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High pressure antagonism of alcohol effects on the main phase-transition temperature of phospholipid membranes: biphasic response

Katsuhiro Tamura *, Yoshiroh Kaminoh, Hiroshi Kamaya and Issaku Ueda

Department of Anesthesia, University of Utah College of Medicine, and Anesthesia Service, Veterans Administration Medical Center, Salt Lake City, UT (U.S.A.)

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The combined effects of high pressure (up to 300 bar) and a homologous series of 1-alkanols (ethanol C_2 to 1-tridecanol C_{13}) were studied on the main phase-transition temperature of dipalmitoylphosphatidylcholine (DPPC) vesicle membranes. It is known that short-chain alkanols depress and long-chain alkanols elevate the main transition temperature. The crossover from depression to elevation occurs at the carbon-chain length about C_{10}–C_{12} in DPPC vesicle membranes coinciding with the cutoff chain-length where anesthetic potency suddenly disappears. Alkanols shorter than C_8 linearly decreased the transition temperature and high pressure antagonized the temperature depression. Alkanols longer than C_{10} showed biphasic dose-response curves. High pressure enhanced the biphasic response. In addition, alkanols longer than the cutoff length depressed the transition temperature under high pressure at the low concentration range. These non-anesthetic alkanols may manifest anesthetic potency under high pressure. At higher concentrations, the temperature elevatory effect was accentuated by pressure. This biphasic effect of long-chain alkanols is not related to the 'interdigitation' associated with short-chain alkanols. The increment of the transition temperature by pressure was 0.0242 K bar^{-1} in the absence of alkanols. The volume change of the transition was estimated to be 27.7 cm^3 mol^{-1}. This value stayed constant to the limit of the present study of 300 bar.

Introduction

Alkanols with short carbon-chain perturb lipid membrane structures and decrease the temperature of the main transition between the solid-gel and liquid-crystal phases. Long-chain alkanols, on the other hand, elevate the main phase-transition temperature. With dipalmitoylphosphatidylcholine (DPPC) membranes, the crossover from depression to elevation occurs between C_{10} and C_{12} at ambient atmospheric pressure [1–3].

In a series of 1-alkanols, the anesthetic potency increases with the increase in the carbon-chain length up to about C_{10}–C_{12}, where the anesthetic activity suddenly disappears [4]. This phenomenon is known as 'cutoff' of the anesthetic potency in a homologous series of alkanols. The cutoff chain-length coincides with the chain-length where the effect on the phase-transition temperature of DPPC membrane changes from depression to elevation. High pressure antagonizes anesthesia and also increases the main transition temperature. Accordingly, anesthetic action has been correlated with its potency to depress the phase transition temperatures.

In this communication, we report pressure effects on the main phase-transition temperatures of DPPC membranes doped with a series of 1-alkanols with chain-length C_2–C_{13}. High pressure changed the monotonous elevation of the transition temperature by long-chain alkanols into depression. At high concentrations, however, they elevated the transition temperature, forming a temperature minimum in the solid-liquid phase diagram.

The biphasic response of the main transition tem-

* Present address: Department of Chemical Science and Technology, Faculty of Engineering, The University of Tokushima, Minami-Josanjima, Tokushima, Japan.

Correspondence: I. Ueda, Anesthesia Service 112A, VA Medical Center, Salt Lake City, UT 84148, U.S.A.

perature to short-chain alkanols was reported by Jain and Wu [1], but not for 1-alkanols with the alkyl chain longer than 5 carbons. Later, the mechanisms of this biphasic effect was attributed to the interpenetration of the apposing lipid layers to form the 'interdigitated' state [5–8]. The formation of the interdigitated state, however, is limited to the interaction with small ligands. In the 1-alkanol series, the chain length must be shorter than 1-butanol to form the interdigitated state [6]. The discrepancy between the biphasic response induced by long-chain alkanols and the interdigitated state will be discussed.

Materials and Methods

Synthetic dipalmitoylphosphatidylcholine and 1-alkanols (1-hexanol, 1-octanol, 1-decanol, 1-undecanol, 1-dodecanol, 1-tridecanol, and 1-tetradecanol) were obtained from Sigma, ethanol from Quantum Chemical USI (Anaheim, CA), and spectrograde 1-butanol from J.T. Baker (Phillipsburg, NJ). Water was purified by two-stage mixed-bed ion-exchanger columns, activated charcoal, and ultrafilter (Sybron/Barnstead, Boston, MA).

