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Effect of dehydroepiandrosterone on phosphatidylserine or phosphatidylcholine bilayers: DSC and X-ray diffraction study

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Abstract

The effect of dehydroepiandrosterone (DHEA) on the thermotropic and structural properties of phosphatidylserine or phosphatidylcholine membranes was investigated by differential scanning calorimetry and X-ray diffraction. At molar fractions of sterol, X (sterol), less than ~ 0.2, DHEA interacts with both types of model membranes, depressing the melting temperature and reducing the enthalpy of melting. At higher concentrations, phase separation of DHEA occurs with appearance of crystallites of the S2 monohydrate form.

Keywords: Dehydroepiandrosterone; Phosphatidylserine; Dipalmitoyl phosphatidylcholine; Differential scanning calorimetry; X-ray diffraction

1. Introduction

Dehydroepiandrosterone (DHEA) is an adrenal steroid precursor. Recently it was found to be synthesized in the brain as well, so by definition it is also a neurosteroid [1]. DHEA has diverse beneficial physiological effects including a positive effect on memory performance [1,2]. It is also considered as a possible therapeutic agent in cases of autoimmune disease [3]. Structurally DHEA is similar to cholesterol in that it has the same rigid four ring structure and a hydroxyl group at position C3 with the same stereochemistry. However, it lacks the hydrophobic alkyl side chain at position C17 and in its place there is a keto group, so the molecule is shorter and more hydrophilic. DHEA in the solid state has been studied by differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction [2]. Chang et al. [2] characterized three polymorphic phases and four solvated crystalline forms.

The presence of cholesterol molecules influences the structure of biological membranes and of model bilayer systems formed from zwitterionic or negatively charged phospholipds [4,5]. This perturbing effect has been shown to be modulated by the length and degree of saturation of the acyl chains of the phospholipid [5]; by the charge and hydrogen bonding capacity of the headgroups [5]; and by the stereochemistry of the hydroxyl group at position C3 of the cholesterol molecule [6]. For example, we have found that the miscibility of cholesterol in bilayers of phosphatidylserine (PS), which is negatively charged at neutral pH, is smaller than in zwitterionic phos-

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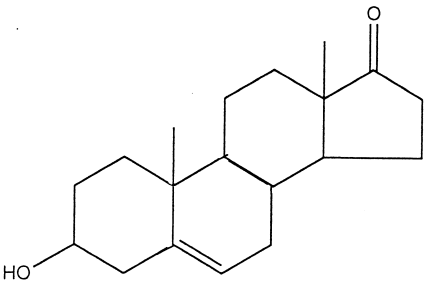
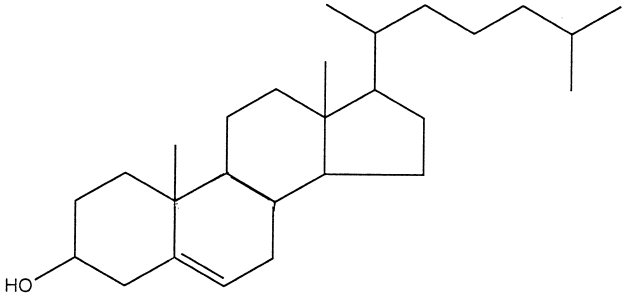
phatidylcholines (PC) [5,7,8]. As DHEA is similar to cholesterol it might also be expected to modify the structure of membranes. By comparing the effects of the two sterols, the influence of the side chain and of the keto group on the nature of the interaction with phospholipid may possibly be evaluated. In this regard, Dicko et al. [9] have recently used infrared spectroscopy to investigate the nature and extent of the interaction of DHEA with membranes of synthetic zwitterionic disaturated dimyristoyl phosphatidylcholine. They have shown that DHEA is less effective than cholesterol in inducing conformational order in the liquid crystalline phase. In the gel state of the phospholipid, DHEA appears to constrain the acyl chains to align closer to the normal to the bilayer surface, but again to a lesser extent than cholesterol.

In the present work we use DSC and X-ray diffraction to characterize the interaction of DHEA with membranes of PS from bovine spinal cord. PS occurs in most biological membranes and especially in neuronal membranes and as such it provides a particularly suitable model for investigation of the effects of DHEA. As it is known that the effect of cholesterol on zwitterionic PCs and anionic PS is completely different [5], this investigation was also carried out on synthetic, zwitterionic dipalmitoyl phosphatidylcholine (DPPC) membranes, for the sake of comparison. The results obtained show that DHEA affects the thermotropic properties of both phospholipids similarly, depressing the melting temperature (T_m) and the enthalpy of melting (ΔH) up to about 4:1 molar ratio phospholipid:DHEA, whereupon phase separation of DHEA takes place, as detected by the appearance of DHEA crystallites.



