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Crystallization of the isobutylphosphocholine–cholesterol–isobutanol (1:3:3) complex and its investigation by X-ray analysis: interaction of phopholipid headgroups with cholesterol

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Abstract

A crystal complex consisting of the isobutyl analog of phosphatidylcholine (PC) (isobutylphosphocholine), cholesterol, and isobutanol with molecular ratio 1:3:3 was obtained and investigated by means of X-ray analysis. The complex was shown to correspond to the monoclinic system (sp. gr. $P2_1$): $a = 16.994(10)$, $b = 11.314(7)$, $c = 28.164(15)$, $\beta = 104.07(3)$, $V = 5252.63$ Å^3, $Z = 2$, $D_{calc} = 1.0273$ g/cm^3. The isobutylphosphocholine molecule is the key component of the complex. Pairs of hydrogen bonds are formed between the $^{-\delta}$O–P–O$^{\delta-}$ group of the isobutylphosphocholine molecule and C–OH groups of two cholesterol and two isobutanol molecules. The third molecules of cholesterol and isobutanol are H-bonded with the $^{-\delta}$O–P–O$^{\delta-}$ group of the isobutylphosphocholine molecule via C–OH groups of isobutanol and cholesterol, respectively. The crystal structure is built up by translation of the complex in multiplicate along the two-fold axis in the direction of axis *b*. It contains bands formed by isobutylphosphocholine molecules alternately changing their direction. They are fixed by virtue of two zones of electrostatic interactions of the type $^{-\delta}$O–P–O$^{\delta-}$...$^{+}$N(CH$_3$)$_3$ and are more or less parallel to the *bc* plane. The structure also contains three-layer domains formed by cholesterol molecules perpendicular to isobutylphosphocholine bands. In the direction of the *c*-axis isobutylphosphocholine bands alternate with the layers of cholesterol molecules herewith reproducing repeated blocks. The obtained structure is compared with that of crystals of phospholipids and cholesterol and its derivatives.

Keywords: Phospholipid analog; Cholesterol; Crystal complex; X-ray analysis; Biomembrane modeling

1. Introduction

Cholesterol is one of the most common lipid molecules in the composition of biomembranes of eukaryotic organisms [1–10]. Its structure contains a cyclopentaneperhydrophenanthrene nucleus (CPP-nucleus), formed by three hexacycles (A, B, C) and one pentacycle (D), and also a side-chain [1]. Cholesterol is alcohol with C–OH-substituent in the third position.

In the biomembrane structure cholesterol is bound with protein, the intensity of binding being dependent on the type of protein participating in such interaction [5–7]. Cholesterol was shown to enter into the composition of detergent-resistant mem-

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brane domains (DRMS), the latter also containing sphingolipids, including sphingomyelin, and cholesterol-binding proteins [6,7]. Cholesterol may contribute almost 50% to the total lipid content of the biomembrane. Particularly high is the cholesterol content in plasmatic membranes, whereas in other types of membranes (endoplasmic reticulum, nucleus membrane, etc.) it is not that significant [5]. Within the bilayer structure of biomembrane both symmetrical and asymmetrical distribution of cholesterol in the outer and inner monolayers may occur [4].

An equimolar ratio of cholesterol and phospholipids (PL) is recorded [2,3] in many membranes, e.g. in myelin membranes. The occurrence of different types of molecules implies a possibility of complex formation between them. In fact, such complexes were discovered in mixed lipid–cholesterol micelles. Normally, for their investigations pure synthetic PL, e.g. dipalmitoyl PC, and different sterols, particularly cholesterol, are used [9]. These investigations showed that in the bilayer lipid membranes the CPP-nucleus and hydrophobic cholesterol side chain are parallel to the PL fatty acid chains. The side chains of cholesterol molecules are localized in the inner region of the bilayer and the C–OH groups of CPP-nuclei are in the vicinity of the ester bonds of PL molecules on the water–membrane boundary [5,10]. The packing pattern of cholesterol molecules in biomembranes is generally assumed as similar to that of cholesterol–PL complexes [6].

The application of the method of electron microscopy to biomembranes treated with antibiotic filipin allowed to reveal periodic structures - filipin–cholesterol complexes [11]. However, up to now there are no precise data on the packing pattern of either cholesterol or its complexes with PL and proteins in biomembranes.

The structure of the crystals of cholesterol molecules and their derivatives as well as of PL molecules is well investigated [8,12]. However, there are practically no data on the structural studies of cholesterol complexes with PL molecules or with their fragments. Of particular interest are interactions arising between PL bipolar groups and cholesterol which are assumed in the zone-block model of biomembrane [13–23].

Earlier [23] we investigated the structure of a PC analog, where the diglyceride radical is replaced by the isobutyl one (isobutyl-2-(trimethylammonium)-ethylphosphate - IPC), the latter forming a complex with isobutanol (IB) and water. The obtained structure allowed to presume the formation of a complex between IPC and cholesterol. The aim of the present work was to obtain IPC complexes with cholesterol, their crystallization, investigation of molecular and crystal structure.

2. Materials and methods

2.1. Materials

To obtain complex crystals we synthesized isobutyl-2-(trimethylammonium)-ethyl phosphate and used cholesterol purchased from Calbiochem. IB (reagent grade) used for growing crystals was preliminarily dried by distillation (the boiling temperature of the collected fraction was 107.5°C). Earlier [23], complex crystals with IPC were grown from that solvent.

2.2. Synthesis of isobutylphosphocholine

IPC was synthesized by the method proposed in [24]. The intermediate cyclic derivative isobutyldioxophospholane was obtained from isobutyloxophosphodichloride and ethylene glycol. Then the cycle was opened with the simultaneous introduction of a trimethylammonium group. Details of the procedure of the chemical synthesis of the compound can be found in [23]. The identification of IPC was carried out by NMR-spectroscopy [20].

2.3. Crystallization of the complexes

Joint crystallization of IPC with cholesterol was performed by evaporation of the solvent IB. Crystals formed at different values of the ratio IPC:cholesterol were examined. Crystals were observed under the microscope equipped with an adapter enabling to regulate temperature of the microscopic stage (Nagema, Germany). Herewith temperature-dependent changes in the aggregative state and birefringence were monitored. Crystals with the properties different from those of IPC and earlier obtained [23] IPC complex with IB were discovered. Thus, the new

crystals manifested phase transition in the temperature range 75–80°C. Their melting temperature was 170°C, which is significantly different from that of IPC (240°C) and cholesterol (149°C).

Samples of IPC (26 mg) and cholesterol (19.4 mg) (molar ratio 2:1) were weighed in 5 ml test tubes (50 mm long, diameter 10 mm). Both components were dissolved together in 400 μl IB heated to 50–60°C. Crystals were grown in a desiccator (capacity 0.5–1l) during 7–10 days at room temperature at relative humidity about 12% by a slow evaporation of the solvent (above 50–100 ml) oversaturated water solution of LiCl). Two or three test tubes were placed into a desiccator at some angle which led to the formation of a slanted meniscus. This increased the surface of evaporation and enhanced crystal formation on the test-tube walls. A small amount of liquid (about 50 μl) was left on the bottom of the test tube. In spite of a two-fold excess of IPC, the ratio IPC:cholesterol in the complexes was shifted towards the latter, being equal to 1:3.

2.4. Determination of the composition and ratio of the components in the complex crystals

Preliminary information on the composition and ratio of the components in crystals is necessary for determination of their structure. The loss of mass caused by heating the crystal samples at 50–70°C to the constant weight was about 15%, which is apparently associated with the evaporation of incorporated IB. The phosphorus content in the crystals is approximately equal to $1.95 \pm 0.02\%$, which testifies to the presence of IPC in the crystal structure. Theoretical calculations revealed that the complex crystals contain three cholesterol and three IB molecules per one IPC molecule. These preliminary results were confirmed by the subsequent determination of the crystal structure.

