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SYNERGETIC EFFECTS OF Ca^{2+} AND Cu^{2+} ON PHASE TRANSITION IN PHOSPHATIDYLSERINE MEMBRANES

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In complexes of divalent metals with large exchange rate constant (K_{H_2O}) of the coordinated H_2O, such as Ca^{2+} and Cu^{2+}, the cubic structure in the ligand field is usually unstable and conformation changes are easily induced. We observed the molecular motion of phosphatidylserine (PS) in an amphipathic solvent (water/methanol/chloroform) by ^{1}H-NMR and ESR using Ca^{2+} and/or Cu^{2+}, which has a similar K_{H_2O} to that of Ca^{2+}. We found that Ca^{2+} did not hinder the molecular movements of PS. However, Cu^{2+} reduced the movements of both headgroups and the double bonds in the fatty acids of PS. By addition of both Ca^{2+} and Cu^{2+}, phase transition to a soft solid phase in the PS membrane was observed at room temperature. The results indicate that the headgroups are clustered in two-dimensional network with each ligand field displaced from the aqueous phase to the water/oil interface. The structure changes of the polar headgroups after the binding of divalent cations are considered to trigger the phase transition of this acidic phospholipid membrane.

Introduction

Calcium (II) binds to acidic phospholipids such as phosphatidylserine (PS) [1–3], and the interactions between PS molecules and divalent cations in membranes may be of physiological significance. Using spin-labels, Ca^{2+} in phosphatidylserine/phosphatidylcholine (PS/PC) bilayer membranes was found to induce a phase transition to a solid phase [4]. NMR and infrared measurements [5] showed that Ca^{2+} was bound to the phosphate and carboxyl groups of PS, and that the movements at the headgroups are not hindered by the binding of Ca^{2+} in an amphipathic solvent.

We suggest that changes of the ligand field of the metal ion-PS complex may contribute to phase transitions in acidic phospholipid membranes. To demonstrate this assumption, we investigated the effects of Cu^{2+}, which has an exchange rate constant of the coordinated water molecules (K_{H_2O}) close to that of Ca^{2+}, on PS membranes, using magnetic resonances. We also studied the phase transition induced by the binding of these two divalent cations to polar headgroups.

Experimental Procedure

Phosphatidylserine (PS) obtained from brain extract type III (Sigma) was purified by silica gel column chromatography and DEAE-cellulose column chromatography. The purity (approx. 99% as free acid) was checked by thin-layer chromatography. About 100 mg of PS was dissolved in 1 ml of $C^2HCl_3/C^2H_3O^2H$ or $CHCl_3/CH_3OH$ (3 : 1, v/v). The solution was shaken with 1 ml ^2H_2O or H_2O containing 100 mM $CuCl_2$ and/or 1 M $CaCl_2$ and the upper phase containing unbound ions was removed. The excess ions in mixed solution were eliminated by extraction with ^2H_2O or H_2O.

ESR spectra were recorded at room temperature, on a Hitachi 771 ESR Spectrometer and ^{1}H-NMR was done with a Hitachi R-24 NMR Spectrometer (60 MHz) at 37°C. The ESR method was especially chosen to investigate any change of the polar headgroups in the aqueous phase of the PS solution (amphipathic solvent: chloroform/methanol/water) containing the paramagnetic ion Cu^{2+}. The effects on the hydrophobic parts of the PS molecules caused by Cu^{2+} or $Cu^{2+} + Ca^{2+}$ and the phase changes of the PS membranes caused by the binding of Ca^{2+} were studied by ^{1}H-NMR.

