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A solid-solution theory of anesthetic interaction with lipid membranes: temperature span of the main phase transition

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Anesthetics (or any other small additives) depress the temperature of the main phase transition of phospholipid bilayers. Certain anesthetics widen the temperature span of the transition, whereas others do not. The widening in a first-order phase transition is intriguing. In this report, the effects of additive molecules on the temperature and its span were explained by the solid-solution theory. By assuming coexistence of the liquid-crystal and solid-gel phases of lipid membranes at phase transition, the phase boundary is determined from the distribution of anesthetic molecules between the liquid-crystal membrane versus water and between the solid-gel membrane versus water. The theory shows that when the lipid concentration is large or when the lipid solubility of the drug is large, the width of the transition temperature increases, and vice versa. Highly lipid-soluble molecules, such as long-chain alkanols and volatile anesthetics, increase the width of the transition temperature when the lipid: water ratio is large, whereas highly water-soluble molecules, such as methanol and ethanol, do not. The aqueous phase serves as the reservoir for anesthetics. Depletion of the additive molecules from the aqueous phase is the cause of the widening. When the reservoir capacity is large, the temperature width does not increase. The theory also predicts asymmetry of the specific heat profile at the transition.

Introduction

Lipid membranes undergo thermotropic phase transition between the ordered solid-gel and disordered liquid-crystal phases. The main phase-transition temperature of lipid membranes is depressed by small additives such as anesthetics. This phenomenon is often analyzed by the van't Hoff model on the freezing point depression. Kaneshina et al. [1] expressed the change in the transition temperature, ΔT_m, by anesthetics as

$$\Delta T_m = -RT_m^2(1-\kappa)x/\Delta H \quad (1)$$

and

$$\Delta T_m = T_\gamma - T_m \quad (2)$$

where T_m and T_γ are the transition temperatures in the absence and presence of anesthetics, x the mole fraction of the additive in the liquid-crystal membrane, ΔH the molar latent heat of the transition, R the gas constant, and κ the partition coefficient of additives between the liquid and gel phases.

The magnitude of the temperature depression by anesthetics correlates extremely well to their clinical potency. Another anesthetic effect on the phase transition is widening of the temperature span between the start and finish of the transition [2–6]. In a first-order phase transition, the transition should be isothermal. The existence of a finite temperature span is intriguing,

Symbols: N_p, total number of phospholipid molecules; N_w, total number of water molecules; N_a, total number of additive molecules; N_{aw}, number of additive molecules in water; N_{am}, number of additive molecules in the lipid membranes; N_{af}, number of additive molecules in the liquid-crystalline (fluid) membrane; N_{ag}, number of additive molecules in the solid-gel membrane ($N_a = N_{aw} + N_{am} = N_{aw} + N_{af} + N_{ag}$); κ, partition coefficient of additive molecules between the liquid-crystalline and solid-gel phases; x_{am}, mole ratio between additive molecules and lipid molecules in the whole system (N_a/N_p); x_{af}, mole ratio between additive molecules and lipid molecules in the liquid-crystalline phase; x_p, mole ratio between lipid and water (N_p/N_w); x_a, mole ratio between additive and water (N_a/N_w); P_{af}, partition coefficient of additive molecules between the liquid-crystalline phase and water; γ, melted fraction of the lipid membrane; C, heat capacity; R, gas constant; T, absolute temperature; T_m, main transition temperature in the absence of additives; T_γ, main transition temperature in the presence of additives; T_B, starting temperature of the phase transition; T_E, finishing temperature of the phase transition.

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and is often attributed to contamination by impurities [5]. To make the problem more complex, addition of some anesthetics, such as ethanol, do not widen the temperature span [2,3,6].

Mountcastle et al. [4] analyzed this problem by applying the cooperative cluster model of Hintz and Sturtevant [5]. At the transition temperature, solid-gel and liquid-crystal domains coexist and the number of phospholipid molecules in a cluster, synchronously changing the state, determines the width of the transition temperature. Nagle [7] criticized, however, that the model is nothing but a simple curve-fitting procedure falling short of explaining mechanisms.