2.5. X-ray analysis

Crystals for X-ray analysis were selected by the photographic method. To avoid decomposing, crystals were placed in the capillary tubes. Data were obtained with a monocrystal diffractometer Syntex $P2_1$ (MoK_α radiation, graphite monochromator) in the range 2–50° of the angle 2θ. In the experiments, 9443 independent reflexes were recorded by the ω-method from a monocrystal $7 \times 1 \times 0.2$ mm. For the subsequent analysis 3159 reflexes meeting the condition $F_{hkl} > 5.00\ \sigma_F$ were taken. Data reduction was performed by the CSD programs [25] and included the long-term instability correction and Lp-correction. The determination of the structure by the direct method and subsequent Fourier syntheses were carried out by means of SIR 97 [26]. The structure was refined in the anisotropic approximation for nonhydrogen atoms by the block–fullmatrix method. The blocks, each containing 300 parameters to be refined, were composed manually. For each block, parameters selected for subsequent refinement procedure exhibited the least correlation coefficients. The position of hydrogen atoms was determined from geometrical considerations (hydrogen atoms of methyl radicals were calculated on the basis of the assumption of non-shielded conformation). Refinement was undertaken within the model of rigid body. The whole procedure was carried out on the basis of SHELX 97 [27]. A list of refined anisotropic thermal parameters is available from the authors. The absorption correction was introduced by the DIFABS program [28]. The structure was refined to $R_F = 0.0691$.

3. Results

3.1. General characterization of the complex and nomenclature

IPC–cholesterol–IB complex was shown to crystallize in the monoclinic system (sp. gr. $P2_1$) with cell dimensions: $a = 16.994(10)$, $b = 11.314(7)$, $c = 28.164(15)$, $\beta = 104.07(3)°$, $V = 5252.63$ Å^3, $Z = 2$, $D_{calc} = 1.0273$ g/cm^3. There are three cholesterol (Chol 1, Chol 2, Chol 3) and three IBs (IB 1, IB 2, IB 3) molecules per one IPC molecule in the independent part of the crystal cell. This suggests that the complex crystal is solvate. Fig. 1 represents the arrangement of molecules in the complex crystal and numbering of atoms in the structure.

Numbering of atoms in IPC and IB molecules is similar to that used in [23]. Numbering of atoms in cholesterol molecules corresponds to the conventional nomenclature [1] with the upper subindex desig-

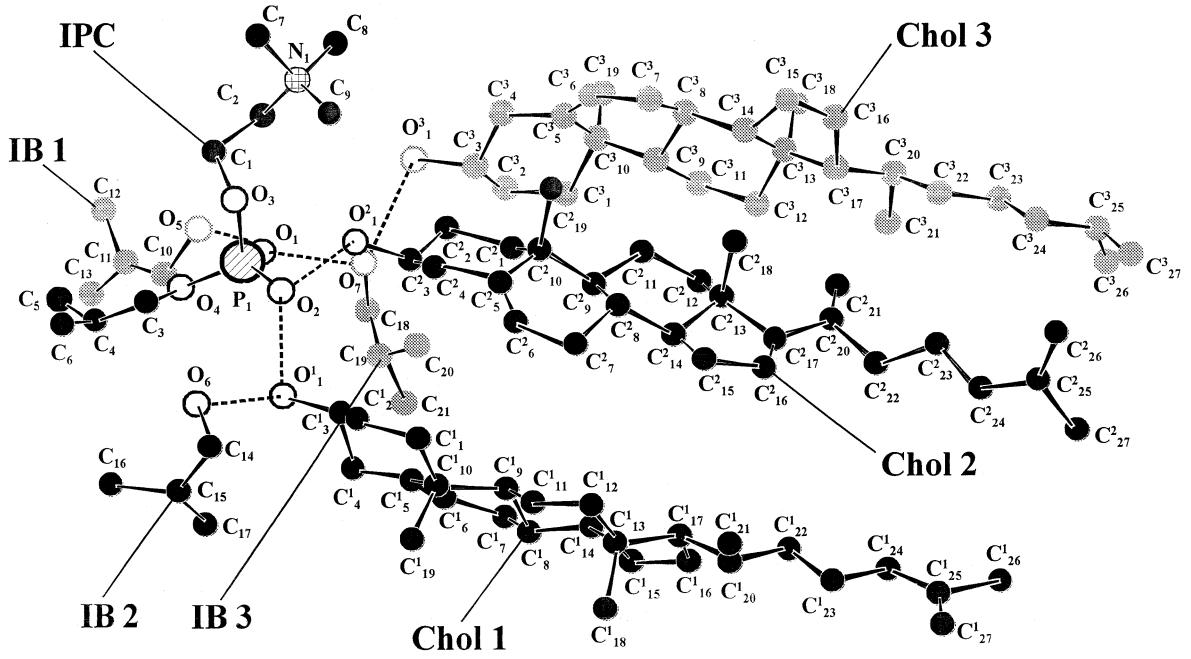


Fig. 1. Isobutylphosphocholine–cholesterol–IB complex (1:3:3): conformation and arrangement of molecules; numbering of atoms in the crystal structure. IPC, isobutylphosphocholine; IB 1–IB 3, isobutanol molecules; Chol 1–Chol 3, cholesterol molecules. Filled circles denote carbon atoms. Carbon atoms of more distant cholesterol and IB molecules are represented by hatched circles.

nating the number of the molecule in the crystal. The coordinates of atoms and equivalent thermal parameters (U_{eq}) are given in Table 1. Geometric parameters of the molecules (distances between the atoms and valence angles) are calculated and available from the authors.

3.2. Structure of the molecules making up the IPC–cholesterol–isobutanol (1:3:3) complex

3.2.1. Isobutylphosphocholine molecule

Aminoethyl and isobutyl branches are bonded to the phosphate group of IPC via ester oxygens O_3 and O_4. Distances P–O for the ester atoms (1.599 Å and 1.561 Å) are significantly larger than for atoms O_1 and O_2 (1.454 Å and 1.471 Å). Closeness of the two latter values testifies to the absence of protons associated with O_1 and O_2. Similar observations were made with the IPC and isobutyl-2-aminoethyl phosphate (isobutylphosphoethanolamine–IPEA) molecules investigated earlier [16,21,23].

The torsion angles for the aminoethyl fragment of IPC in the IPC–cholesterol–IB complex are given in Table 2. For comparison, data on other PL analogs investigated earlier, i.e. IPEA–HCl (molecules A′ and A) [21], IPEA [16], IPC–H_2O and IPC–H_2O–IB [23] are also provided. As seen from Table 2, these angles are most close to those observed in the molecule A′ (IPEA–HCl), where atoms P_1 and C_2 also have a gauche conformation similar to the considered compound. In other molecules, containing the amino group (IPEA–HCl, molecule A; IPEA), these atoms exhibit *trans*-conformation, whereas in the molecules with the choline group the conformation is intermediate. Interatomic distances and valence angles in the aminoethyl fragment of the complex are also closer to those observed in the molecule A′ [21].

As seen from Fig. 1, the isobutyl branch is almost perpendicular to the aminoethyl one. It was presumed in [21] that the *trans*-conformation of the aminoethyl branch is energetically not favored and, hence, is compensated for by high values of thermal oscillations of the isobutyl branch. This results in a substantial reduction of interatomic distances. That is why we expected that, owing to the gauche conformation of the aminoethyl branch of the IPC molecule of the complex, thermal oscillations and interatomic distances in the isobutyl branch would assume values usual for similar compounds. Though

the distance C_3–C_4 is slightly larger than similar distances in the molecules A (IPEA–HCl) and IPC, studied earlier [21,23], our assumption about the relationship presumably existing between the geometry and dynamics of the isobutyl fragment, on one hand, and the conformation of the aminoethyl fragment, on the other, proved false.

3.2.2. Cholesterol molecules

In the investigated complex the conformation of the cycles composing CPP-nuclei of the cholesterol molecules (Chol 1, Chol 2, Chol 3) is close to the standard one. Cycles A and C have chair, cycle B, semichair and cycle D, envelope conformation. The conformation of the cycle B in each molecule is accounted for by the presence in the cycle of the double bond between C_5^i and C_6^i atoms (upper index i designates the cholesterol molecule assuming values 1, 2, 3). As a consequence, C_{10}^i, C_5^i, C_6^i and C_7^i lie in one plane while C_8^i and C_9^i atoms are situated on the opposite sides of the plane. Angular methyl groups associated with C_{10}^i and C_{13}^i atoms are directed to one side from the plane of the CPP-nucleus.

