© 1988 The Chemical Society of Japan Bull. Chem. Soc. Jpn., 61, 2337—2341 (1988)

Molecular and Crystal Structure of the Bilayer-Forming Lipid-Model Amphiphile, Ditetradecyldimethylammonium Bromide Monohydrate

Kenji Okuyama,* Naomi Iijima, Kiyoshi Hirabayashi, Toyoki Kunitake,† and Masami Kusunoki††
Faculty of Technology, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184
†Faculty of Engineering, Kyushu University, Fukuoka 812
††Crystallographic Research Center, Institute for Protein Research,
Osaka University, Suita 565
(Received February 2, 1988)

The conformation and molecular packing of ditetradecyldimethylammonium bromide monohydrate ($2C_{14}N^+2C_1Br^- \cdot H_2O$), $C_{30}H_{62}NBr \cdot H_2O$, has been determined by the X-ray diffraction method. The crystals are triclinic with the space group $P\bar{1}$, $Z=2$, $a=2.9827(9)$, $b=0.7847(3)$, $c=0.7480(3)$ nm, $\alpha=104.69(4)$, $\beta=90.64(7)$, and $\gamma=90.84(6)°$. The structure was determined by direct methods and refined by the least-squares procedure: $R=0.07$ ($R_w=0.07$) for 3335 observed reflections. Two amphiphile molecules of the unit cell are related by centrosymmetry and pack tail to tail in a bilayer structure. The hydrocarbon chains tilt about 45° with respect to the bilayer normal. There are statistically four water molecules with half occupancies in a unit cell. Water molecules and bromide anions make hydrogen-bonding networks along the *c*-axis in the hydrophilic layer. The above molecular and crystal structure of $2C_{14}N^+2C_1Br^- \cdot H_2O$ was quite similar to those of $2C_{18}N^+2C_1Br^- \cdot H_2O$.

The fundamental organization of biomembrane is based on the bilayer structure of lipid molecules. Although lipid molecules in biomembranes are in the form of liquid crystal, the bilayer structures of this form are closely related to those of the gel (crystal) form. For better understanding of the bilayer structure and its properties, it is essential to accumulate detailed knowledge about preferred molecular and crystal structures of various types of bilayer-forming amphiphiles. It has, however, been difficult to get single crystals of amphipathic molecules suitable for X-ray structure analyses which could provide the most detailed and accurate information about molecular and crystal structures.

Since one of the authors (T. Kunitake) succeeded in formation of the biomembrane-like bilayer structure from totally synthetic amphiphiles,[1,2] a large number of other long-chain mono-, di-, and trialkyl amphiphiles were shown to form bilayer structures. These synthetic bilayer membranes, especially dialkylammonium membranes, has been intensively investigated their structures and physicochemical properties by means of electronmicroscopy, X-ray diffraction, differential scanning calorimetry, and so on.[3,4] Recently, we succeeded in growing single crystals of dialkyldimethylammonium bromides[5] and the detailed crystal structure of dioctadecyldimehylammonium bromide has been determined.[6] According to the structure of this compound, two amphiphile molecules pack tail to tail in a bilayer structure with tilting hydrocarbon chains by about 45° to the bilayer surface. In the hydrophilic layer bromide anions make hydrogen-bonding networks along the *c*-axis with water molecules contained statistically in a unit cell.

In this paper we discuss detailed structure of ditetradecyldimethylammonium bromide, $2C_{14}N^+2C_1Br^-$, which has the shortest alkyl chains among the homologs crystallized so far. Because of the short alkyl chains, the crystal liquid-crystal transition temperature, T_c, is rather low, 16 °C in dilute aqueous solution.

Experimental and Structure Determination

Ditetradecyldimethylammonium bromide, $2C_{14}N^+2C_1Br^-$, was prepared by step-wise alkylation of dimethylamine. Detailed procedures have been reported elsewhere.[1] $2C_{14}N^+2C_1Br^-$ (50 mg) was dissolved in 1 ml of dichloromethane. After complete dissolution, 4 ml of hexane was added. Transparent, plate-like crystals formed by keeping the solution for several days at about 8 °C. The size of the crystal used for the intensity measurement was about 0.8 mm×0.5 mm×0.01 mm.

