[Chem. Pharm. Bull.
27(8)1887—1892(1979)]

UDC 547.426.1.02 : 541.63.08

Conformational Analysis of Glycerophosphate

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(Received March 15, 1979)

As a part of our studies on the conformational analysis of calcium glycerophosphate, empirical potential energy calculations were carried out to investigate the conformational features of α- and β-glycerophosphates. The energy terms included in the total energy expression consist of nonbonded, electrostatic and torsional energies. In this calculation, the values of torsion angles are parameterized and optimized by means of the Powell algorithm. We obtained four stable conformations for α-glycerophosphate and three for β-glycerophosphate. The conformational features of glycerophosphates could be summarized as follows; the conformation of α-*d*-glycerophosphate is characterized by the glycerol backbone chain ($\Psi 2$, $\Psi 3$) corresponding to *trans-gauche*(+), and that of β-glycerophosphate is characterized by the linkage of glycerol and phosphate groups ($\Phi 5$) corresponding to *gauche*(−).

Keywords——α-glycerophosphate; β-glycerophosphate; conformational analysis; computer calculation; PPF method; torsion angle; Powell algorithm

Recently, many studies have been carried out by potential energy analyses in order to elucidate the conformations of biologically important molecules. Sasisekharan *et al.* used the PPF(partitioned potential energy function) method for calculating the conformations of nucleic acids and obtained useful results.[2] Since PPF calculation is an effective method to obtain the preferred conformation, we applied this method to the conformational analysis of glycerophosphate.

Calcium glycerophosphate has five crystal forms[3]: three in the α-form and two in the β-form, which might be attributed to conformational flexibility. Therefore, it is of interest to elucidate the characteristics of the conformation. Glycerophosphate has three isomers: the *d*(+)- and *l*(−)-forms of α-glycerophosphate $HOCH_2CH(OH)CH_2OPO(OH)_2$ and β-glycerophosphate $(HOCH_2)_2CHOPO(OH)_2$. In this study, we investigated α-*d*(+)-glycerophosphate-(α-GP) and β-glycerophosphate(β-GP).

Experimental

1. Model Building——The crystal structures of α- and β-GP have not yet been determined. We therefore obtained the atomic coordinates of these molecules by X-ray analyses of disodium *dl*-glycerol-3-phosphate hexahydrate[4] and disodium β-glycerophosphate pentahydrate.[5] The atomic numbering and the torsion angles are presented in Fig. 1 and 2.

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[2] A.V. Lakshminarayanan and V. Sasisekharan, *Biopolymers*, **8**, 475 (1969); *idem, ibid.*, **8**, 489 (1969); *idem, ibid.*, **8**, 505 (1969).

[3] M. Nakagaki and M. Inoue, Abstracts of the 88th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1968, p. 483.

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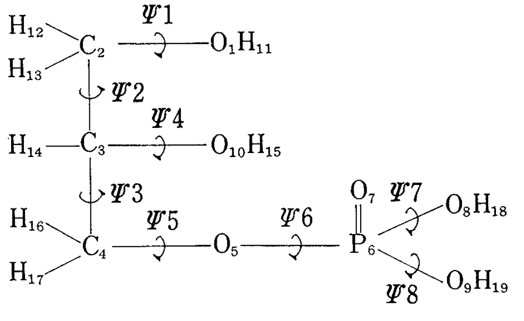
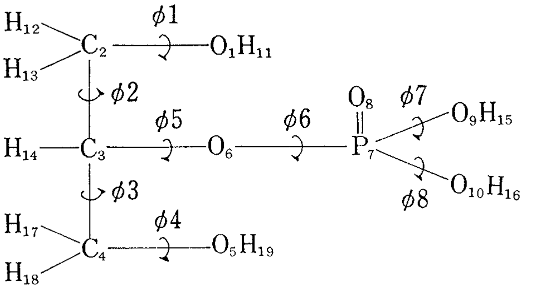


$\Psi 1$: C_3–C_2–O_1–H_{11}
$\Psi 2$: C_4–C_3–C_2–O_1
$\Psi 3$: O_5 C_4–C_3–C_2
$\Psi 4$: C_4–C_3–O_{10}–H_{15}
$\Psi 5$: C_3–C_4–O_5–P_6
$\Psi 6$: C_4–O_5–P_6–O_7
$\Psi 7$: O_5–P_6–O_8–H_{18}
$\Psi 8$: O_5–P_6–O_9–H_{19}

Fig. 1. The Notation of Structure, Numbering and Torsion Angles for α-GP

All torsion angles A–B–C–D are measured clockwise from A to D when viewed along B–C.



