# DSC study of phase transition of anhydrous phospholipid DHPC and influence of water content*

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**Abstract** The phase transition behavior of 1, 2-di-*n*-heptadecanoyl phosphatidylcholine (DHPC) with and without water has been studied by use of differential scanning calorimetry. It was found by experiment that the glass transition occurred at first during the first heating of a sample of DHPC without water and then the sample underwent melting as an ordinary crystal. Therefore the sample of DHPC without water was a glassy crystal. However, the DHPC sample crystallizing from melt was an ordinary crystal. From the relationship between the total melting enthalpy $Q_f$ of freezable water and the water content $h$, it was concluded that the water contained in the DHPC samples might exist in three states recognizable thermodynamically. The water in the first state was an unfreezable water. It was the water bound directly with the head groups of the phospholipid, i.e. the primary hydration water. Every head group might bind seven such molecules of water. The water in the second state was the secondary hydration water, its melting point was close to that of pure water and its specific melting enthalpy $dQ_f/dh$ was lower than that of pure water. Every molecule of DHPC might possess at most about six such molecules of water. With the further increasing of the water content, the bulk water appeared in the system, its melting point and specific melting enthalpy were close to those of pure water. The experiments demonstrated that the melting peak of DHPC shifted toward the side of lower temperature with the increasing of water content. At $h > 16.2$ (percent by weight), the temperature $T_{tr}$ and the enthalpy $\Delta H_{tr}$ of the gel-liquid crystal phase transition of the lipid were unchanged substantially with the increasing of water content. The experimental values of the quantities, $T_{tr}$ and $\Delta H_{tr}$, and phase transition entropy $\Delta S_{tr}$ were in good agreement with those calculated by the set of Huang equations (1981).

**Keywords: 1, 2-di-*n*-heptadecanoyl phosphatidylcholine, phase transition, differential scanning calorimetry.**

The amphipathic phospholipids are the important components of biomembranes and membranous organellae. In the presence of water, the combination of hydrophilic and hydrophobic interactions causes the lipid molecules to form a bilayer structure; the water fills in the interspace between the two bilayers and contacts with the hydrophilic head groups. Only when a sufficient proportion of liquid crystal phase exists in a lipid bilayer can the biomembrane possess certain fluidity and hence the cells grow and propagate. Therefore the investigation of the phase transitions in biomembranous lipid bilayer, especially the gel-liquid crystal phase transition, is always very attractive in scientific researches. Ladbrooke and Chapman[1] gave the information about phase transitions of anhydrous phospholipids, but all the acyl chains in the phospholipids contained the even number of carbon atoms. In addition, Kodama *et al.*[2] gave the different thermal data. Furthermore, if the information of the phase transition of phospholipid with odd-carbon acyl is obtained, the knowledge of the rules known about the phospholipid phase transition may be perfected further. Therefore, the present work undertaken aims at investigating the rules about the phase transition of anhydrous phospholipid and the influence of water content and studying the thermodynamic states of hydration water in phospholipid using the representative, 1, 2-di-

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*n*-heptadecanoyl phosphatidylcholine (DHPC), by means of differential scanning calorimetry (DSC).

## 1 Materials and method

### 1.1 Materials

The phospholipid DHPC (P5014, Lot 91H8422) used was purchased from the Sigma Chemical Company. Before use it was evacuated for 3 h by a machine vacuum pump, and then dried in a desiccator over the $P_2O_5$ powder for at least 3 d.

### 1.2 Instrumental

The instrument used was a Model DSC-2C differential scanning calorimeter equipped with a Model 3700 Data Station from the Perkin-Elmer Company. Before use it was calibrated by highly pure indium and lead. Their melting points obtained from the onset points by extrapolation were 429.66 and 600.57 K, respectively. Their melting enthalpies were 28.45 and 22.84 J/g, respectively. All these indicated that the calorimetric system used had the thermometric accuracy within $\pm 0.1$ K and the enthalpy-measuring accuracy within $\pm 1\%$.