Dehydroepiandrosterone



Cholesterol

Fig. 1. Schematic formulae of cholesterol and dehydroepiandrosterone.

2. Materials and methods

DPPC and DHEA (>99% pure) were purchased from Sigma (St. Louis, MO, USA). PS from bovine spinal cord (grade 1) was purchased from Lipid Products (South Nuttfield, UK). Cholesterol was from Nu Chek Prep (Elysian, MN, USA). The schematic formulae of cholesterol and of DHEA are presented in Fig. 1. The phospholipids and the sterols were dissolved in chloroform-methanol (2:1 v/v) and mixed at appropriate ratios. The solvents were driven off by a stream of nitrogen and the samples were kept under high vacuum for 3 h.

2.1. Differential scanning calorimetry

The phospholipids or phospholipid-sterol mixtures (1–2 mg) were weighed directly into the aluminum pans of the calorimeter. An excess of solution of 500 mM NaCl in 10 mM Tris-HCl buffer (pH 7.4) was added. The higher salt concentration allowed comparison with the X-ray diffraction experiments where well ordered samples are only obtainable at high ionic strength [10]. The pans were sealed and incubated for 1 h at 50–65°C and the experiments were performed on a Du Pont 990 Thermal Analyzer (Du Pont Instruments, Wilmington, DE, USA) equipped with cell base two, at a scanning rate of

5°C/min. Each sample was scanned three times to verify reproducibility and the enthalpy values are mean values of the three scans.

2.2. X-ray diffraction

The dry phospholipids or phospholipid-sterol mixtures were introduced directly into 1.5 mm diameter quartz capillaries. An excess of 500 mM NaCl in 10 mM Tris-HCl buffer (pH 7.4) was added and the samples were incubated for 1 h at 50–60°C. In some experiments the lipid mixtures were dispersed in the salt solution at a concentration of about 10 mg/ml, incubated, centrifuged in an Eppendorf centrifuge and the precipitate loaded into the capillaries. The experimental protocol used for the low angle X-ray diffraction is described in [6]. Two-dimensional wide angle diffraction patterns were obtained as in [11]. Profiles of intensity vs. scattering angle were generated by averaging the two-dimensional data about the pattern center. All experiments were performed at room temperature.

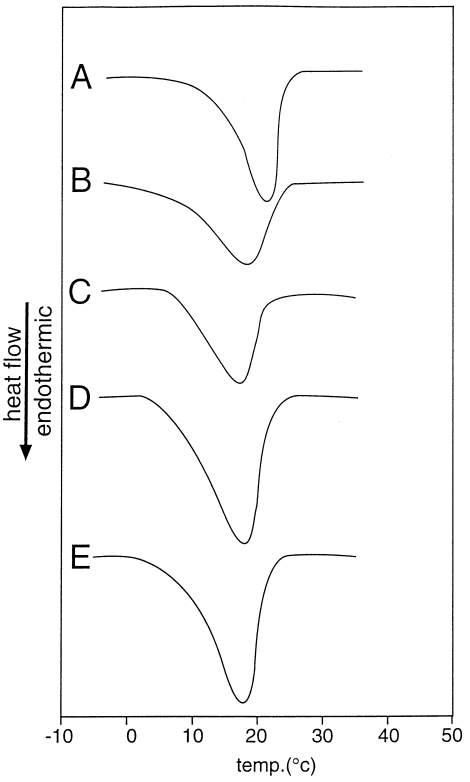


Fig. 2. Thermograms of bovine spinal cord PS in 500 mM NaCl, 10 mM Tris-HCl buffer (pH 7.4) with X(DHEA): A-0; B-0.17; C-0.33; D-0.42; E-0.51. Measurements performed as described in Section 2.