$\Phi 1$: C_3–C_2–O_1–H_{11}
$\Phi 2$: C_4–C_3–C_2–O_1
$\Phi 3$: O_5–C_4–C_3–C_2
$\Phi 4$: C_3–C_4–O_5–H_{19}
$\Phi 5$: C_2–C_3–O_6–P_7
$\Phi 6$: C_3–O_6–P_7–O_8
$\Phi 7$: O_6–P_7–O_9–H_{15}
$\Phi 8$: O_6–P_7–O_{10}–H_{16}

Fig. 2. The Notation of Structure, Numbering and Torsion Angles for β-GP

All torsion angles A–B–C–D are measured clockwise from A to D when viewed along B–C.

2. **Energy Calculation**——The energy (E) of a molecule was calculated using the equation

$$E = E_{\text{nb}} + E_{\text{el}} + E_{\text{t}} \tag{1}$$

where E_{nb}, E_{el} and E_{t} are the nonbonded, electrostatic and torsional contributions to the total energy, respectively. These quantities can be computed as follows;

$$E_{\text{nb}} = \sum_{i<j}\sum (A_{ij}R_{ij}^{-6} + B_{ij}R_{ij}^{-12}) \tag{2}$$

$$E_{\text{el}} = \sum_{i<j}\sum 332 Q_i Q_j R_{ij}^{-1} \varepsilon^{-1} \tag{3}$$

$$E_{\text{t}} = \sum_{k=1}^{8} \frac{V_{o,k}}{2}(1+\cos 3\theta_k) \tag{4}$$

All energy units are in kilocalories per mole. In Eq. (2)—(4), R_{ij} is the distance in angstroms between atoms i and j, and A_{ij} and B_{ij} are parameters in the Lennard–Jones "6—12" potential function. Q_i is the Coulombic charge on atom i, calculated by the CNDO/2 method for α- and β-GP, and ε is the dielectric constant. $V_{o,k}$ is the barrier to internal rotation for the k-th torsion angle, and θ_k is the value of that angle. The parameters, A_{ij}, B_{ij} and $V_{o,k}$ were taken from the literature.6)

Minimization of the energy, with the torsion angles as variable parameters, was done using the Powell algorithm.7) The minimization was carried out by the parabola approximation with 4° intervals and no angles was permitted to vary by more than 12° at each step. This method requires much less computer time and gives a result similar to that obtained by the trial and error routine within an accuracy of 1°.

3. **Precedure**——Because of the large number of sterically allowed conformations, the calculations were divided into two steps:

First Step: All eight torsion angles were rotated simultaneously to find approximate stable conformations. The most reasonable values for each starting torsion angle were as follows for α-GP; $\Psi 1$: 180°, $\Psi 2$: 180°.

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6) A.V. Lakshminarayanan and V. Sasisekharan, *Biopolymers*, **8**, 475 (1969); *idem, ibid.*, **8**, 489 (1969); V. Renugopalakrishnan, A.V. Lakshminarayan, and V. Sasisekharan, *ibid.*, **10**, 1159 (1971); N. Yathindra and M. Sundaralingam, *ibid.*, **12**, 297 (1973).

7) M.J.D. Powell, *Computer J.*, **7**, 155 (1964).

$\Psi3$: 60°, 180°, 300°, $\Psi4$: 180°, $\Psi5$: 60°, 180°, 300°, $\Psi6$: 60°, 180°, 300°, $\Psi7$: 180°, $\Psi8$: 180°. For β-GP; $\Phi1$: 180°, $\Phi2$: 60°, 180°, 300°, $\Phi3$: 60°, 180°, 300°, $\Phi4$: 180°, $\Phi5$: 60°, 180°, 300°, $\Phi6$: 60°, 180°, 300°, $\Phi7$: 180°, $\Phi8$: 180°. These starting values were based on various reports.[8] In the first step, 27 different sets were calculated for α-GP and 81 for β-GP.

