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The interaction of radical scavenging compounds with biomembranes studied by ^{31}P nuclear magnetic resonance

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Abstract. It is well known that carotenoids and their derivatives (xanthophylls) are present in human blood serum. However, the location of these compounds in the biomembrane and their interactions with the phospholipid bilayer are still a subject of debate. In the present study, we have examined the interactions of these compounds with phosphatidylcholine-small unilamellar vesicle (PC-SUV) liposomes, using ^{31}P-nuclear magnetic resonance (^{31}P-NMR).

Keywords: Carotenoid; Liposome; Radical scavenger; Xanthophyll; ^{31}P-NMR

1. Introduction

In addition to quenching singlet oxygen [1], it has been reported that carotenoids are also able to trap free radicals [2,3]. However, recent clinical studies show that β-carotene supplements are not effective in free radical-related disease prevention. Liebler et al. [4] also reported that β-carotene is ineffective as an antioxidant on microsomal lipid peroxidation.

On the other hand, carotenoid derivatives having some oxygen groups (xanthophylls) such as astaxanthin and canthaxanthin are extremely effective in rat microsomal lipid peroxidation by radical initiation [5].

In the present study, we investigated the effect of carotenoids/xanthophylls on the fluidity and permeability of model membranes, using ^{31}P-NMR.

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2. Materials and methods

2.1. Materials

L-α-Phosphatidylcholine (PC), DL-α-tocopherol, β-carotene, canthaxanthin, ubiquinone-10, and praseodymium nitrate were purchased from Wako Pure Chemical Industries (Osaka, Japan). Astaxanthin was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO). All other reagents were of analytical grade and obtained from Wako or Sigma-Aldrich.

2.2. Preparation of liposome

Multilamellar vesicles (MLV) were prepared by the following procedure. Chloroform solutions of PC (0.2 mmol) were evaporated *in vacuo*, and the residual solvent was removed by overnight drying under high vacuum. MLV dispersions (0.1 M) were prepared by vortexing the dry lipid with 2 mL of 0.15 M KCl/50 mM Tris–HCl buffer (pH 7.4).

Small unilamellar vesicles (SUV) were obtained by sonication of the MLV dispersions in a cooled bath-type sonicator until translucence (30 min–1 h). The SUV solutions were then centrifuged and passed through a Millipore filter (0.2 μm) to remove large liposomes. All operations were carried out under argon.

Mixed SUV solutions were prepared in a same manner by first co-dissolving both compounds in $CHCl_3$. The lipid-to-compound molar ratio used was 5:1.

2.3. ^{31}P-NMR studies

^{31}P-NMR spectra of SUVs in Tris–HCl buffer were obtained with a JEOL JNM A-500 (500 MHz) spectrometer operating at 202.35 MHz in the pulse Fourier transform mode. Chemical shifts (δ) were referenced to external 85% H_3PO_4 at 0 ppm. In all these experiments a pulse width of 10 μs and pulse delay of 1.0 s were used at a temperature of 27 °C. Initial spectra were taken and 50 μL of 0.1 M $Pr(NO_3)_3 \cdot 5H_2O$ in the preceding buffer solution was then added and subsequent spectra taken. The rate of infusion of Pr^{3+} across the SUVs was proportional to the rate of disappearance of the high field ^{31}P-NMR signal [6–8]. Using 0.6 mL of vesicle solution gave a Pr^{3+} concentration of 7.7 mM.

3. Results and discussion

Generally, pure lipid membranes are not able to transport ions from the outer to the inner water phase and *vice versa*. The ^{31}P-NMR technique used in this study, therefore, provided useful information on the nature of interaction of carotenoids/xanthophylls with PC-SUV model membrane systems. We have used praseodymium nitrate as a lanthanide [10], and kinetic analysis of paramagnetic Pr^{3+} infusion into PC-SUV bilayers has been performed using ^{31}P-NMR. The rate of infusion of Pr^{3+} across the bilayer was proportional to the rate of disappearance of the high field ^{31}P-NMR signal.

Fig. 1 shows the reduced rates of intermembrane phosphorus ^{31}P-NMR signals relative to their initial values *vs*. time. The kinetic data from Fig. 1 were quantified in Table 1. Either α-tocopherol or β-carotene incorporation into PC-SUVs extremely increased the permeability