The side chains of cholesterol molecules possess almost identical conformation which is confirmed by the values of torsion angles (Table 3). This table and Fig. 1 clearly illustrate, that in all three cholesterol molecules the side chain is fully extended. The values of angles between the perpendiculars to the plane passing through the atoms of the D-cycle and that passing through C_{17}^i, C_{20}^i, C_{22}^i, C_{23}^i, C_{24}^i, and C_{25}^i atoms of the side chain are close to each other and vary in the range 43.7°–47°. The side chains and CPP-nuclei are extended along one direction.

3.3. Structure of the complex

As seen from Figs. 1 and 2a, free oxygens of the phosphate group ($^{-\delta}O_1$–P–$O_2^{\delta-}$) of the IPC molecule in the independent part of the crystal cell contribute to the formation of hydrogen bonds with cholesterol and IB molecules (Table 4). Thus, the C_{10}–O_5H-group of the IB 1 molecule and the C_{18}–O_7H-group of the IB 3 molecule form such a bond with the O_1 oxygen of the $^{-\delta}O_1$–P–$O_2^{\delta-}$ group. Distances O_5–O_1 and O_7–O_1 are equal to 2.69(2) Å and 2.61(2) Å, respectively. Angle O_5–O_1–O_7 is equal to 92.8(6)°. One of the above atoms (O_7) forms a hydrogen bond with the C_3^3–O_1^3H-group of a cholesterol molecule (Chol 3). Distance O_7–O_1^3 is equal to 2.65(2) Å and the value of angle O_1^3–O_7–O_1 is 109.3(6)°. Oxygen O_2 of the phosphate group participates in the formation of two hydrogen bonds with the C_3^1–O_1^1H–group of the Chol 1 molecule and C_3^2–O_1^2H-group of the Chol 2 molecule. Distances O_2–O_1^1 and O_2–O_1^2 are equal to 2.67(2) and 2.71(2) Å, respectively, and the value of the angle at O_2 is 101.8(5)°. Though the planes of the CPP-nuclei of the two cholesterol molecules and the side chains (Fig. 1) are approximately parallel, the molecules are turned to about 180° with respect to the plane of the figure, so that the angular methyl groups are oriented in the opposite directions. The same is true for the methyl groups of the C_{21}^1 and C_{21}^2 side chains.

The C_3^1–O_1^1H-group of the Chol 1 molecule is bonded with the C_{14}–O_6H-group of IB 2 by a hydrogen bond, distance O_6–O_1^1 being equal to 3.03(3) Å. Thus, atom O_1^1 as well as atoms O_2 and O_7 contribute to two hydrogen bonds leading to the value of 105.5(6)° for the angle O_2–O_1^1–O_6. Hence, as seen from Fig. 1, two cholesterol molecules (Chol 1 and Chol 2) interact directly with the oxygen atom O_2 of the IPC phosphate group, whereas the third cholesterol molecule (Chol 3) is bound with the IPC molecule via the C_{18}–O_7H-group of the IB 3 molecule. Two IB molecules (IB 1 and IB 3) are directly associated with the oxygen atom O_1 of the IPC phosphate group. The third molecule (IB 2) is bound to IPC via the C_3^1–O_1^1H-group of the Chol 1 molecule. Therefore, each free oxygen of the $^{-\delta}O_1$–P–$O_2^{\delta-}$ group of the IPC molecule participates in the formation of two hydrogen bonds.

All three molecules are extended along axis c of the cell. Angles between the planes Chol 1–Chol 2, Chol 1–Chol 3, Chol 2–Chol 3 of cholesterol molecules assume the following values: −9.4°, −74.8° and −71.1°, respectively. Thus, the planes of Chol 1 and Chol 2 are nearly parallel, while the plane of the third molecule (Chol 3) is almost perpendicular to the first two planes.

3.4. Packing of the molecules

As seen in Fig. 2a, oxygen $O_2^{\delta-}$ of the phosphate group of one IPC molecule from the basal complex of the crystal cell is localized in the proximity of the

Table 1
Coordinates of the atoms and equivalent thermal parameters (U_{eq}) of the IPC–cholesterol–IB (1:3:3) complex

Atom	*x/a*	*y/b*	*z/c*	U_{eq}
1	2	3	4	5
IPC				
P_1	0.6870(4)	0.0816(6)	0.4969(2)	0.087(2)
O_1	0.6930(8)	−0.0436(14)	0.5089(4)	0.115(4)
O_2	0.7191(7)	0.1689(12)	0.5355(4)	0.109(4)
O_3	0.5938(8)	0.1177(10)	0.4770(4)	0.094(4)
O_4	0.7234(8)	0.0908(15)	0.4512(4)	0.115(4)
N_1	0.4363(9)	0.0176(14)	0.4989(5)	0.083(4)
C_1	0.5376(11)	0.0386(21)	0.4462(6)	0.103(7)
C_2	0.4945(15)	−0.0425(22)	0.4767(7)	0.121(8)
C_3	0.7304(17)	0.2001(28)	0.4285(8)	0.151(10)
C_4	0.7618(24)	0.1995(32)	0.3855(11)	0.211(16)
C_5	0.7533(35)	0.0982(35)	0.3546(12)	0.374(40)
C_6	0.7545(31)	0.3149(34)	0.3627(13)	0.296(26)
C_7	0.3745(17)	0.0740(26)	0.4600(8)	0.176(11)
C_8	0.3976(17)	−0.0737(28)	0.5218(9)	0.176(12)
C_9	0.4780(14)	0.1005(21)	0.5388(7)	0.141(9)
Molecules of Cholesterol				
Chol 1				
O_1^1	0.8642(8)	0.2663(23)	0.5652(5)	0.185(9)
C_1^1	0.9034(10)	0.3923(20)	0.6930(6)	0.119(8)
C_2^1	0.8815(15)	0.3899(24)	0.6364(7)	0.151(10)
C_3^1	0.8865(14)	0.2676(24)	0.6175(7)	0.144(10)
C_4^1	0.9678(14)	0.2170(21)	0.630(7)	0.144(10)
C_5^1	0.9890(16)	0.2158(19)	0.6932(7)	0.142(10)
C_6^1	1.0186(17)	0.1193(23)	0.7178(8)	0.169(13)
C_7^1	1.0431(17)	0.1078(18)	0.7734(8)	0.169(11)
C_8^1	1.0545(11)	0.2261(14)	0.7988(6)	0.096(6)
C_9^1	0.9891(9)	0.3159(14)	0.7732(5)	0.085(6)
C_{10}^1	0.9856(9)	0.3329(15)	0.7178(6)	0.092(6)
C_{11}^1	0.9963(10)	0.4283(15)	0.8026(5)	0.087(6)
C_{12}^1	0.9980(10)	0.4169(16)	0.8563(5)	0.089(6)
C_{13}^1	1.0623(9)	0.3277(14)	0.8816(5)	0.071(5)
C_{14}^1	1.0499(11)	0.2134(15)	0.8519(6)	0.096(6)
C_{15}^1	1.1082(15)	0.1265(17)	0.8853(7)	0.148(9)
C_{16}^1	1.0988(14)	0.1605(17)	0.9376(6)	0.127(8)
C_{17}^1	1.0525(9)	0.2846(13)	0.9316(5)	0.073(5)
C_{18}^1	1.1472(8)	0.3784(18)	0.8859(6)	0.106(7)
C_{19}^1	1.0544(12)	0.4129(20)	0.7098(6)	0.125(8)
C_{20}^1	1.0832(9)	0.3584(14)	0.9778(5)	0.075(5)
C_{21}^1	1.0444(12)	0.4814(14)	0.9738(6)	0.103(6)
C_{22}^1	1.0702(10)	0.2946(17)	1.0229(6)	0.094(6)
C_{23}^1	1.1070(12)	0.3497(21)	1.0711(6)	0.124(8)
C_{24}^1	1.0934(13)	0.2864(19)	1.1149(7)	0.121(7)
C_{25}^1	1.1287(23)	0.3221(27)	1.1642(7)	0.233(18)
C_{26}^1	1.1150(18)	0.2520(23)	1.2038(7)	0.176(11)
C_{27}^1	1.1508(27)	0.4476(26)	1.1770(9)	0.267(23)
Chol 2				
O_1^2	0.6363(8)	0.2784(11)	0.5940(3)	0.093(4)
C_1^2	0.6568(10)	0.1546(14)	0.7208(5)	0.081(5)
C_2^2	0.6239(10)	0.1662(15)	0.6655(5)	0.079(5)
C_3^2	0.6640(9)	0.2666(15)	0.6458(5)	0.069(5)

