EFFECT OF *n*-PROPYL GALLATE ON THERMOTROPIC BEHAVIOUR OF LIPID MIXTURES WITH DIFFERENT DEGREE OF UNSATURATION IN PHOSPHATIDYLCHOLINE

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ABSTRACT

Phosphatidylcholine/sphingomyelin/cholesterol (PC/SM/CHOL) ternary mixtures were investigated using differential scanning calorimetry (DSC). Palmitoyl-oleoyl phosphatidylcholine (POPC) and palmitoyl-docosahexaenoyl (ω-3) phosphatidylcholine (PDPC) were used to study the effect of the number of double bonds at the sn-2 position in PC molecule on the phase behavior of PC/SM/CHOL mixtures. The PDPC-containing mixture showed a lower cooperativity of the transition than the POPC mixture. Moreover, in the PDPC/SM/CHOL mixture more pronounced phase separation between the two co-existing phases was detected. The antioxidant n-propyl-gallate (nPG) exhibited a fluidizing effect on both types of mixtures. This thermotropic effect might be implicated in its mechanism of antioxidant protection.

Keywords: *differential scanning calorimetry (DSC), docosahexaenoic acid (DHA), n-propyl gallate (nPG), polyunsaturated fatty acids (PUFA)*

INTRODUCTION

Polyunsaturated fatty acids (PUFAs) are a key target of many current investigations because of their essential role in brain function [1]. The most abundant PUFA of membrane phospholipids in the central nervous system (CNS) is docosahexaenoic acid (DHA) [2], belonging to the family of ω-3 fatty acids. DHA is known for its important role in the prevention of neurodegenerative diseases like Alzheimer's disease [3] and dementia [4]. It is also related to the process of aging [5]. Omega-3 fatty acids are also reported to reduce cardiovascular mortality [6] and prevent atherosclerosis [7]. Besides, they influence some behavioral manifestations and neuropsychiatric disorders such as stress, depression, suicide [5]. So the importance of ω-3 fatty acids for human health may have therapeutic value in a number of medical conditions.

It is proposed that PUFAs exert their effects by modulating the organization of biological membranes. Thus they may alter the composition and functionality of lipid micro-domains known in literature as lipid rafts [8, 9]. This hypothesis could explain their involvement in cell signaling processes [10, 11]. According to literature data however phospholipids containing fatty acids with more than two double bonds are more prone to oxidation [12]. Therefore their phase behavior in model systems should be studied in the presence of an antioxidant species. In the current experiments we used the free radical scavenger *n*-propyl gallate (*n*- propyl 3, 4, 5-trihydroxybenzoat) which is a synthetic derivative of the naturally occurring antioxidant gallic acid, a natural component of many plant cells [13, 14].The use of *n*-propyl gallate could attenuate possible artifacts due to lipid peroxidation [15, 16].

MATERIALS AND METHODS

Materials

L-α-phosphatidylcholine-β-palmitoyl-γ-oleoyl, L-α-phosphatidylcholine-β-palmitoyl-γ docosahexaenoyl and egg-yolk sphingomyelin were obtained from Avanti Polar Lipids, Alabaster, AL and used without further purification. The distribution of fatty acids in egg sphingomyelin is 84% C16:0, 6% C18:0, 2% C20:0, 4% C22:0 and 4% C24:0. Cholesterol was from Sigma-Aldrich.

The buffer of 0.5 mM Hepes, pH 7.4 (conductance σ = 59 μS/cm) and the antioxidant *n*-propyl gallate (propyl 3, 4, 5-trihydroxybenzoat) were also purchased from Sigma.

Differential Scanning Calorimetry

Multilamellar lipid vesicles were prepared by dispersing lipids in required amounts of buffer solutions, 0.5mM Hepes, pH 7.4. The lipid concentrations were 3 mg/ml. In some samples n-propyl gallate was added to the buffer phase in varying concentrations, 0.5-5mM. Calorimetric measurements were performed using high-sensitivity differential adiabatic scanning micro calorimeter DASM-4 (Biopribor, Pushchino, Russia) with sensitivity > 4.10^{-6} cal/K and a noise level < 5.10^{-7}W. Egg SM samples were loaded into the calorimetric cell at 20°C, and then heating were started with a rate of 0.1°C/min. The binary and ternary lipid mixtures were loaded into the calorimetric cell at 20°C, then cooled to 12°C and reheated to 60°C. The cooling step, together with the equilibration of the instrument for the heating run, takes 30 min.