There have been many trials which analyzed the phase transition of lipid membranes by estimating the molecular conformation of phospholipids and calculating the intermolecular interaction energies quantitatively. O'Leary [8] applied the decorated dimer model proposed by Nagle [9] and reproduced the effects of nonpolar anesthetics upon the shift of the main transition temperature and the change in the membrane volume. His analysis, however, did not explain the widening of the temperature span caused by anesthetics. Izuyama and Akutsu [10] also used Nagle's model and proposed that phase transitions in membranes may be of the pseudo-first-order, originating from the finite chain length of the phospholipid. De Verteuil et al. [11] applied the lipid hydrocarbon chain dynamics in bilayers described by Caille et al. [12] and estimated the anesthetic-induced changes in the transition temperature, enthalpy and entropy of the transition.

Although the theory based on the conformation of lipid tails is rigorous within its extent, it is not easy to extend the model to quantitatively include the effects of small additive molecules on the contour of the specific heat profile. It is important to devise a tractable model which can be used to treat the interrelationship between the microscopic interaction energy of lipid molecules and the cooperativity of macroscopic phase transition, and at the same time to analyze impurity effects upon the changes in the transition temperature and the width of the temperature span.

In this report, we shall analyze the relationship between the depression and the broadening of the transition temperature as an integral event by assuming a solid solution for the membrane/additive interaction. It will be shown that width of the transition temperature is determined by the distribution of additives among solid-gel, liquid-crystal, and aqueous phases, and not by the cooperativity of the membrane molecules in the putative clusters.

Theory

In a liposome suspension, anesthetics (or any additive molecules) are partitioned between the membrane and the aqueous phase. Additives change the transition temperature as stated in Eqn. 1. At the transition temperature, the liquid-crystalline and solid-gel phases coexist [4–6,13]. When the lipid molecules and additive molecules form solid solution, the phase transition proceeds in a state where the membrane is partially melted. Mastrangelo and Dornte [14] expressed the melted fraction by γ in this partially melted phase. The factor γ is determined as a function of temperature, T_γ. They analyzed the calorimetric data by this parameter for two-component system as follows:

$$T_\gamma = T_m - \frac{RT_m^2}{\Delta H}(1-\kappa)x_{af} \tag{3}$$

where x_{af} is the mole ratio between additives and lipid molecules in the liquid-crystalline phase.

We define N_p, as the number of lipid molecules, and N_{am} and N_{af} as the numbers of additive molecules in the whole membrane and in the liquid-crystalline (fluid) membrane, respectively. By definition, the partition coefficient, κ, is written

$$\kappa = \left(\frac{N_{am} - N_{af}}{(1-\gamma)N_p}\right)\left(\frac{N_p\gamma}{N_{af}}\right) \tag{4}$$

The equilibrium distribution of additive molecules between the membrane and the aqueous phase is now considered. Let N_w and N_a be the number of water molecules and the total number of additive molecules in the whole system, respectively. Then the following equation holds.

$$x_{af} = \frac{N_{af}}{N_p\gamma} = P_{af}\left(\frac{N_a - N_{am}}{N_w}\right) \tag{5}$$

where P_{af} is the partition coefficient of additives between the liquid domain of the membrane and the aqueous phase. From Eqns. 4 and 5, we obtain

$$x_{af} = \frac{x_{am}}{\kappa + \gamma(1-\kappa) + \dfrac{1}{P_{af}x_p}} \tag{6}$$

where

$$x_{am} = N_a/N_p \tag{7}$$

$$x_p = N_p/N_w \tag{8}$$

In deriving Eqn. 4, we assumed $N_w \gg N_p \gg N_a$. By combining Eqns. 3 and 6, the following equation is obtained.

$$\Delta T_m = -\frac{RT_m^2}{\Delta H}(1-\kappa)x_{am}\frac{1}{\kappa + \gamma(1-\kappa) + \dfrac{1}{P_{af}x_p}} \tag{9}$$

From Eqn. 9, we define the beginning (T_B) and ending (T_E) temperatures of the transition. We also define $\Delta T_B = T_B - T_m$ and $\Delta T_E = T_E - T_m$. The values for ΔT_B and ΔT_E, respectively, are determined by putting $\gamma = 0$ and $\gamma = 1$ in Eqn. 9.