Table 1 (*continued*)

Atom	*x*/*a*	*y*/*b*	*z*/*c*	U_{eq}
1	2	3	4	5
C_4^2	0.6538(10)	0.3785(13)	0.6701(5)	0.067(5)
C_5^2	0.6808(8)	0.3696(13)	0.7257(4)	0.049(4)
C_6^2	0.7315(8)	0.4502(12)	0.7502(5)	0.056(4)
C_7^2	0.7618(9)	0.4509(14)	0.8053(5)	0.071(5)
C_8^2	0.7115(8)	0.3736(11)	0.8304(4)	0.049(4)
C_9^2	0.6948(8)	0.2535(12)	0.8035(4)	0.051(4)
C_{10}^2	0.6464(8)	0.2687(13)	0.7488(4)	0.056(4)
C_{11}^2	0.6563(10)	0.1683(14)	0.8335(4)	0.080(5)
C_{12}^2	0.7004(10)	0.1560(13)	0.8873(5)	0.077(5)
C_{13}^2	0.7091(7)	0.2777(13)	0.9126(4)	0.058(4)
C_{14}^2	0.7549(8)	0.3543(12)	0.8834(4)	0.051(4)
C_{15}^2	0.7820(9)	0.4624(12)	0.9158(4)	0.065(4)
C_{16}^2	0.7979(9)	0.4133(12)	0.9688(5)	0.068(5)
C_{17}^2	0.7674(8)	0.2811(12)	0.9642(4)	0.060(4)
C_{18}^2	0.6279(8)	0.3314(16)	0.9148(5)	0.081(5)
C_{19}^2	0.5572(8)	0.2878(18)	0.7456(6)	0.097(6)
C_{20}^2	0.7399(9)	0.2435(13)	1.0097(4)	0.062(4)
C_{21}^2	0.7081(11)	0.1173(17)	1.0046(6)	0.109(8)
C_{22}^2	0.8045(8)	0.2606(16)	1.0570(4)	0.068(4)
C_{23}^2	0.7791(9)	0.2451(19)	1.1034(5)	0.090(6)
C_{24}^2	0.8405(10)	0.2641(19)	1.1498(5)	0.093(6)
C_{25}^2	0.8224(13)	0.2389(27)	1.1975(6)	0.154(12)
C_{26}^2	0.7518(16)	0.3028(45)	1.2030(7)	0.304(32)
C_{27}^2	0.8920(13)	0.2599(27)	1.2410(6)	0.170(11)
Chol 3				
O_1^3	0.5648(8)	−0.1785(13)	0.6106(4)	0.115(4)
C_1^3	0.6222(11)	−0.2666(18)	0.7431(6)	0.106(7)
C_2^3	0.6104(12)	−0.2757(15)	0.6882(6)	0.100(6)
C_3^3	0.5716(11)	−0.1672(16)	0.6620(5)	0.088(6)
C_4^3	0.4929(10)	−0.1468(19)	0.6724(6)	0.098(6)
C_5^3	0.4996(10)	−0.1464(16)	0.7268(7)	0.087(6)
C_6^3	0.4631(10)	−0.0607(17)	0.7455(6)	0.090(6)
C_7^3	0.4603(10)	−0.0466(15)	0.7986(6)	0.094(6)
C_8^3	0.4909(9)	−0.1541(15)	0.8295(5)	0.077(5)
C_9^3	0.5651(9)	−0.2075(14)	0.8133(5)	0.080(5)
C_{10}^3	0.5424(10)	−0.2451(14)	0.7582(5)	0.074(5)
C_{11}^3	0.6069(11)	−0.3047(17)	0.8478(6)	0.102(6)
C_{12}^3	0.6264(9)	−0.2726(16)	0.9018(6)	0.093(6)
C_{13}^3	0.5500(8)	−0.2298(13)	0.9178(5)	0.067(5)
C_{14}^3	0.5141(9)	−0.1271(13)	0.8834(5)	0.073(5)
C_{15}^3	0.4520(10)	−0.0697(17)	0.9074(7)	0.102(6)
C_{16}^3	0.4909(10)	−0.0892(15)	0.9635(6)	0.092(6)
C_{17}^3	0.5655(8)	−0.1731(12)	0.9681(5)	0.066(5)
C_{18}^3	0.4871(10)	−0.3294(15)	0.9140(6)	0.094(6)
C_{19}^3	0.4891(13)	−0.3537(16)	0.7482(7)	0.126(8)
C_{20}^3	0.5816(9)	−0.2456(14)	1.0155(5)	0.070(5)
C_{21}^3	0.6509(10)	−0.3319(15)	1.0166(6)	0.094(6)
C_{22}^3	0.6009(10)	−0.1677(15)	1.0603(6)	0.086(6)
C_{23}^3	0.6116(10)	−0.2243(17)	1.1090(6)	0.092(6)
C_{24}^3	0.6306(12)	−0.1388(22)	1.1512(7)	0.137(9)
C_{25}^3	0.6490(17)	−0.1860(25)	1.2006(8)	0.165(12)
C_{26}^3	0.7029(18)	−0.2836(23)	1.2116(9)	0.175(11)

Table 1 (*continued*)

Atom	*x*/*a*	*y*/*b*	*z*/*c*	U_{eq}
1	2	3	4	5
C_{27}^3	0.6675(17)	−0.0935(24)	1.2408(8)	0.193(14)
Molecules of IB				
IB 1				
O_5	0.6845(14)	−0.2395(21)	0.4540(7)	0.221(10)
C_{10}	0.7520(14)	−0.2566(34)	0.4336(11)	0.211(17)
C_{11}	0.7328(15)	−0.2841(44)	0.3862(10)	0.239(24)
C_{12}	0.6563(18)	−0.3252(53)	0.3609(11)	0.361(42)
C_{13}	0.7923(11)	−0.2980(24)	0.3625(6)	0.274(24)
IB 2				
O_6	0.8622(11)	0.4847(24)	0.4979(6)	0.398(29)
C_{14}	0.9213(11)	0.4645(24)	0.5225(6)	0.790(132)
C_{15}	0.9989(11)	0.4928(24)	0.4949(6)	0.665(115)
C_{16}	1.0764(11)	0.4791(24)	0.5105(6)	0.464(58)
C_{17}	0.9887(11)	0.5745(24)	0.4639(6)	0.730(136)
IB 3				
O_7	0.7081(10)	−0.1645(22)	0.5894(7)	0.201(10)
C_{18}	0.7770(17)	−0.2352(28)	0.6079(15)	0.326(32)
C_{19}	0.8260(17)	−0.1448(30)	0.6317(13)	0.346(42)
C_{20}	0.8150(36)	−0.0859(62)	0.6736(24)	0.630(89)
C_{21}	0.8939(16)	−0.2051(40)	0.6417(13)	0.327(30)

Note: The atomic fractional coordinates in the unit cell with the error indicated in parentheses.

nitrogen atom of the $N_1^+(CH_3)_3$-group of another IPC molecule related to the first one by rotation about the screw axis 2_1. The distance between the above atoms is equal to 4.71(2) Å (Table 4). Subsequent translation of the two molecules along axis *b* of the unit cell results in the formation of a continuous chain of IPC molecules with aminoethyl fragments having antiparallel orientation. The aminoethyl fragments lie in a plane approximately parallel to plane *ab*, whereas isobutyl fragments are roughly perpendicular to that plane. Since IPC molecules are interrelated by a double screw axis and two of them are present in the unit cell, two lines of isobutyl fragments parallel to axis *b* emerge. The isobutyl fragments have a different orientation with respect to the plane of aminoethyl fragments.