The thermograms were corrected for the instrumental baseline. The calorimetric enthalpy (ΔH) of the transition was determined as the area under the excess heat capacity curve.

RESULTS AND DISCUSSION

In the two ternary lipid mixtures of interest, POPC/SM/CHOL 85/10/5 and PDPC/SM/CHOL 85/10/5, egg sphingomyelin has the highest melting temperature (Tm), undergoing highly cooperative transition with enthalpy equal to 7.9 kcal / mol and heat capacity (C_p) 4.1 kcal / mol.°C, while the Tm of used phosphatidylcholines is known to be below 0°C. The temperature of the main transition of pure SM suspended in Hepes buffer was 39.7°C. The presence of *n*PG in the aqueous phase of the egg SM suspension led to a gradual shift of the phase transition temperature to lower values, broadening of the transition peak (reduced cooperativity of the phase transition), reduced heat capacity and enthalpy. 1 mM *n*PG decreased the transition temperature to 37.7°C. Further increasing *n*PG concentration to 2, 3 and 4 mM lowered the temperature of the phase transition as follows: 31.6°C, 28.3°C and 25.5°C.

5 mM *n*PG inhibited the phase transition and no transition peak was traced (Fig.1, Panel A).

The addition of 10 mol % cholesterol reduced the cooperativity and shifted the main transition of SM to lower temperatures (37.9°C) compared to pure lipid suspension. A decrease in ΔH of the process was detected (ΔH= 1.3 kcal / mol) as well as a decrease in heat capacity (C_p = 0.3 kcal / mol.°C). Two phases were observed - a CHOL-deficient phase I, corresponding to the first transition peak, and phase II enriched in CHOL, corresponding to the second peak. Addition of 1 mM *n*PG to the SM/CHOL 90/10 mixture further reduced Tm to 33.3°C, ΔH to 0.6 kcal / mol and C_p to 0.2 kcal / mol.°C (Fig.1, Panel B), Thus, the fluidizing effect of the antioxidant was more pronounced in the presence of cholesterol than in pure egg SM (Fig.1, Panels A and B).