Results

ESR measurements and absorption spectra

The relaxation time T_{2C} ($\Delta\nu = 1/T_2$; $\Delta\nu$ is the linewidth of the ESR signal with motional narrowing) and the g_C of the Cu^{2+}- PS complex in the amphipathic solvent (Fig. 1a) differed from the values, T_{2aq} and g_{aq}, which were obtained in Cu^{2+} aqueous solution. T_{2aq} was compared with T_{2C}, which was converted into the value in aqueous solution. We obtained $T_{2aq} \simeq 2.4T_{2C}$, $g_C \simeq 2.18$, and $g_{aq} \simeq 2.27$. In both systems, the ESR signals (Fig. 1 a and b) showed motional narrowing, so that g_C and g_{aq} were averaged by tumbling motion; the ligand field, therefore, has a cubic structure (O_h-symmetry). However, the tumbling motion in the Cu^{2+}-PS complex decreased compared with that of the Cu^{2+} aqueous solution ($T_{2aq} \simeq 2.4T_{2C}$). The molar ratio of PS to Cu^{2+} in the Cu^{2+}-PS complex was found to be 2 : 1 using an atomic absorption spectrometer.

When the two metal ions Ca^{2+} and Cu^{2+} were added to the PS organic solution (Ca^{2+} to the Cu^{2+}-PS complex or Cu^{2+} to the Ca^{2+}-PS complex), a static Jahn-Teller distortion in the Cu^{2+} ligand field was observed at room temperature. The g factor of Cu^{2+} split into a parallel ($g_{\parallel}$) and a perpendicular ($g_{\perp}$) component on the z-axis in the ligand field (Fig. 1c) with $g_{\parallel} \simeq 2.38$ and $g_{\perp} \simeq 2.05$.

These results indicate that a tetragonal distortion (D_{4h}-symmetry) serves to remove the orbital degeneracy. Further, the movements of the headgroups of PS are hindered in the Ca^{2+}-Cu^{2+}-PS complex. The average g factor was about 2.18, nearly equal to $(1/3)\cdot(g_{\parallel} + 2g_{\perp}) \simeq 2.16$. This small difference is within the error of the measurement of the magnetic field.

The d-d transition, Δ, of Cu^{2+} in the Ca^{2+}-Cu^{2+}-PS complex appeared at about 15 673 cm^{-1} (Fig. 2). The covalency rate of Cu^{2+} in this complex was calculated to be about 10% by using Δ.

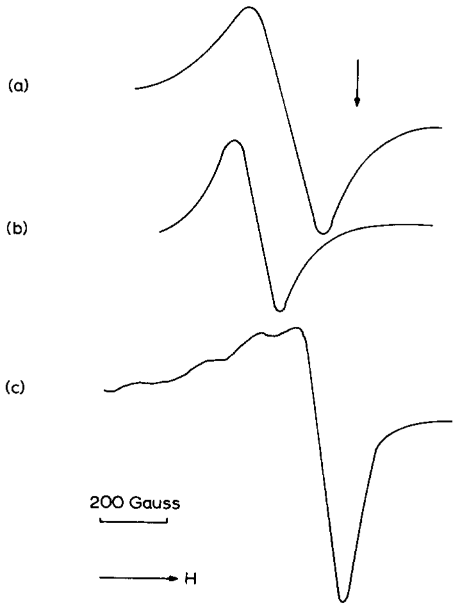

Fig. 1. ESR spectra of Cu^{2+} at room temperature. (a) Cu^{2+}-PS complex in the amphipathic solvent, (b) Cu^{2+}-PS complex in aqueous solution, and (c) Ca^{2+}-Cu^{2+}-PS complex in the amphipathic solvent. Arrow ↓ indicates position of 1,1-diphenylpicryl-2-hydrazyl (DPPH).