Another free oxygen atom ($^{-\delta}O_1$) of the phosphate group from the IPC molecule, related to the basal one by the axis 2_1 (Fig. 2a), is associated with the nitrogen atom of the trimethylammonium group of the basal molecule. The distance between these atoms (5.41(2) Å) is somewhat larger than the distance O_2–N_1. The presence of the opposite charges of the groups $^{-\delta}O_1$–P–$O_2^{\delta-}$ and $N_1^+(CH_3)_3$ together with the absence of shielding groups allow to interpret their interaction as electrostatic or ionic. Thus, two

Table 2
Comparison of the torsion angles in the of PL analogs

Torsion angles	IPC–Cholesterol–IB	IPEA–HCl mol.A′	IPEA–HCl mol.A	IPEA	IPC–H_2O	IPC–H_2O–IB
C_6–C_4–C_3–O_4	−171.9°	170.3°	175.5°	180.0°	46.6°	-69.0°
C_4–C_3–O_4–P_1	177.7°	−179.3°	−159.6°	−143.4°	150.0°	−179.5°
C_3–O_4–P_1–O_3	66.5°	53.0°	62.8°	62.3°	76.4°	−73.3°
O_4–P_1–O_3–C_1	−73.0°	62.9°	58.3°	73.2°	60.5°	−59.9°
P_1–O_3–C_1–C_2	91.8°	−100.5°	−176.9°	−157.0°	−131.5°	131.9°
O_3–C_1–C_2–N_1	70.4°	67.7°	62.6°	59.2°	72.0°	−79.1°
C_1–C_2–N_1–C_8	−175.1°	163.3°	−48.0°	42.8°	−178.0°	177.5°

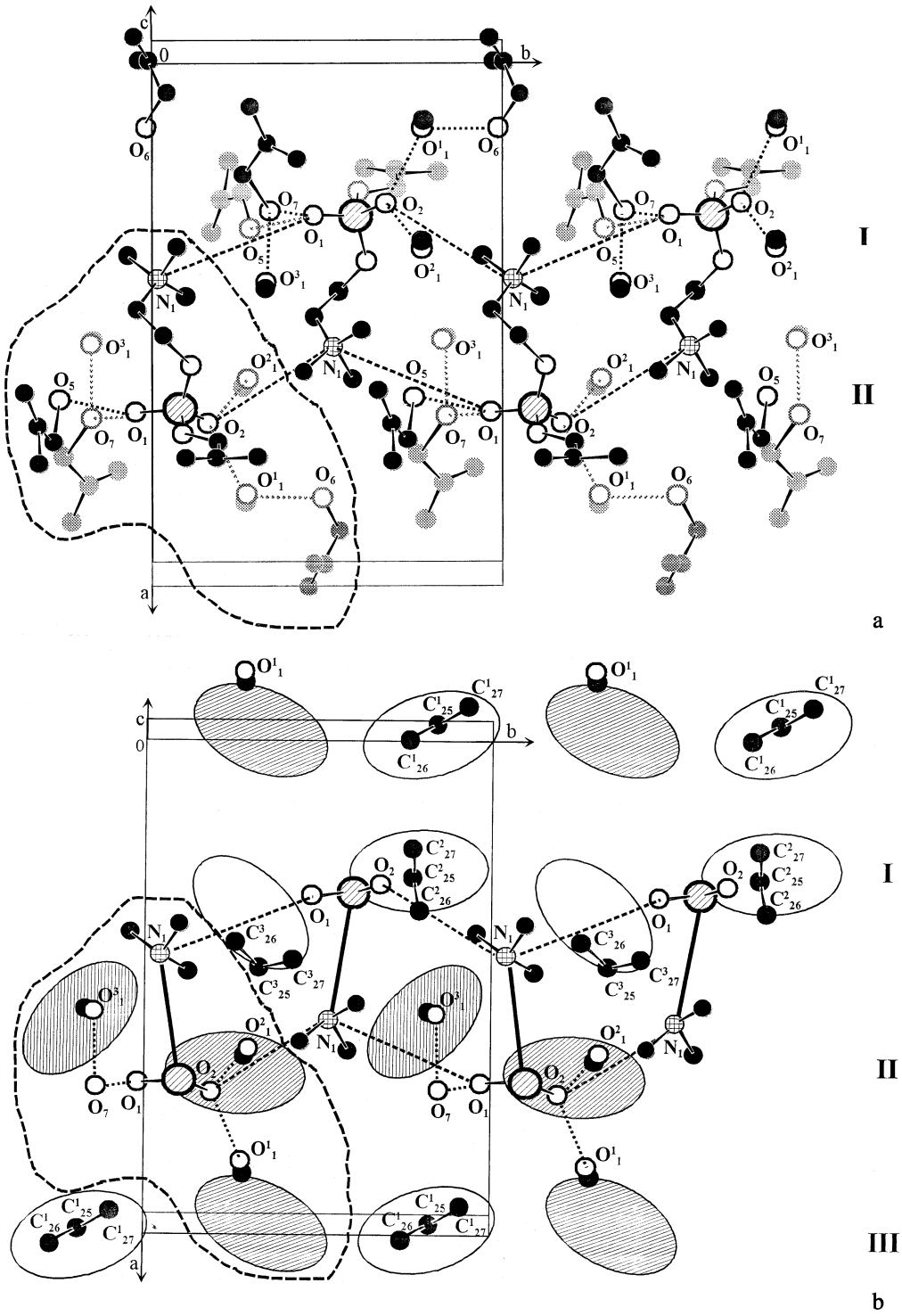


Fig. 2. Projection of the crystal structure isobutylphosphocholine–cholesterol–IB complex (1:3:3) on the plane *ab*. (a) Cross-section along axis *c* from 0.35–0.65; hydrogen bonds - dotted lines and ionic (electrostatic) bonds - dashed lines. Only C–O of cholesterol molecules are shown. Filled circles denote carbon atoms. Carbon atoms of IB situated behind the line of isobutylphosphocholine molecules are represented by hatched circles. The complex from the independent part of the cell is encircled with a dashed line. (b) Packing of cholesterol molecules: cross-section (0.5–1.0) along axis *c*. Dashed and blank ellipses correspond to cholesterol molecules turned towards the line of isobutylphosphocholine molecules with C–OH-groups and side chains, respectively.