$$\Delta T_B = -\frac{RT_m^2}{\Delta H}(1-\kappa)x_{am}\frac{1}{\kappa + \frac{1}{P_{af}x_p}} \tag{10}$$

$$\Delta T_E = -\frac{RT_m^2}{\Delta H}(1-\kappa)x_{am}\frac{1}{1 + \frac{1}{P_{af}x_p}} \tag{11}$$

Although we did not show the explicit form of γ as a function of temperature T_γ, it can be seen from Eqn. 9 that γ increases monotonically from 0 to 1 with downward convex curvatures. The enthalpy h at γ is written as

$$h = \gamma \Delta H \tag{12}$$

By ignoring the ΔH change of the system before and after the phase transition, the heat capacity, C, becomes

$$C = \frac{\partial h}{\partial T} = \Delta H \frac{\partial \gamma}{\partial T} \tag{13}$$

Origin of the width of phase transition

We shall determine the phase boundaries and evaluate thermal quantities such as transition temperature and heat capacity. In Eqns 10 and 11, the temperatures of beginning and ending of the phase transition were shown. When the factor $P_{af}x_p$ in Eqns. 10 and 11 is large compared to unity, ΔT_B and ΔT_E become,

$$\Delta T_B = -\frac{RT_m^2}{\Delta H}(\kappa^{-1} - 1)x_{am} \tag{14}$$

$$\Delta T_E = -\frac{RT_m^2}{\Delta H}(1-\kappa)x_{am} \tag{15}$$

This result is equivalent to that of Mastrangelo and Dornte [14]. It means that almost all additive molecules are adsorbed to the membrane and the number of the free additive monomers in the aqueous phase is nearly depleted. The temperature span is a function of the degree of the depletion. The larger the depletion, the wider the temperature span becomes.

Next, we analyze the opposite case where $P_{af}x_p$ is small compared to unity. Then, ΔT_E becomes equal to ΔT_B as follows.

$$\Delta T_B = \Delta T_E = -\frac{RT_m^2}{\Delta H}(1-\kappa)P_{af}x_a \tag{16}$$

where

$$x_a = \frac{N_a}{N_w} \tag{17}$$

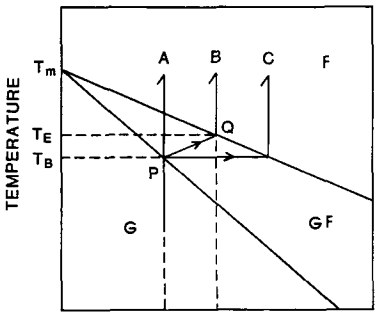

Fig. 1. Schematic drawing of the phase diagram of lipid membranes in the presence of additives. Abscissa is the fraction of additives in the membrane and ordinate is the temperature. G, gel phase; F, fluid (liquid-crystal) phase; GF, mixture of the gel and liquid-crystal phases; T_m, phase transition temperature in the absence of additives; T_B, the temperature where the phase transition starts; T_E, the temperature where the phase transition finishes. See text for explanation.

Here, the transition temperature is depressed without affecting the temperature span.

Fig. 1 is the graphic representation of the above result. In this diagram, there are three possible routes for the transition to travel when the temperature is raised from the gel phase. In Route A, the transition temperature is decreased and the temperature span is broadened. This route represents Eqns. 14 and 15. In Route C, the temperature span does not change. Here, the aqueous phase acts as a buffer reservoir for the additive molecules during the phase transition and the depletion of the additive molecules from the aqueous phase is minimal (Eqn. 16). The additives are highly soluble to water and the size of the aqueous phase is much larger than the lipid phase. Route B represents the course between Route A and C.

Depression of the transition temperature without increasing the temperature span has been observed with *n*-alcohols with shorter alkyl chain [2,3,6]. In contrast, long-chain *n*-alcohols increased the temperature span. When the water solubility of the additives is high and the lipid-water partition coefficient is low, the temperature span does not increase. Although the sharpness of the phase transition has generally been attributed to the cooperativity of the microscopic molecular interactions, the present analysis shows that the membrane-water partition coefficient of the additives and the size of the aqueous phase determine the sharpness of the transition.