### 1.3 Method

Weighing was carried out by use of a Model AD-2Z electromagnetic ultramicrobalance from the Perkin-Elmer Company. Weighing error was $\pm 0.01$ mg or still lower. With anhydrous lipid, the sample was encapsulated by the volatile aluminium pan. After weighing rapidly and immediately sealing up, a small hole with diameter $< 0.5$ mm was bored at the centre of the top of the aluminium cover, and then the aluminium pan with lipid was placed into a desiccator with $P_2O_5$. After drying for 1 d it was used for DSC determination. For the lipids containing water, the stainless steel capsules were used. After loading a certain amount of anhydrous lipid, the stainless steel pan was covered immediately by a sheet of double-directionally stretched and weighed film (Parafilm), and then the weight of the loaded lipid was weighed accurately. According to the water content required, the corresponding amount of distilled water was sucked and injected with a microsyringe onto the inside wall of the pan, which was immediately covered by a stainless steel cover with a Viton O-ring and then sealed. The weight of water added was weighed accurately. Every sample with water was heated and cooled between 30℃ (20℃ less than the temperature of main phase transition of DHPC given by McElhaney[3]) and 120℃ (slightly higher than the melting point of anhydrous DHPC known first by this work), cycled so five times and then used for DSC determination. It was found by the experiments that the head groups of the DHPC insoluble but swollen in water could be hydrated sufficiently by the treatment mentioned above, and the heating curves obtained in two successively determinations showed good reproducibility. All the DSC determinations were carried out in highly pure $N_2$. The reference was an empty stainless steel capsule of the same type. Unless especially stated otherwise, the scanning rate of 5.00 K/min was used throughout.

## 2 Results and discussion

The five DSC curves obtained by successively heating and cooling a sample of anhydrous

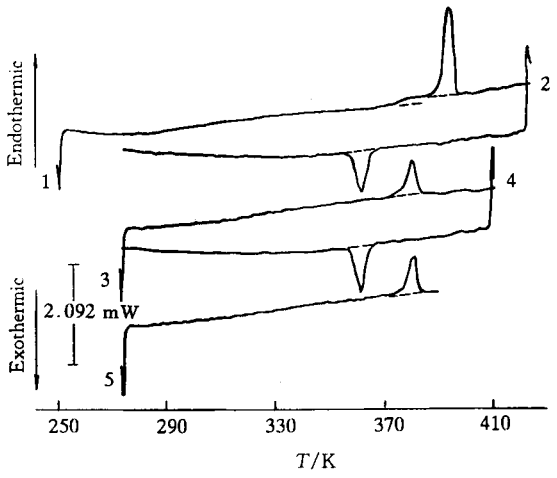


Fig. 1. DSC curves of anhydrous DHPC. Curves 1, 3 and 5 are heating curves; curves 2 and 4, cooling curves.

DHPC are presented in fig. 1. It can be seen from curve 1 of fig. 1 that the DHPC underwent a glass transition at 375.39 K (midpoint of the transition) and showed 1.02 J/g·K step of specific heat capacity, and then was melted at 390.42 K (the expolated onset point of its melting peak). These facts show that the anhydrous DHPC is a glassy crystal. Curves 2 and 4 show that the melted DHPC has crystallized exothermically on cooling and the undercooling extent has amounted to 26 K or so. Curves 3 and 5 show that the DHPC crystal having crystallized from melt has the lower melting point and melting enthalpy. The latter two sets of curves show good reproducibility. The cooling curves begin to deflect gradually toward the endothermic side from 340 K, which shows that the crystal having crystalized from melt is changing gradually into another crystal form. Consequently, when the DHPC having crystallized from melt remelts, the area of remelting peak is less than the area of exothermic crystallizing peak. Weighing before and after the DSC determinations shows that the weight of the lipid sample does not decrease, although it had been heated to about 150℃. Ladbrooke and Chapman[1] indicated that the monohydrates of 1,2-diacyl phosphatidylcholines lost the water to be removed most difficultly above 120℃, and gave a large endothermic peak in the case of being loaded into an open vessel. The curves in fig. 1 do not show the new endothermic peak above 120℃. In addition, the weight of the sample does not change before and after the DSC determinations. Therefore, the results obtained in this work are the thermodynamic parameters of melting process of the anhydrous lipid DHPC, and are presented in table 1 in detail. According to the thermal parameters of fusion of the anhydrous lipids given by Ladbrooke and Chapman[1], the melting point of the anhydrous DHPC obtained by the first heating is higher than the melting point 115℃ of the $\beta$ crystal of 1,2-distearoyl phosphatidylcholine (DSPC), a homologue of DHPC, and much higher than the melting point 95℃ of $\alpha$ crystal of DSPC. As to another homologue 1,2-dipalmitoyl phosphatidylcholine (DPPC), the melting point of anhydrous DPPC obtained by Kodama and chapman[2] is 97.8℃, which is also higher than 93℃ given by Ladbrooke and Chapman[1], but lower than the melting point of DHPC in this work. Therefore, by comparing the thermal parameters of fusion of DHPC obtained in this work with the literature data, it could be said that the melting points increase with the increasing number of carbon atoms of acyl chain in the homologous series of 1,2-dialkanoyl phosphatidylcholines, which signifies that the melting processes of the lipids are related to the melting of the acyl chains. Moreover, the glassy crystal, a more specific crystal form, exists also in this homologous series, in addition to the polymorphism such as $\alpha$ crystal and $\beta$ crystal.

