117.6	0.6
Adenosine 5'-phosphate	Kraut & Jensen (1963)	0.1610	0.1566	0.1514	0.1495	0.0008	106.5	108.7	105.7	110.2	106.9	118.2	0.4
Adenosine 3'-phosphate	Sundaralingam (1966)	0.1612	0.1579	0.1486	0.1477	0.0003	105.5	103.4	110.7	112.3	107.1	117.2	0.1
Hydrolysed co-carboxylase	Karle & Britts (1966)	0.1607	0.1570	0.1519	0.1498	0.0014	105.6	104.8	109.6	111.8	107.5	116.9	0.8

* Second ester bond.

Table 3. Comparison of bond lengths (nm) and angles (°) with their average estimated standard deviations of glycerol moieties

The numbering system used here is not necessarily that given in the original paper.

Material	Reference	O-1-C-1	C-1-C-2	C-2-C-3	C-3-O-3	C-2-O-2	A _v . estimated s.d.	O-1-C-1-C-2	C-1-C-2-C-3	C-2-C-3-O-3	C-1-C-2-O-2	C-3-C-2-O-2	A _v . estimated s.d.	Ester bond angle	Estimated s.d.
DL-Glycerol 3-phosphate	Present work	0.1436*	0.1495	0.1510	0.1428	0.1441	0.0012	113.0	111.0	111.0	112.7	111.0	0.7	121.9	0.6
Glycerol 2-phosphate	Ul-Haque & Caughlan (1966)	0.1439	0.1509	0.1477	0.1341	0.1368*	0.0024	113.8	113.7	110.9	107.8	108.0	1.1	120.3	0.9
L-Glycerol 3-phosphoryl- choline†	Sundaralingam & Jensen (1966)	0.141	0.151					108	112					118	
L-Glycerol 3-phosphoryl- choline	Abrahamsson & Pascher (1965)	0.1390*	0.1553	0.1538	0.1449	0.1433	0.0008	109.1	109.6	111.6	107.6	107.5	0.5	115.8	0.5
Sulpholipid	Okaya (1964)	0.145*	0.146	0.152	0.139	0.144	0.003	114	111	113	111	110	3.0	117	3.0
Diglyceride of thiado- decanoic acid	Larsson (1963)	0.150	0.149	0.158	0.149	0.147			100		111	111			

* Ester bond oxygen.

† Average bond lengths and angles.

Table 4. *Relative energies of the possible conformations of glycerol as given by Koningsveld (1968)*

Conformation	$\alpha \alpha$	$\alpha \gamma$	$\gamma \gamma$	$\beta \alpha$	$\beta \gamma$	$\beta \beta$
Energy (kJ/mol)	0	2.51	5.86	18.00	20.50	36.00

Fig. 3. *Torsion angles (°) in D-glycerol 3-phosphate*

Summary of crystal data

Formula: $\text{Na}^+, \text{CH}_2\text{OH}-\text{CH}(\text{OH})-\text{CHOPO}_3^{2-}, 6\text{H}_2\text{O}$

Formula weight: 323 kg/kg-atom

Density measured: $1.606 \times 10^3 \text{ kg}^{-3}$

Density calculated: $1.619 \times 10^3 \text{ kg}^{-3}$

Crystal system: Monoclinic

Space group: $C 2/c$

Unit-cell parameters: $a = 2.410 \text{ nm}$, $b = 0.835 \text{ nm}$,
 $c = 1.380 \text{ nm}$, $\beta = 105.38^\circ$

Unit-cell volume: 2.8727 nm^3

Number of formula units in unit-cell: 8

X-radiation used: $\text{Cu } K\alpha$

Wavelength of radiation: 0.154178 nm

Density measurement was by the flotation method and the cell parameters were measured by applying the method of least squares to six reflexions whose reflexion angles were measured on a Stöe four-circle diffractometer.

