Biochemical and Biophysical Research Communications 435 (2013) 361–366

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Preparation of quercetin and rutin-loaded ceramide liposomes and drug-releasing effect in liposome-in-hydrogel complex system

Soo Nam Park*, Min Hye Lee, Su Ji Kim, Eun Ryeong Yu

Department of Fine Chemistry, Cosmetic R&D Center, College of Energy and Biotechnology, Seoul National University of Science and Technology, 232, Gongneung-ro, Nowon-gu, Seoul 137-743, South Korea

ARTICLE INFO

Article history:
Received 17 April 2013
Available online 10 May 2013

Keywords:
Ceramide-3
Cellulose hydrogel
Quercetin
Rutin
Drug release

ABSTRACT

In this study, we developed a 2-step delivery system to enhance transdermal permeation of quercetin and its glycoside rutin, an antioxidant. Liposome-in-hydrogel complex systems were prepared by incorporating ceramide liposomes, which consist of biocompatible lipid membranes, into cellulose hydrogel. We evaluated the encapsulation efficiency, *in vitro* release behavior, and skin permeability of formulations that remained stable for over 3 weeks. Rutin had greater encapsulation efficiency and better *in vitro* release properties than quercetin. However, quercetin demonstrated greater skin permeability than rutin. We also found that liposome-in-hydrogel complex systems (quercetin, 67.42%; rutin 59.82%) improved skin permeability of quercetin and rutin compared to control (phosphate buffer, pH 7.4) (quercetin, 2.48%; rutin, 1.89%) or single systems of hydrogel (quercetin, 31.77%; rutin, 26.35%) or liposome (quercetin, 48.35%; rutin, 37.41%). These results indicate that liposome-in-hydrogel systems can function as potential drug delivery systems to enhance transdermal permeation of the water-insoluble antioxidants quercetin and rutin.

1. Introduction

Stratum corneum is the outermost layer of the skin, which functions as primary barrier to protect the skin from potentially harmful environmental agents. In addition, by inhibiting the loss of moisture to the outside, intercellular lipids in the stratum corneum help maintain homeostasis of the skin, protecting the skin from dehydration. However, the skin barrier can be adversely affected when a drug is delivered via the transdermal route. Therefore, there has been an increase in research investigating a variety of drug delivery systems aimed at promoting better skin permeation of active materials [1–4].

Hydrogels are 3-dimensional networks that, consist of hydrophilic polymers that swell in aqueous solution and retain a large amount of water without dissolving. Recently, the development of cellulose-based hydrogels has been actively pursued. Hydrogels based on cellulose have biodegradable properties, high permeation of active materials, a high degree of swelling, and no associated toxicity or irritation. In particular, owing to high biocompatibility, hydrogels are widely used in the medical and pharmaceutical fields as drug delivery vehicles [5–7].

Liposomes have a phospholipid composition that is similar to the lipid bilayer of cell membranes in the body. Moreover, both hydrophilic and hydrophobic drugs can be loaded within the internal space of liposomes. Liposomes have low toxicity and are highly biocompatible. For these reasons, liposomes are generally used as a drug delivery system [8].

Quercetin and its glycoside, rutin are typical flavonoids that are reported to act as strong antioxidants [9]. These flavonoids, have been widely used as anti-oxidants in cosmetics, but their use is limited because of poorly water solubility Therefore, research has been focused on ways to improve their solubility in water.

In this study, a 2-step delivery system consisting of ceramide liposomes composed of biocompatible membranes and porous cellulose hydrogel, was designed to enhance transdermal permeation of quercetin and rutin. We also studied the effects of interactions between the 2 delivery systems by conducting a skin permeation test using a Franz diffusion cell.

2. Materials and methods

2.1. Materials

Cellulose (microcrystalline, powder), (±)-epichlorohydrin (⩾99.0%, ECH), L-α-phosphatidylcholine (from egg yolk, ⩾60%, egg PC), cholesterol (⩾99.0%, Chol), oleic acid (⩾99.0%, OA), quercetin and rutin were purchased from Sigma (USA). Ceramide-3 (DS-CERAMIDE Y30) was obtained from Doosan Co. (Korea). Solvents such as 1,3-butylene glycol, methanol, ethanol,

* Corresponding author.
E-mail address: snpark@seoultech.ac.kr (S.N. Park).

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http://dx.doi.org/10.1016/j.bbrc.2013.04.093

chloroform, and acetone, were of analytical grade. Preparation of the hydrogel required the use of a stirrer (HSD 120-03P, Misung Scientific Co., Ltd., Korea) and a centrifuge (2236R, Gyrozen Co., Ltd., Korea). Preparation of liposomes required the use of a rotary evaporator (Buchi, Switzerland) and a probe sonicator (Branson, USA). UV Spectrometer (Cary 100, Agilent, USA) and HPLC (Shimadzu, Japan) were used to determine the concentration of quercetin and rutin.