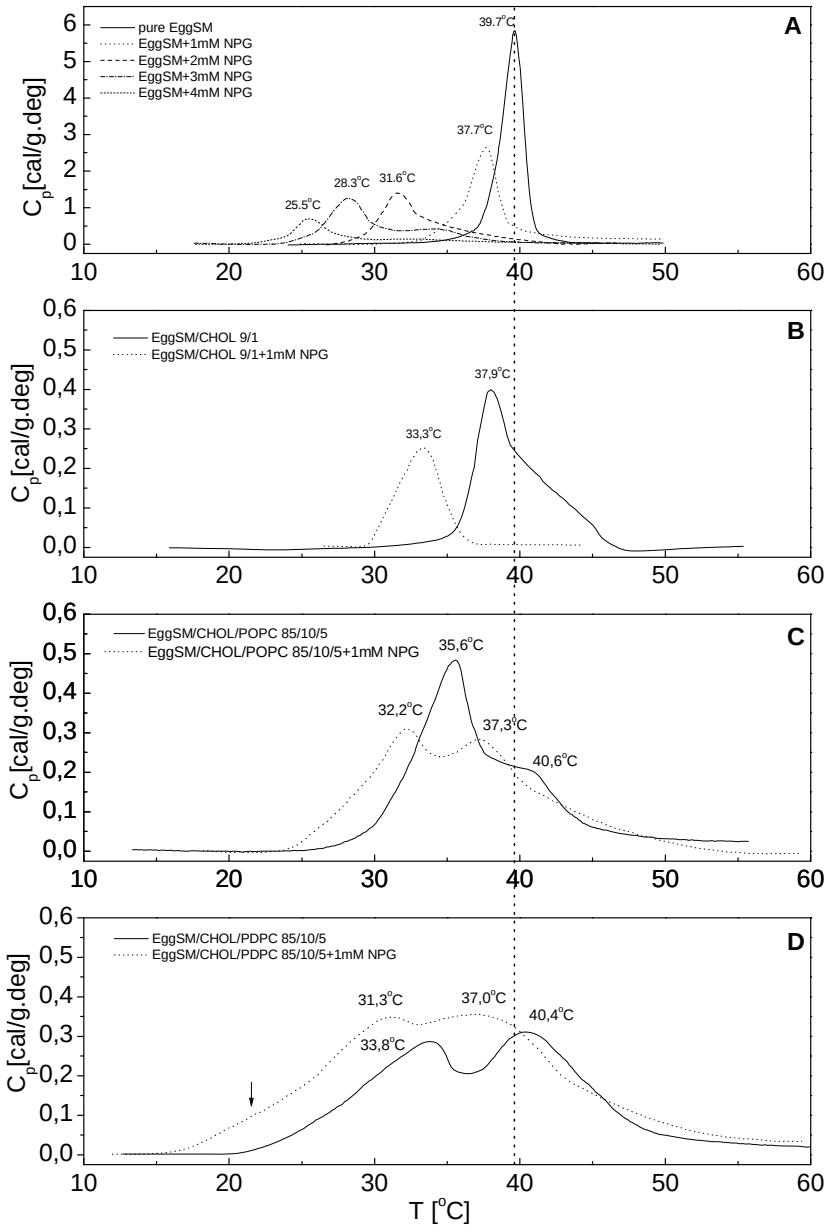

Figure 1. DSC output curves, illustrating the main transition of pure egg SM in the absence as well as in the presence of different concentrations (1, 2, 4, 5 mM) of the radical scavenger *n*PG (Panel A), the phase transition peaks of SM/CHOL 90/10 (Panel B), POPC/SM/CHOL 85/10/5 (Panel C) and PDPC/SM/CHOL 85/10/5 (Panel D) without *n*PG and on addition of 1mM *n*PG. Scanning rate was 0.1°C/min in the temperature range 12-55°C. Dot lines correspond to mixtures with *n*PG but straight lines correspond to control conditions.

Both types of ternary mixtures displayed the coexistence of two different phases. The thermotropic phase transition for POPC/SM/CHOL mixture started from 25°C and ended at about 45°C, leading to a clear separation of two peaks – one at 35.6°C and the other, corresponding to the more ordered phase, at 40.6°C. The presence of POPC in the ternary mixture led to a slight shift of Tm for the two co-existing phases compared to the binary mixture SM/CHOL 90/10. Tm for phase I was 34.9°C, while for phase II it was 41.2°C. ΔH of the transition was 1.6 kcal / mol for phase I and 0.6 kcal / mol for phase II. The transition of phase I had C_p equal to 0.2 kcal / mol.°C , phase II- 0.1

kcal / mol.°C (Fig1. Panel C). The effect of PDPC was a reduction in ΔH of phase I (1.5 kcal / mol) and an increase in ΔH of phase II (1.7 kcal / mol) compared with the control mixture SM/CHOL 90/10. Cp was 0.2 kcal / mol.°C for the transitions of both phases (Fig.1, Panel D). This mixture formed a phase (II) characterizing with higher melting temperature and enthalpy compared with POPC one containing only one double bond in the fatty acid at *sn*-2 position (Fig1. Panel C and D). The width of the transition peak for the PDPC mixture was about 2.9 times greater than that of the POPC-containing one. This means a greater difference in physicochemical properties between the two coexisting phases when PDPC is a component of the mixture. This fact suggests that the presence of a fatty acid with six double bonds in raft mixtures reduces the miscibility of SM and CHOL. The fluidizing effect of *n*PG on both ternary mixtures was detected as it is demonstrated in Fig. 1 (Panel C and D). This effect is more pronounced for PDPC mixtures compared to POPC.

CONCLUSIONS

The present experimental results show that omega-3 fatty acids promote the formation of more ordered phase at physiological temperature. DSC scans clearly demonstrate the increasing heterogeneity of the membrane bilayer in the presence of an omega-3 fatty acid. This suggests that omega-3 fatty acids could participate indirectly in the process of raft domain formation by creating a phase with a higher Tm and ΔH than the one formed in the presence of monounsaturated phosphatidylcholines. We showed that the antioxidant *n*PG exhibited strong fluidizing effect on pure egg sphingomyelin and cholesterol containing mixtures. Moreover, we present a more interesting aspect of its role in lipid systems not only as a free radical scavenger but also as a modulator of lipid phase behavior. This finding could be considered as one of the first steps in clarifying the mechanism of NPG antioxidant action.

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