Second Step: In order to investigate the preferred conformation in more detail, two flexible torsion angles were rotated, with the remaining six angles fixed at the values suggested by the first step. In the second step, the ($\Psi5$, $\Psi6$) map for α-GP and ($\Phi2$, $\Phi3$) map for β-GP were calculated at intervals of 30°. The most favorable conformations were obtained from the computations of steps 1 and 2.

Results

1. α-GP

The five stable conformations (**A**—**E**) obtained from the first step are presented in Table I. Table I indicates that stable conformations might occur for the following values of torsion angles; $\Psi1=180°$, $\Psi2=180°$, $\Psi3=60°$, $\Psi4=180°$, $\Psi5=60°$ and 180°, $\Psi6=60°$, 180° and 300°, $\Psi7=180°$, $\Psi8=180°$. These results suggest that $\Psi5$ and $\Psi6$ are the critical angles to be explored further in this study. Thus, the ($\Psi5$, $\Psi6$) energy map was made. $\Psi5$ and $\Psi6$ (the linkages between the glycerol and phosphate groups) were rotated, while the remaining angles were fixed at the values assigned by the first step. An energy contour map for $\Psi5$ *versus* $\Psi6$ is shown in Fig. 3.

Table I. The Initial and Final Torsion Angles of α-GP

Set No.	Initial angles (°)								Final angles (°)								Energy (kcal/mol)
	$\Psi1$	$\Psi2$	$\Psi3$	$\Psi4$	$\Psi5$	$\Psi6$	$\Psi7$	$\Psi8$	$\Psi1$	$\Psi2$	$\Psi3$	$\Psi4$	$\Psi5$	$\Psi6$	$\Psi7$	$\Psi8$	
A	180	180	60	180	60	180	180	180	179	185	51	180	69	162	181	177	−12.90
B	180	180	60	180	180	60	180	180	179	185	66	181	167	49	183	177	−12.57
C	180	180	60	180	180	300	180	180	179	185	66	181	166	298	183	176	−12.50
D	180	180	60	180	180	180	180	180	179	185	66	181	166	179	184	176	−12.35
E	180	180	60	180	60	300	180	180	179	183	49	179	73	276	185	177	−12.08

It can be seen from Fig. 3 that the regions near $\Psi5=180°$ are relatively stable irrespective of the value of $\Psi6$. There are three energy minima at $\Psi6$ values near 60°, 180° and 300° for $\Psi5=180°$. The energy differences among the three minima are very small (0.2 kcal/mol). This indicates that the preferred value of $\Psi5$ is 180° and that for $\Psi6$, three regions are energetically favorable. Table II shows these minimum values for $\Psi5$–$\Psi6$ and the corresponding conformations.

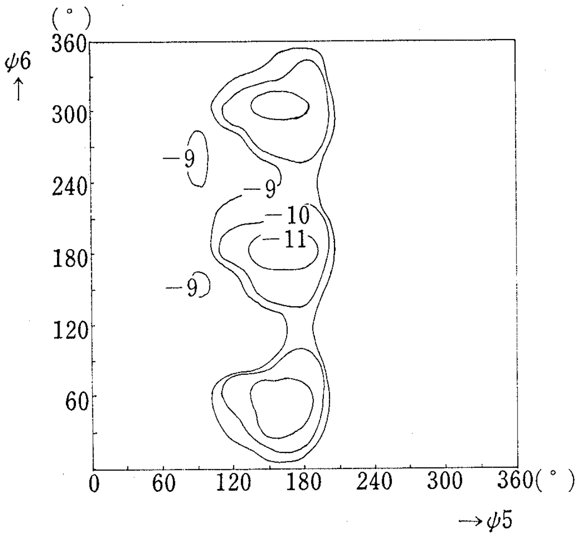

Fig. 3. Minimized Energy Contour Map of α-GP, for the Torsion Angle $\Psi5$ *vs.* $\Psi6$

Other torsion angles are fixed at $\Psi1=180°$, $\Psi2=180°$, $\Psi3=60°$, $\Psi4=180°$, $\Psi7=180°$, $\Psi8=180°$. The contours are drawn at energy values of −9, −10 and −11 kcal/mol, respectively.

