Phase Changes Induced by Cyclopropane of Phosphatidylserine Bilayers in the Presence and Absence of Calcium

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Received September 22,1975

SUMMARY

The effects of calcium on the thermodynamic parameters of dipalmitoyl-phosphatidylserine (DPS) bilayers in the presence of 1 atm nitrogen and cyclopropane was investigated calorimetrically. It found that at pH 3.1, in the absence of calcium, cyclopropane produces two endothermic phase transitions, at temperatures 63.0° and 60.5°, as compared to the single endothermic transition that was found in the presence of nitrogen. The enthalpies of the cyclopropane transition were also reduced.

In the presence of calcium and nitrogen the transition temperature was 66.5°. Upon substituting cyclopropane for nitrogen two endothermic transitions were observed. The main peak remained at 66.5° while the second peak melted at 58.0°. This lower peak melts at about the same temperature as the calcium free dispersion in the presence of cyclopropane. Thus, in the presence of an inert gas, the ionic environment of a lipid can moderate the effect a particular inert gas may have. Moreover, the presence of an inert gas can cause calcium to apparently dissociate from the bilayer to form a separate calcium free phase.

INTRODUCTION

The interaction of inert gases with homogeneous dispersions of lipid

bilayers are of interest to physiologists, because of the anesthetic properties of these gases (1), as well as to physical chemists, for the interaction of an inert gas with a two-dimensional liquid such as a lipid bilayer (2) has the possibility of being well understood. The same argument may be cited for calcium. There is, however, very little available data with biological molecules under controlled conditions interacting with gases in the presence and absence of Ca^{++} (3). We have chosen, therefore, to examine the effects of the inert gases N_2 and C-C_3H_6 on bilayer dispersions of 1,2-L-α-dipalmitoyl-phosphatidylserine (DPS) in the presence and absence of Ca^{++}. Phosphatidylserine (PS) was chosen as the lipid to study these effects as it is the most common negatively charged lipid found in the brain and other parts of the nervous system (4).

Previous investigations of the interactions of Ca^{++} with PS monolayers and bilayers have shown that Ca^{++} reduces the surface potential (5), decreases membrane fluidity (6), induces phase separation in lipid dispersions of PS and phosphatidylcholine (6), and competitively inhibits H^+ ions (5).

Recent experimental evidence has demonstrated that the "gel"-liquid crystal phase transition of PS is very sensitive to changes in ionic strength, pH, and divalent ion concentration (7, 8).

An initial step in the production of general anesthesia may be the partitioning of inert gas molecules into the lipid bilayer region of a membrane (9). We wished to test the hypothesis that not all lipid bilayer membranes, (or all parts of biological membranes), are equally receptive to the inert gases so that models of anesthesia involving lipid bilayers will have to take into account such variables as ionic strength, pH, mono and divalent ion concentrations. Moreover we wish to show that membrane inhomogeneity may be induced in a homogeneous dispersion under the appropriate conditions by an inert gas.

MATERIAL AND METHODS

DPS was prepared according to the method of Baer and Maurukas (10) and

gave a single spot on thin layer chromatography. Water was doubly distilled. $CaCl_2$ was washed in chloroform to remove non-polar impurities. Cyclopropane and nitrogen were used as obtained.

The calorimetry scans were performed on a Dupont 900 base with a DSC cell (Differential Scanning Calorimetry). The calibration of the calorimeter is described elsewhere (11). Each experiment was repeated three times. The lipid was weighed into the bottom part of a volatile sample pan. Aqueous solutions which were equilibrated with N_2 or C-C_3H_6 were then added to the DPS (in a glove box with the appropriate gas in it). The amount of water, usually three to four times the weight of the DPS, was added to insure that the system was in the excess water region of the phase diagram (12).

The pH of the aqueous solutions were adjusted with HCl. The pH was not appreciably changed upon the addition of DPS to the sample. Upon sealing the sample chamber, the chamber was heated to 95°C for a period of about two hours to insure that the water penetrated into the DPS lattice (12).

The transition temperature, Tm, was chosen to be the maximum of the endothermic peak, as it was found that this point rather then the onset or end of melting was more reproducible. The transition enthalpy, ΔH_m, was calculated by measuring the area(s) under the endothermic peak(s) and the transition entropy was calculated via $\Delta S_m = \frac{\Delta H_m}{T_m}$. It also has been shown by others that the $P\Delta V$ term that arises when equating the chemical potentials in the two phases at the transition may be neglected when compared to the enthalpy necessary to create "kinks" and to separate the hydrocarbon chains (13). Thus without appreciable error we may consider the pressure of the gas to be 1 atm. The samples were heated at 2°C/minute.

RESULTS

Figure 1 shows the calorimetric scans of four different samples:

DPS/N_2, DPS/C-C_3H_6, DPS + Ca^{++}/N_2, DPS + Ca^{++}/C-C_3H_6

all at pH 3.1. Note that all peaks are endothermic. Below are the thermodynamic data derived from these scans.

