Calcium binding to phospholipid: structural study of calcium glycerophosphate

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Abstract To consider possible interaction of the phospholipid membrane with calcium ions, crystal structures of calcium *dl*-α- and β-glycerophosphates (α- and β-CaGs, respectively) were investigated by X-ray diffraction methods. After many attempts, relatively large single crystals of β-CaG were prepared from the aqueous solution containing HCl, while crystals of $CaHPO_4 \cdot 2H_2O$ were obtained from α-CaG solution under the same crystallization conditions. The crystal structure of β-CaG is orthorhombic with space group $Pna2_1$ and cell dimensions of a = 8.251(1), b = 13.038(3), c = 25.483 (10) Å, V = 2741.5 (13) Å^3 and Z = 16 [four molecules (A to D) in an asymmetric unit]. Molecules of A to D took, as a whole, similar extended conformations, although A and B were different from C and D in the orientation about a glycerol C–C bond. Four independent β-glycerophosphates commonly act as two types of bidentate ligands, where one is the coordination to the calcium ion by the glycerol O(1) and phosphate O(22) atoms, and the other by the phosphate O(22) and O(23) atoms, thus forming the calcium coordination of a distorted square plane, respectively. Each of four independent calcium ions forms the same coordination geometry of a distorted pentagonal bipyramid. Infinite double layers consisting of alternate A/B molecules and of alternative C/D ones and sandwiching calcium ions were arranged face-to-face along the *b*-direction and were piled up in the *a*-direction, thus forming the stacked bilayer unit with the thickness of d_{002} = 12.75 Å. The elaborate networks of calcium coordinations and hydrogen bondings were formed among the layers and stabilized the crystal structure. Based on the structural parameters of the present β-CaG crystal, a possible interaction model of phospholipid with calcium ions was proposed. – **Inoue, M., Y. In, and T. Ishida.** Calcium binding to phospholipid: structural study of calcium glycerophosphate. *J. Lipid Res.* 1992. **33:** 985–994.

Supplementary key words crystal structure • calcium coordination

The cell membrane regulates the passage of materials between the cell and its surroundings, and, in some tissues, is involved in intercellular communication mediated by the chemical transmitter or metal ion. In addition to the skeletal constituent, calcium ions interact with the surfaces of many types of cells and play important roles in various biological processes (1, 2).

Concerning the calcium ion–cell membrane interaction, little is known about types of interactions that may be specifically involved, although they are generally presumed to be of the simple electrostatic or ionic type. Therefore, the model study on the calcium–membrane constituent interaction has a direct bearing on understanding the specific permeation of calcium ions through the cell membrane (calcium channel). As glycerophosphates are biologically important molecules and are a main constituent (*l*-α-form) of the membrane phospholipid, physicochemical properties of calcium *dl*-α- and β-glycerophosphates (called α-CaG and β-CaG, respectively) have been investigated (3, 4). The present report deals with the crystal and molecular structure of β-CaG determined by X-ray single-crystal analysis and with a possible interaction model between the phospholipid and calcium ions, based on the structural similarity between the α- and β-CaG crystals.

The preparation of CaG single crystals large enough to carry out the X-ray analysis is not straightforward, because CaGs are only slightly soluble in water and are apt to exist as mixtures of anhydrate, monohydrate, and dihydrate for α-CaG and of anhydrate and monohydrate for β-CaG (4). This is the reason why the crystal structures of CaGs remain to be analyzed in spite of their biological importance, while those of sodium (5–8) and cadmium (9) salts of glycerophosphates were already analyzed in the 1960–1970s.

EXPERIMENTAL PROCEDURES

Syntheses of CaGs

Sodium salts of *dl*-α- or β-glycerophosphates (Sigma Co., St. Louis, MO) were dissolved in water. An equimolar amount of $CaCl_2$ was added to the solution at room temperature, and the mixture was stirred for 2 h. The precipitate of α- or β-CaG was filtered off and washed using 45% aqueous ethanol until no Cl^- was detected with

Abbreviations: CaG, calcium glycerophosphate.

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silver nitrate. The anhydrate crystals of α- and β-CaGs (microcrystalline states) were then prepared by heating the samples at 150°C for 1 h; the purities were estimated as > 95% by the periodic acid method (10).

X-ray powder diffractions of α- and β-CaG crystals

The prepared anhydrate crystals of CaGs were thoroughly powdered, and their X-ray diffraction patterns were measured at 20°C with an X-ray diffractometer (Rigaku Denki, Japan) using graphite-monochromated CuKα radiation (λ = 1.5418 Å) at a scan rate of 1°/min in 2θ and with a 0.1-mm receiving slit. For the hydration experiments of CaG anhydrate powders, the previously described method (4) was used.

Preparation of β-CaG single crystals

In contrast to sodium or cadmium salts of glycerophosphates, the single crystals of CaGs suitable for the X-ray analysis are generally difficult to prepare because CaGs are only slightly soluble in water. Thus, various attempts were made to prepare CaG crystals complexed with compounds such as citric acid or HCl, which increases the solubilities of CaGs. After many failures, transparent platelet single crystals of β-CaG were obtained from the aqueous solution containing dilute HCl by vapor diffusion at room temperature. The content of HCl in the sample solution was a crucial factor in the preparation of single crystals, and the same crystallization conditions for α-CaG solution led to single crystals of $CaHPO_4 \cdot 2H_2O$. This could be interpreted as a result of different susceptibility against the hydrolysis of the P-O-C bond at α- and β-CaG molecules.

Crystal analysis of β-CaG

Single-crystal X-ray data for β-CaG were collected at room temperature (20°C) using a Rigaku AFC-5 diffractometer and a rotating anode generator with graphite-monochromated CuKα radiation. A crystal with the dimensions of 0.2 × 0.1 × 0.4 mm^3 was mounted on the diffractometer, where the *c*-axis was nearly parallel to the φ-axis. Cell parameters were determined by a least squares refinement of the scattering parameters of 45 reflections with 30° < 2θ < 60°. The crystal data and parameters for data collection are summarized in **Table 1,** where the crystal density was measured by the flotation method using a benzene-chloroform mixture. The peak counts of reflections were corrected with background counts for 5 sec measured at both ends of the scan range. Four standard reflections were monitored at every 100 reflections throughout the data collection and showed no significant deterioration (< ±3%). The observed intensities were corrected for Lorentz and polarization effects. Absorptional corrections for respective reflections were also made using the averaged intensity variation of (00*l*) reflections with the φ scan at χ = 90°.