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[8] S. Abrahamsson and I. Pascher, *Acta Crystallogr.*, **21**, 79 (1966); H.V. Koningsveld, *Rec. Trav. Chim.*, **87**, 243 (1968); R.H. Fenn and G.E. Marshall, *J. Chem. Soc.*, (*D*), **1971**, 984; T. Taga, M. Senma, and K. Osaki, *ibid.*, (*D*), **1972**, 465; M. Sundaralingam and L.H. Jensen, *Science*, **150**, 1035 (1965); M. Ul-Haque and C.N. Caughlan, *J. Am. Chem. Soc.*, **88**, 4124 (1966).

Table II. Three Major Minimum Energy Regions of $\Psi5$, $\Psi6$ for α-GP

Region ($\Psi5$–$\Psi6$)	Torsion angle (°) ($\Psi5$, $\Psi6$)	Corresponding set No. from Table I	Energy (kcal/mol)
t-*g*(+)	160, 55	B	−11.2
t-*t*	160, 180	D	−11.0
t-*g*(−)	160, 300	C	−11.2

t, *g*(+) and *g*(−) refer to *trans*, *gauche*(+) and *gauche*(−).

2. β-GP

From 81 different sets in the first step, the eight stable conformations (**H**—**O**) are given in Table III. The preferable values of torsion angles for these conformations can be summarized as follows; $\Phi1=180°$, $\Phi2=60°$, 180° and 300°, $\Phi3=60°$, 180° and 300°, $\Phi4=180°$, $\Phi5=300°$, $\Phi6=60°$ and 180°, $\Phi7=180°$, $\Phi8=180°$. $\Phi5$ is the most important angle to specify the conformation, because deviation of $\Phi5$ from near 300° results in unstable energy unequivocally, irrespective of the remaining torsion angles.

Table III. The Initial and Final Torsion Angles of β-GP

Set No.	Initial angles (°)								Final angles (°)								Energy (kcal/mol)
	$\Phi1$	$\Phi2$	$\Phi3$	$\Phi4$	$\Phi5$	$\Phi6$	$\Phi7$	$\Phi8$	$\Phi1$	$\Phi2$	$\Phi3$	$\Phi4$	$\Phi5$	$\Phi6$	$\Phi7$	$\Phi8$	
H	180	180	60	180	300	180	180	180	181	190	60	180	296	193	180	181	−16.12
I	180	300	180	180	300	60	180	180	180	300	179	180	297	69	178	182	−15.76
J	180	60	60	180	300	300	180	180	179	64	61	180	295	328	178	183	−15.75
K	180	180	60	180	300	60	180	180	181	193	60	180	293	77	176	182	−15.73
L	180	180	300	180	300	180	180	180	181	190	302	180	296	193	179	181	−15.73
M	180	60	60	180	300	60	180	180	179	64	61	180	295	76	178	182	−15.72
N	180	60	60	180	300	180	180	180	179	64	61	180	296	191	178	182	−15.71
O	180	300	300	180	300	60	180	180	180	299	301	180	297	69	178	182	−15.67

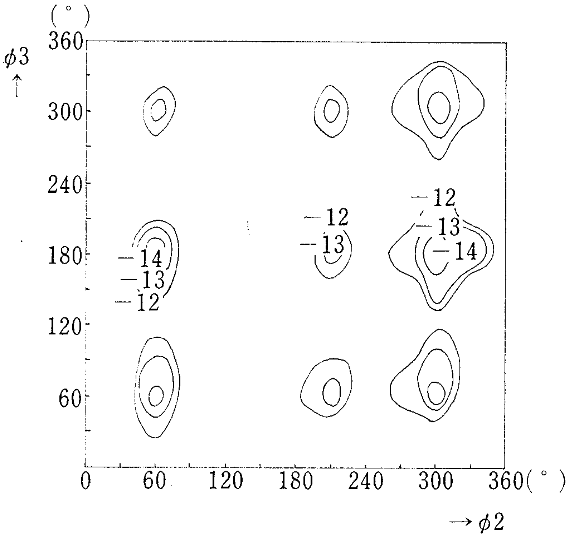

Fig. 4. Minimized Energy Contour Map of β-GP, for the Torsion Angle $\Phi2$ *vs.* $\Phi3$