The typical DSC heating curves of DHPC with different water contents are presented in fig. 2, the thermal parameters obtained are summarized in table 1. Every $T_{tr}$ of phase transition in table 1 is the temperature of the extrapolated onset point of peak, because of the extrapolated onset

points of the melting peaks of highly pure indium and lead were taken as their melting points in the temperature calibration and the temperature of peak top can be changed with changing the sample weight and scanning rate. Every specific enthalpy $\Delta H_{tr}$ of phase transition is calculated by the total area of peak. Except for the determination by the first heating of anhydrous DHPC and the cooling determinations by the samples with water, the results of the rest DSC determinations are the averages of two successive determinations. Given in the rightest column of table 1 are the values of the ratio $\Delta H_{vH}/\Delta H_{cal}$ for the phase transition peaks of the lipid during the second heating of anhydrous DHPC and the heating of the samples with water. Each van't Hoff enthalpy $\Delta H_{vH}$ here is calculated by the formula $2R^{\frac{1}{2}}T_P(M\Delta C_p^{max})^{\frac{1}{2}}$ [4], where $T_p$ is the temperature of peak top; $M$ is the molecular weight (762.1 for DHPC); $\Delta C_p^{max}$ is the maximal peak height relative to the baseline of peak and measured by the unit of specific heat capacity; $\Delta H_{cal}$ is the molar enthalpy of phase transition of DHPC obtained by calorimetry.

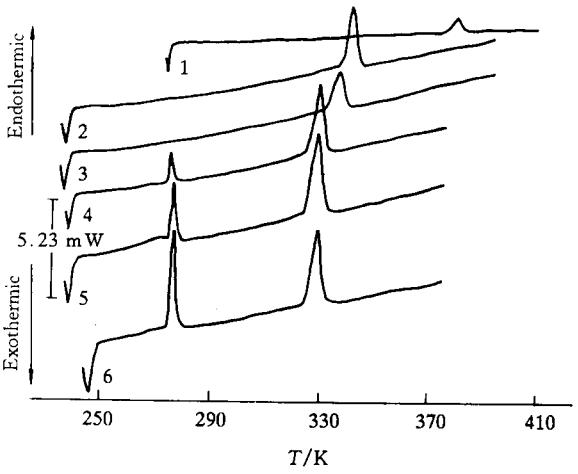


Fig. 2. Typical DSC heating curves of DHPC with various water contents. 1, The second heating curve of anhydrous DHPC; 2, 6.1% by weight of water content; 3, 10.8% by weight; 4, 16.2% by weight; 5, 18.0% by weight; 6, 23.6% by weight.

Table 1 Results of DSC determinations of DHPC with various water contents

| Dry lipid weight/mg | Water weight/mg | Scanning rate/K·min$^{-1}$ | $T_{tr}$/K | $\Delta H_{tr}$/J·g$^{-1}$ lipid | $\Delta H_{vH}/\Delta H_{cal}$ |
|---|---|---|---|---|---|
| 1.24 | 0.00 | +5.00 | 390.42 | 66.07 | |
| | | −5.00 | 364.29 | −38.83 | |
| | | +5.00 | 376.84 | 31.63 | 6.72 |
| 3.94 | 0.254 | +5.00 | 336.90 | 42.72 | 5.23 |
| | | −5.00 | 338.70 | −40.12 | |
| 2.58 | 0.312 | +5.00 | 331.58 | 47.28 | 4.42 |
| | | −5.00 | 333.76 | −46.19 | |
| 3.72 | 0.718 | +5.00 | 274.17 | 5.02 | |
| | | | 324.87 | 54.14 | 4.31 |
| | | −5.00 | 326.22 | −52.63 | |
| 4.81 | 1.055 | +5.00 | 274.52 | 11.55 | |
| | | | 323.91 | 55.23 | 4.09 |
| | | −5.00 | 324.03 | −54.39 | |
| 4.23 | 1.31 | +5.00 | 274.56 | 29.50 | |
| | | | 324.13 | 54.98 | 4.18 |
| | | −5.00 | 324.28 | −51.71 | |
| 3.59 | 1.66 | +5.00 | 274.50 | 79.04 | |
| | | | 323.98 | 54.48 | 4.09 |
| | | −5.00 | 324.65 | −51.51 | |
| 3.70 | 2.25 | +5.00 | 274.15 | 127.1 | |
| | | | 323.42 | 52.93 | 4.16 |
| | | −5.00 | 323.98 | −49.41 | |

According to the viewpoint of Sturtevant[5], the value of the ratio $\Delta H_{vH}/\Delta H_{cal}$ represents the size of one cooperative unit involved in the phase transition, i.e. $\Delta H_{vH}/\Delta H_{cal}$ molecules of DHPC constituting one cooperative unit undergo the cooperative phase transition.