TABLE 1. Summary of β-CaG crystal data and data collection

Formula	$C_8H_7O_6PCa$
M_r	210.1
Space group	$Pna2_1$
a, Å	8.251(1)
b, Å	13.038(3)
c, Å	25.483(10)
V, Å^3	2741.5(13)
Z	16 [4 molecules per asymmetric unit]
D(measd), g • cm^{-3}	2.009
D(calcd), g • cm^{-3}	2.028
μ(Cu Kα), cm^{-1}	101.56
F(000)	1728
Data collection method	ω-2θ scan
Scan speed in 2θ, deg • min^{-1}	4
Scan range in ω, deg	1.50 + 0.15 tan θ
Data range measd, deg	$2 < 2\theta < 130$
Data collected	*h k l*
No. of unique data measd	2426
No. of data with |Fo| > 0.0 (= M)	2344
No. of variables (= N)	531
R	0.084
Rw	0.114
S	0.9779

Systematic absent spectra, (0*kl*) with $k + l = 2n + 1$ and (*h*0*l*) with $h = 2n + 1$, suggested $Pna2_1$ or *Pnma*[2] as a possible space group. The space group was finally determined as $Pna2_1$ from the statistical distribution of normalized structure factors and by the structure determination. The structure was determined by a combination of the heavy atom and direct methods using the MULTAN87 program (11). The obtained positional parameters of non-H atoms were then refined by the block-diagonal least-squares analysis with anisotropic temperature factors. The geometrically reasonable positions of H atoms were calculated and included in the last refinement with isotropic temperature factors. The function minimized was $\Sigma w(|Fo| - |Fc|)^2$, where |Fo| and |Fc| are the observed and calculated structure factors, respectively. In the last refinement, the weight was used as $w = 1.0/[\sigma(Fo)^2 - 1.35101|Fo| + 0.04737|Fo|^2]$, where $\sigma(Fo)^2$ is the standard deviation of the intensity based on counting statistics. The discrepancy indices, $R(= \Sigma||Fo| - |Fc||/\Sigma|Fo|)$ and $Rw(= [\Sigma w(|Fo| - |Fc|)^2/\Sigma wFo^2]^{1/2})$ were reduced to 0.084 and 0.114, respectively, for 2344 reflections of $|Fo| > 0.0$; the goodness-of-fit, $S(= [\Sigma w(|Fo| - |Fc|)^2/(M - N)]^{1/2})$, was 0.9779. For all crystallographic computations, the UNICS program system (12) was used, and the atomic scattering factors were taken from the literature (13). Final atomic coordinates and equivalent isotropic thermal parameters are listed in **Table 2.**[3] All numerical calcula-

[2]By exchanging *b*- and *c*-axes, the systematic absent spectra indicate the space group of *Pnma*, i.e., (0*kl*) with $k + l = 2n + 1$ and (*hk*0) with $h = 2n + 1$.

[3]Tables of anisotropic thermal parameters of non-H atoms, atomic positions and isotropic thermal parameters of H-atoms and observed and calculated structure factors have been deposited at the Cambridge Crystallographic Data Centre.

TABLE 2. Fractional atomic coordinates and isotropic thermal parameters (B_{eq}) with their e.s.d.s in parentheses

Atom	x	y	z	Beq
Ca(1)	0.7248(2)	−0.44169(8)	0.80429(4)	1.67(5)
Ca(2)	0.7458(2)	0.05944(9)	0.80200(5)	2.03(5)
Ca(3)	0.5817(2)	−0.18572(8)	0.74482(5)	1.78(5)
Ca(4)	0.6104(2)	0.30427(8)	0.74759(5)	1.88(5)
C(1)A	0.882(1)	−0.3903(6)	0.9287(3)	3.5(4)
O(1)A	0.9079(8)	−0.4302(4)	0.8801(2)	3.4(2)
C(2)A	0.8993(9)	−0.2730(5)	0.9294(3)	2.9(3)
O(2)A	0.7885(6)	−0.2311(3)	0.8937(2)	2.4(2)
P(2)A	0.8376(2)	−0.1850(1)	0.83772(6)	1.70(6)
O(21)A	0.9988(6)	−0.1302(3)	0.8426(2)	2.4(2)
O(22)A	0.8457(6)	−0.2722(3)	0.7984(1)	2.2(2)
O(23)A	0.6991(5)	−0.1158(3)	0.8248(2)	1.8(2)
C(3)A	0.870(2)	−0.2275(6)	0.9812(3)	4.6(4)
O(3)A	0.910(1)	−0.1226(5)	0.9843(3)	5.6(3)
C(1)B	0.906(1)	0.1036(6)	0.9331(3)	3.5(4)
O(1)B	0.884(1)	0.0489(4)	0.8875(3)	5.0(3)
C(2)B	0.957(1)	0.2131(5)	0.9226(3)	3.9(4)
O(2)B	0.8271(6)	0.2623(3)	0.8927(2)	2.2(2)
P(2)B	0.8676(2)	0.3122(1)	0.83661(6)	1.81(6)
O(21)B	1.0321(5)	0.3623(3)	0.8417(2)	2.5(2)
O(22)B	0.8683(6)	0.2257(3)	0.7960(2)	2.3(2)
O(23)B	0.7304(5)	0.3814(3)	0.8249(2)	2.1(2)
C(3)B	0.978(1)	0.2723(6)	0.9718(3)	3.8(4)
O(3)B	1.016(1)	0.3798(4)	0.9601(3)	6.5(4)
C(1)C	0.425(1)	−0.1327(5)	0.6172(3)	3.8(4)
O(1)C	0.3954(9)	−0.1827(4)	0.6674(2)	4.3(3)
C(2)C	0.4039(9)	−0.0226(5)	0.6198(2)	2.5(3)
O(2)C	0.5268(6)	0.0149(3)	0.6561(2)	2.3(2)
P(2)C	0.4746(2)	0.0649(1)	0.71099(6)	1.65(6)
O(21)C	0.3229(5)	0.1261(3)	0.7008(2)	2.4(2)
O(22)C	0.4530(6)	−0.0223(3)	0.7508(2)	2.5(2)
O(23)C	0.6222(6)	0.1286(3)	0.7259(2)	2.5(2)
C(3)C	0.451(1)	0.0288(6)	0.5678(3)	3.7(4)
O(3)C	0.3325(7)	0.0022(4)	0.5299(2)	4.0(3)
C(1)D	0.476(1)	0.3611(6)	0.6164(3)	4.4(4)
O(1)D	0.4717(9)	0.3030(4)	0.6650(2)	4.5(3)
C(2)D	0.409(1)	0.4654(4)	0.6235(3)	2.8(3)
O(2)D	0.5211(6)	0.5204(3)	0.6570(2)	2.5(2)
P(2)D	0.4737(2)	0.5611(1)	0.71357(6)	1.60(6)
O(21)D	0.3005(5)	0.5994(3)	0.7126(2)	2.6(2)
O(22)D	0.4937(5)	0.4741(3)	0.7537(2)	1.7(2)
O(23)D	0.6051(6)	0.6418(3)	0.7247(1)	2.4(2)
C(3)D	0.413(1)	0.5258(6)	0.5727(3)	3.8(4)
O(3)D	0.331(2)	0.6240(8)	0.5791(4)	4.6(6)
O(3')D	0.332(2)	0.475(1)	0.5364(5)	8.7(9)