From Eqns. 9 and 13, we obtain the heat capacity, C, as follows:

$$C = RT_m^2 \frac{x_{am}}{\Delta T_m^2} \tag{18}$$

The profile of C as a function of T is schematically expressed in Fig. 2, where the shape of the peak is asymmetric, in agreement with calorimetry data. The

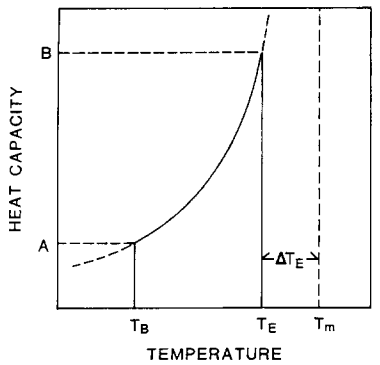

Fig. 2. Schematic drawing of the heat capacity, C, as a function of temperature, T. $A = kT_m^2 N_a/\Delta T_E^2$. $B = kT_m^2 N_a/\Delta T_B^2$. T_B and T_E are the temperature of the start and finish of the phase transition in the presence of additives, respectively, and T_m is the transition temperature in the absence of additives. ΔT_B and ΔT_E are given by Eqns. 11 and 12, respectively.

actual profile of the specific heat, however, is smoothed by the finite scanning rate and the impurity of the phospholipid membrane.

We now numerically analyze the halothane effect in the dipalmitoylphosphatidylcholine (DPPC) vesicle membranes. In the study of Mountcastle et al. [4], the lipid concentration was 12 to 14 mM. Kamaya et al. [15] estimated that the apparent partition coefficient, $P_{af}(1 - \kappa)$, to be 2570 at the transition temperature. By radioactive tracer method, Simon et al. [16] estimated the κ value to be about 1/4 to 1/3 in the DPPC vesicle membranes. Then, the right-hand side of the denominators of Eqns. 10 and 11 becomes

$$P_{af} x_p = 0.843 \tag{19}$$

where we have used the values $\kappa = 1/3.5$ and 13 mM for the lipid concentration. The value 0.843 is close to unity. Then, the transition pathway is between the two extremes of Route A and Route C in Fig. 1 and becomes B-like. The lipid concentration of about 13 mM is the level where halothane broadens the temperature span of the transition.

The temperature width of the transition has been analyzed on the data obtained mainly by differential scanning calorimetry (DSC). Although DSC is probably the only direct method to measure the first-order phase transition, high lipid concentrations are required to maintain reasonable sensitivity to detect the heat flow. The lipid concentration is typically 10 mM and above. Compared to DSC, optical methods use low lipid concentrations, typically 0.1 mM and below. Single-shelled liposomes can be used with these methods. Although fluorescence anisotropy is popular for this purpose, incorporation of probe molecules into the membrane tends to perturb the membrane structure. It measures the property of microscopic region around the probe molecule in a macroscopic change in the integral membrane structure. Hitzemann [17] reported that the effect of short-chain alkanols on the fluidity of the lipid membranes differs between core-binding and near-surface-binding fluorophores. In this context, electron spin resonance is not free from the problem of the perturbation induced by the probe molecules.

Turbidimetry measures the change in the refractory index of the membrane at the main transition. The method eliminates the perturbation by the probe molecules incorporated into the membrane structure. It is a macroscopic method and reveals the integrated property of the membrane. In addition to these advantages, it is simpler and can be performed with inexpensive spectrophotometers.

To critically examine the above model, the main phase transition of dipalmitoylphosphatidylcholine (DPPC) single-shelled vesicle membrane was measured by the turbidimetry method.

Experimental

Synthetic 1,2-dipalmitoyl-*sn*-3-phosphorylcholine was obtained from Sigma (St. Louis, MO), and halothane from Ayerst (New York, NY). Water was purified by a Sybron-Barnstead Nanopure system (Boston, MA). DPPC was sonicated in a Cuphorn of a Branson Sonifier Model 185 (Danbury, CT). The DPPC concentration was 0.1 mM. Halothane was vaporized in a Copper Kettle of an anesthesia machine by nitrogen gas. The anesthetic concentration was estimated by the vaporizer temperature and the ratio between the gas flow through the vaporizer and the diluting gas flow, and verified by a Shimadzu Gas-chromatograph (Columbia, MD) with a flame ionization detector, and a 1/8 inch × 6 ft stainless steel tubing packed with Porapak Q 80/100 mesh at operating temperature of 160 °C.