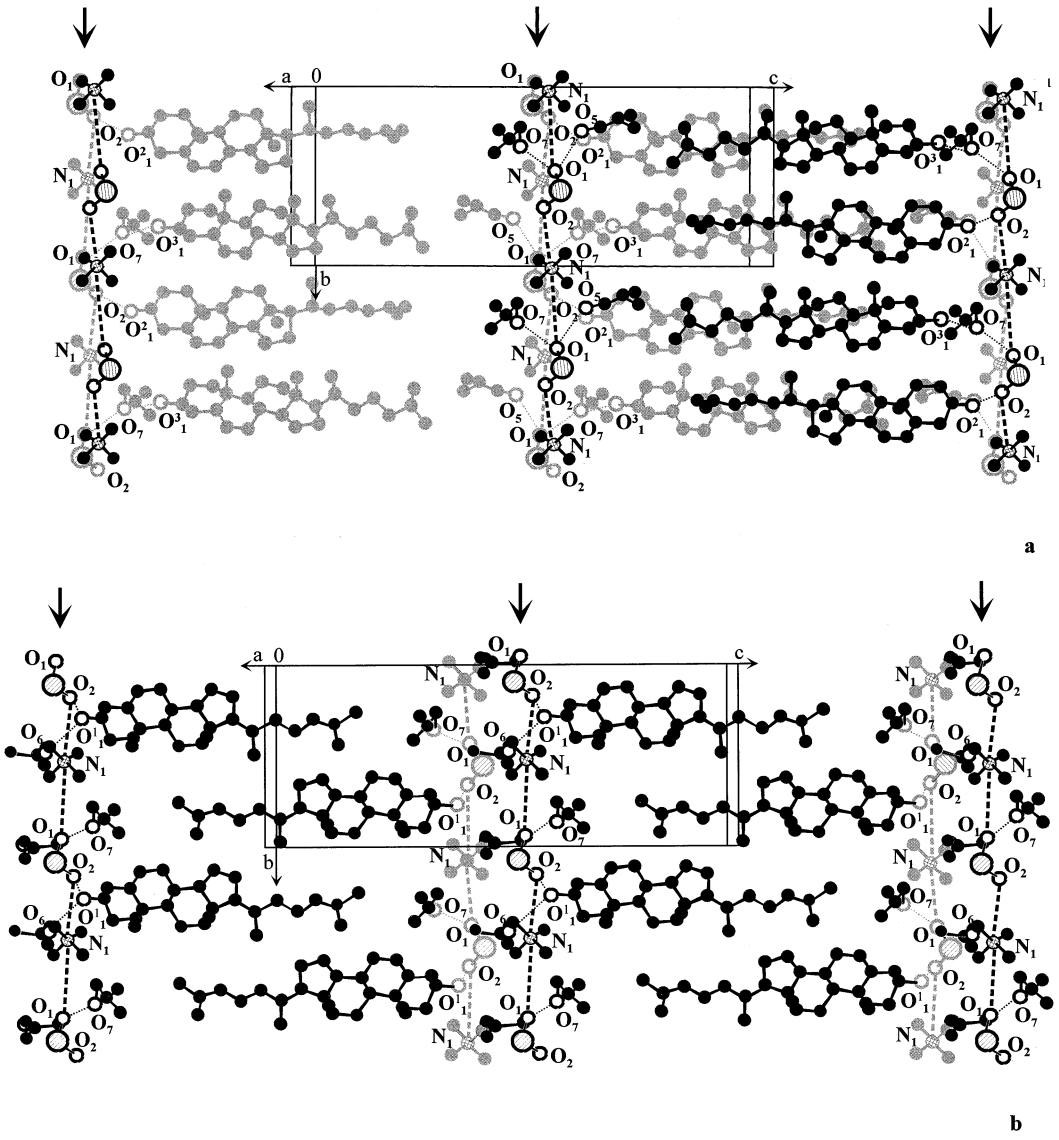


Fig. 3. Projection of the crystal complex isobutylphosphocholine–cholesterol–IB (1:3:3) on the plane *bc*. (a) Layers of cholesterol molecules localized along the axis *a* from 0.45–0.75 of the unit cell. In the left part, the upper layer of cholesterol molecules is removed to show the layer situated below (carbon atoms are represented by hatched circles). (b) The layer of cholesterol molecules localized along the axis *a* from 0.90–1.10 of the unit cell. Periodically repeated blocks are marked with arrows.

systems of ionic interactions can be distinguished in the plane of amino phosphate fragments, which provides for the association of IPC molecules along the *b*-axis (Fig. 2a,b, dotted lines).

Cholesterol molecules are connected with the bilayer via the above described system of hydrogen bonds (see Section 3.3). As seen in Fig. 3, all cholesterol molecules are roughly perpendicular to the plane of the bilayer. The axis of the cholesterol molecule passing through the aliphatic side chain and planes of the cycles is almost parallel to the *c*-axis.

All three cholesterol molecules from the basal complex of the crystal cell associated with one IPC molecule have the side chains oriented downwards with respect to the plane of the figure (Fig. 2b). Two of them (Chol 2 and Chol 3) are situated on approximately the same level in *bc* plane, whereas the third molecule (Chol 1) lies on a different level. The screw axis 2_1, having the direction [010] and passing

through the point 1/2, 0, 1/2 multiplies the basal complex turning the orientation of the cholesterol side chains to the opposite direction from the bilayer plane (non-hatched ellipses).

Thus, three layers of cholesterol molecules perpendicular to *ab*- and parallel to *bc*- plane emerge in the cell. The first two layers (Fig. 2b layers I and II) consist of the molecules possessing the same orientation within each particular layer, the orientation of the molecules belonging to the two layers being opposite. This can be better seen from Fig. 3a showing a cross-section (0.25–0.45) of the crystal cell perpendicular to axis *a* and passing through the layer of cholesterol molecules with the same orientation of the side chains. The third layer contains cholesterol molecules with alternating orientation (Fig. 3b, the cross-section 0.95–1.05). In this layer one cholesterol molecule belongs to the basal complex and the other to the complex from the cell situated below. While the first two layers contain IPC molecules, the latter are absent from the third layer.

Systems of hydrogen bonds surrounding IPC molecules from all sides are insulated by the regions of Van der Waals' interactions and ionic groups (Fig. 2a,b). This is essentially different from the situation observed in crystals of IPEA and IPC [16,21,23], where systems of hydrogen bonds unite molecules of PL analogs into continuous two-dimensional structures.

As seen from Fig. 3a,b, the cholesterol molecules of both cross-sections from the adjacent cells penetrate each other for a distance equal to the length of the side chains, whereas the regions of the CPP-nuclei do not overlap. Contacts between the side chains and cyclic fragments of the cholesterol molecules from the cells neighboring in the plane *ab* take place due to Van der Waals' interactions. These interactions also contribute to the contacts between the cells neighboring in the plane *bc*. In the layers I and II with the same orientation of cholesterol molecules only axes of these molecules are parallel, whereas planes passing through the cyclic fragments of the molecules are not. On the contrary, the angle between the perpendiculars to the planes of the CPP-nuclei in the layer III with the alternating orientation of the molecular axes is about 180°, i.e. the molecules are more or less parallel. However, their angular methyl groups have opposite directions. The four-residue fragment with two angular CH_3-groups of the side chain is complementary to the five-residue cycle of the adjacent cholesterol molecule. Therefore, the parameter *c* of the unit cell is determined by thickness of the bilayer and doubled length of the CPP-nucleus.

As seen from Figs. 1 and 3a, IB molecules surround IPC molecules from three sides, being localized in the lower part of the bilayer and forming a kind of solvate medium. The IB 1 molecule is roughly parallel to the isobutyl branch of IPC,

Table 3
Torsion angles for side chains of cholesterol molecules

Torsion angles	Values of torsion angles		
	Chol 1	Chol 2	Chol 3
C_{13}–C_{17}–C_{20}–C_{21}	56.4	52.5	52.5
C_{13}–C_{17}–C_{20}–C_{22}	−178.2	−176.9	−177.2
C_{15}–C_{16}–C_{17}–C_{20}	150.3	153.3	154.4
C_{16}–C_{17}–C_{20}–C_{21}	−179.4	−174.7	−174.2
C_{16}–C_{17}–C_{20}–C_{22}	55.3	54.7	61.2
C_{17}–C_{20}–C_{22}–C_{23}	−170.4	−167.9	−176.1
C_{20}–C_{22}–C_{23}–C_{24}	−178.6	−178.5	−178.4
C_{21}–C_{20}–C_{22}–C_{23}	62.3	61.4	59.6
C_{22}–C_{23}–C_{24}–C_{25}	−177.4	−178.6	−177.3
C_{23}–C_{24}–C_{25}–C_{26}	13.3	23.3	45.5
C_{23}–C_{24}–C_{25}–C_{27}	−177.0	−175.0	−173.7

Table 4
Hydrogen bonds and ionic interactions in the crystal of the IPC–cholesterol–IB (1:3:3) complex

Symmetry operator[a]	Atoms	Interatomic distances, Å
Hydrogen bonds		
	O_1...O_5	2.69(2)
	O_1^3...O_7	2.65(2)
	O_1...O_7	2.61(2)
	O_2...O_1^2	2.71(2)
	O_1^1...O_2	2.67(2)
	O_1^1...O_6	3.03(3)
Ionic interactions		
[2 1 −1 1]	$O_2^{\delta-}$...$^{+}N_1$	4.71(2)
[2 1 0 1]	$O_1^{\delta-}$...$^{+}N_1$	5.41(2)

The operator: 2: $-x$, 1/2+ y, $-z$

[a]The numbers in square brackets show that the ionic bond contains an atom the coordinates of which are obtained from the initial ones (Table 2) by transforming the symmetry operator (first digit) and subsequent translation along axes *a*, *b*, and *c* (respectively, the second, third and fourth digits).

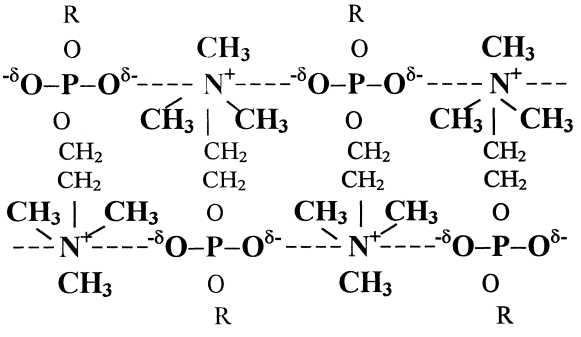
whereas IB 2 and IB 3 molecules have the same orientation as the aminoethyl branch of IPC. These molecules play an important role in the formation of the systems of hydrogen bonds generating the molecular complex, though producing no essential effect on the packing of such complexes.