Experimental

DL-Glycerol 3-phosphate was obtained from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K.; it was originally recrystallized from an aqueous solution containing alcohol, but this resulted in poor-quality crystals with ill-defined faces. Superior-quality crystals were produced when the aqueous solution was placed in a small sample tube which had a restricted orifice of approx. 3 mm^3 . The tube was immersed in alcohol, which was allowed to diffuse into the solution over a period of several weeks. These crystals took the shape of truncated trapeziums with the crystal axes bisecting the apices and having approximately equal dimensions of 0.3 mm .

These crystals were investigated by using standard X-ray-diffraction techniques. Seven layers of data containing 1700 unique non-zero reflexions were recorded by the equi-inclination Weissenberg method by using one pack of films per layer. Systematically

Fig. 4. *Hydrogen bonding between molecules in a single crystal of disodium DL-glycerol 3-phosphate hexahydrate*

Symbols used are: ----, hydrogen bonds; —○—, molecules of glycerol 3-phosphate; ○, oxygen atoms; ●, sodium atoms.

Fig. 5. *Sodium co-ordination angles (°) in single crystals of disodium DL-glycerol 3-phosphate hexahydrate*

Table 5. *Sodium co-ordination and probable hydrogen-bonding scheme*

The oxygen and sodium numbering scheme is given in Fig. 2 and Fig. 4.

Oxygen no.	Co-ordinated to sodium no.	Co-ordination bond lengths (nm)	Hydrogen bonded to oxygen no.	Hydrogen bond length (nm)
11	1	0.240	14	0.287
12	2	0.236		
	3	0.239		
13	2	0.247		
	3	0.245		
14	3	0.240	11	0.295
15	1	0.256		
	3	0.239		
16			12	0.271
			13	0.280
1			12	0.282
			15	0.279
2			15	0.280
			14	0.277
			16	0.271
3			13	0.283
			6	0.266
			5	0.266
4			11	0.280
			16	0.268
5	1	0.238	4	0.266
	3	0.240		
6	2	0.236	3	0.266
	3	0.252		

absent reflexions indicated the space group to be either *Cc* or *C2/c*. The data were estimated visually and those data from the lower three films in each pack were scaled up to those from the top film before Lorentz and polarization corrections were applied. A correction for spot extension (Phillips, 1954) was also made but, since the linear absorption coefficient was only 3.0mm^{-1} , no absorption correction was made. The layers of data were finally placed on an approximately absolute scale by using M. T. G. Powell's development (personal communication) of the Wilson method (Wilson, 1942).

A Patterson map failed to show any conclusive evidence of the phosphorus position and use of the minimum function was equally unsuccessful. Data sharpened by the Wunderlich equation (Wunderlich, 1965) enabled a Patterson map to be produced in which one peak predominated. By comparison with other parts of the map it was possible to fit the phosphate around this position and all other phosphorus-phosphorus vectors that would be expected if the space group were *C2/c*. The space-group ambiguity was thus resolved. By using the positions of the phosphate groups, structure factors and an electron-

density Fourier map were calculated and a large portion of the remainder of the molecule was found. Subsequent electron-density maps combined with least-squares refinement of the positional co-ordinates and isotropic temperature parameters showed the remainder of the molecule and six water oxygen positions. Refinement with anisotropic thermal parameters coupled with a weighting scheme which reflected the accuracy of the original data, and refinement of the scales of the seven layers of data, decreased the residual *R* value $[(\sum ||F_{\text{obs.}}| - |F_{\text{calc.}}||)/\sum |F_{\text{obs.}}|]$ to $12\frac{1}{2}\%$. A difference map showed the position of most of the hydrogen atoms bonded to the molecule, but since the background 'noise' was up to 500 electrons/nm³ it was not possible to find the remaining hydrogen atoms. The fractional atomic co-ordinates and thermal parameters are shown in Tables 6 and 7, and the observed and calculated structure factors have been deposited as Supplementary Publication 50010 at the National Lending Library for Science and Technology, Boston Spa, Yorks. LS23 7BQ, U.K.