Phospholipid vesicles were prepared by suspending DPPC in water at $1.01 \cdot 10^{-3}$ molal, with a Branson Sonifier Model 185 (Danbury, CN) at 318 K for 10 min. The fresh preparation after sonication is composed of vesicles of various sizes. To form uniform size distribution, the liposome was stored at 277 K for several days [9]. 1-Alkanols longer than C_8 were sparingly soluble to water, hence, they were added to the vesicle suspension and mixed for 30 s by sonication [10].

The main phase-transition of DPPC vesicles was measured by the optical method [10,11]. The cell compartment assembly of a Hitachi Perkin-Elmer 139 UV-visible spectrophotometer was replaced with a high-pressure vessel (Fig. 1) with quartz optical windows. The sample solution was introduced into the sliding cell (inner quartz cell, Fig. 1), thereby avoiding contamination of the sample solution by the metal body of the high-pressure vessel. Pressure was generated by a hand-operated hydraulic pump and measured with an accuracy of ± 0.1 bar by a digital pressure transducer system (Autoclave Engineering Model DPS-0021, Erie, PA). The solution temperature was monitored with 0.01 K resolution by a digital thermometer (Cole Parmer Model 8502-16, Chicago, IL) and a thermistor probe inserted into the high-pressure vessel. The calibration curve between the temperature of the sample solution in the optical cell and that of the high pressure vessel body was constructed and the sample temperature was estimated from the observed temperature of the vessel.

The temperature was scanned at 0.45 K/min by circulating water from a water bath through the jacket

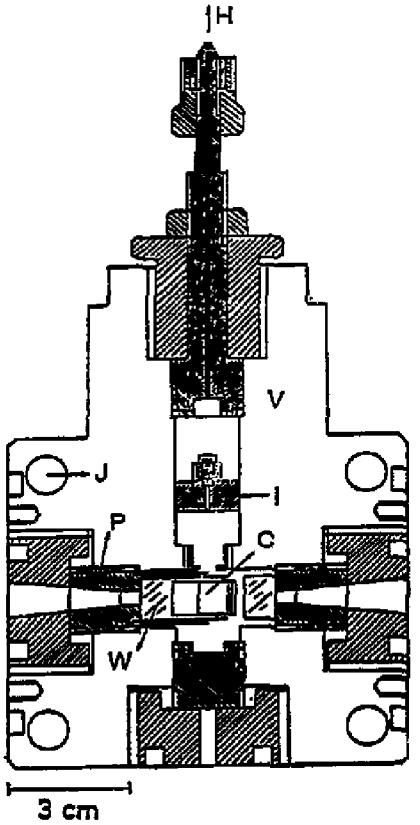

Fig. 1. Block diagram of the high pressure cell. W, quartz window; P, plug; C, quartz sliding cell; I, fluid-separator piston; V, high pressure vessel; H, to a high pressure pump; and J, jacket.

of the high-pressure vessel. Phase transition was monitored by the sudden change in the absorbance at 400 nm. The optical and temperature signals were A-D converted by a Nicolet 3091 digital oscilloscope (Madison, WI) and recorded in a floppy disc of an IBM-PC. The midpoint between the beginning and the end of the change was taken as the transition temperature.

All concentrations are expressed by molality (m, mole solute in 1000 g solvent) because the numerical value of molarity (M, mole solute in 1000 ml solution) changes according to the temperature and pressure.