4. Discussion

Three aspects of the presented investigation will be discussed below: packing of isobutylphosphocholine molecules, packing of cholesterol molecules; role of IB molecules. Properties of the investigated complex will be compared with: the packing pattern of bipolar headgroups in the crystals of PL molecules; the structure of cholesterol crystals and its derivatives; solvate crystals. Other aspects, particularly those related to the biomembrane structure will be dealt with in subsequent works.

4.1. Packing of isobutylphosphocholine molecules

Above (Section 3.4.) it was shown that negatively charged groups $^{-\delta}O_1$–P–$O_2^{\delta-}$ of IPC molecules interact with positively charged groups $N_1^+(CH_3)_3$ of neighboring IPC molecules which have the opposite (antiparallel) orientation. Isobutyl branches of these molecules are situated on the opposite sides of a band composed by phosphocholine groups. Hence, two parallel zones emerge (Scheme 1, bold; R, isobutyl residue) due to ionic interactions. Distances N^+–$^{-\delta}O$ are equal to 4.71 Å and 5.41 Å which is rather long for ionic bonds. Normally, the length of such bonds is in the range 3.7–4.5 Å [12,23].



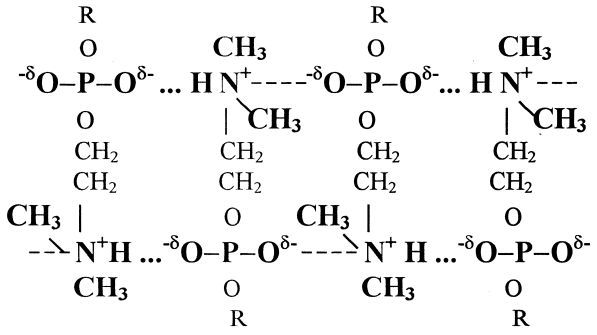
Scheme 1.

Apart from ionic interactions there are also other factors contributing to the stability of the above structure in the investigated crystal. Thus, due to hydrogen bonds between C–OH-groups of Chol 1 and Chol 2 molecules and O_2 atom of IPC phosphate group, bipolar heads are retained in the perpendicular position with respect to cholesterol. The packing pattern of cholesterol molecules consisting in stacking and side-by-side Van-der-Waals' interactions is also important for stabilization of the structure.

Similar interdigitating and antiparallel arrangement of phosphocholine groups from adjacent monolayers was observed in the crystals of 3-octadecyl-2-methyl-D-glycero-1-phosphocholine monohydrate (OMPC) [29]. In the crystals of 3-palmitoyl-D-glycero-1-phosphocholine monohydrate (PPC) and 3-hexadecyl-D-glycero-1-phosphocholine chloroform solvate (HPC) [12] such a packing pattern was shown only for the dimers of the molecules which belong to the adjacent monolayers.

In the crystals of 2,3-dimyristoyl-D-glycero-1-phosphocholine dihydrate (DMPC) [30,31], 2-deoxy-3-lauroyl-glycero-1-phosphocholine monohydrate (deoxyLPC) [30,32], IPC monohydrate and complex of IPC monohydrate with IB [23] an essentially different packing pattern of bipolar groups takes place. Thus, in DMPC crystals ionic bonds were shown to emerge between $^{-\delta}O$–P–$O^{\delta-}$- and $N^+(CH_3)_3$-groups of the neighboring molecules belonging to one monolayer. In addition, $^{-\delta}O$–P–$O^{\delta-}$-groups of DMPC are H-bonded with each other within one monolayer via water molecules. Close to each other zig-zag ribbons of hydration water molecules were observed in the monolayers of molecules of deoxyLPC [30,32], IPC monohydrate and in the complex of IPC monohydrate with IB [23]. In the above crystals interaction of headgroup dipoles is observed both within the monolayer and bilayer with the inward orientation of the bipolar groups. Interaction of antiparallel phosphocholine groups of pairs of molecules from the adjacent monolayers accounts for the formation of the bilayer.

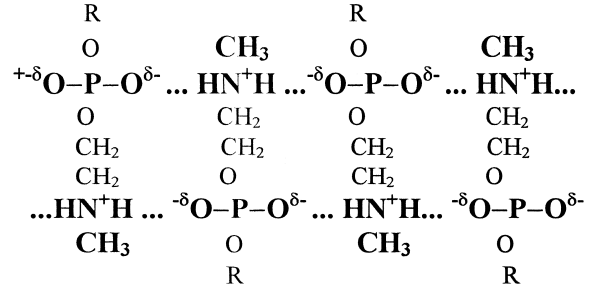
Zones of interdigitating headgroups were discovered not only in PC crystals but also in the crystals of dimethyl- and monomethyl-substituted phosphatidylethanolamine (PE). Such zones contain ionic and hydrogen bonds, e.g. in the crystals of 2,3-dilauroyl-DL-glycero-1-phospho-*N*,*N*-dimethylamine



Scheme 2.

(DLPEM$_2$) [33] systems, shown in Scheme 2 (R, 2,3-dilauroyl-DL-glycerol radical). In the zones (Scheme 2, bold) alternating dipole pairs form hydrogen bonds of the type C–N$^+$H...$^{-\delta}$O–P–O$^{\delta-}$ and ionic bonds, similar to those in the complex analyzed in our work. In the crystals of 2,3-dilauroyl-DL-glycero-1-phospho-*N*-monomethylamine (DLPEM$_1$) [12] quasi one-dimensional systems of hydrogen bonds formed by $^{-\delta}$O–P–O$^{\delta-}$- and C–N$^+$H-groups of bipolar heads (Scheme 3; R, 2,3-dilauroyl-DL-glycerol radical) can be observed. Apparently, the formation of the above shown systems of hydrogen bonds is connected with the replacement of a methyl group in DLPEM$_2$ by the hydrogen atom. Under certain conditions, pairs of two-dimensional layers containing systems of alternating C–N- and O–P–O-groups will probably be discovered in PEA crystals, where the nitrogen atom retains three hydrogen atoms.

Formation of the double zones of ionic or hydrogen bonds from interdigitating antiparallel bipolar heads in PL crystals and in the investigated structure is not related to biomembranes on the conventional basis [34]. However, from the view-point of the zone-block model [13–23] such zones situated in the central region of the biomembrane structure provide for



Scheme 3.

the lateral transfer of charge (signal) between the blocks of protein, thus playing an important role in the intramembrane communication [15,19].

4.2. Packing pattern of cholesterol molecules

The main peculiarity in the structural organization of cholesterol in the investigated structures suggests that cholesterol, IPC and IB molecules make up a complex. The base fragment consists of one IPC molecule, three cholesterol molecules and three IB molecules (Fig. 1). As was shown above (3.3), two cholesterol molecules (Chol 1 and Chol 2) form hydrogen bonds directly with the O_2-atom of the phosphate group of IPC, whereas the third molecule (Chol 3) is connected with the O_1-atom of the IPC molecule via the C–OH-group of IB 3. Molecules Chol 1 and Chol 2 directly bonded to IPC are situated practically one above the other and their planes are almost parallel. The mutual orientation of the angular methyl groups of these molecules is almost opposite (Fig. 1). Molecule Chol 3, like Chol 1 and Chol 2, has a C–OH-group directed towards IPC. The angle between the plane of the CCP-nucleus of Chol 3 and that of the two other Chol molecules is over 70°.

IPC molecules together with IB molecules associated with them form a continuous band. Cholesterol molecules are bound to it and their main axes are perpendicular to the band (Figs. 1 and 3). These molecules form layers with varying orientation within each layer (Fig. 2b and 3).