It is shown by fig. 2 and the data in table 1 that the melting peak of ice did not appear during heating the two samples with the water contents less than 16.2% by weight, although they had been cooled down to 235 K. With further increasing the water content, the melting peak of DHPC with water shifts toward the side of lower temperature, the specific enthalpy of phase transition increases. When the water content is increased stepwise from 16.2% by weight, the melting peak of freezable water begins to appear at about 274 K, the peak of gel-liquid crystal phase transition of DHPC with water begins to appear at about 323 K. The relationship between the total melting enthalpy $Q_f$ of the ice in per gram of lipid and water content $h$ is shown in fig. 3. It may be seen from fig. 3 that not only $Q_f$ is a function of $h$, but also the $dQ_f/dh$ is a function of $h$. At $h > 0.310$ gram of water/gram of lipid, the water in the sample system may exist in three states. The water in the first state is unfreezable bound water or primary hydration water. Its maximal quantity amounts to 0.165 gram water/gram lipid (at the point intercepted with the abscissa axis by linear line 2). This indicates that the phosphocholine head group of each DHPC molecule binds with seven water molecules. The seven molecules of primary hydration water were not transformed into ice structure even though the water had been cooled down to 235 K. The second kind of water is freezable water, but its specific melting enthalpy $dQ_f/dh$ is 201.08 J/gram of water, much lower than the specific melting enthalpy of pure water. This kind of water is the secondary hydration water of the phospholipid. Owing to the polarization by the polar groups, especially by the phosphocholine head groups, the ice formed on freezing has been distorted and hence different from the hexagonal ice structure of pure water. Therefore, the ice does not possess the specific melting enthalpy of hexagonal ice. Its maximal quantity is (0.310—0.165) gram water/gram lipid. Consequently, the molecule number of the secondary hydration water is at most about 6 moles of water/mole of DHPC. Interestingly, the secondary hydration water of the phospholipid is similar to the secondary hydration water of proteins. The specific melting enthalpy $dQ_f/dh$ of the secondary hydration water of the DHPC is intermediate between that of collagen 180 J/g and that of Onozuka R-10 cellulase 222 J/g[6], and is lower than those of other proteins. This indicates that the polarization imposed by the phosphocholine head groups upon the water molecules is very strong. At $h > 0.31$ gram water/gram lipid, the water in the third state appears in the sample system, its quantity is equal to $(h - 0.310)$ gram water/gram lipid. The melting point of this water is close to that of pure water, its specific melting enthalpy 329.36 J/gram of

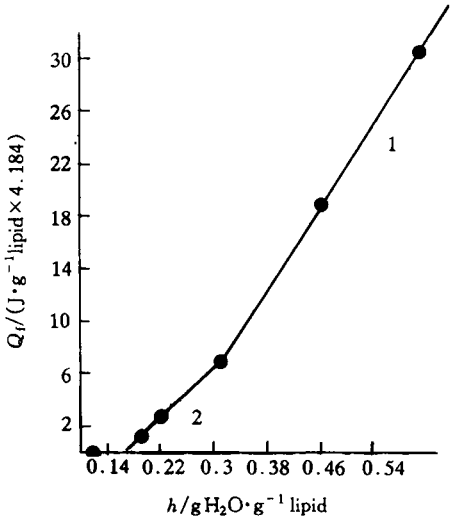


Fig. 3. Relationship between the total melting enthalpy $Q_f$ of freezable water and the water content $h$ in the samples of DHPC containing water. Line 1, $Q_f = -73.18 + 329.36\,h$, $0.310 \leqslant h \leqslant 0.608$, correlation coefficient $r = 1.000$; line 2, $Q_f = -33.22 + 201.08\,h$, $0.165 \leqslant h \leqslant 0.310$, $r = 0.999$.