Results

The effects of 1-alkanols between C_2 and C_8 on the main phase-transition temperature are shown in Fig. 2 under various hydrostatic pressure. The plot between the transition temperature (T_c) and the alkanol concentration (C_A) decreased linearly with the increase in the alkanol concentrations. The ability of alkanols (C_{12} and C_{13}) to depress the transition temperature increased with the elongation of the carbon chain; the

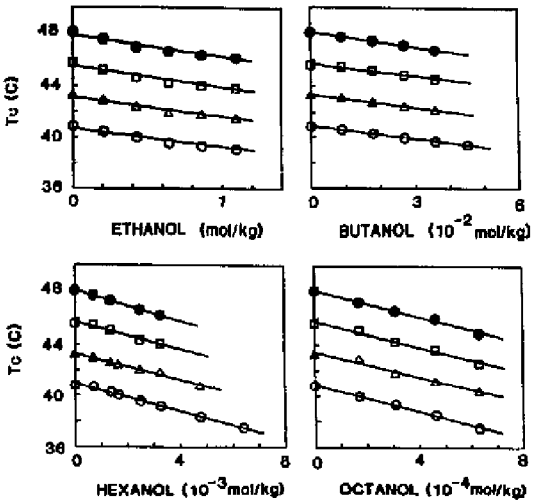

Fig. 2. The effects of pressure and 1-alkanols on the main phase-transition temperature of DPPC. Ordinate: The main phase-transition temperature. Abscissa: The alkanol concentrations in molality (mole solute in 1000 g solvent). The standard errors were within the symbol. Symbols: ambient pressure (○), 100 bar (△), 200 bar (□), and 300 bar (●).

alkanol concentrations that depressed the transition temperature 1.0 K were $C_2 = 4.85 \cdot 10^{-1}$, $C_4 = 3.16 \cdot 10^{-2}$, $C_6 = 1.81 \cdot 10^{-3}$, and $C_8 = 1.92 \cdot 10^{-4}$ molal. High pressure shifted the dose-response (T_c vs. C_A) plot toward the higher temperature range approximately parallel to the control curve.

When the chain-length exceeded C_{10}, the response of the transition temperature to alkanols became biphasic as we reported previously [3]. Fig. 3 shows the results on C_{10} to C_{13}. With C_{10}, T_c decreased in the

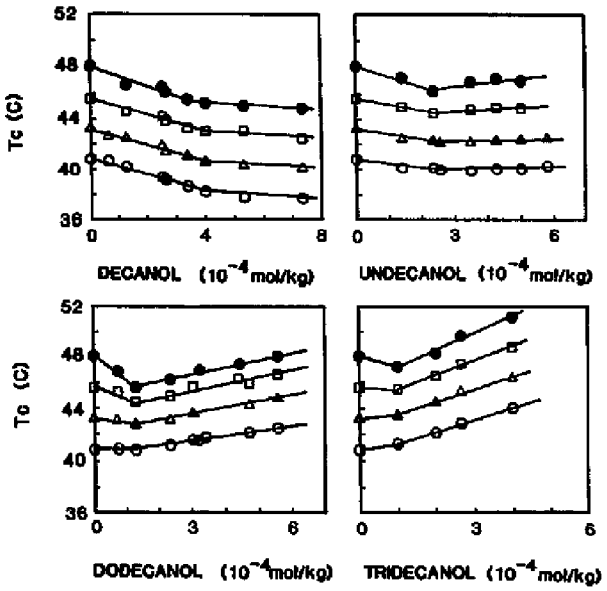

Fig. 3. Same as Fig. 2.

TABLE I

Alkanol concentrations (expressed in molality) that changed the main phase transition temperature 1.0 K and those at the breaking points (see Fig. 3) at 1 bar

	Alkanol (mol/kg)	
	$d[A]/dT_c$	Breaking point
Ethanol	$-4.85 \cdot 10^{-1}$	
1-Butanol	$-3.16 \cdot 10^{-2}$	
1-Hexanol	$-1.81 \cdot 10^{-3}$	
1-Octanol	$-1.92 \cdot 10^{-4}$	
1-Decanol	$-1.56 \cdot 10^{-4}$	$4.02 \cdot 10^{-4}$
1-Undecanol	$-3.01 \cdot 10^{-4}$	$2.40 \cdot 10^{-4}$
1-Dodecanol	0	$1.29 \cdot 10^{-4}$
1-Tridecanol	$2.08 \cdot 10^{-4}$	$9.97 \cdot 10^{-5}$

total concentration range with a break at $4.02 \cdot 10^{-4}$ molal where the slope of the T_c vs. C_A plot became less steep in the higher concentration range. With C_{11}, the break was at $2.40 \cdot 10^{-4}$ molal. C_{12} and C_{13} no longer decreased the T_c at low concentration ranges and increased it steeply with the break at $1.29 \cdot 10^{-4}$ (C_{12}) and $9.97 \cdot 10^{-5}$ molal (C_{13}). The alkanol concentrations at the break point and those decreased the main transition temperature 1.0 K at low concentration ranges are summarized in Table I.

