Effect of cholesterol into liposome on the stabilization of incorporated retinol

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To investigate the effect of cholesterol in liposome on the stability of incorporated retinol, the physico-chemical experiments for various amounts of cholesterol-containing liposomes were performed. Liposome with retinol containing cholesterol was prepared as multilamella vesicles(MLVs) by dehydration/rehydration method. The incorporation efficiency of retinol into liposome was maximized as 99.31 % at 50:50 (phosphatidylcholine/cholesterol) at pH 9. The stability of incorporated retinol at low storage temperature was enhanced with increasing cholesterol content than at high storage temperature. For example, incorporated retinol in liposome at glycine buffer(pH 9) was degraded slowly during storage at 4. The degradation of retinol in liposomes was slower at pH 9 than at pH 7. These results supported that cholesterol in liposome increased largely the stability of incorporated retinol.

Keywords : liposome, retinol, cholesterol, incorporation efficiency, stability

1. Introduction

Vitamin A is crucial for morphogenesis, vision, immune function, reproduction, neuronal and neural development, and maintenance of differentiation functions[1]. Vitamin A is an essential micronutrient that is found naturally in animal foods as preformed retinol and in plant foods as vitamin A precursors, e.g., -carotene. Both preformed retinol and provitamin A are fat soluble and sensitive to air, oxidizing agents, ultraviolet light, and low pH values[2,3]. As with all kinetic reactions, preformed retinol and provitamin A loss are catalyzed by increasing temperature and the presence of mineral ions.

Retinol is the most common and most biologically active form of preformed vitamin A is reported to be the predominant form of vitamin A in the liver and flesh of freshwater fish[4]. Retinol is currently the vitamin A ingredient of choice in cosmetics. When stabilized in formula, by protecting it from breakdown from oxygen and light, it is a clinically useful addition to skin care products. In one study, application of retinol to human skin in vivo resulted in increased epidermal thickness as well as increased mRNA levels of cellular retinoic acid-binding proteins and cellular retinol-binding proteins[5].

Cholesterol is the major sterol component in most mammalian membranes. It is nonhomogeneously distributed among different organelles. The cholesterol content in the eucaryotic plasma membrane is usually rather high(e.g., larger than 20 wt% in plasma membranes), whereas it is much less in internal membranes[6]. Cholesterol acts as a precursor of the active form of vitamin D[7]. Moreover, cholesterol can participate in cellular signaling both directly by modulating the physical properties of the plasma membrane thereby affecting the activity of receptors and enzymes residing in it,

or directly as a regulator of enzymes in the cholesterol metabolic pathways[7,8,9]. Properties of lecithin-cholesterol model membranes were recently reviewed[10]. It appears that in the case of 1,2-dimyristoyl-sn-glycero-3-phosphatidylcholine(DMPC) below the gel-to-liquid transition temperature, the molecular organization of the mixture with cholesterol was strongly dependent on the sterol content[11]. Also, at low lipid concentrations, both in vivo and in vitro, small unilamellar vesicles composed only of egg lecithin(no cholesterol) can rapidly disintegrate in plasma, the lipid components being redistributed among the lipoproteins[12]. Incorporation of cholesterol into the egg lecithin membrane partially inhibits disintegration, and sphingomyelin SUVs containing 30 mol% cholesterol or more remain intact with complete retention of aqueous contents[13].

Liposomes are single or multi-layered vesicles that completely enclose an aqueous phase within a phospholipid bilayer membrane. An important aspect of liposomes is the protection that they afford as an encapsulating agent against potentially damaging conditions in the external environment[14]. In previous study, we investigated the effect of liposome incorporation on the kinetics of retinol degradation in aqueous buffers[15]. It appears that the protective effect of incorporation of retinol into liposomes was greater at lower temperatures, at neutral ,high pH, and in the dark. In this presentation, the effects of cholesterol into liposomes on the stability of incorporated retinol under a variety of conditions of pH(7 and 9) and temperature(4,25,37,and 50) were determined.

2. Experimental

2.1. Materials

All *trans* retinol, L-α-phosphatidylcholine isolated from soybeans and cholesterol were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals and reagents were of analytical grade.

2.2. Instruments

The UV-visible spectra of solutions were determined using a UV –VIS spectrophotometer (UV 1601, Shimadzu, Japan). Mean size of the liposome in the aqueous dispersions was examined with particle size analyzer (LS230 Small Volume Module, Coulter Co., USA). Liposome pellet was collected by centrifugation to examine the effect on the incorporation efficiency with Hitachi preparative ultracentrifuge (SCP 55H, Hitachi Koki Co. Ltd., Japan).

