EFFECT OF STEROLS ON DIPHENYLHEXATRIENE FLUORESCENCE IN LECITHIN VESICLES

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1. Introduction

Cholesterol is a major component of many mammalian membranes and in some membranes it is present at a molar phospholipid to sterol ratio of 1 : 1 [1]. Although its exact function is not understood, many studies have indicated that it can affect the packing of paraffin chains of lipid in model and biological membranes [2]. Its effect, however, depends on the physical state of phospholipids [2]; when lipids are in the liquid-crystalline state cholesterol decreases the chain mobility [3] but when in the gel state, cholesterol increases the chain mobility [4]. In order to establish the molecular features of cholesterol that are necessary for interaction with lipids, the effect of cholesterol analogs lacking the 3β-hydroxyl group have been evaluated [1,5,6]. Butler et al. [5] studying multilayers prepared from brain phospholipids and using ESR reported that cholesterol interacted with lipids to promote order and that 5-α-cholestan-3-one caused a low degree of order, but that 5-α-cholestane caused no ordering of phospholipids. In monolayers of 1-oleoyl-2-stearoylphosphatidylcholine 5-α-cholestan-3-one increased the rigidity of the alkyl chain more at 22°C than at 37°C [1]. Using high-resolution proton-NMR McDonald et al. [6] reported that 5-α-cholestane and 5-α-cholestan-3-one were as effective as cholesterol in reducing spin-lattice relaxation times of protons in the paraffinic groups of the phospholipid systems studied. These apparent discrepancies prompted us to undertake the present studies in which we have found that interaction of these sterols with lecithin depends on the physical state and structure of the paraffinic portion of the phospholipid.

2. Materials and methods

Egg phosphatidylcholine (EPC), Type III-E, L-α-dipalmitoylphosphatidylcholine (DPPC), cholesterol, 5-α-cholestane, 5-α-cholestan-3-one and 1,6-diphenyl-1,3,5-hexatriene (DPH) were purchased form Sigma Chemical Company. The sterols were recrystallized three times before use. Phospholipids were shown to be pure by thin-layer chromatography [1]. Vesicles were prepared by sonication according to Andrich and Vanderkooi [7] except that sonication time was increased to 5 min. Vesicles were prepared in 50 mM Na phosphate buffer, pH 7.2. Concentration of phospholipid was 1 mM, sterol 1 mM and DPH 0.001 mM. Steady state fluorescence polarization was determined at different temperatures according to Andrich and Vanderkooi [7]. Each polarization value was repeated 3 times.

3. Results and discussion

Polarization of DPH fluorescence in membranes is a function of fluidity and degree of order in lipid chains [7]. Polarization of DPH fluorescence in vesicles of DPPC, or DPPC plus cholesterol, 5-α-cholestane, or 5-α-cholestan-3-one over the temperature range of 15 to 50°C is shown in fig.1. DPH demonstrates a phase transition from gel to liquid-crystalline in DPPC vesicles [7]. Below 35°C polarization values of ~0.35 ± 0.1 were obtained indicating that the lipid is in the crystalline state. As the temperature was raised, there was a sharp decrease in polarization so that at 45°C the value was 0.12 indicating nearly complete depolarization of the fluorescence due to

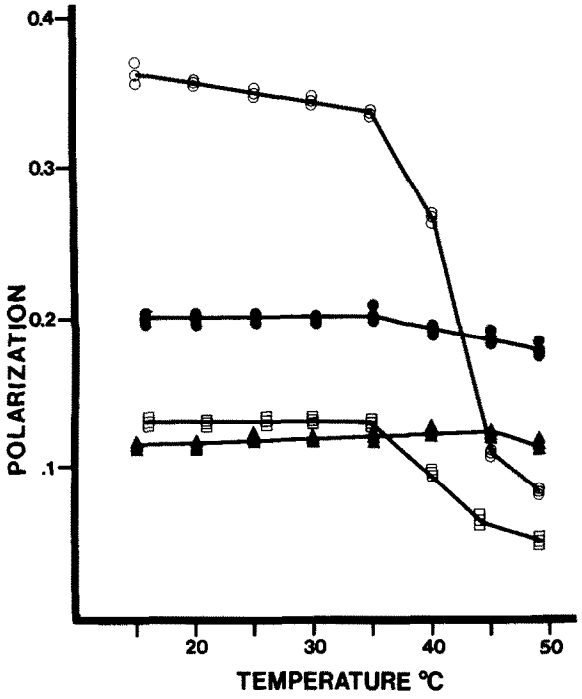

Fig.1. Effect of sterols on DPH fluorescence polarization in vesicles of DPPC at different temperatures. ○, DPPC only; ●, DPPC plus cholesterol; □, DPPC plus 5-α-cholestane; ▲, DPPC plus 5-α-cholestan-3-one. Sterol to DPPC molar ratio was 1 : 1.