3. Results and discussion

3.1. Interaction of DHEA with PS membranes

In Fig. 2 are presented thermograms of PS alone and in the presence of increasing concentrations of DHEA. It has been shown previously that DHEA alone in solution has no thermotropic transition in the range of temperatures −40 to 85°C [2] and we have verified this finding for 0.5 M NaCl. The endothermic peak seen in the profiles is due to the melting of the acyl chains (gel-liquid crystal transition) of the phospholipid. As PS is a natural product containing acyl chains of different lengths and degrees of unsaturation (70% of the chains are unsaturated), the melting peak is broad. The melting temperature (T_m) of PS alone is 20.5°C. At X(DHEA)≈0.17 (trace B) T_m decreases by about 3°C and almost does not change with further addition of DHEA. By integrating peak areas in thermograms, the enthalpy of melting was calculated. With addition of DHEA, the enthalpy decreases by about 20%, levelling off when X(DHEA) is approximately 0.2, a change which is larger than any experimental error. This may be compared with the ∼85% decrease in enthalpy found for PS with X(chol)=0.5 [7]. Such behaviour indicates that the interaction of PS with DHEA is weak and that phase separation of DHEA occurs. Indeed, in X-ray diffraction patterns of PS/DHEA mixtures, diffraction from DHEA crystallites was detected at X(DHEA)≈0.2 and higher. The positions of the observed reflections (Fig. 3) are consistent with those of S2, the most stable form of DHEA in water [2]. The interbilayer spacing d was measured at room temperature (liquid crystalline phase) for the PS-DHEA mixtures and compared to that of PS-cholesterol mixtures, also above T_m (Fig. 4). The onset of phase separation of cholesterol in PS/cholesterol mixtures occurs at X(chol)=0.3 [8]. Assuming that the thickness of the water layer in these PS membrane systems is controlled osmotically by the high concentration of NaCl, as found previously [11], the increase in the value of d for PS-cholesterol mixtures in the liquid crystalline phase may be attributed

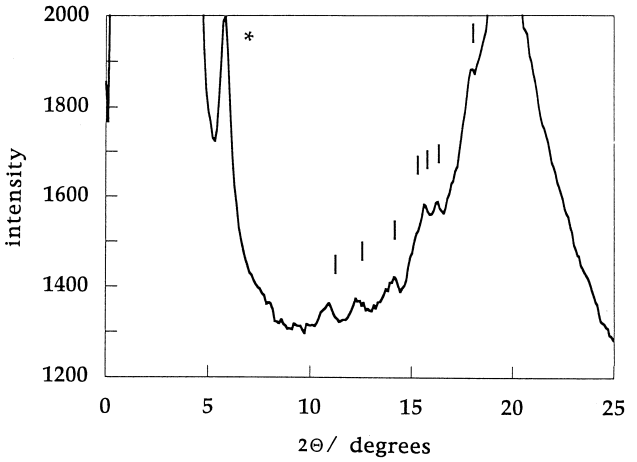


Fig. 3. X-ray diffraction profile of bovine spinal cord PS/DHEA mixture, X(DHEA) = 0.2 in 500 mM NaCl, 10 mM Tris-HCl buffer (pH 7.4) at room temperature. Measurements performed as described in Section 2. Positions of diffraction peaks of the S2 form of DHEA as found in [2] are marked by |. * denotes a lamellar diffraction peak.

to straightening of the PS chains by the interacting cholesterol. In the case of PS-DHEA mixtures, the d spacing initially decreases with added sterol by approx. 2 Å. Such behaviour suggests that, due to the absence of the hydrocarbon chain at C17 and the presence of the keto group, the penetration of DHEA into the phospholipid bilayer is superficial. The interbilayer spacing returns to its sterol-free value after phase separation of DHEA begins.

The idea that DHEA and cholesterol interact (data not shown) independently with PS membranes is supported by experiments with sterol mixtures, which showed that the presence of DHEA does not promote phase separation at molar ratios of cholesterol to PS smaller than those found in the absence of DHEA. Conversely cholesterol does not promote crystallization of DHEA.

3.2. Interaction of DHEA with DPPC membranes

In Fig. 5 are presented the thermograms of DPPC alone and in the presence of increasing concentrations of DHEA. In trace A (DPPC only) two endothermic peaks are seen. The lower one – the pretransition – is attributed to the transition of the L_β phase to the P_β phase and the higher temperature peak is due to the chain melting transition – P_β to L_α. As DPPC is a synthetic disaturated phospholipid the chain melting transition is sharp: the half width at the scan rate used (5°C/min) is 1°C. Upon addition of DHEA several changes are observed. The pretransition disappears: either it vanishes or it is included in the main peak which becomes very broad. T_m as determined by the main peak decreases. The maximal decrease of the cooperativity of melting as judged by the broadening of the peak is seen at X(DHEA) $\approx$ 0.2 where the half width is approximately 7°C. With further addition of DHEA the peak sharpens and at 1:1 molar ratio its half width is about 2.5°C, indicating an increase in cooperativity. At the same time, T_m increases. From the thermograms of Fig. 5 the enthalpy of melting was calculated (not shown). As in the case of PS, the decrease of enthalpy is small, about 25%, and it levels off at X(DHEA) $\approx$ 0.2 indicating phase separation. At higher ratios of DHEA to DPPC, ΔH slightly increases, paralleling the increase of cooperativity and increase of T_m. The cause of this behavior is not clear. We do observe crystals above X(DHEA) $\approx$ 0.2 in the gel state of the lipid, and it is possible that as these large aggregates of DHEA are formed, the interaction of the sterol with the tightly packed DPPC decreases, In the case of interaction with PS this effect is not seen (Fig. 2) perhaps because the presence of double bonds would render the gel state of PS more expanded. A second possibility is that a complex of DPPC-DHEA is formed, analogous to what was sug-