Other than those programs specially written for this work (calculation of minimum function and

Table 6. Fractional co-ordinates and thermal parameters for those atoms in disodium DL-glycerol 3-phosphate hexahydrate which have been refined anisotropically

x, y and z are the three-dimensional co-ordinates of the atoms referred to the axes of the unit-cell and B_{ij} 's are the standard parameters used to describe the anisotropic thermal factor with the expression: $\exp(-\sum_i \sum_j B_{ij} h_i h_j)$, where $i \leq j$ and h_i is the i^{th} reflexion index, and from which the thermal motion of the atom may be obtained.

	x/a	y/b	z/c	B_{11}	B_{12}	B_{13}	B_{22}	B_{23}	B_{33}
P	0.1266	-0.1444	0.5113	0.00032	-0.00028	0.00079	0.00068	0.00028	0.00085
O-1	0.1159	-0.1603	0.3895	0.00082	-0.00155	0.00182	0.00587	-0.00111	0.00161
O-2	0.1849	-0.2167	0.5607	0.00056	0.00077	0.00074	0.00476	0.00112	0.00239
O-3	0.0763	-0.2387	0.5274	0.00065	-0.00199	0.00200	0.00492	-0.00092	0.00293
O-4	0.1243	0.0305	0.5323	0.00062	-0.00014	0.00123	0.00334	-0.00041	0.00195
O-5	0.1585	0.1870	0.3907	0.00093	-0.00053	0.00044	0.00012	-0.00046	0.00208
O-6	0.0673	0.2560	0.2153	0.00073	0.00160	0.00135	0.00576	-0.00026	0.00199
C-1	0.0790	0.0968	0.2531	0.00019	-0.00039	0.00208	0.00049	0.00134	0.00348
C-2	0.1418	0.0770	0.3076	0.00046	-0.00054	0.00085	0.00427	-0.00074	0.00116
C-3	0.1551	-0.0933	0.3383	0.00091	0.00033	0.00291	0.00015	0.00243	0.00286
Na-1	0.2500	0.2500	0.5000	0.00049	-0.00015	0.00087	0.00788	0.00099	0.00178
Na-2	0.0000	0.4372	0.2500	0.00046	0.00000	0.00152	0.00343	0.00000	0.00312
Na-3	0.1370	0.4510	0.3217	0.00053	0.00010	0.00086	0.00543	0.00047	0.00257
O-11	0.2123	-0.2275	0.1441	0.00120	0.00058	0.00218	0.00510	0.00086	0.00308
O-12	0.0661	-0.3666	0.2286	0.00103	-0.00105	0.00094	0.00717	0.00081	0.00337
O-13	0.0660	0.4707	0.4193	0.00130	-0.00013	0.00176	0.00574	-0.00188	0.00245
O-14	0.1996	0.4428	0.2127	0.00120	-0.00084	0.00148	0.01000	-0.00056	0.00228
O-15	0.2188	-0.4616	0.4502	0.00069	0.00008	0.00117	0.00350	-0.00052	0.00370
O-16	0.0398	0.2329	0.5436	0.00073	0.00024	0.00136	0.00724	-0.00195	0.00359

Table 7. *Fractional co-ordinates for some hydrogen atoms in disodium DL-glycerol 3-phosphate hexahydrate*

x , y and z are the three-dimensional co-ordinates of the atoms referred to the axes of the unit-cell. These atoms were refined isotropically with the isotropic temperature-factor set at 0.03 nm^2 .

	x/a	y/b	z/c
H-1	0.069	0.25	0.148
H-2	0.053	0.09	0.305
H-3	0.066	0.02	0.194
H-4	0.144	0.14	0.448
H-5	0.160	0.09	0.256
*H-6	0.200	-0.07	0.367

* This position would not refine.

application of the sharpening function) the programs used were from the Crystal 69 Suite (M. T. G. Powell, A. Griffiths and J. H. Cross of this Department) written in Fortran IV for a computer having a magnetic-core store of 32K 24-bit words.

We thank the Governors of this Polytechnic for providing research facilities and also the computing staff for running the computer programs. We are grateful to Professor D. C. Phillips of the University of Oxford and to our colleagues in Portsmouth for helpful discussions.

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