acyl chains being in a fluid or disordered state [7]. This transition which takes place over a temperature range of 35 to 45°C is similar to that detected by differential scanning calorimetry for vesicles of DPPC prepared by sonification [8]. In vesicles containing cholesterol or 5-α-cholestan-3-one no phase transitions were detected. Furthermore, while in vesicles containing polarization values ranged between 0.19 to 0.2 over the temperature range of 15 to 50°C, the corresponding value for DPPC vesicles containing 5-α-cholestan-3-one was 0.12 indicating that this sterol is even more effective than cholesterol in inducing disorder in paraffinic chains of DPPC and allowing a greater freedom of rotation of the probe.

Polarization values of DPPC vesicles containing 5-α-cholestane below 35°C were close to those of vesicles containing cholestan-3-one. Above this temperature there was a further decrease in polarization so that at 45°C the value was 0.05. It is clear that 5-α-cholestane also interacts with DPPC paraffinic chains to create disorder permitting a greater degree of freedom of rotation of DPH leading to the observed fluorescence polarization. Since DPH molecules are known to be located deep in the lipid-bilayer [7], then cholestane and cholestan-3-one must at least interact with DPPC in the inner core of the bilayer. Thus the 3β-hydroxyl group is not required for such an interaction.

We then considered the possibility that interaction of the sterols with lecithin may depend on the structure of the paraffinic chain of the phospholipid. Therefore, effects of the sterols on polarization of DPH in vesicles of egg lecithin (EPC) were also determined over the temperature range of 15–50°C (fig.2). The results were different from those obtained with DPPC. While cholestan-3-one had no effect on the polarization of the phospholipid which ranged from 0.11 to 0.15 over the temperature range studied, the corresponding values for vesicles containing cholesterol was 0.21 to 0.23 and for those containing cholestane was 0.05 to 0.07. Therefore, cholesterol induces order in the acyl chains of EPC, cholestan-3-one has no effect and cholestane creates further disorder leading to the observed depolarization of the fluorescence. EPC is in the liquid-crystalline state at a temperature above 0°C [8]. Its fatty acid composition consists of 50% palmitate plus stearate with the rest consisting of oleate and polyunsaturated fatty acids [9]. This difference in acyl chain composition

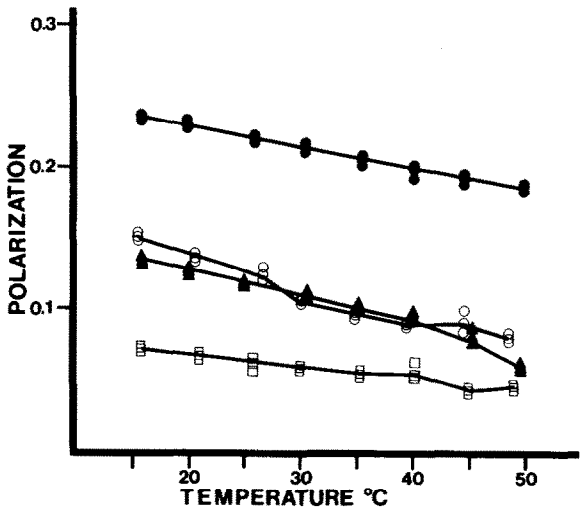

Fig.2. Effect of sterols on DPH fluorescence polarization in vesicles of EPC at different temperatures. ○, EPC only; ●, EPC plus cholesterol; □, EPC plus 5-α-cholestane; ▲, EPC plus 5-α-cholestan-3-one. Sterol to EPC molar ratio was 1 : 1.

then accounts for a pattern of interaction with sterols different from that observed with DPPC.

Two types of interactions have been speculated between cholesterol and lecithin, van der Waals' forces between the sterol nucleus and acyl chains and hydrogen bonding between the 3β-hydroxyl group and oxygen anchored to phosphorus of lecithin [1]. Cholestane cannot have hydrogen-bonding with lecithin phosphorus. Therefore, it must be deeply buried in the bilayer where it increases disorder and fluidity irrespective of the nature of the acyl chain. Cholestan-3-one cannot hydrogen-bond with phosphorus of lecithin either but because of the polar nature of 3-keto group, it cannot be as deeply buried in the lipid-bilayer as cholestane. Its interaction with lecithin is then governed by conformation of the acyl chains. Where the chains are rigid as in DPPC it produces disorder but where they are already in a disordered state as in EPC, it has no effect.

From these studies, we conclude that the requirement for 3β-hydroxyl group of the sterol for interaction with lecithin and pattern of interaction depend on the structure of the fatty acyl chains and physical state of the phospholipid.

Acknowledgements

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