2.3. Analytical method

Retinol in liposomes was analyzed using a colorimetric assay [16]. A 0.15 mL of liposome solution containing retinol was mixed with 0.45 mL of a chloroform/methanol solvent mixture (2:1, v/v). The mixture was centrifuged for 3 min at 5,000 rpm. A 0.1 mL aliquot of the organic solvent layer was then transferred to the test tube and 1 mL of a 20 % $SbCl_3$ solution was added and the absorbance at 620 nm was measured immediately.

2.4. Preparation of liposomes containing retinol

The method was based on the dehydration-rehydration procedure of Kirby and Gregoriadis[17, 18] with slight modification. Retinol was added to phospholipid at a ratio

of 0.01:1 (wt:wt). Cholesterol was added proportionally that the ratio of soybean phosphatidylchline at wt:wt is 100:0, 90:10, 80:20, 70:30, 60:40, and 50:50, respectively.

2.5. Stability of incorporated retinol in liposomes during storage

Liposome suspension of incorporated retinol were placed in glass vials and saturated with oxygen by equilibrating against the atmosphere for 2h in the dark. Vials for storage in the dark were wrapped in aluminum foil.

3. Results and Discussion

3.1. Incorporation efficiency of retinol and particle sizes into liposomes

The incorporation efficiency of retinol into liposomes showed a trend to increase with added cholesterol content at pH 7 and pH 9 (Table Ⅰ). At pH 7, incorporation efficiency of retinol was maximized as 98.51 % at 50:50 (phosphatidylcholine/cholesterol, wt/wt), whereas incorporation efficiency of retinol was maximized as 99.31 % at 50:50 (phosphatidylcholine/cholesterol, wt/wt) at pH 9. Mean size of liposome was larger increasingly with increasing cholesterol content(Table Ⅱ). This results agreed that size of liposome was increased with increasing cholesterol content[21].

3.2. Stability of incorporated retinol into liposome

The time dependent degradation of incorporated retinol into liposome in the aqueous solution was compared with a variety of conditions of pH, temperature for ten days. The results are plotted as % of the remaining retinol versus time. Figure 1 shows the stability of retinol in 10 mM phosphate buffer (pH 7). The stability of incorporated retinol at low storage temperature was enhanced with increasing cholesterol content

than at high storage temperature. Retinol in liposomes degraded very slowly during storage at 4 over 90% remaining after ten days at 50:50 (phosphatidylcholine/cholesterol, wt/wt). While the degradation of retinol in liposomes was retarded at pH 9 compared to at pH 7 (Fig. 2). Retinol degradation in liposomes were also quite similar at pH 7 with nearly 90% of the retinol remaining after ten days of storage at 4 at 50:50 (phosphatidylcholine/cholesterol, wt/wt). Also, we observed that the stability of incorporated retinol at low storage temperature was enhanced with increasing cholesterol content than at high storage temperature. In general, the protective effect of the liposome incorporation was well shown at low temperatures, at pH 9, and at a ratio of 50:50 (phosphatidylcholine/cholesterol, wt/wt). These results indicate that cholesterol in liposome affect largely to the stability of incorporated retinol.

4. Conclusion

Retinol was efficiently incorporated into liposome at pH values that ranged from 7 to 9. Incorporation efficiency of retinol into liposome were increased with increasing cholesterol content. Also, the stability of incorporated retinol was greater at 50:50(wt:wt) phospholipid:cholesterol. The protective effect was greatest at low temperature storage than high temperature storage, and at pH 9. Furthermore, the stability of incorporated retinol into liposome containing cholesterol was increased with increasing cholesterol content. It considered that phospholipid containing cholesterol was partially inhibits disintegration of lipid bilayer and releasing of incorporated retinol was reduced from membrane inside to aqueous media. These data means that liposome incorporation

containing cholesterol is an effective method to improve the shelf-life of foods and to elevate stability of cosmetic materials and drug delivery systems.

5. References

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Table Ⅰ. Incorporation of retinol into liposomes depends on cholesterol and pH.

Ratio[a]	Incorporation efficiency (%)[b]	
	pH 7	pH 9
100 : 0	95.46 0.27	97.50 0.22
90 : 10	95.60 0.24	97.87 0.35
80 : 20	96.74 0.23	98.23 0.33
70 : 30	97.51 0.39	98.55 0.27
60 : 40	97.85 0.27	98.96 0.20
50 : 50	98.51 0.01	99.31 0.15

[a] Weight ratio of phosphatidylcholine to cholesterol.