The suffix letters A to D correspond to molecules A to D of β-glycerophosphates. $B_{eq} = 4/3\ (B_{11}a^2 + B_{22}b^2 + B_{33}c^2)$.

tions were carried out at the Computation Center, Osaka University of Pharmaceutical Sciences.

RESULTS AND DISCUSSION

Molecular dimensions and conformation of β-glycerophosphate

The molecular conformations of four independent β-glycerophosphates are shown in **Fig. 1**, together with the atomic numbering used. Possible intramolecular hydrogen bonds are shown by dotted lines. The bond lengths and angles are listed in **Table 3**, and torsion angles in **Table 4.** The γ-hydroxy oxygen atom of molecule D was disordered into O(3) and O(3') positions, in which respective occupancies were treated as 0.5. Thus, the bond lengths and angles concerning this OH group are somewhat imprecise.

Compared with the reported data (5–9), no unusual values were observed concerning the bonding parameter. The O(2)–P(2) bond distance ranging from 1.586 to 1.607 Å is in agreement with the P-O ester bond of the nucleotide, 1.60 to 1.63 Å (14). The other three P-O bonds are in the range of 1.479 to 1.537 Å and have the characteristics of a monosubstituted phosphate having a dianion state ($-PO_3^{2-}$). The O-P-O bond angle varies considerably from 102.8 to 116.3°. Such a large angle distribution is also found in the related compounds, and could be mainly caused by environmental influences such as the charge–charge repulsion of geminal oxygen atoms and/or the participation in the hydrogen bondings or coordinations (discussed later).

Conformational torsion angles for four β-glycerophosphates are all in the region usually observed in related compounds, and belong to one of the energetically stable conformations (3); the conformational feature of glycerophosphate is characterized by a linkage between the glycerol and phosphate group, and the present torsion angles of C(1)–C(2)–O(2)–P(2) (= χ_2)/C(3)–C(2)–O(2)–P(2) are all close to ± 120° and correspond to a stable orientation frequently observed in other glycerophosphates (5). On the whole, four independent molecules take similar extended conformations. The only difference was observed in the torsion angle (χ_3) around the C(2)–C(3) bond. The O(3) atoms of molecules C and D (one of two disordered conformers) take *trans* orientation with respect to the O(2) atom, while those of molecules A, B, and D (the other of disordered conformers) are all in a *gauche* region. This *gauche* orientation is primarily due to the electrostatic interaction (including partial hydrogen bond) of O(3) with phosphate O(2) and O(21) atoms. This interaction force appears to be weaker than that of O(1) atom with phosphate O(2) and O(22) atoms, because the *gauche* orientation (χ_1) of O(1) with respect to the O(2) atom is commonly observed in all molecules. Another characteristic commonly observed in molecules A to D is that the torsion angles of χ_1, χ_2 and χ_4 are all close to 60°, −120°, and 90°, respectively. This is primarily attributable to the behavior of β-glycerophosphate as a bidentate ligand, where both O(1) and O(22) atoms coordinate to a calcium atom (discussed later).

Crystal structure and molecular interaction

A stereoscopic view of β-CaG molecular packing is shown in **Fig. 2,** where the calcium ions and oxygen atoms are represented by filled and open circles, respectively. Intra- and intermolecular short contacts (< 3.3 Å)