Lattice parameters and diffraction intensities were measured on a Rigaku four-circle diffractometer with Ni-filtered Cu *Kα* radiation ($\lambda=0.1542$ nm). The lattice parameters were refined by the least-squares fit using 24 reflections in the 2θ range of 11°—35°. By using the ω-scan mode at a scan rate of 6° min^{-1}, intensities of 5037 reflections were measured up to 120° in 2θ. The scan width was $\Delta\omega=(1.8+0.15 \tan\theta)°$. Three reference reflections monitored every 53 reflections showed no significant intensity deterioration during the data collection. Corrections were made for the Lorentz and polarization factors, but not for absorption and extinction effects. A total of 3344 reflections greater than $3\sigma(F_o)$ were used in the following analysis.

Density was measured by flotation method using aqueous solution of sodium chloride. Since the specimen dissolved a little during the measurement, the value of the obtained density was not accurate and reliable. However, we could not find out more stable solution than aqueous sodium chloride for this compound. On the basis of the measured density and a unit cell volume, the unit cell contains two $2C_{14}N^+2C_1Br^-$ molecules. In the process of X-ray structure analysis, the monohydrated structure was revealed as shown in the following section.

Crystal Data: $C_{30}H_{64}NBr\cdot H_2O$, F.W.=534, triclinic, space group $P\bar{1}$, $Z=2$, $a=2.9827(9)$, $b=0.7847(3)$, $c=0.7480(3)$ nm, $\alpha=104.69(4)$, $\beta=90.64(7)$, $\gamma=90.84(6)°$, $V=1.693(1)$ nm^3, $D_x=0.91$, $D_m=1.01$ g cm^{-3}, $\mu(\mathrm{Cu}\ K\alpha)=18.5$ cm^{-1}.

Determination and Refinement of the Structure

The direct method with the MULTAN 78 program[7] was applied for $P1$ and $P\bar{1}$, space groups. For the both space groups, two planar zigzag alkyl chains could be readily discerned. In the former space group, the revealed structure consisted of two amphiphile molecules related by a centrosymmetry, which supported $P\bar{1}$ space group for the $2C_{14}N^+2C_1Br^-\cdot H_2O$ crystal. After several cycles of block-diagonal least-squares refinement starting from the coordinates of all the non-hydrogen atoms of the amphiphile obtained by the direct method, difference Fourier syntheses revealed two peaks, which were assumed to be oxygen atoms of water molecules. After another several cycles including these newly found water positions, a full-matrix least-squares calculation was performed to investigate occupancies of two water oxygen atoms, since the short distances between these oxygens (OW1···OW2, 0.18 nm and OW2···OW2, 0.22 nm) made it impossible to accomodate both of them simultaneously in the same unit cell. It was found from this calculation that two water molecules with half occupancies were most preferable and they were accomodated statistically in an asymmetric unit.

At this stage, all the magnitudes of the observed structure factors for the (h00) reflections with $h\leqq 9$ were consistently smaller than those of the calculated ones. This systematic disagreement was attributable to the absorption effect originated from the plate-like crystal shape and its long lattice constant a directed normal to the plate surface, and fairly large absorption coefficient of this compound. Therefore, these 9 reflections were excluded from the reflection data set during the following calculations. After an anisotropic refinement of non-hydrogen atoms, hydrogen atoms located on their calculated positions with isotropic temperature factors were included in the refinement. The quantity minimized was $\sum w$

Table 1. Fractional Coordinates and Equivalent Isotropic Temperature Factors[13] for Non-Hydrogen Atoms of $2C_{14}N^+2C_1Br^-\cdot H_2O$ with Estimated Standard Deviations in Parentheses