Prior to the comparative analysis of the investigated complex and crystals of cholesterol molecules and its derivatives let us underline some structural peculiarities of this compound. Cholesterol molecules have a very conservative CCP-nucleus, which is practically invariant in all previously investigated crystals of cholesterol and its derivatives [8]. On the contrary, the aliphatic fragment of the molecule is very labile and often it is difficult to fix it due to a big amplitude of temperature oscillations. As a rule, a reliable fixation of these atoms can be achieved at low temperature [35]. Therefore, it is clear why the little discrepancy we observed in the values of distances and angles of the CCP-nuclei of the investigated structure is within the accuracy of the determination of the positions of atoms. This also refers to the difference

between the investigated structure and the structures of other compounds containing cholesterol and its derivatives.

Let us compare packing patterns of cholesterol molecules in different compounds. It should be noted that formation of crystals with several molecules in the independent part of the lattice cell is typical for cholesterol and its derivatives, e.g. anhydrous cholesterol [36], cholesterol monohydrate [37] and other substances [38] contain eight independent molecules each in the cell and cholesterol hemiethanolate [39] has even twice this number. The number of cholesterol molecules in the independent part of the lattice cell of all structures known to us is even. However, in the cell of the structure investigated by us this number is equal to three. Together with the occurrence of other bulky molecules in the investigated structure, this accounts for a peculiar packing pattern.

Due to the fact that the number of molecules is even, it is always possible to distinguish a pair of independent molecules which fully determine the packing pattern in the structure and may explain its pseudosymmetry. Thus, in the structure of cholesteryl acetate [35] there are two independent cholesterol molecules. They have antiparallel arrangement on the adjacent screw axes and lie approximately in one plane perpendicular to the binary screw axis. This results in a stack packing of the molecules. The stacks of molecules form parallel continuous layers. In such an arrangement the tail fragments of one molecule are directed towards the acetyl radicals of a molecule from the neighboring stack and lying approximately in one plane perpendicular to the screw axis. In the next plane the tail fragment of one molecule and the acetyl radical from the neighboring stack are antiparallel to those considered above. Such a pattern was observed in cholesteryl iodide [40].

CCP-nuclei of cholesterol can be situated in parallel planes or form different angles with each other. Several types of packing can be obtained with cholesterol molecules whose CCP-nuclei lie in the planes approximately parallel to each other. Stacking may take place with the cholesterol molecules which are parallel to each other. Stacks can form layers, where adjacent cholesterol molecules are situated at the same height. The stacks involved in the formation of such a structure may have two types of mutual orientation. In the first case the tail of one molecule is directed towards hydroxyl of the other. In the second case the arrangement pattern is alternating, i.e. in one pair of the adjacent stacks it is tail to tail whereas in the other it is hydroxyl to hydroxyl. Two neighboring stacks can be shifted relative to each other in height. In this case interpenetration of stacks and overlapping of a part of one cholesterol molecule with another molecule can be observed in the projection of the structure on the CCP-nucleus plane. The antiparallel arrangement of molecules mentioned earlier [35] can be regarded as the realization of complete interpenetration of adjacent stacks. Theoretically other types of packing of cholesterol molecules can also be conceived [8].

Another pattern suggests that the main element of the crystal structure is formed by a pair of molecules which have CCP-nucleus planes practically perpendicular to each other. This pattern is obtained with such compounds as laurate, nonanoate and decanoate of cholesterol [41–43]. The arrangement of molecules in their crystals is such that the CCP-nucleus of one molecule is situated just opposite the ester radical of the other molecule. This fragment is further repeated via two-fold screw axes.

In the compound investigated by us, the structural fragment, consisting of three cholesterol molecules (see above), possesses many common features with both structures composed by parallel pairs and perpendicular pairs of cholesterol molecules. Thus, layers I and II in the crystal (Fig. 2b and 3a) consist of cholesterol molecules with the parallel orientation. However, the layers themselves are oriented in the opposite direction, so that pairs of molecules situated one above the other (Chol 2 and Chol 3) have antiparallel orientation forming nearly a direct angle. In the layer III, molecules Chol 1 have antiparallel orientation. In the stacks of molecules from the layer II, the parallel orientation of molecules Chol 1 and Chol 2 alternates with the antiparallel arrangement (the angle equal to 70°) of molecules Chol 1 and Chol 3.

Due to the occurrence of a continuous layer consisting of phosphocholine residues, approximately perpendicular to the axes of cholesterol molecules, the repeated structural blocks in the investigated crystal are divided into two regions containing IPC molecules and cholesterol molecules with interpenetrating tail fragments. This structure resembles that

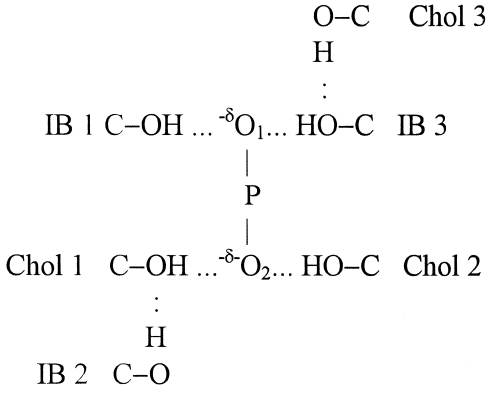
of cholesteryl myristate [44] which also reveals two distinct regions. However, in the latter case myristyl radicals interdigitate, whereas in the structure we investigated, this zone is filled with IPC and IB molecules.

4.3. The role of IB molecules

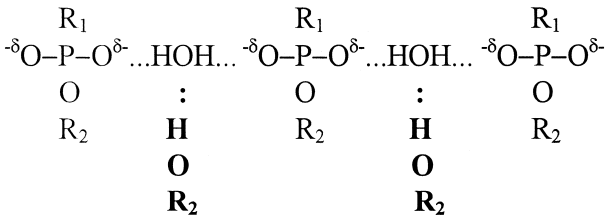
IB molecules undoubtedly play an essential role in the structural organization of the investigated crystal complex. Thus, evaporation of IB from the crystal results in turbidity and amorphization of the latter. As can be seen from Fig. 3, IB and IPC molecules are localized in the same region. In the biostructures, the side chain of the amino acid serine can be regarded as an analog of the IB molecule [23]. In the structure of the complex, IB plays a role which is complementary to that of cholesterol, i.e. IB molecules fill in the space inaccessible for cholesterol molecules. Besides, IB contributes to the formation of an antisymmetric system of hydrogen bonds (Scheme 4).

As follows from Scheme 4, C–OH-groups of IB 1 and IB 3 molecules are bonded to O_1 of the phosphate group, whereas the third C–OH-group, incorporated in this system, belongs to the Chol 3 molecule. C–OH-groups of Chol 1 and Chol 2 molecules are bonded to the O_2 atom, whereas the third C–OH-group of this system belongs to IB 2. Therefore, the two systems are related to each other by transition IB ← → Chol.

It is worth mentioning that incorporation of solvent was observed both in some crystals of PLs, e.g. HPC [12], and cholesterol (cholesteryl hemiethanolate) [39]. In this connection, crystals of the complex



Scheme 4.



Scheme 5.

of IPC monohydrate with IB, we investigated earlier [23], are of particular interest. Schematically elements of this complex are shown in Scheme 5 (R_1: choline radicals, R_2: isobutyl radicals in IPC, $\mathbf{R}_2$: isobutyl radicals in IB). It is seen in the scheme that phosphate groups and water molecules form a system of hydrogen bonds. In the lower part of the scheme, IB molecules (bold) are 'suspended' from water molecules by means of hydrogen bonds. They wedge in between isobutyl radicals of IPC. Just on the basis of this structure we obtained the complex of IPC with cholesterol and IB.

Thus, in the present article crystals of the complex of IPC with cholesterol and IB were obtained and their molecular and crystal structure was studied. It was shown that in the complex IPC molecules are packed into continuous bands formed by antiparallel interdigitating bipolar heads. They generate double zones of $C–N^+(CH_3)_3$ and $^{-\delta}O–P–O^{\delta-}$-groups. The structure also contains three-layer domains formed by cholesterol molecules perpendicular to isobutylphosphocholine bands. Since the packing pattern of bipolar groups of IPC is very much similar to that shown for PL molecules in crystals, one should not exclude that in the future some of its elements, particularly zones of bipolar headgroups of PLs, can be discovered in biomembranes.

By and large, the developed approach opens new prospects in the study of complexes of PL analogs with cholesterol and its derivatives, e.g. creating complexes with plant sterols, steroid hormones, incorporating cholesterol-binding proteins or their fragments and so on.

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