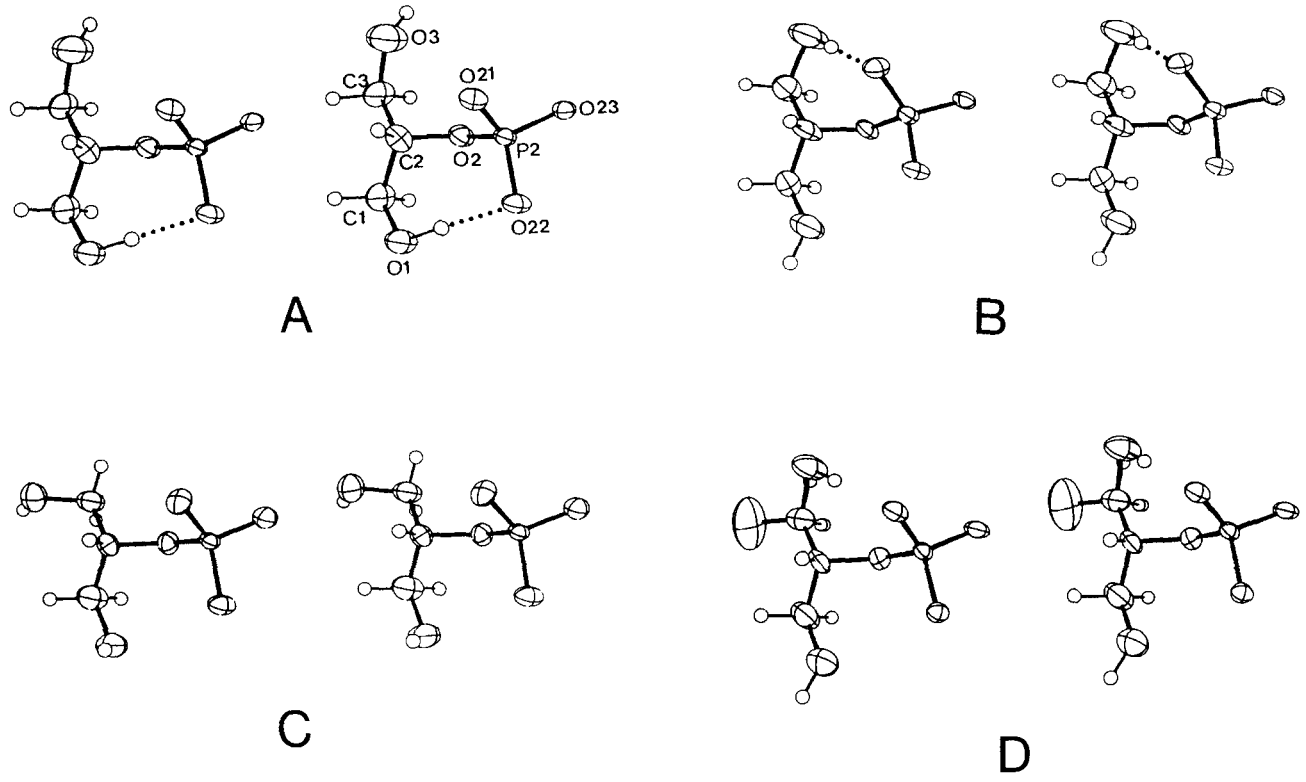

Fig. 1. The stereoscopic views of four independent β-CaG molecular conformations. The letters A to D correspond to molecules A to D of β-glycerophosphates, respectively. The O(3) atom of molecule D is in the disordered state and its two positions are given. Atomic numbering used is shown in molecule A.

TABLE 3. Bond lengths (Å) and angles (°) of β-glycerophosphate molecules A to D with their e.s.d.s in parentheses

	A	B	C	D
Bond lengths				
C(1)-C(2)	1.536(10)	1.512(11)	1.448(10)	1.479(11)
C(1)-O(1)	1.361(9)	1.377(10)	1.456(10)	1.453(10)
C(2)-C(3)	1.467(12)	1.480(12)	1.536(10)	1.516(11)
C(2)-O(2)	1.400(8)	1.464(9)	1.457(8)	1.448(8)
O(2)-P(2)	1.602(5)	1.607(5)	1.602(5)	1.586(5)
P(2)-O(21)	1.514(5)	1.513(5)	1.507(5)	1.514(5)
P(2)-O(22)	1.517(4)	1.529(5)	1.533(5)	1.536(4)
P(2)-O(23)	1.492(4)	1.479(4)	1.523(5)	1.537(4)
C(3)-O(3)	1.409(11)	1.467(11)	1.419(10)	1.457(15)
C(3)-O(3')				1.318(19)
Bond angles				
O(1)-C(1)-C(2)	112.1(4)	112.2(5)	112.5(4)	111.4(4)
C(1)-C(2)-O(2)	108.6(4)	107.5(4)	106.1(4)	106.7(4)
C(1)-C(2)-C(3)	113.4(5)	112.0(5)	111.2(5)	111.3(5)
C(2)-O(2)-P(2)	124.0(3)	119.2(3)	120.2(3)	122.9(3)
O(2)-P(2)-O(21)	109.0(2)	106.6(2)	106.8(2)	109.2(3)
O(2)-P(2)-O(22)	108.5(2)	107.7(2)	107.9(2)	109.3(2)
O(2)-P(2)-O(23)	103.3(2)	105.6(2)	103.0(2)	102.8(2)
O(21)-P(2)-O(22)	110.0(2)	108.5(2)	114.2(2)	110.8(2)
O(21)-P(2)-O(23)	111.7(2)	111.9(2)	114.7(2)	116.3(2)
O(22)-P(2)-O(23)	113.9(2)	116.1(2)	109.4(2)	107.9(2)
C(2)-C(3)-O(3)	113.9(5)	110.6(5)	107.8(4)	110.5(6)
C(2)-C(3)-O(3')				109.2(7)
O(3)-C(3)-O(3')				106.4(10)

including hydrogen bonds are summarized in **Table 5.** Bonding parameters concerning calcium ions are given in **Table 6.**

Two kinds of layers consisting of alternating molecules A and B and of molecules C and D are arranged face-to-face and are infinitely expanded along the *b*-direction. These layers sandwich four independent calcium ions and are piled up to the *a*-direction, thus forming a stacked bilayer unit with the thickness of 12.75 Å (= d_{002}), as can be seen in Fig. 2. A structural characteristic of the β-CaG crystal is the antiparallel alignment of these hydrophilic units perpendicular to the *c*-axis. The repeated arrangements of four independent β-glycerophosphates and calcium ions form a planar bilayer, as is shown in **Fig. 3.** The α-hydroxy O(1) and phosphate O(22) atoms of β-glycerophosphates coordinate to one calcium ion, and the phosphate O(22) and O(23) atoms to another one. Thus, β-glycerophosphate acts as two kinds of bidentate ligands[4]. On the other hand, calcium ions lying in a *zigzag* chain with

[4]These two bidentate chelations to the calcium ion are the first X-ray examples in the metal-glycerophosphate complexes.

TABLE 4. Conformational torsion angles (°) of four β-glycerophosphates with their e.s.d.s in parentheses

	A	B	C	D
O(1)-C(1)-C(2)-O(2):χ_1	58.5(5)	61.3(6)	63.6(5)	68.0(5)
O(1)-C(1)-C(2)-C(3)	179.2(7)	178.9(8)	174.8(6)	- 180.0(7)
C(1)-C(2)-O(2)-P(2):χ_2	- 105.5(5)	- 122.8(5)	- 133.9(5)	- 116.3(5)
C(3)-C(2)-O(2)-P(2)	130.9(5)	116.6(5)	129.1(5)	126.3(5)
C(1)-C(2)-C(3)-O(3)	170.7(8)	- 175.9(7)	70.2(6)	174.0(9)
C(1)-C(2)-C(3)-O(3')				57(1)
O(2)-C(2)-C(3)-O(3):χ_3	- 68.6(7)	- 58.2(6)	- 176.5(6)	- 71.8(7)
O(2)-C(2)-C(3)-O(3')				171(1)
C(2)-O(2)-P(2)-O(21)	- 37.0(4)	- 38.7(4)	- 37.7(4)	- 37.6(4)
C(2)-O(2)-P(2)-O(22):χ_4	84.9(4)	81.5(4)	85.5(4)	83.8(4)
C(2)-O(2)-P(2)-O(23)	- 158.5(4)	- 162.7(4)	- 158.9(4)	- 161.7(4)

the averaged separation distances of 3.752 Å [Ca(1)-Ca(3) = 3.851(2), Ca(3)-Ca(2) = 3.765(2), Ca(2)-Ca(4) = 3.655(2) and Ca(4)-Ca(1) = 3.735(2) Å] are connected in such a way that alternating four-membered rings are formed with neighboring phosphate atoms [O(22)A/O(23)D, O(23)A/O(22)C, O(22)B/O(23)C, and O(23)B/O(22)D] bridging two calcium ions, and give rise to an infinite chain along the *b*-direction; this kind of edge-sharing of the calcium polyhedron is also frequently found in the geometry of calcium coordination in the complexes with the amino acid (15) or nucleic acid base (16).

