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Original Research Paper

A hydrophobic peptide fraction that enhances the water dispersibility of curcumin

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ABSTRACT

The present study describes the complexation between curcumin (Cur) and a peptide mixture (Pep). Pep was prepared by enzymatic hydrolysis of casein and used as an excipient for poorly water-soluble Cur. An aqueous solution of Pep and an acetone solution of Cur were mixed and lyophilized to obtain a white-yellow powder of the peptide and Cur complex (Cur-Pep). The water dispersibility of Cur was enhanced by the complexation with Pep. Pep was fractionated using ammonium sulfate precipitation and ultrafiltration to identify which peptides preferentially interact with Cur. Relatively hydrophobic peptides with high molecular weights (>5 kDa) were more effective in enhancing the water dispersibility of Cur than other fractions. Cur-Pep dispersed under acidic and neutral conditions, at which amphoteric Pep is positively or negatively charged. Cur-Pep exists as a hydrocolloid with particle size 160–330 nm in aqueous media.

1. Introduction

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, Cur, Fig. 1) is a polyphenol compound derived from turmeric root (Curcuma longa) and has attracted attention as a potential chemotherapeutic agent [1,2]. It has a wide range of biological and pharmacological effects including antioxidant, antitumor, anti-inflammatory, and anticancer properties [3–5]. However, absorption of Cur in the gastrointestinal tract is poor because it is not highly soluble [6]. Its maximum solubility is reportedly 11 ng/ml in a pH 5.0 aqueous buffer [7]. Therefore, various techniques have been used to increase its water solubility and/or water dispersibility and improve its oral bioavailability [8–10].

Amphiphilic block copolymer micelles have been used as vehicles for the solubilization and stabilization of Cur [11,12]. Cur has also been incorporated into micellar aggregates of cross-linked and random copolymers of *N*-isopropylacrylamide with *N*-vinyl-2-pyrrolidone, and poly(ethyleneglycol)monoacrylate. The resulting nanoparticles (about 50 nm in diameter) were dispersed in aqueous media [13]. Complex formation between Cur and cyclodextrins (CDs) also increases the water solubility of Cur [7,13]. The hydrolytic stability of Cur improved after

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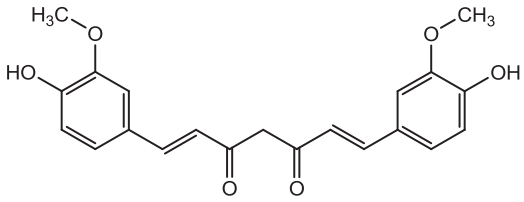

Fig. 1 – Molecular structure of Cur.

complexation with CDs, but its photodecomposition rate increased compared with a Cur solution in an organic solvent. Formation of a 2:1 complex between β-CD and Cur has also been reported [14]. Water-soluble polymers are also used to increase the water solubility of Cur [15], and binding with polyvinylpyrrolidone [16] and hyaluronic acid [17] has also been studied. Incorporation into an emulsion is also an effective way to enhance the oral administration of Cur [18–20].

Casein micelles have also been developed as nanocarriers for Cur [21–25]. Complex formation of Cur with a casein micelle and its use for delivery to cancer cells was investigated [21]. The complex was analyzed using fluorescence spectroscopy [21,26,27]. Encapsulation of Cur in a camel β-casein micelle has also been studied [28], as well as the binding of curcumin to α_{s1}-casein, which was analyzed by fluorescence spectroscopy and circular dichroism [29,30]. Recently, encapsulation of Cur in casein by spray-drying has been developed [31]. Soy protein isolate can also be used as a complexation agent for Cur to improve its water solubility [32].