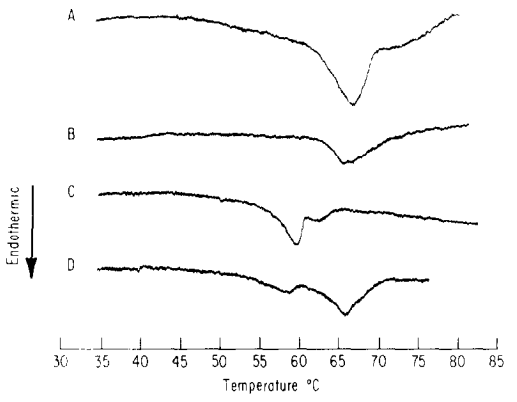

Figure 1

Differential scanning calorimetry for DPS. Heating rate 2°/minute: thermogram A, B are approximately 1/8 DPS/H_2O (w/w); C, D are approximately 1/6 DPS/electrolyte (w/w).

(A)	DPS - H_2O	pH 3.1	1 atm N_2
(B)	DPS - $CaCl_2$	pH 3.1	1 atm N_2
(C)	DPS - H_2O	pH 3.1	1 atm C-C_3H_6
(D)	DPS - $CaCl_2$	pH 3.1	1 atm C-C_3H_6

Under N_2, but in the absence of Ca^{++}, DPS exhibits a near symmetric phase transition with an enthalpy of 8.6 $\frac{Kcal}{mole}$ and a peak temperature of 67.5°C. Substitution of cyclopropane for nitrogen results in the appearance of two thermal events both at lower temperatures than the DPS/N_2 transition; one at 60.5°C with enthalpy of 3.3 $\frac{Kcal}{mole}$ and a smaller one at 63°C with an enthalpy of 1.0 Kcal/mole. The presence of two peaks in a DSC scan is normally taken to indicate the presence of two phases (11).

In the presence of N_2 and Ca^{++}, DPS exhibits an asymmetric phase transitior centered at 66.5°C with an enthalpy of 4.4 Kcal/mole. It is evident that Ca^{++} has a small effect on T_m but a substantial effect on ΔH_m.

Substitution of C-C_3H_6 fpr N_2 when Ca^{++} is present causes the appearance

of a small peak at 58°C with a ΔH_m of 1.5 $\frac{K_{cal}}{mole}$ but the major peak remains at 66.5°C with a ΔH_m of 6.1 $K_{cal/mole}$.

DISCUSSION

Nitrogen was used as control gas because of its basic unreactivity and the fact that it is not a general anesthetic at 1.0 atm. Cyclopropane was chosen because: it is anesthetic at 1.0 atm, has a zero permenent dipole moment (14), and an oil-water partition coefficient of 35:1 (15). These properties suggest that its effects on bilayers are due its penetration in the non-polar regions of the bilayer.

The reduction of T_m by cyclopropane (relative to N_2) for Ca^{++}-free dispersions cannot, because of the presence of two phases, be explained simply as a freezing point depression of a pure component by a solute as was done by Hill for a variety of anesthetics with the lipid dipalmitoylphosphatidylcholine (16). Nevertheless cyclopropane lowered the transition temperature and enthalpy which may be interpreted to mean that the cyclopropane must have increased the entropy of the bilayer, perhaps differentially for the two phases.

Upon comparisons of dispersions under N_2 with and without Ca^{++} we found in agreement with Jacobson and Papahadjopolos (7), who studied brain phosphatidylserine at a higher pH in the presence of Ca^{++}, that the enthalpy of a transition is diminished.

In the presence of Ca^{++} it was again found that cyclopropane induced two endothermic peaks in the DCS scan. In this case, however, one peak remained at the same temperature as the N_2-Ca^{++} peak and the other (the smaller one) was found about the temperature of the major peak of the C-C_3H_6 free dispersion. The appearance of the latter peak suggest phase separation of a portion of PS that has lost its affinity for Ca^{++}. Thus the anesthetic, cyclopropane, may cause Ca^{++} to be dissociated from lipid bilayer membranes. In the presence of both Ca^{++} and C-C_3H_6 there appears to exist a Ca^{++}-DPS phase which includes C-C_3H_6 and a C-C_3H_6-DPS which excludes Ca^{++}. As a result we may state even in a model membrane system, depending on the type of lipid and ionic environment,

the partition of an inert gas may be very different in one part of the system than another.

We are aware of the implications of inert gas dissociation of calcium from biological membranes that relate to possible anesthesia mechanisms (17).

Acknowledgements: This work was funded by grants HL-12157 and ONR contract number N0014-67-A-02151-0022. We would like to thank Drs. A.A. Lev and W. Stone for their useful criticisms.

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