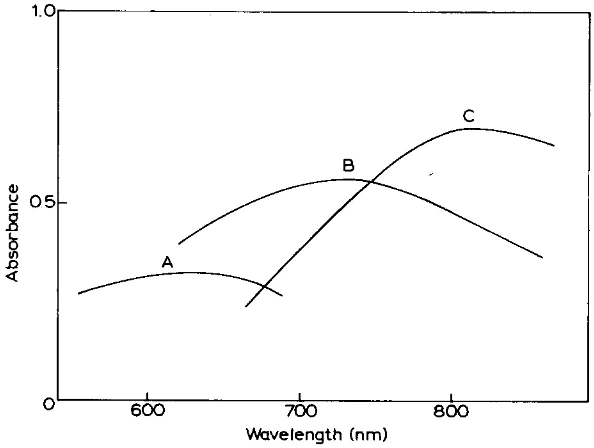

Fig. 2. Visible light absorption spectra (A) Cu^{2+}-PS complex with Ca^{2+}. (B) Cu^{2+}-PS complex (A, B: in the amphipathic solvent). (C) Cu^{2+} in aqueous solution.

^1H-NMR measurements

The ^1H-NMR spectrum of the PS molecules in $C^2HCl_3/C^2H_3O^2H$ (3 : 1, v/v) is shown in Fig. 3a. The ^1H signals are identified according to the Chapman and Morrison [8]. The spectrum of the Ca^{2+}-PS complex (Fig. 3c) closely resembles that of PS in $C^2HCl_3/C^2H_3O^2H/^2H_2O$ (Fig. 3b). A slight reduction in the linewidths of ^1H signals of the headgroups was observed in the amphipathic solvent compared with the spectrum in organic solvent, which is shown in Fig. 3a. In the Cu^{2+}-PS complex (Fig. 3d), ^1H signals of the -$(CH_2)_n$- chains and of the -CH_3 terminals in fatty acid did not have broadening of the linewidths and also did not show paramagnetic effects, whereas the signals of part of the polar headgroups of PS disappeared. From the ESR results (Fig. 1a), this disappearance is mainly caused by the μ^2_{eff} of Cu^{2+} (μ_{eff} is the effective magnetic moment). However, Cu^{2+} reduced the movements of both headgroups and the double bonds in the fatty acids of PS. The NMR spectrum of the Ca^{2+}-Cu^{2+}-PS complex (Fig. 3e) agrees with that of the Cu^{2+}-PS complex in Fig. 3d. The broadening of ^1H signals at D, E and F of Fig. 3e was induced by the μ^2_{eff} of Cu^{2+} and by hindrance of molecular motion in the headgroups. In the Ca^{2+}-Cu^{2+}-PS complex, ^1H signals of the hydrophobic parts (-$(CH_2)_n$- and -CH_3) were narrow and paramagnetic shifts were not observed. However, the signal (H in Fig. 3) of the double bonds showed more broadening than that in the Cu^{2+}-PS complex.

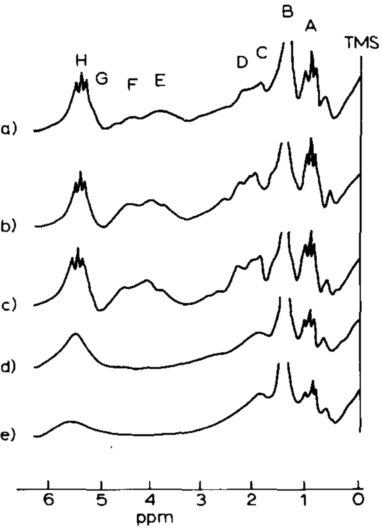

Fig. 3. ^1H-NMR spectra of PS in $C^2HCl_3/C^2H_3O^2H$ (3:1, v/v) (a), and in $C^2HCl_2/C^2H_3O^2H/^2H_2O$ (b–e) at 37°C. (b) PS, (c) Ca^{2+}-PS complex, (d) Cu^{2+}-PS complex, and (e) Ca^{2+}-Cu^{2+}-PS complex. A, CH_3; B, $(CH_2)_n$; C, $C\underline{H}_2CH_2=$; D, CH_2CO; E, CH_2OCO; F, CH_2OP; G, $CHOCO$; H, $HC=CH$. TMS: $(CH_3)_4Si$.