In the present study, a peptide mixture (Pep) was prepared by enzymatic hydrolysis of casein, and was used as a complexation agent for Cur. Recently, researchers have found that peptide mixtures obtained as protein hydrolysate were effective in enhancing the water solubility and/or water dispersibility of poorly water-soluble materials. The water solubility of a poorly water-soluble drug, indomethacin, was enhanced by complexation with Pep [33,34]. The resulting complex between indomethacin and Pep is quite small and can pass through ultrafilter membranes. Additionally, complexation with albumin hydrolysate as a peptide mixture enhanced the water dispersibility of extremely poorly water-soluble coenzyme Q_{10} [35]. The particle size of the complex between coenzyme Q_{10} and albumin hydrolysate was 170–280 nm, suggesting that the complex is present as a hydrocolloidal material in aqueous media. Furthermore, the water dispersibility of paclitaxel was also enhanced by the complexation with Pep [36].

Peptide mixtures prepared from protein hydrolysate will contain diverse peptides with different molecular weights and amino acid sequences. Some of these exhibit affinity to poorly water-soluble materials on the basis of various interactions, such as hydrophobic and/or electrostatic interactions. For example, heme-iron-enriched polypeptide (digested hemoglobin, HIP), which is obtained by hydrolysis of hemoglobin using protease and subsequent enrichment by ultrafiltration, is known to be complex between poorly water-soluble heme iron and peptide fragments [37–40]. As the peptidic fraction of hemoglobin hydrolysate confers hydrophilicity to heme iron, HIP is apparently soluble in aqueous media and is more readily absorbed by the human body than heme iron alone [41]. These examples demonstrate that complexation with Pep could increase the water solubility, and consequently the oral bioavailability, of Cur. In this study, casein was used as the resource to prepare Pep as it is an edible protein. Additionally, casein is a family of four phosphoproteins α_{s1}-casein, α_{s2}-casein, β-casein, and κ-casein, which show different hydrophilic/hydrophobic balances, resulting in diverse peptides [42,43]. Therefore, Pep was fractionated using ammonium sulfate precipitation followed by ultrafiltration in order to specify and use the peptides that are effective for enhancing the water dispersibility of Cur [36].

Pep was prepared by enzymatic hydrolysis of casein and fractionated by precipitation using ammonium sulfate solution and ulfrafiltration [36]. The complex between Cur and Pep was prepared by mixing an acetone solution of Cur and an aqueous solution of Pep, followed by removal of acetone *in vacuo* and lyophilization [33,35]. The water dispersibility of Cur-Pep was compared with Cur alone and with other excipients. To estimate the structure of Cur-Pep in aqueous media, an aqueous suspension of Cur-Pep was fractionated by centrifugation followed by successive membrane filtrations. The particle size of Cur-Pep and the distribution of Cur in the fractions were analyzed. The zeta potential distribution of Cur-Pep and Pep was also measured to evaluate the surface charge of the materials. Cur-Pep was characterized using scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and powder X-ray diffraction (XRD). The results were used to determine how Cur is incorporated into Cur-Pep.

2. Materials and methods

2.1. *Materials*

Cur, milk casein (Hammarsten grade), polyethylene glycol 4000 (PEG 4000, with average molecular weight 4000 g/mol), α-cyclodextrin, methyl-β-cyclodextrin, γ-cyclodextrin, glucose, and sucrose were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). α-Chymotrypsin from bovine pancreas, pepsin from porcine gastric mucosa, and pancreatin from porcine pancreas were purchased from Sigma-Aldrich Japan K.K. (Tokyo, Japan). All other reagents were analytical grade. Disposable membrane filters (DISMIC®, pore size: 0.80 μm (25CS080AN), 0.45 μm (25CS045AN), or 0.20 μm (25CS020AN)) made of cellulose acetate were purchased from Advantec Toyo (Tokyo, Japan). The following ultrafiltration membranes made of regenerated cellulose were purchased from Merck Milipore (Billerica, MA, USA) and used for fractionation of the peptides: Ultracel® 5 kDa, molecular weight cut-off of 5000; Ultracel® 3 kDa, molecular weight cut-off of 3000; and Ultracel® 1 kDa, molecular weight cut-off of 1000.

2.2. *Preparation and fractionation of Pep*

Pep was prepared by enzymatic hydrolysis of casein according to similar procedures described previously [31]. Casein (25 g) was hydrated in distilled water (500 ml) and the pH was