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Investigation of the Incorporation Efficiency of β-Carotene into Liposomes

– Research Note –

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Abstract

Chemical and photochemical processes during food storage and preparation rapidly degrade β-carotene, the most active form of carotenoids. We investigated the possibility of liposomes as a tool to preserve β-carotene. Liposomes with β-carotene were prepared as multilamellar vesicles by using soybean phosphatidylcholine, in terms of the ratio of β-carotene to phospholipid and pH. Incorporated efficiency was 99.7% at 1 : 0.05 of phospholipid : β-carotene and at pH 9.0. As the concentration of β-carotene increased, the incorporated efficiency increased progressively. pH did not affect the incorporation efficiency greatly.

Key words: liposome, β-carotene, phospholipid, incorporated efficiency

INTRODUCTION

Liposomes are single or multi-layered vesicles involving the complete enclosure of an aqueous phase within a phospholipid-based membrane. These vesicles form spontaneously when phospholipids are dispersed in an aqueous media. A portion of the aqueous media becomes enclosed in the lipid membrane which then serves as a controlled release particle for the active material dispersed in the lipid or aqueous phase of the particle. Liposomes are, therefore, capable of delivering both lipophilic and aqueous-based active materials (1).

Liposomes have been extensively investigated and developed in the biomedical field as drug delivery systems (2) An important aspect of this application is the protection of food ingredients by encapsulation against potentially damaging conditions in the extracapsular environment (3-6). Liposomes have been used as vitamin carriers in the food field (7-9), and for the purpose of controlling exposure of the encapsulated ingredients to the bulk phase.

β-Carotene is one of many carotenoids contained in the diet that possess antioxidant activity (10). The carotenoids are categorized as one of the important groups of natural pigments, especially in the plant kingdom. Some carotenoids are important as precursors of vitamin A. They are fat soluble compounds that are found in flowers and fruits of higher plants and are also produced by many nonphotosynthetic microorganisms (11). Due to its hydrophobic property, β-carotene is usually found in a complex with lipid droplets or micelles in foods, where the primarily linear molecule probably associates with the extended hydrocarbon acyl chains of the lipid components. Such an environment, which is expected to protect β-carotene from degradative reactions, can be readily approximated in the laboratory using a system of multilamellar liposomes dispersed in aqueous buffer. Therefore, we investigated the incorporation efficiency of β-carotene into liposomes as a function of the ratio of β-carotene to phospholipid and various pH values as preliminary research to investigate the effect of liposome incorporation on the kinetics of β-carotene degradation in aqueous buffers.

MATERIALS AND METHODS

Materials

β-Carotene was purchased from Wako Pure Chemical, Ltd. (Osaka, Japan) and L-α-phosphatidylcholine isolated from soybeans was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade.

Preparation of liposomes containing β-carotene

A 1.0 g quantity of L-α-phosphatidylcholine, appropriate concentration of β-carotene, and 100 ml of chloroform/methanol solvent mixture (2:1, v/v) were put into a 250 ml round-bottom flask. The solvent was evaporated on a rotary evaporator to deposit a dry lipid film on the wall of the flask. The flask was removed from the evaporator and 100 ml of 10 mM glycine buffer (containing 0.115 M NaCl) and 0.5 g of glass beads were added into flask to assist hydration of the lipids. The solution was then mixed on the rotary evaporator (without vacuum) to hydrate the lipids, which forms multilamellar vesicles. The solution was centrifuged for 1 hr at 80,000×g and then the supernatant removed. The pellet was washed with 100 ml of the buffer and centrifuged again for 1 hr at 80,000×g. The supernatant was again removed, and the liposome pellet containing β-carotene was diluted with 100 ml of the appropriate buffer (12).

Analytical methods

The β-carotene in liposomes was analyzed using a colorimetric assay. 0.2 ml of liposome solution containing retinol was

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mixed with 0.6 ml of a chloroform/methanol solvent mixtrue (2:1, v/v). A 0.1 ml aliquot of the organic solvent layer was then transferred to the test tube and diluted with 0.9 ml of a chloroform/methanol solvent mixtrue and then the absorbance at 453 nm was measured immediately. The concentration of β-carotene was determined by comparison of the absorbance with a standard curve prepared using pure β-carotene (13).

RESULTS AND DISCUSSION

To study the protection of β-carotene incorporated into liposome, we investigated the effect of the ratio of phospholipids to β-carotene and pH of the hydration buffer. The incorporated efficiency of β-carotene into liposomes of soybean phosphatidylcholine was 99.85% at a ratio of 0.05 g of β-carotene to 1.0 g phospholipid. Incorporation of β-carotene into liposomes decreased slightly with the decrease in the concentration of β-carotene (Table 1). The incorporated efficiency of β-carotene into liposomes was 99.79% when the mixture was hydrated at a buffer solution of pH 3.0 and with a ratio of 0.05 g of β-carotene to 1.0 g of phospholipid (Table 2). However, the effect of the pH on incorporated efficiency was not great. β-Carotene was most readily incorporated into liposomes at the ratio of phospholipid to β-carotene (1 : 0.05), showing 99.85% incorporated efficiency. The incorporated efficiency was greater than 94% for all pH values (Table 1), except for 1.0 wt% of β-carotene. These results were similar to the incorporated efficiency of retinol into liposomes (9).

The structure of β-carotene complex with liposomes is unknown. However, it is supposed that β-carotene binds to lipid bilayers in a manner similar to that found for the low molecular weight fluorescent probe diphyenyl hexatriene (DPH), a small molecule that contains two aromatic phenyl groups attached to each end of a linear conjugated triene double bond system. Although an ambiguity remains, it is now generally accepted that DPH intercalates into the hydrophobic bilayer in two orientations (14): at the planar interface between the hydrophobic acyl chains as well as within each leaflet with its long axis parallel to the phospholipid acyl chains. Thus, we expect that β-carotene is distributed within the hydrophobic core of the liposomes at both the planar interface between lipid leaflets and within each acyl chain region.

We previously conducted research on the stabilization of retinol by incorporation into liposomes (9). Through the research, we knew that incorporation into multilamellar liposomes of retinol significantly protects retinol against chemical degradation under a variety of solution conditions. Thus, it is expected that β-carotene incorporated into liposomes will be stable against degradation under various pHs and temperature conditions.

Table 1. Effect of phospholipid : β-carotene weight ratio on incorporated efficiency of β-carotene into liposomes at pH 9.0

Ratio[1)]	Incorporated efficiency (%)
1 : 0.01	73.62±13.71[2)]
1 : 0.02	94.47± 3.50
1 0.03	97.75± 2.49
1 : 0.04	99.66± 0 24
1 : 0.05	99.85± 0.13

[1)]Weight ratio of phospholipid to β-carotene.
[2)]Mean±standard deviation of triplicate measurements.

Table 2. Effect of various solution pHs on incorporated efficiency of β-carotene into liposomes (phospholipid . β-carotene = 1 : 0.05)

pH	Incorporated efficiency (%)
3	99.79±0.04[1)]
5	99.56±0.05
7	99.56±0.27
9	99.71±0.03
11	99.72±0.11

[1)]Mean±standard deviation of triplicate measurements.

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