Conformation change of PS membranes by the binding of Ca^{2+} and Cu^{2+}

A PS molecule is expected to bind more than one metal, because it has three polar groups, amino, carboxyl, and phosphate, in its head part. Ca^{2+}

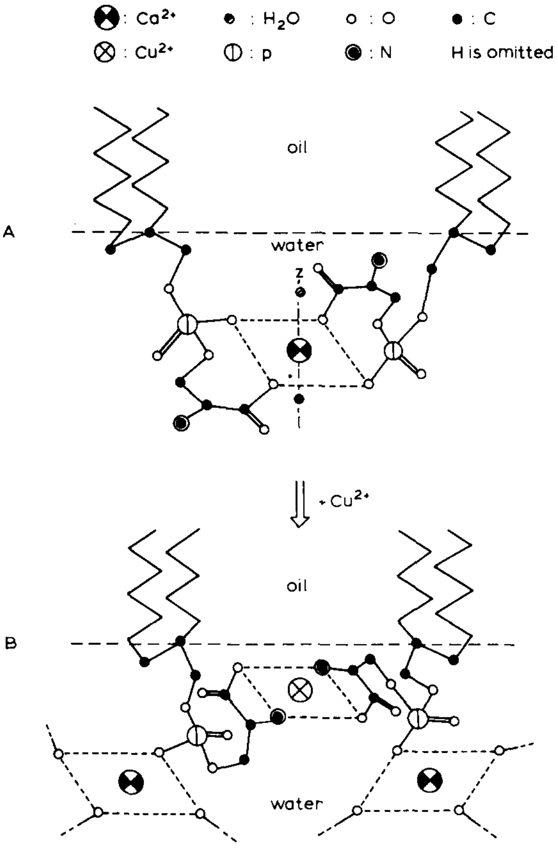

Fig. 4. Model of conformation changes upon binding of Cu^{2+} to the Ca^{2+}-PS complex in a water/oil emulsion. (A) Ca^{2+}-PS complex. The ligand field of Ca^{2+} with O_h symmetry is in the aqueous phase. Ca^{2+} is bound with two H_2O on the z-axis and with oxygens of carboxyl and phosphate groups. (B) Addition of Cu^{2+}. Cu^{2+} forms a complex with amino and carboxyl groups and Ca^{2+} is bound to phosphate groups around PS molecules. Both ligand fields show D_{4h} symmetry and are displaced to the water/oil interface.

binds to PS in the stoichiometric ratio of 1 Ca^{2+} to 2 PS molecules; the primary binding sites are the phosphate groups, and the carboxyl groups also contributes positively [5]. As the molar ratio of Cu^{2+} to PS was 1 : 2 in our system, the binding sites of Cu^{2+} may be the amino and carboxyl groups. From these findings, the carboxyl group may be bound competitively with Ca^{2+} and Cu^{2+}; the binding affinity depends on the pH of the aqueous phase of the emulsion system. In our experiment, the molar ratio of Ca^{2+} to PS with Cu^{2+} proved to be about 1 : 5 using an atomic absorption spectrometer. At 100 mM $CaCl_2$, however, Ca^{2+} was not easily bound to the Cu^{2+}-PS complex. From the above results, and because the affinity of Cu^{2+} to carboxyl and amino groups is larger than that of Ca^{2+}, Cu^{2+} may be bound to them, and Ca^{2+} to the phosphate groups of nearby PS molecules (Fig. 4). Thereby, many PS molecules may be cluster together and move to the water/oil interface of the emulsion by the binding of these two cations, Ca^{2+} and Cu^{2+}. We think this structure change is induced by synergetics caused by the S_N-1 mechanism in Ca^{2+} and by the Jahn-Teller effect in Cu^{2+}, because of their exchange rate constants, which are both large and quite similar (K_{H_2O} approx. 10^{-8} s^{-1}) [6,7].