Under high pressure, the response to C_{11}–C_{13} became clearly biphasic with depression of the transition temperature followed by elevation according to the increase in the alkanol concentrations. The T_c-depressant action of alkanols in the low concentration range was clearly demonstrated under high pressure. High pressure, however, did not alter the break point in the alkanol concentration coordinate.

Discussion

The depression of the main phase-transition temperature of lipid membranes by anesthetics and its elevation by high pressure are well documented. The present data agree with the previous reports [10,12–15]. The reversal of the alkanol-induced temperature elevation to depression under high pressure at low concentration range of C_{12} and C_{13}, however, has never been recognized and is intriguing. The presence of minimum temperature in the liquid-solid diagram indicates a nonideal mixing between the alkanol and lipid molecules. Although a possibility cannot be ruled out that the minimum point has a finite width to form eutectic mixtures, all alkanols appeared to form uniform mixtures with the phospholipid. The minimum has a similar meaning to azeotropes in a gas-liquid equilibrium of a nonideal mixture of volatile liquids [3].

1. Biphasic response

The biphasic effect of short-chain 1-alkanols on the main transition temperature of phospholipid mem-

branes was reported by Jain and Wu [1]. The temperature minima of the main transition occurred at 3.0 M for methanol, 1.0 M for ethanol, 0.4 M 1-propanol, and 0.2 M for 1-butanol. The temperature elevation by short-chain alkanols is attributable to the formation of the interdigitated states (discussed later).

Jain and Wu [1] reported that C_{11} and higher alcohols did not affect the transition significantly even at 15 mM concentrations. Their failure to detect the alcohol effect may be caused by the differential scanning calorimetry (DSC) they used to monitor the phase transition. Although DSC is probably the only direct method to measure the first-order phase transition (in the sense that it measures the first derivative of the enthalpy change) high lipid concentrations are required to maintain reasonable sensitivity to detect the heat flow. The lipid concentration is typically 10 mM and above. In their experiment, it was 75 mM. When the lipid concentration is large, highly lipophilic compounds, such as long-chain alkanols, are absorbed to the membrane and are depleted from the water phase. The equilibrium concentration in the aqueous phase becomes much smaller than the added nominal concentrations. In the 75 mM DPPC liposome suspension, the equilibrium aqueous concentrations of 15 mM C_{10} and C_{12} become 0.053 mM and 0.0034 mM, respectively. Because of these low equilibrium concentrations, Jain and Wu [1] did not observe any effect [16,17].

Compared to DSC, optical methods use low lipid concentrations, typically 1 mM and below. The low lipid content makes it possible to use unilamellar liposomes in contrast to DSC where multilamellar system is often used. By the optical absorbance technique, we [3] have shown that 1-alkanols longer than C_{10} affected the main transition temperature of phosphatidylcholine vesicle membranes biphasically, and those longer than C_{12} monotonously increased the main-transition temperature. Yi and Macdonald [18] evaluated the absorbance method for monitoring lipid phase transition.

2. Optical absorbance: cooperativity of the transition

Despite the above advantages of the optical method, it is often criticized that the optical absorbance method may not follow the main phase-transition temperature profile with fidelity, and that analysis of the configuration of the excess enthalpy peak in the DSC scan should be more informative in understanding the additive effect. On the contrary, we [16,17] have shown that the additive-induced distortion of the temperature profile of the main transition and the increase in the temperature span are an illusion associated mainly with the DSC experiments.

It is known that volatile anesthetics and long-chain alkanols increase the temperature span. The increased temperature span is often analyzed by the concept of the 'cooperativity unit' [19,20]: at the transition temperature, solid-gel and liquid-crystal domains coexist, and the number of phospholipid molecules in a cluster, synchronously fluctuating between the two phases, determines the temperature profile [19,20]. The smaller the number of lipid molecules in a cluster, the wider the temperature span becomes. The theory, however, was criticized strongly [21] that it is only a simple curve-fitting procedure.