2.2. Preparation of ceramide liposomes

Liposomes were prepared using the thin-film hydration method [10,11]. Egg PC, Cer, Chol, OA, quercetin, and rutin were dissolved in 25 mL of a chloroform–methanol mixture (4:1) using a fixed molar ratio (Table 1). The mixtures were evaporated in a rotary evaporator to remove traces of solvent and also to form a film. To prevent separation of phospholipid membrane by hydrolysis, the film was hydrated with phosphate buffer (pH 9.0) for 1 h at a temperature that was above the lipid transition temperature, and the product was maintained as a liposome solution at pH 7.0 ± 0.5. The vesicle suspension was then homogenized using a probe sonicator, passed through a 0.45 μm filter (Minisart CA 26 mm), and stored until use.

2.3. Stability test of ceramide liposomes

Stability of unloaded ceramide liposomes and quercetin or rutin loaded ceramide liposomes was evaluated by measuring the average particle size and distribution. Size and distribution in a liposome solution was assessed 70 times, with 3 repeated measurements using a particle size analyzer (Otsuka ELS-Z2, Otsuka Electronics, Japan) at 25 °C, with a scattering angle of 165° using an Argon laser. The average particle size was determined by cumulative analysis, and distribution was resolved using the Contin method [12].

2.4. Encapsulation efficiency of quercetin or rutin in ceramide liposomes

Unloaded quercetin or rutin was recovered from a 2 mL volume of liposome solution by passing the liposome solution through a 0.45 μm filter (Minisart, CA 26 mm), and degrading the solution with 15 mL of ethanol. The ethanol was evaporated in a rotary evaporator and quercetin or rutin was then redissolved in 2 mL of ethanol. The concentration of quercetin and rutin was determined using HPLC, and the following Eq. (1) was used to calculate the encapsulation efficiency of quercetin or rutin into liposomes:

$$EE = \frac{C_e}{C_i} \times 100\ (\%) \quad (1)$$

EE encapsulation efficiency (%)
C_i initial concentration of drug (μM)
C_e concentration of encapsulated drug (μM)

2.5. Preparation of cellulose hydrogel

We prepared cellulose hydrogel using a modification of the method used by Ciolacu et al. [13]. Cellulose powder (0.5 g) was dispersed into 13.45 mL of 9% NaOH solution at room temperature, and the sample was dissolved by freezing at −70 °C for 24 h. After thawing, 11.55 mL of distilled water was added to the cellulose solution, followed by the drop wise addition of 3 mL of ECH, with continuously stirring. The crosslinking reaction was subsequently performed at 80 °C for 8 h. When the reaction was completed, the cellulose hydrogel product was washed with distilled water to remove the NaOH and NaCl that had formed, followed by an acetone wash to remove excess ECH. Solvent was removed from the sample by centrifugation, and the sample was dried in a vacuum at room temperature prior to storage.

2.6. Preparation of a ceramide liposome-in-cellulose hydrogel complex

The complex was prepared by incorporating 5 mL of ceramide liposome solution into 0.1 g of dried cellulose hydrogel. The ceramide liposome penetrated into and was absorbed within the matrices of swollen hydrogel.

2.7. Incorporating efficiency of ceramide liposome into cellulose hydrogel and degree of water uptake of cellulose hydrogel

Dried cellulose hydrogel (0.1 g) was swollen in 5 mL of a ceramide liposome solution at 37 °C for 24 h in order to measure incorporation efficiency. To assess the amount of incorporated liposome, the concentration of quercetin or rutin in liposomes was determined, and for this, liposome solutions before and after incorporation were analyzed using HPLC.

The following Eq. (2) was used to calculate the concentration of quercetin or rutin in liposomes incorporated into matrices of cellulose hydrogel:

$$IE = \frac{v_1 c_1 - v_2 c_2}{v_1 c_1} \times 100\ (\%) \quad (2)$$

IE incorporation efficiency (%); v_1 volume of initial liposome solution (mL); c_1 initial concentration of the drug (μg/mL); v_2 remaining

Table 1
Physical stability of unloaded liposomes assessed by particle size according to different formulations.

Sample		Liposome formulation (molar ratio)				Mean particle size	Particle size distribution
		PC[a]	Cer[b]	Chol[c]	OA[d]		
Unloaded liposome	L-a	–	1	1	1	Aggregation (N.D.)	
	L-b	1	–	1	1	200.57 ± 83.16[e]	131.63 ± 47.67
	L-c	0.25	0.75	1	1	366.45 ± 5.75	206.15 ± 90.25
	L-d	0.5	0.5	1	1	517.49 ± 270.76	564.28 ± 367.98
	L-e	0.75	0.25	1	1	240.45 ± 36.12[e]	187.98 ± 39.88
	L-f	0.75	0.25	0.5	1	140.48 ± 8.86[e]	71.88 ± 16.77
	L-g	0.8	0.2	1	1	136.55 ± 12.40[e]	69.71 ± 5.17
	L-h	0.8	0.2	0.5	1	139.49 ± 17.17[e]	77.31 ± 9.29

(N.D.: No data).
[a] L-α-Phosphatidylcholine (from egg yolk, ⩾60%).
[b] Ceramide-3 (DS-CERAMIDE Y30).
[c] Cholesterol.
[d] Oleic acid (unsaturated fatty acid, C18).
[e] Formulation sustained stable condition for more than 3 weeks.