The stereochemistry of the Ca(1) coordination shell is shown in **Fig. 4**; three other calcium ions also take nearly the same coordinations (see Table 6). Each calcium ion is surrounded by a shell composed of seven oxygen atoms and forms a distorted pentagonal-bipyramidal environment. The pentagonal plane is formed by coordinations to Ca(1) through the O(1) and O(22) atoms of molecule A, the O(22) and O(23) atoms of molecule D, and the O(23) atom of molecule B; these ligand atoms lie within ± 0.23

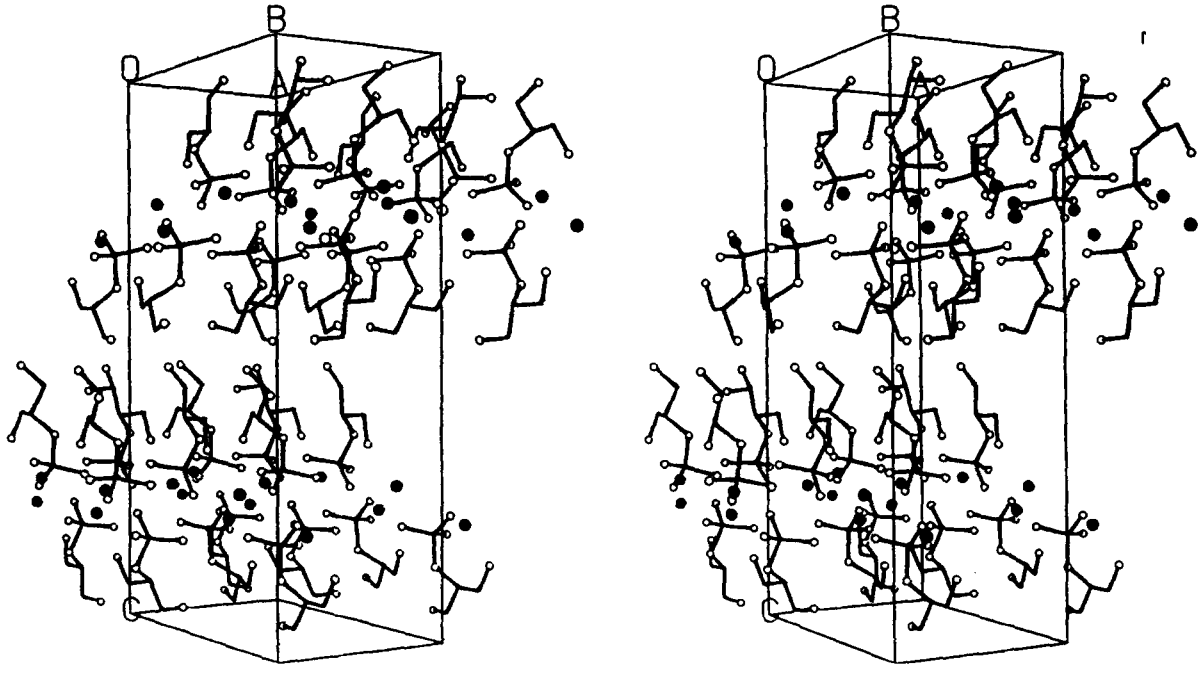

Fig. 2. A stereoscopic view of β-CaG crystal packing. The calcium ions and oxygen atoms of β-glycerophosphate are shown by filled and open circles, respectively.

TABLE 5. Molecular hydrogen bonds or short contacts of less than 3.3 Å with their e.s.d.s in parentheses

[1] Intramolecular hydrogen bonds or short contacts

O(1)A--O(2)A	2.798(7) Å	O(1)A--O(22)A	2.973(7) Å	O(3)A--O(2)A 2.887(8) Å
O(1)B--O(2)B	2.825(8)	O(3)B--O(2)B	2.778(8)	O(3)B--O(21)B 3.028(8)
O(1)C--O(2)C	2.810(7)	O(1)C--O(22)C	3.020(7)	
O(1)D--O(2)D	2.870(8)	O(1)D--O(22)D	3.179(7)	O(3)D--O(2)D2.87(1)

[2] Intermolecular hydrogen bonds or short contacts

(a) Possible hydrogen bonds

Atom 1 Atom 2	Symmetry[a]	Distance (Å)
O(1)A---O(21)B	*x,y − 1,z*	3.053(7)
O(1)A---O(23)B	*x,y − 1,z*	3.186(7)
O(1)A---O(23)A	*x + 1/2, − y − 1/2,z*	2.849(7)
O(3)A---O(3)C	*− x + 1, − y,z + 1/2*	2.794(9)
O(3)A---O(3')D	*− x + 3/2,y − 1/2,z + 1/2,z + 1/2*	2.81(2)
O(1)B---O(21)A	*x,y,z*	3.080(8)
O(1)C---O(21)D	*x,y − 1,z*	3.164(7)
O(1)C---O(23)D	*x,y − 1,z*	3.219(7)
O(1)C---O(23)D	*x − 1/2, − y + 1/2,z*	2.855(7)
O(3)C---O(3)B	*− x + 3/2,y − 1/2,z − 1/2*	2.698(9)
O(1)D---O(21)C	*x,y,z*	2.767(8)
O(1)D---O(23)C	*x,y,z*	3.020(8)
O(1)D---O(21)C	*x + 1/2, − y + 1/2,z*	3.175(8)

(b) Short contacts

O(2)A---O(21)A	*x − 1/2, − y − 1/2,z*	3.268(6)
O(21)A---O(22)D	*x + 1/2, − y + 1/2,z*	3.047(6)
O(21)A---O(23)D	*x + 1/2, − y + 1/2,z*	3.135(6)
O(22)A---O(23)D	*x,y − 1,z*	2.955(6)
O(22)A---O(22)C	*x + 1/2, − y − 1/2,z*	3.072(6)
O(2)B---O(21)B	*x − 1/2, − y + 1/2,z*	3.203(6)
O(21)B---O(22)B	*x + 1/2, − y + 1/2,z*	3.220(6)
O(21)B---O(22)C	*x + 1/2, − y + 1/2,z*	3.184(6)
O(21)B---O(23)C	*x + 1/2, − y + 1/2,z*	3.045(6)
O(22)B---O(23)C	*x,y,z*	2.987(6)
O(22)B---O(22)D	*x + 1/2, − y + 1/2,z*	3.003(6)
O(2)C---O(21)D	*y + 1/2, − y + 1/2,z*	3.065(6)
O(21)C---O(22)B	*x − 1/2, − y + 1/2,z*	3.125(6)
O(21)C---O(23)B	*x − 1/2, − y + 1/2,z*	3.255(6)
O(22)C---C(23)B	*x − 1/2, − y + 1/2,z*	3.211(6)
O(21)D---O(22)A	*x − 1/2, − y + 1/2,z*	3.161(6)
O(21)D---O(23)A	*x − 1/2, − y + 1/2,z*	2.987(6)
O(21)D---O(22)C	*x − 1/2, − y + 1/2,z*	3.047(6)
O(22)D---O(23)B	*x,y,z*	2.927(6)

[a]This represents the symmetry of Atom 2 with respect to Atom 1 at *x,y,z*.