Atom	x	y	z	$B_{eq}\times 10^4/\mathrm{nm}^2$
Br	0.0656(0)	0.2466(1)	0.6782(1)	700
N	0.0745(2)	−0.4446(8)	0.2456(8)	566
C(1)	0.0344(2)	−0.5667(12)	0.2294(11)	714
C(2)	0.0643(3)	−0.3061(11)	0.1407(11)	720
C(31)	0.0822(2)	−0.3566(9)	0.4511(9)	557
C(32)	0.1214(3)	−0.2258(9)	0.4917(10)	589
C(33)	0.1351(3)	−0.1783(10)	0.6972(10)	686
C(34)	0.1542(2)	−0.3305(10)	0.7655(10)	594
C(35)	0.1975(2)	−0.4049(10)	0.6799(9)	605
C(36)	0.2120(2)	−0.5642(10)	0.7467(10)	593
C(37)	0.2560(3)	−0.6404(10)	0.6704(10)	648
C(38)	0.2689(3)	−0.8047(10)	0.7332(10)	646
C(39)	0.3132(3)	−0.8806(10)	0.6657(10)	656
C(310)	0.3257(3)	−1.0444(10)	0.7282(11)	701
C(311)	0.3704(3)	−1.1192(11)	0.6622(11)	697
C(312)	0.3830(3)	−1.2822(11)	0.7271(11)	739
C(313)	0.4279(3)	−1.3537(12)	0.6600(13)	860
C(314)	0.4414(4)	−1.5133(14)	0.7264(16)	1159
C(41)	0.1147(2)	−0.5470(10)	0.1594(9)	571
C(42)	0.1281(2)	−0.7002(9)	0.2386(10)	582
C(43)	0.1715(2)	−0.7768(9)	0.1629(9)	551
C(44)	0.1846(2)	−0.9362(9)	0.2317(10)	576
C(45)	0.2293(2)	−1.0132(9)	0.1643(10)	571
C(46)	0.2407(2)	−1.1765(9)	0.2305(10)	577
C(47)	0.2854(2)	−1.2539(9)	0.1662(10)	596
C(48)	0.2965(2)	−1.4182(10)	0.2287(10)	596
C(49)	0.3410(2)	−1.4961(10)	0.1651(11)	632
C(410)	0.3521(3)	−1.6598(10)	0.2279(11)	651
C(411)	0.3975(3)	−1.7323(10)	0.1683(11)	703
C(412)	0.4094(3)	−1.8972(11)	0.2327(12)	744
C(413)	0.4543(3)	−1.9655(13)	0.1776(15)	955
C(414)	0.4668(4)	−2.1260(15)	0.2435(17)	1129
OW1	−0.0001(5)	0.0763(14)	0.2959(18)	1020
OW2	−0.0292(6)	0.0501(18)	0.0826(22)	1337

$B_{eq}=4\times(B_1\times a^2+B_2\times b^2+B_3\times c^2)/3$, where B_1, B_2, and B_3 are diagonal components of the anisotropic thermal parameters.

$(|F_o|-|F_c|)^2$ with $w=1.0$ for all reflections. After several cycles some hydrogen atoms with unacceptable geometry were excluded from further calculations. The final R value was 0.070 ($R_w=0.067$) for all non-hydrogen atoms and 61 hydrogen atoms. The final fractional coordinates and equivalent thermal parameters of the non-hydrogen atoms are given in Table 1.[8)]

The atomic scattering factors were taken from International Tables for X-Ray Crystallography, Vol.IV.[9)] Computations were done on an ACOS 700 S computer at the Crystallographic Research Center, Institute for Protein Research, Osaka University and on a HITAC M-280 H computer at the Computer Center, University of Tokyo with the help of the programs in UNICS III.[10)]