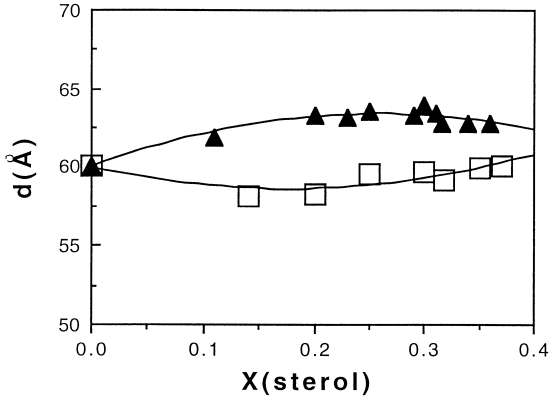


Fig. 4. Interbilayer spacing d for bovine spinal cord PS/DHEA and PS/cholesterol mixtures (this work and [11]) as a function of molar fraction of sterol at room temperature. ▲, PS/cholesterol; □, PS/DHEA. Measurement uncertainty is ±1 Å, due to detector channel width.

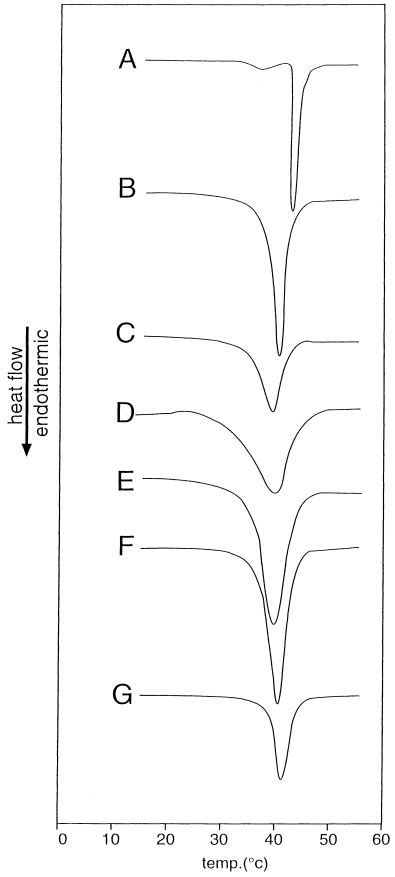


Fig. 5. Thermograms of DPPC in 500 mM NaCl, 10 mM Tris-HCl buffer in the presence and absence of DHEA with *X*(DHEA): A-0, B-0.05, C-0.097, D-0.19, E-0.33, F-0.42, G-0.53. Measurements performed as described in Section 2.

gested for epicholesterol-dielaidoylphosphatidylethanolamine mixtures [6].

McMullen et al. have performed a comparative investigation of the interaction of cholesterol and androstenol with DPPC membranes [12]. Androstenol has almost the same structure as cholesterol but it lacks the hydrocarbon chain at position C17. Using DSC, they found that androstenol has a smaller effect than cholesterol on the cooperativity and enthalpy of melting of DPPC. At a 1:1 molar ratio of cholesterol to DPPC the enthalpy of melting vanishes whereas in the case of androstenol even at this ratio a small melting peak is seen. They found that addition of androstenol at a molar fraction of 0.45 reduces the enthalpy of melting by 80%. Based on DSC, FTIR and ^{31}P-NMR studies McMullen et al. [12] concluded that in DPPC bilayers, both in the gel and in the liquid crystalline states, the solubility of androstenol is small. If we compare their results with those presented above for DHEA, we see that DHEA has an even weaker effect on the thermotropic properties of DPPC than androstenol. We may attribute this to the presence of the polar keto group at position 17, which reduces still further the solubility of the sterol in the membrane.

DHEA is soluble in model membranes of bovine spinal cord PS or DPPC until a molar fraction of 0.2, at which point phase separation is detected by DSC and X-ray diffraction. DHEA has a disordering effect on the acyl chains of gel phase PS and DPPC which is weaker than that caused by cholesterol. In liquid crystalline PS, DHEA initially disorders the acyl chains, opposite to what is observed for cholesterol. These results are consistent with the more hydrophilic character of DHEA. Although cholesterol is clearly more soluble in DPPC than in PS membranes, no such difference is detected in the case of DHEA.

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