TABLE 6. Coordination distances (Å) and angles (°) of calcium ions with their e.s.d.s in parentheses

	Symmetry[a]	Distance		Symmetry[a]	Distance
[a] Distance		(Å)			(Å)
Ca(1)---O(1)A	*x,y,z*	2.456(6)	Ca(2)---O(1)B	*x,y,z*	2.467(7)
O(22)A	*x,y,z*	2.429(4)	O(22)B	*x,y,z*	2.397(5)
O(22)D	*x,y − 1,z*	2.551(4)	O(22)C	*x,y,z*	2.945(4
O(23)D	*x,y − 1,z*	2.505(4)	O(23)C	*x,y,z*	2.370(5)
O(23)B	*x,y − 1,z*	2.366(4)	O(23)A	*x,y,z*	2.389(4)
O(21)A	*x − 1/2, − y − 1/2,z*	2.305(5)	O(21)B	*x − 1/2, − y + 1/2,z*	2.274(5)
O(22)C	*x + 1/2, − y − 1/2,z*	2.371(4)	O(22)D	*x + 1/2, − y + 1/2,z*	2.427(4)
Ca(3)---O(1)C	*x,y,z*	2.502(6)	Ca(4)---O(1)D	*x,y,z*	2.395(6)
O(22)C	*x,y,z*	2.386(4)	O(22)D	*x,y,z*	2.419(4)
O(22)A	*x,y,z*	2.808(4)	O(22)B	*x,y,z*	2.665(4)
O(23)A	*x,y,z*	2.434(4)	O(23)B	*x,y,z*	2.423(4)
O(23)D	*x,y − 1,z*	2.316(4)	O(23)C	*x,y,z*	2.358(5)
O(21)D	*x + 1/2, − y + 1/2,z*	2.280(4)	O(21)C	*x + 1/2, − y + 1/2,z*	2.307(4)
O(22)A	*x − 1/2, − y − 1/2,z*	2.441(4)	O(22)B	*x − 1/2, − y + 1/2,z*	2.380(4)

TABLE 6. *Continued*

	Symmetry[a]	Distance	Symmetry[a]	Distance
[b] Angle[b]				
	Ca(1)	Ca(2)	Ca(3)	Ca(4)
O(1)-Ca-O(22)	75.0(2)°	84.9(2)°	76.3(2)°	82.7(2)°
O(1)-Ca-O(22)	152.1(2)	138.7(2)	150.1(2)	141.8(2)
O(1)-Ca-O(23)	148.5(2)	160.8(2)	154.1(2)	155.8(2)
O(1)-Ca-O(23)	82.7(2)	78.8(2)	83.8(2)	78.9(2)
O(1)-Ca-O(21)	98.0(2)	89.5(2)	101.2(2)	84.9(2)
O(1)-Ca-O(22)	88.6(2)	92.8(2)	87.4(2)	93.1(2)
O(22)-Ca-O(22)	131.9(1)	130.2(1)	132.3(1)	129.8(1)
O(22)-Ca-O(23)	73.6(1)	77.6(2)	77.8(1)	74.4(1)
O(22)-Ca-O(23)	153.2(2)	161.5(2)	156.8(2)	157.0(2)
O(22)-Ca-O(21)	89.3(2)	87.1(2)	86.2(2)	88.6(2)
O(22)-Ca-O(22)	79.6(2)	77.0(1)	79.1(2)	77.5(2)
O(22)-Ca-O(23)	58.9(1)	54.7(1)	55.5(1)	57.1(1)
O(22)-Ca-O(23)	73.0(1)	68.3(1)	69.7(1)	72.7(1)
O(22)-Ca-O(21)	77.5(2)	73.9(1)	76.0(1)	77.5(2)
O(22)-Ca-O(22)	102.6(1)	113.7(1)	104.9(1)	111.5(2)
O(23)-Ca-O(23)	127.7(1)	119.6(2)	121.1(2)	125.2(2)
O(23)-Ca-O(21)	81.2(2)	81.9(2)	78.5(2)	87.0(2)
O(23)-Ca-O(22)	86.2(2)	90.9(2)	86.2(1)	89.4(2)
O(23)-Ca-O(21)	108.5(2)	101.3(2)	109.5(2)	103.3(2)
O(23)-Ca-O(22)	85.4(2)	95.0(1)	88.4(2)	89.8(2)
O(21)-Ca-O(22)	165.2(2)	163.7(2)	160.7(2)	166.1(2)

[a]This represents the symmetry operation of ligand atom with respect to calcium ion at *x,y,z*.
[b]The atom order for the coordination angle coincides with that in each column used for distance [a].