water is also close to that of pure water. It may be seen from fig. 2 and table 1 that the presence of unfreezable water lowers the melting point of DHPC at $h < 0.165$ gram water/gram lipid, and that the molecule number in one cooperative unit (i.e. the value of the ratio $\Delta H_{vH}/\Delta H_{cal}$) lowers during the phase transitions. At $h \geqslant 0.165$ gram water/gram lipid, the temperatures and enthalpies of the gel-liquid crystal phase transitions of the DHPC with various water contents are substantially the same. Therefore, the corresponding values of the last five samples in table 1 are averaged to obtain the temperature of the gel-liquid crystal phase transition of DHPC, which is 324.06 K or 50.9℃; the enthalpy of the phase transition, which is 54.35 J/g or 41.42 kJ/mol, and the entropy of the phase transition $\Delta S_{tr}$, which is 127.8 J/K·mol. In this phase transition, one cooperative unit contains 4.17 DHPC molecules on average. The temperature of main phase transition of DHPC given by McElhaney[3] is 49.8℃ at the water content over 99%, while that given by Chen *et al.*[7] is 48.5℃. Therefore the agreement between the $T_{tr}$ values is satisfactory. Mason and Huang[8] collected the thermal parameters of the gel-liquid crystal phase transitions of the five homologues of 1,2-di-*n*-alkanoyl phosphatidylcholines, in those molecules every acyl is an even-carbon acyl and contains 12—22 carbon atoms; and presented by linear regression analysis a set of equations, which correlated these thermal parameters of the main phase transitions with a perturbation parameter $P = 3.5 \times 100/(2N - 5.5)$. They are: $T_{tr} = -7.77P + 145$ (correlation coefficient $r = 0.999$); $\Delta H_{tr} = -1.31P + 26.4$ ($r = 0.994$); $\Delta S_{tr} = -3.66P + 75.3$ ($r = 0.998$). The number of carbon atoms in every acyl chain of DHPC molecule $N$ is equal to 17, i.e. its perturbation parameter $P$ is 12.3. By use of 1 cal = 4.184 J, it may be calculated for the DHPC that $T_{tr} = 49.4$℃, $\Delta H_{tr} = 43.0$ kJ/mol, and $\Delta S_{tr} = 126.7$ J/K·mol. These calculated values are in good agreement with the average values mentioned above. This indicates that the thermal parameters of gel-liquid crystal phase transition for the DHPC samples with freezable water are amenable to the linear tendencies observed by the homologues with even-carbon acyl, although the DHPC molecules contain odd-carbon acyls. The set of Huang equations has been verified further and expanded in the number of applicable objects by this work.

Recently, McElhaney and Lewis[9] found the temperature of gel-liquid crystal phase transition of the 1,2-diheptadecanoyl phosphatidylethanolamine (DHPE) to be 70.5℃, and the enthalpy of the phase transition to be 39.3 kJ/mol. Compared with the averages of this work, it may be seen that changing the group $-N^+(CH_3)_3$ in the head group of DHPC into the group $-N^+H_3$, which shows the only difference between the molecular structures of DHPC and DHPE, can cause the temparature of gel-liquid crystal phase transition to increase by 19.6℃ and the enthalpy of the phase transition to decrease by 2.1 kJ/mol. Except that the differences among the numbers of carbon atoms in the acyl chains and between the head groups can influence the thermal parameters of gel-liquid crystal phase transition discussed above, the types and unsaturation extent of the acyl chains are also the important factors. From the literature data, the following situations may be seen. When the normal alkanoyl chain containing 17 carbons is isomerized, the temperature of gel-liquid crystal phase transition of 1, 2-diisoheptadecanoyl phosphatidylcholine is 28.4℃[10] or 27℃[11], which is much lower than 50.9℃ of DHPC. For the antiisobranched isomer 1,2-diantiisoheptadecanoyl phosphatidylcholine, its temperature of main phase transition is 8℃ (racemic isomer) or 7.6℃ (levo-isomer)[12]. When one double bond is introduced into the ninth position of heptadecanoyl chain, the temperature of the main phase transition of 1, 2-di-*cis*-9-

heptadecenoyl phosphatidylcholine is $-27.6$℃[13]. We consider that all the structural changes mentioned above cause the $R_2$ regions, which were divided together with the regions $R_1$ and $R_3$ in the molecular structural formulae of the 1, 2-diacyl phosphatidylcholines by Mason and Huang[8] and were the parts of the alkanoyls or alkenoyls really taking part in the main phase transitions, to have very different structures between the DHPC and its isomers. The two parallel extended straight chains exist in region $R_2$ of DHPC, this should cause the more regular arrangement to be formed easily. Therefore the stability of the gel state of DHPC should be higher, and its $T_{tr}$ is higher, too.

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