To compensate for the escape of anesthetics into air during the measurements, halothane was continuously delivered to the liposome by bubbling into the cuvette. A Perkin-Elmer Model 554 spectrophotometer (Norwalk, CT) was used to measure the light absorbance of the liposome at 400 nm. The cuvette temperature was scanned at 0.5 C°/min by a programmable digital temperature controller and a Peltier heat-exchanger. The temperature of the sample was monitored by a thermistor probe inserted into the cuvette slightly above the light-path and a Digitec thermometer Model 5810 (Dayton, OH) with 0.01 C° resolution. The sample solution was continuously mixed with a direct-drive Spectrostir (Oreland, PA) during the temperature scan. The spectrophotometer and temperature signals were monitored by a Nicolet digital oscilloscope (Madison, WI) and stored in a floppy disk of an IBM-PC.

TABLE I

Halothane effects on the main phase transition temperature and the width of the transition temperature

Halothane concentrations are expressed by the partial pressure (atm) in the gas phase in equilibrium with the liposome solution. (Mean ± S.D., $n = 3$).

	Halothane partial pressure (10^{-2} atm)				
	0.00	1.24	2.39	4.44	6.24
T_γ (°C)	41.0 ±0.04	40.4 ±0.20	39.6 ±0.14	38.6 ±0.13	38.1 ±0.21
Half-height width (C°)	0.19±0.02	0.18±0.06	0.17±0.01	0.19±0.02	0.20±0.02

Results and Discussion

Fig. 3 shows typical tracings of the control without halothane and in the presence of $6.24 \cdot 10^{-2}$ atm. Table I shows the main phase transition temperatures at various halothane concentrations together with the widths of the temperature span. The data on the temperature depression agree with those reported by Kamaya et al. [15] and Mountcastle et al. [4].

At $6.24 \cdot 10^{-2}$ atm halothane partial pressure, the main transition temperature decreased 2.9 °C, but the temperature span was essentially unchanged when the depletion of the halothane concentration in the aqueous phase was prevented in the present study. The DSC data by Mountcastle et al. [4] show that at halothane partial pressure of $4.7 \cdot 10^{-2}$ atm, the main transition temperature decreased 1.75 °C and the temperature span increased 3.8-fold.

The width of the transition temperature span depends upon the size of the aqueous phase that functions as a buffer reservoir, as shown by the above discrepancy of DPPC concentrations between the DSC (12–14 mM) and the optical method (0.1 mM). In the present study, the factor $P_{af}x_p$ is $6.48 \cdot 10^{-3}$, and ΔT_B and ΔT_E in Eqns. 10 and 11 become almost identical. When the decrease of the equilibrium anesthetic concentration in the aqueous phase by partition into lipid membranes is significant, the temperature width increases. Thus, not only the concentrations of additives but those of lipids also affect the transition characteristics. Under high lipid concentrations, it may be possible to decrease the width of the temperature span by supplementing the anesthetic depletion with continuous gas flow.

Recently, Sturtevant [18] reported that the temperature span is influenced by the cooperativity mechanism plus the buffer action of the aqueous phase. The model proposed by de Verteuil et al. [11] does not include aqueous phase, but they mentioned the importance of the partition of anesthetics in aqueous phase to determine the phase boundaries. Despite different approaches, the same conclusion of the buffering action of the water phase was reached. It is probably imperative to include the water phase in describing membrane properties because bilayers are supported by the hydrogen-bonded water matrix. The previous results on the increased temperature span in the presence of additives may have been the consequence of the high phospholipid concentrations required for DSC measurements.

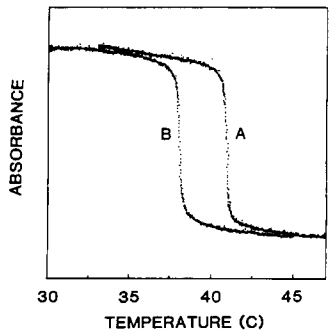

Fig. 3. The main phase transition of the DPPC liposome measured by turbidimetry. The spectrophotometer and temperature outputs were AD converted and recorded in a floppy disk. Normalized tracings of the heating scan without (A) and with halothane $6.24 \cdot 10^{-2}$ atm (B) are shown. Ordinate is the absorbance at 400 nm in arbitrary scale.

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