Other torsion angles are fixed at $\Phi1=180°$, $\Phi4=180°$, $\Phi5=300°$, $\Phi6=180°$, $\Phi7=180°$, $\Phi8=180°$. The contours are drawn at energy values of −12, −13 and −14 kcal/mol, respectively.

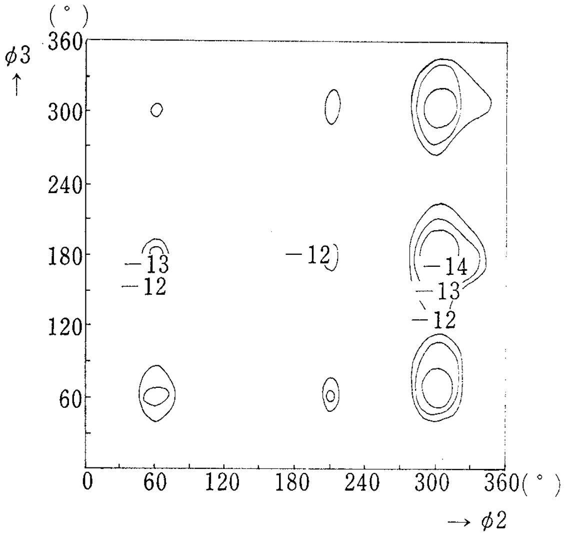

Fig. 5. Minimized Energy Contour Map of β-GP, for the Torsion Angle $\Phi2$ *vs.* $\Phi3$

Other torsion angles are fixed at $\Phi1=180°$, $\Phi4=180°$, $\Phi5=300°$, $\Phi6=60°$, $\Phi7=180°$, $\Phi8=180°$. The contours are drawn at energy values of −12, −13 and −14 kcal/mol, respectively.

On the other hand, $\Phi2$, $\Phi3$ and $\Phi6$ were optimized at two or three different values. To analyze the conformation in more detail, the ($\Phi2$, $\Phi3$) maps ($\Phi2$ and $\Phi3$ form the backbone chain of the glycerol unit) were calculated for both $\Phi6=180°$ and $\Phi6=60°$. The energy contour maps for $\Phi2$ *versus* $\Phi3$ in Fig. 4 ($\Phi6=180°$) and Fig. 5 ($\Phi6=60°$) were made in the same way as for α-GP.

As shown in Fig. 4, five energy minima occurred in the $\Phi2$–$\Phi3$ map at $\Phi6=180°$. These minimum values and the corresponding conformations are listed in Table IV.

Table IV. Five Major Minimum Energy Regions of $\Phi2$, $\Phi3$ for β-GP

Region ($\Phi2$–$\Phi3$)	Torsion angle (°) ($\Phi2$, $\Phi3$)	Corresponding set No. from Table III	Energy (kcal/mol) Fig. 4	Energy (kcal/mol) Fig. 5
g(+)-*g*(+)	60, 60	J, M, N	−14.9	−13.7
g(+)-*t*	60, 180		−14.5	−13.5
g(−)-*g*(+)	290, 60		−14.9	−15.0
g(−)-*t*	290, 180	I	−15.3	−15.5
g(−)-*g*(−)	290, 300	O	−15.3	−15.5

t, *g*(+) and *g*(−) refer to *trans*, *gauche*(+) and *gauche*(−).

On the other hand, as shown in Fig. 5, three minima occurred in the $\Phi2$–$\Phi3$ map at $\Phi6=60°$. Both figures (Fig. 4 and 5) indicate that in regions of low energy for $\Phi3$, values of $\Phi2$ near 300° are energetically preferable irrespective of the value of $\Phi6$.