We [16,17] have shown that the width of the temperature span is determined not by the putative cooperative unit but by the ratio of the partition coefficients of the solute between liquid-crystal phase vs. water and solid-gel phase vs. water. The water phase serves as a buffer, and the depletion of the solute from the water phase increases the temperature span. When the lipid concentration is large or the lipid solubility of the solute is large, the depletion effect becomes significant and the temperature span increases. This is why the strongly hydrophilic methanol and ethanol do not increase the temperature span, whereas the more hydrophobic long-chain alkanols and volatile anesthetics increase the span. We [17] have shown that halothane does not increase the temperature span when measured by the optical absorbance method, in contrast to more than 3-fold increase in the span reported by Mountcastle et al. [20] with the DSC experiment. Their DSC data were obtained at DPPC 12–14 mM whereas our optical data [17] were obtained at DPPC 0.1 mM. Thus, the distortion of the temperature profile is characteristic of DSC experiments because the method uses high lipid content, as discussed above. In the present study, the temperature profile of the transition did not vary from the control even with the highest concentration of C_{13}.

There is a tendency to assume that more expensive instrumentation leads to more accurate data. Fluorescence yield or fluorescence anisotropy are often considered to be superior than the simple absorbance. Fluorescence measures the property of a microscopic domain around the fluorophore in the macroscopic phenomenon of the integral membrane structure. The effect of ethanol on the fluidity of the lipid membrane was reported [22] to differ between the surface-binding and core-binding fluorophores. The core-binding fluorophore showed an increase in fluidity by ethanol, whereas the surface-binding fluorophore showed a decrease. Depending upon the site of residence, a fluorophore may report entirely opposite information. Phase transition is an integral property of the whole membrane, hence, the property probed by fluorophores does not necessarily represent the macroscopic phase transition. On the other hand, optical absorbance measures the property of the whole membrane. It is also free from the perturbation by the probe molecules in the membrane structure. We [3,17]

have shown that the main phase-transition temperature and the temperature profile measured by the optical absorbance method agreed well with DSC in the absence of hydrophobic solute.

3. Interdigitation and biphasic response

McIntosh et al. [5] reported that methanol, ethanol, and chlorpromazine decreased the thickness of the DPPC bilayer. The acyl chains of DPPC interpenetrate each other to form interdigitated gel phase, now commonly known as $L_{\beta I}$ phase. Despite the original report that the phenomenon is limited to amphipathic small ligands that localize themselves solely at the interface, the interdigitated phase attracted a number of reports. An anonymous referee contended that the present biphasic effect of long-chain alkanols is caused by the interdigitation. Despite the criticism, Simon et al. [6] conclusively demonstrated that long-chain alkanols do not interdigitate bilayers.

Lipid membranes cannot be formed without water. These structures are supported by the hydrogen-bonded water matrix. Bilayers exist by the balance between the lipid-lipid cohesive force and the lipid-water adhesive force. When the lipid-water adhesive force is weakened, membranes are disordered and expand. Further decrease in the lipid–water interaction force breaks the balance and lets the lipid-lipid cohesion force to dominate. The lipid tails interpenetrate each other and form all-trans conformation. The interdigitation is limited to small amphipathic compounds that interact only to the interface. Nonpolar molecules such as alkanes and long-chain alkanols that penetrate into the membrane core do not induce interdigitation [6]. Although high pressure also favors the interdigitated state, the interdigitation occurs at about 1500 bar [23], which is much higher than the present study at 300 bar.

Thus, the elevation of the main transition temperature by the high concentration of long chain alkanols is not caused by interdigitation. By using three phosphatidylcholines with varying acyl chain lengths (distearoyl-, dipalmitoyl-, and dimyristoyl-phosphatidylcholines), we have shown [3] that when the hydrocarbon chain length of alkanol becomes close to that of the phospholipid, the long lipid-chain of alkanols increases the packing efficiency of the lipid core of the membrane by the interaction between similar lipid chains. The nonideal mixing in the lipid-alkanol binary system generates the temperature minimum in the solid-liquid equilibrium.