[b] Mean ± standard deviation of triplicate measurements.

Table Ⅱ. Effect of cholesterol content in liposome on the size of retionol incorporated liposomes.

Ratio[a]	Mean size of liposome(nm)	
	pH 7	pH 9
100 : 0	4.54	8.33
90 : 10	4.49	10.78
80 : 20	8.34	11.38
70 : 30	41.14	11.63
60 : 40	63.66	25.92
50 : 50	89.53	41.42

[a] Weight ratio of phosphatidylcholine to cholesterol.

Fig. 1. The stability of retinol in liposome depend on temperature and cholesterol content in liposome. Liposomes were stored in 10 mM phosphate buffer (pH 7.0) at (A) 4 , (B) 25 , (C) 37 , and 50 . Liposomes were composed of phosphatidylcholine and cholesterol with ratio (wt:wt) of 100:0 (○), 90:10(●), 80:20(△), 70:30(▲), 60:40(□), and 50:50(■), respectively.

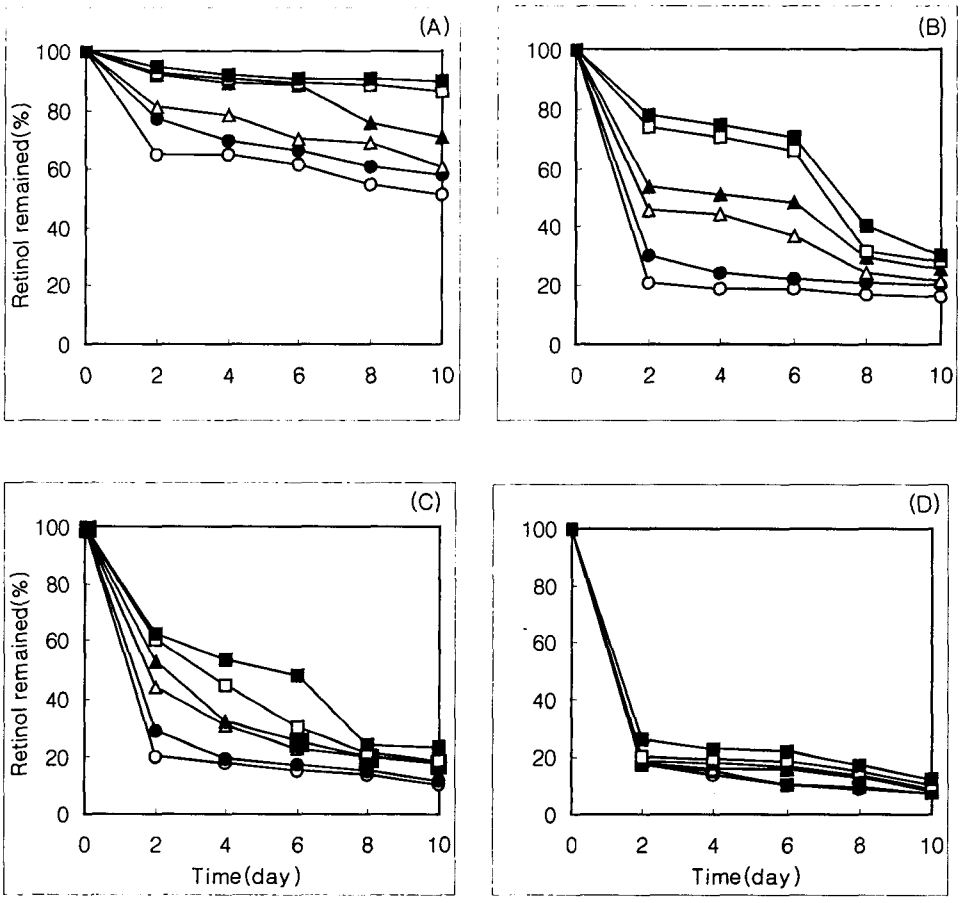

Fig.2. The stability of retinol in liposome depend on temperature and cholesterol content in liposome. Liposomes were stored in 10 mM glycine buffer (pH 9.0) at (A) 4 , (B) 25 , (C) 37 , and 50 . Liposomes were composed of phosphatidylcholine and cholesterol with ratio (wt:wt) of 100:0 (○), 90:10(●), 80:20(△), 70:30(▲), 60:40(□), and 50:50(■), respectively.