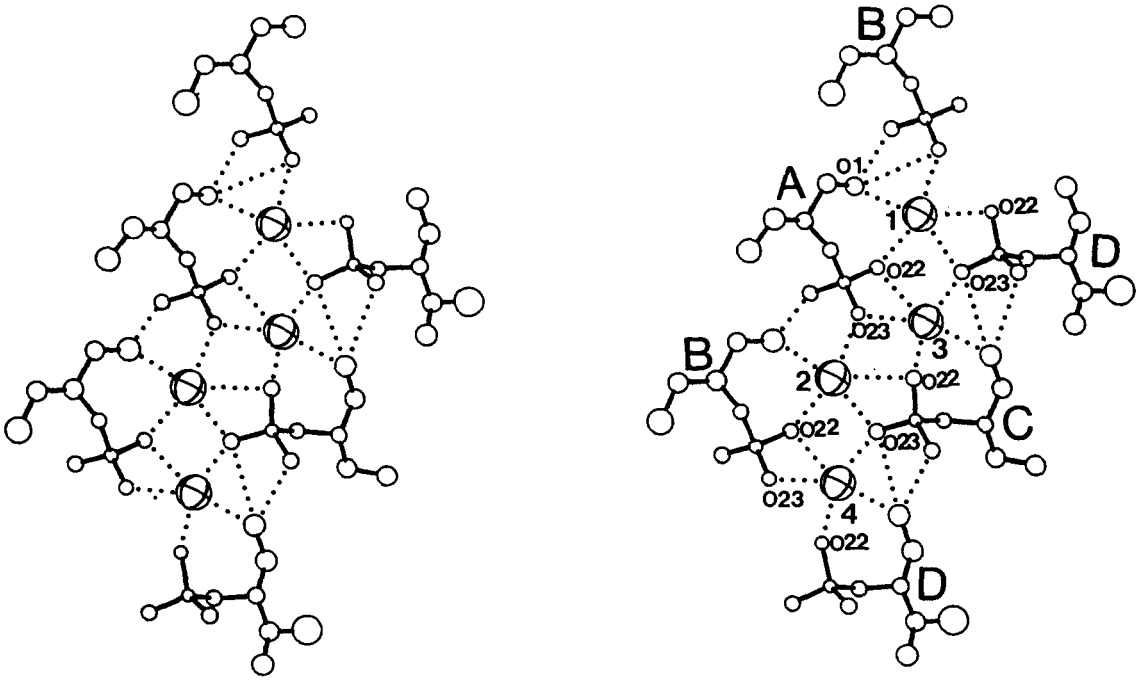

Fig. 3. A stereoscopic view of a part of bilayer unit consisting of molecules A and B and of molecules C and D, respective layers of which are arranged face-to-face and are infinitely expanded to the *b*-direction. These layers sandwich calcium ions marked by the ellipsoidal circles. The intermolecular hydrogen bonds and the coordinations to calcium ions are represented by dotted lines. Four independent calcium ions are denoted by numbers 1 to 4, respectively.

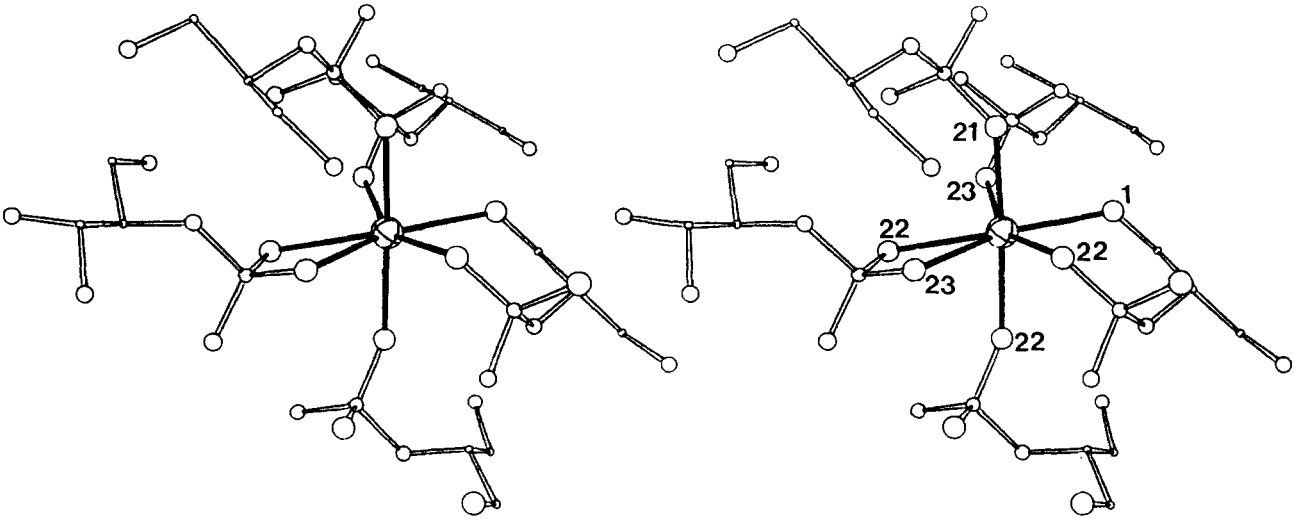

Fig. 4. A stereoscopic view of Ca(1) coordination shell. The coordination bonds are represented by solid lines. Similar distorted pentagonal-bipyramidal environments are observed in the coordinations of three other calcium ions.

Å from the best fit plane. The O(21)A and O(22)C atoms coordinate to the calcium ion as the distorted axial donors of the fourth and fifth ligands, respectively.

In addition to coordinations of these ligand atoms to calcium ions, the intermolecular hydrogen bonds and short contacts listed in Table 5 further stabilize the stacked bilayer units. The very low-temperature factors of many atoms listed in Table 2 are a result of these elaborate hydrogen-bonding and calcium-coordinating networks formed within the unit structures. In addition to this tightly knit bilayer unit, the neighboring units are linked with three hydrogen bonds [O(3)A–O(3)C, O(3)A–O(3')D, and O(3)B–O(3)C] and many short contacts (Table 5), thus contributing to the stability of β-CaG crystals.

Possible molecular packing of α-CaG and interaction model of phospholipid with calcium ions

Despite its biological importance, the crystal structure of α-CaG has not yet been analyzed. This is mainly due to the difficulty in preparing single crystals large enough

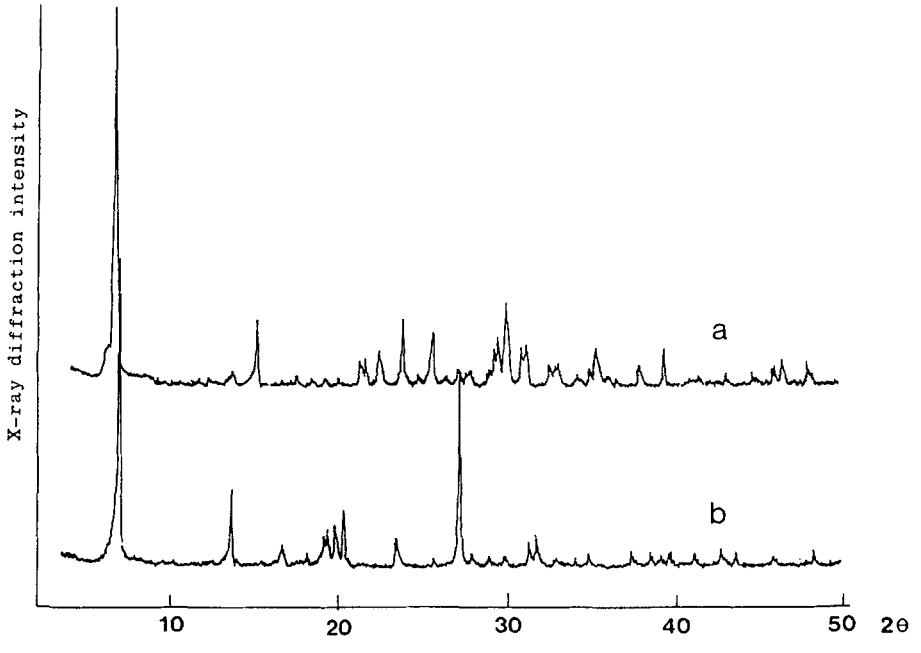

Fig. 5. X-ray diffraction patterns of β-CaG (a) and α-CaG (b) anhydrate powders.