Results and Discussion

Molecular Conformation of $2C_{14}N^+2C_1Br^-$. Figure 1 shows the molecular structure of $2C_{14}N^+2C_1$ viewed parallel (*a* and *b*) and perpendicular (*c*) to the zigzag plane. It also shows bond lengths, bond angles and dihedral angles together with the atom numbering following that of $2C_{18}N^+2C_1Br^-$.[6)] The average C–C bond distance and C–C–C bond angle in the molecule are 0.151(1) nm and 114(1)°, respectively. The average bond distance is shorter by 0.002 nm and the average bond angle is wider by 2° than the corresponding values of $2C_{18}N^+2C_1Br^-$. These are still in good agreement with those previously reported for the long hydrocarbon chains of the amphiphilic compounds.[11,12)] All the dihedral angles are within the range of 180°±2°, with exception in the connection parts between the head portion (N, C(31), C(32), C(33)) and the zigzag plane, C(33)–C(34)–C(35)–C(36) (−176°) and N–C(41)–C(42)–C(43) (−172°). One of the hydrocarbon chains (C(31), C(32), C(33), ···, C(314)) is bent at the C(33) atom at almost right angles. Since the quaternary nitrogen atom can be regarded as a carbon atom in the geometrical point of view, the molecular structure may be taken as one hydrocarbon chain from C(414) to C(314) folding at the bilayer surface just like polyethylene folding at the surface of the single crystal. Short atomic interactions between two chains in the same molecule are 0.405 (C(35)···C(42)), 0.407 (C(37)···C(44)), 0.407 (C(39)··· C(46)), 0.409 (C(311)··· C(48)), and 0.413 nm (C(313)···C(410)). The values of equivalent isotropic temperature factors (B_{eq}) of the chain carbon atoms gradually increase along the hydrocarbon chains. This kind of disorder was commonly observed in the bimolecular structures built up with amphiphilic molecules.[6,11,12)] The detailed molecular structure of $2C_{14}N^+2C_1$ is quite similar to that of $2C_{18}N^+2C_1$, including the folding pattern (G, G, T, G, G) in the head portion.

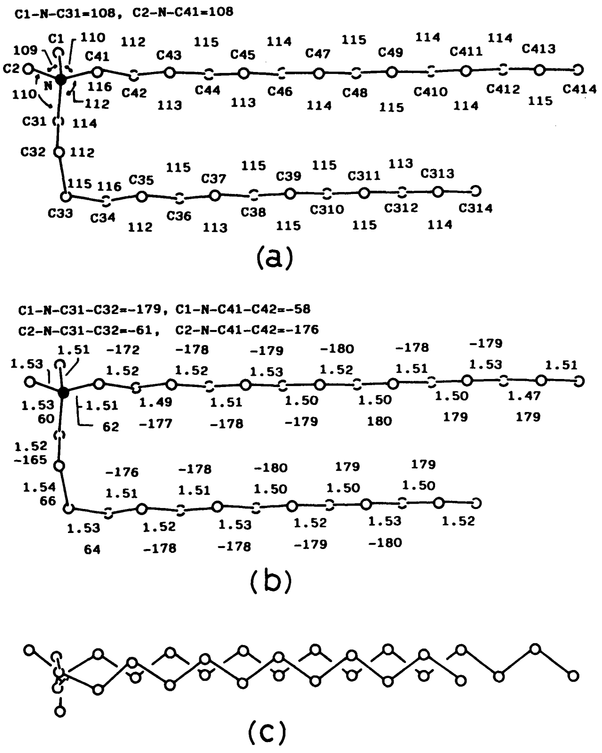

Fig. 1. (a) Atomic labelling and bond angles (/°), and (b) bond lengths (1×10/nm) and dihedral angles (/°) in $2C_{14}N^+2C_1Br^-$. Maximum estimated standard deviations for bond lengths, bond angles and dihedral angles are 0.002 nm, 1° and 1°, respectively. (c) Molecular conformation viewed from the direction normal to the zigzag plane.

Crystal Structure of $2C_{14}N^+2C_1Br^-$. The packing arrangement of $2C_{14}N^+2C_1Br^-$ viewed along the *c*-axis is shown in Fig. 2 together with short interatomic distances between neighbouring hydrocarbon chains and those between a bromide anion and the head portion of the amphiphile. There are two amphiphile molecules in a unit cell, arranged tail to tail fashion in a bilayer structure with tilting long hydrocarbon chains by about 45° to the layer surface. The molecules of the two opposite bilayer halves are related by centrosymmetry. The short intermolecular contacts between carbon atoms at the tail portions are 0.378 (C(413)···C(413)), 0.401 (C(314)···C(414)), and 0.427 nm (C(413···C(313)). While in the hydrophilic region, the bromide anion is in contact with many atoms with fairly short distances, 0.382 (C(41)), 0.391 (C(42)), 0.394 (C(31)), 0.409 and 0.388 (C(1)), 0.411 (C(2)), 0.401 (C(32)), 0.399 (C(33)), 0.345 (OW2), and 0.342 and 0.322nm (OW1). Though the direction of *a*-axis is slightly different from each other, the above detailed packing structure of $2C_{14}N^+2C_1Br^-$ is quite similar to that of $2C_{18}N^+2C_1Br^-$,[6)] including hydrogen bonding networks between the bromide anions and water oxygens, mentioned below.