4. High pressure and transition volume

To estimate the volume change at the phase transition, the pressure effect on the transition temperature is replotted from Fig. 3 for dodecanol and is shown in Fig. 4. The pressure effects at two C_{12} concentrations are shown; one at the temperature-decreasing side

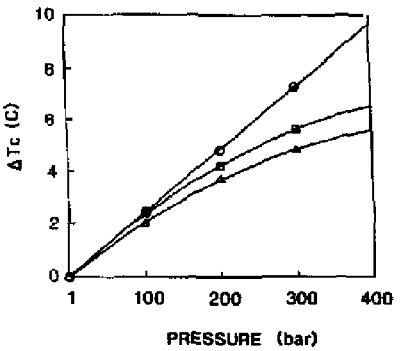

Fig. 4. The pressure effect on the transition temperature at two C_{12} concentrations. Ordinate: The change in the main phase-transition temperature. Abscissa: the pressure in bar. Symbols: control without C_{12} (○), C_{12} $1.29 \cdot 10^{-4}$ molal (△), $5.58 \cdot 10^{-4}$ molal (□).

($1.29 \cdot 10^{-4}$ molal), and the other at the temperature-increasing side ($5.58 \cdot 10^{-4}$ molal).

In the absence of alkanols, the response of the main transition temperature to pressure was linear, but it became nonlinear when alkanols were present. The slope, dT_c/dP was 0.0242 K bar^{-1} for the control. The slopes in the presence of $1.29 \cdot 10^{-4}$ and $5.58 \cdot 10^{-4}$ molal C_{12} were 0.0225 K bar^{-1} and 0.0255 K bar^{-1}, respectively, at ambient pressure, and 0.00987 K bar^{-1} and 0.0117 K bar^{-1}, respectively, under 300 bar.

According to the Clausius-Clapeyron equation

$$\frac{dT_c}{dP} = \frac{\Delta V \cdot T_c}{\Delta H} = \frac{\Delta V}{\Delta S}$$

where V, H, and S are the volume, enthalpy, and entropy, respectively. The ΔV of the main phase transition was 27.7 cm^3 mol^{-1} when estimated by the value $\Delta H = 36.4$ kJ mol^{-1} [3,24]. The ΔV value agreed well to the $\Delta T_c/\Delta P$ value of 28 cm^3 mol^{-1} reported by Mountcastle et al. [20] and also to the dilatometry data 27.2 cm^3 mol^{-1} reported by Nagle and Wilkinson [25] and 27.0 cm^3 mol^{-1} by Macdonald [13].

By approximating the excess enthalpy under high pressure, ΔH_p, as

$$\Delta H_p \approx \Delta H + \Delta V \cdot P$$

the ΔV_p value (the transition volume change at high pressure) at 300 bar was 27.7 cm^3 mol^{-1}. Because the rate of the change in the transition temperature was equal to the change in the ΔH, the ΔV and ΔS of the phase transition stayed constant at least up to 300 bar.

5. High pressure and biphasic response

When the carbon-chain length was shorter than eight carbon atoms, the response of the transition temperature to alkanols was a simple monotonous depression, approximately linear to the alkanol concen-

tration in the present experimental condition. It became biphasic when the chain length exceeded C_{10}. The biphasic response became stronger under high pressure.

A significant finding was that C_{12} and C_{13} depressed the transition temperature under high pressure at low concentrations. At ambient pressure, these long-chain alkanols do not depress the transition temperature [3]. If one accepts the theory that the anesthetic effect is accompanied by the depression of the main phase-transition temperature, the present result suggests that these non-anesthetic long-chain alkanols may manifest anesthetic potency under high pressure.

The changes in the T_c under high pressure indicate that either ΔS or ΔV or both is altered by the incorporation of alkanols. Presumably, the change occurs in the ΔS at ambient pressure and in the ΔV under high pressure. These parameters assume the minimum values at the break-point. In the presence of long-chain alkanols, the properties of the solid-gel and the liquid-crystal states became closer compared with the alkanol-free lipid membranes. Under high pressure, the affinity of the alkanols longer than C_{10} to the liquid-crystalline phase apparently exceeded the solid-gel phase at the low alkanol concentrations.

Acknowledgments

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