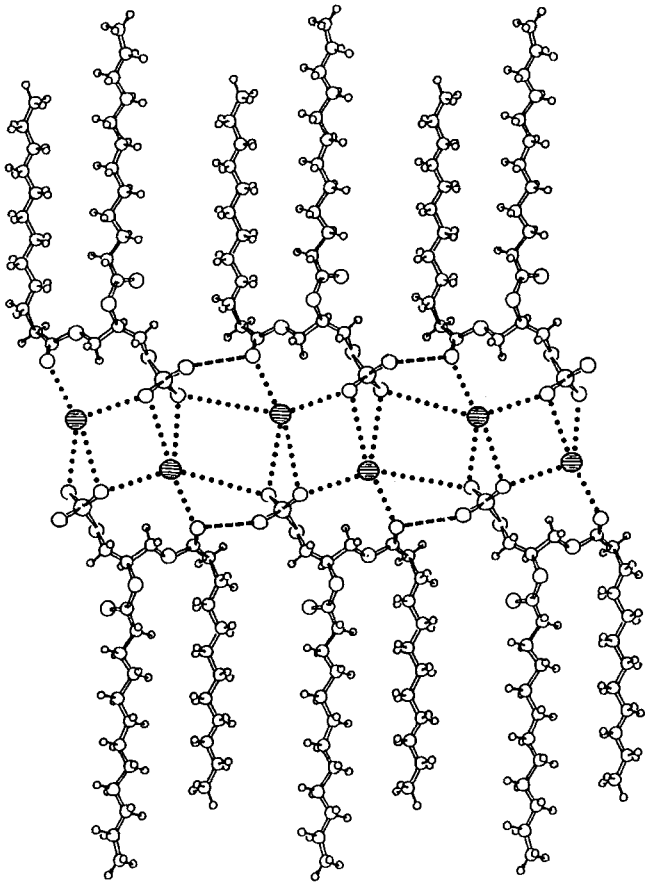

Fig. 6. A schematic interaction model of phospholipid with calcium ions. The broken and dotted lines indicate the intermolecular hydrogen bonds and the coordinations to calcium ions (shown by shaded circles), respectively.

to carry out X-ray analysis. In order to estimate the possible molecular packing of α-CaG crystal from the present β-CaG crystal, the X-ray diffraction patterns of α- and β-CaG anhydrate microcrystalline powders prepared under the same conditions were compared (**Fig. 5**). Characteristically, an intense peak was observed near 2θ (Bragg angle) = 7.0° and is in contrast to the weak diffractions of remaining peaks. Although the diffraction pattern of α-CaG was not exactly identical to that of β-CaG, some structural similarities were observed between both crystals. Among these, the strongest X-ray peaks observed at 2θ = 7.0° (d = 12.63 Å) for β-CaG and 7.1° (d = 12.45 Å) for α-CaG would reflect the whole molecular packing pattern rather than the individual molecular conformation itself. These peaks could correspond to the (002) reflection, as judged from the present crystal structure of β-CaG [d_{002} = 12.94 Å for β-CaG]; the X-ray intensity of this reflection is largely dependent on the whole molecular packing in the crystal rather than the individual molecular conformation of β-CaG itself. This makes us imagine that the molecular packing mode of α-CaG crystal is very similar to that of β-CaG crystal, where α-CaG molecules form the stacked bilayer and the calcium ions are sandwiched between the layers arranged face-to-face within the bilayer unit. Similar molecular packing mode between the α- and β-CaG crystals was further suggested from the hydration experiments of both the anhydrate crystals. By the monohydration of these crystals, the intense peaks of 2θ = 7.1° (α-CaG) and 7.0° (β-CaG) were both shifted to 6.6° (d = 13.39 Å), while the remaining X-ray diffraction peaks showed no noticeable changes. Characteristically, these changes are reversible and return to their original states by dehydration. This also indicates the similar interaction mode between both the CaG crystals.

In the crystal structure of β-CaG, none of the γ-OH groups in the four independent β-glycerophosphates characteristically participated in the coordinations with calcium ions (Table 6). This means that an interaction model similar to β-CaG is also possible for α-CaG simply by replacing the β-phosphate and α-OH groups. Since the phospholipid is mainly composed of the esterification of α-glycerophosphate β- and γ-OHs with fatty acids, it therefore appears reasonable to consider the possible interaction model of phospholipid with calcium ions, based on the present crystal analysis of β-CaG.

A binding model of calcium ions for the bilayer or lamella structure formed by dilauroylphosphatidic acid (DLPA), a phospholipid, is proposed in **Fig. 6**, in which torsional parameters used for the linkage between the fatty acid and *l*-α-glycerophosphate were cited from the crystal structure of DLPA (17). As judged from the behavior of X-ray diffraction profiles of α-CaG powders against the hydration, the hydrophilic bilayer consisting of the face-to-face polar regions of phospholipids would have considerable elasticity concerning the separation distance between these polar regions[5]; the variation of about 2.0 Å may be possible concerning the width of the hydrophilic path formed by the face-to-face phosphate groups and calcium ions. The coexistence of water molecules would widen the unit structure in Fig. 6 and weaken the direct interactions of glycerophosphate regions of phospholipids with calcium ions, as could be presumed from the fact that α-CaG crystals are overwhelmingly obtained as dihydrate under the crystallization condition at 35°C (4). The interrelated coordinations of glycerophosphate and water ligands may regulate the passage of calcium ions through the membrane.

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[5]By the thorough hydration, α-CaG anhydrate crystals were changed to the dihydrate, and the strongest peak of 2θ = 7.1° (d = 12.45 Å) shifted to 2θ = 6.1° (d = 14.49 Å).

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