There are statistically four water oxygen atoms with half occupancies in a unit cell. Because of the short atomic contacts of OW1···OW2 (0.177 nm) or OW2···

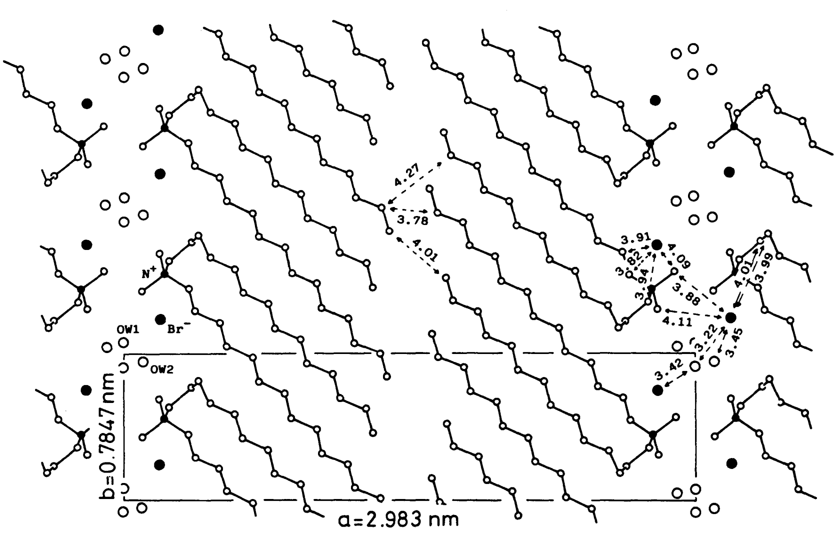

Fig. 2. Packing of $2C_{14}N^{+}2C_{1}Br^{-} \cdot H_2O$ projected onto the *ab*-plane (ORTEP[14] drawing). Four water oxygen atoms, OW1 and OW2 and their centrosymmetrical mates, are located on their positions in the unit cell. Distances (1×10/nm) between arrowed atoms are indicated short contacts in the structure.

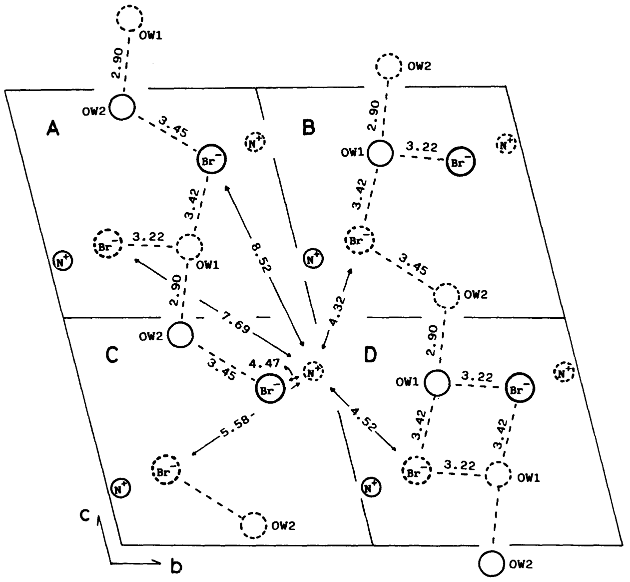

Fig. 3. Schematic illustration of hydrophillic layer of $2C_{14}N^{+}2C_{1}Br^{-} \cdot H_2O$ projected onto the *bc*-plane. Two water oxygen atoms are accomodated in a unit cell. Each unit cell, A, B, C, and D, has a different pair of water oxygens and hydrogen bonding scheme. Hydrogen bonds between bromide anions and water oxygen atoms are indicated by broken lines together with their bond lengths (1×10/nm). Solid and broken circles represent atoms on the upper and lower side of the hydrophillic layer, respectively. The unit cell is shifted by 0.5 along the *b*-axis for the comparison with that of $2C_{18}N^{+}2C_{1}Br^{-}$.