Discussion

The phase transitions of acidic phospholipid membranes are induced by the aggregation of the lipid molecules bridged by Ca^{2+} chelation [4]. In an amphipathic solvent, the binding of Ca^{2+} alone has no effect on movements of the headgroups of PS, in agreement with the results of ^{13}C-NMR [5]. Our experiments with Cu^{2+} demonstrate, however, that the movements of the headgroups and at the part of double bonds in alkyl chains of PS are hindered to some extent at room temperature (Figs. 1a and 3d) and that are almost suppressed at 77 K. The reason may be that since our system is 20–25% H_2O (v/v), it can be taken to have uncondensed monolayer membranes, and also that the molar ratio of PS/Ca^{2+} is 4–5 : 1 (without Cu^{2+}) at pH nearly equal to that of the metal ion solution (pH ≃ 5.5–5.6). In the Ca^{2+}-Cu^{2+}-PS complex, the movements of the headgroups were hindered at room temperature, so that the polar headgroups are thought to form a two-dimensional network on the water/oil interface of the emulsion (Fig. 4) and to change from the fluid phase to the soft solid one.

Here, to clarify the synergetics of the divalent cations, we have described a dynamic model (submitted for publication) in terms of classical mechanics. These synergetics can be explained by the bifurcation of a potential of a molecular system (Fig. 5) due to temperature change and/or the addition of metal ions such as Ca^{2+}. An equation of motion may be simplified by an approximation of the overdamped motion [9] which describes strong interactions. Expanding the potential ($U(Q)$ at an equilibrium point ($Q \simeq 0$), we obtained the following equation containing an anharmonic term:

$$\gamma \frac{dQ}{dt} = \frac{dQ}{dt'} = -\text{grad}\, U(Q) \simeq -K_1 Q - K_3 Q^3 \quad (t \equiv \gamma t') \qquad (1)$$

where γ is the damping factor and Q is the normal coordinate in a vibrational state. The equilibrium points are obtained at $Q = 0$, and if the force constant $K_1 > 0$ ($K_3 > 0$), $Q = 0$ ($U(Q) = U(Q^0)$) and if $K_1 < 0$, $Q^{\pm} = \pm\sqrt{|K_1|/K_3}$ ($U(Q) = U_{\min}(Q^{\pm})$, Fig. 5).

When the phase changes are induced by the binding of divalent cations, K_1 can be described as:

$$K_1 = K(T) - K(T_C;\beta) = \alpha_0 \kappa (T - T_C) \qquad (2)$$

where T_C is the transition temperature, α_0 is a constant related to the circular frequency ω at 0 K, β is a shift parameter of changing T_C, and $\kappa(T - \beta T_C)$ is a function representing the transition at

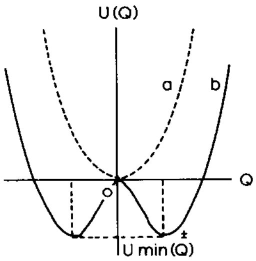

Fig. 5. The bifurcation of a potential $U(Q)$ by the change of the sign of a force constant K_1 in Eqn. 2. (a) $K_1 > 0$, $K_3 > 0$; (b) $K_1 < 0$, $K_3 > 0$.

$T = \beta T_C$. As β increases ($\beta > 1$), the phase transitions can be detected at higher temperatures βT_C, while the thermotropic phase transition is generally observed at $T \ll T_C$. Eqn. 2 suggests that K_1 is related to K_{H_2O} (in preparation).

We assume that in our experiments using Ca^{2+} and Cu^{2+}, β is larger than that of one metal alone. So, the distance between the metal and the binding sites may be shortened, which would stabilize the movements of the headgroups at room temperature. This phenomenon probably caused by the synergetic effect of the S_N-1 mechanism in the case of Ca^{2+} and the Jahn-Teller effect in the case of Cu^{2+}. Also, movements of fatty acid chains are considerably hindered by the synergism of Ca^{2+} and Cu^{2+}, as mentioned before. It is, therefore, expected that the hindrance of the motion at the polar head groups of PS play a role as a trigger to phase transition in phospholipid membranes.

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