Discussion

As a whole, several stable conformations could be considered for α-GP and β-GP, because the differential energies of sterically allowed conformations were only slightly different (0.1—0.3 kcal/mol). Detailed analysis of the results, however, showed that both molecules have particular conformational features. In the α-GP molecule, the stable conformations may be characterized by $\Psi2$ and $\Psi3$ (the backbone chain of glycerol), while they are not significantly

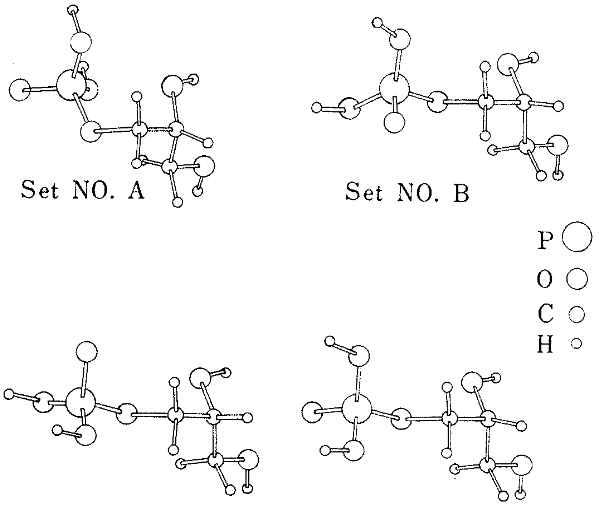

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Fig. 6. Calculated Models of α-GP having energetically Stable Conformations viewed along the *y*-Axis

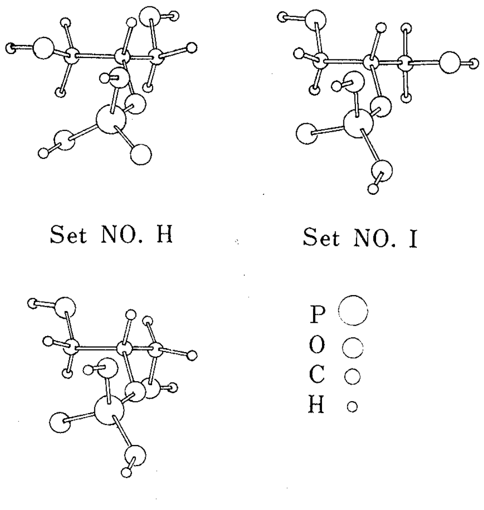

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Fig. 7. Calculated Models of β-GP having energetically Stable Conformations viewed along the *y*-Axis

affected by $\Psi 1$, $\Psi 4$, $\Psi 7$ and $\Psi 8$. The α-GP molecule is most stabilized, when $\Psi 2$–$\Psi 3$ correspond to *trans-gauche*(+). There are some difficulties in discussing $\Psi 5$ and $\Psi 6$. As shown in Fig. 3, the *trans* regions of $\Psi 5$ are larger and energetically more stable than *gauche*(+). That is to say, the B, C and D conformations having *trans* regions for $\Psi 5$ are thought to be more stable than the A and E ones having *gauche*(+). Corresponding values for the four conformations (B, C, D and E) have also been found in other analogs.[9] Among the preferred conformations obtained from the results of first and second step computation, we present A (the most stable in this calculation), B, C and D as proposed models of α-GP(Fig. 6).

In the β-GP molecule, the stable conformations are mainly characterized by the $\Phi 5$ torsion angle (the linkage between glycerol and phosphate), while $\Phi 1$, $\Phi 4$, $\Phi 7$ and $\Phi 8$ do not significantly affect the stability. When the value of $\Phi 5$ is near *gauche*(−), the conformation of β-GP is energetically most stabilized.

On the other hand, two ($\Phi 2$, $\Phi 3$) maps (Fig. 4 and 5) suggest that $\Phi 2$ is stabilized near the *gauche*(−) region and that the values of $\Phi 3$ and $\Phi 6$ are less important. Fig. 7 shows the preferred models of β-GP (H, I and O conformations); the most stable one is H.

Acknowledgement We are extremely grateful to Professor K. Tomita and Dr. S. Fujii, Faculty of Pharmaceutical Sciences, Osaka University for providing the PPF program and for valuable advice.

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