OW2 (the centrosymmetric mates at the origin) (0.219 nm), only four types of arrangement for water molecules are possible (Fig. 3). To make a comparison between $2C_{14}N^{+}2C_{1}Br^{-}$ and $2C_{18}N^{+}2C_{1}Br^{-}$, the unit cell in Fig. 3 was translated by 0.5 along the *b*-axis. Type A and B contain two water oxygen atoms, OW1 and OW2, with full occupancies. While type C or D contains a pair of centrosymmetrically related OW2 or OW1, respectively. In these arrangement, the water oxygen OW1 links to two Br^- and OW2 by hydrogen bonds with bond length of 0.342 and 0.322, and 0.290 nm, respectively. While the other water OW2 links to a bromide anion and OW1 by hydrogen bonds with 0.345 and 0.290 nm, respectively. Depending on the combination of the unit cell type, hydrogen-bonding networks are continuous (AA, BB, BD, DA) along the *c*-direction.

Both ammonium cations and bromide anions make a plane parallel to the bilayer surface (Fig. 2 and Table 1). There are two such planes in a hydrophilic layer. An ammonium cation is surrounded by three bromide anions, two in the same plane (0.432 and 0.452 nm) and one in the other plane (0.447 nm) (Fig. 3). Since distances to the other bromide anions are larger than 0.55 nm, the plus charge on an ammonium cation seems to be exclusively cancelled by these bromide anions.

From the analyses of $2C_nN^{+}2C_{1}Br^{-}$ (n=18,14) single crystal, it was found that the bilayer structure of $2C_nN^{+}2C_{1}Br^{-}$ is stabilized by the hydrogen bonding networks between water molecules and bromide anions, the electrostatic fources between bromide anions and ammonium cations, and the hydrophobi c interactions between long alkyl chains. It is suggested that single crystals of $2C_nN^{+}2C_{1}Br^{-}$ could be obtained by the above stabilizing fources.

References

1) T. Kunitake, Y. Okahata, K. Tamaki, F. Kumamaru

and M. Takayanagi, *Chem. Lett.*, **1977**, 387.

2) T. Kunitake and Y. Okahata, *J. Am. Chem. Soc.*, **99**, 3860 (1977).

3) T. Kajiyama, A. Kumano, M. Takayanagi, Y. Okahata and T. Kunitake, "Contemporary Topics in Polymer Science," ed by W. J. Bailey and T. Tsuruta, Plenum (1984), Vol. 4, pp. 829—854.

4) Y. Okahata, R. Ando and T. Kunitake, *Ber. Bunsenges. Phys. Chem.*, **85**, 789 (1981).

5) K. Okuyama, Y. Soboi, K. Hirabayashi, A. Harada, A. Kumano, T. Kajiyama, M. Takayanagi, and T. Kunitake, *Chem. Lett.*, **1984**, 2117.

6) K. Okuyama, Y. Soboi, N. Iijima, K. Hirabayashi, T. Kunitake and T. Kajiyama, *Bull. Chem. Soc. Jpn.*, **61**, 1485 (1988).

7) P. Main, S. E. Hull, L. Lessinger, G. Germain, J. P. Declercq, and M. M. Woolfson, MULTAN 78. "A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data," Univs. of York, England, and Louvain, Belgium (1978).

8) Tables of anisotropic thermal parameters, coordinates of hydrogen atoms, and observed and calculated structure factors are kept at the Chemical Society of Japan, Document No. 8807

9) International Tables for X-ray Crystallography, Vol. IV, Kynoch Press, Birmingham (1974), p. 71.

10) T. Sakurai and K. Kobayashi, *Rep. Inst. Phys. and Chem. Res.*, **55**, 69 (1978).

11) S. Abrahamsson, B. Dahlen, and I. Pascher, *Acta Crystallogr., Sect. B*, **33**, 2008 (1977).

12) I. Pascher and S. Sundell, *Biochim. Biophys. Acta*, **855**, 68 (1986).

13) W. C. Hamilton, *Acta Crystallogr.*, **12**, 609 (1959).

14) C. K. Johnson, ORTEP II, Report ORNL-5138, Oak Ridge National Laboratory, Tennessee (1976).