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Drug solubilization mechanism of α-glucosyl stevia by NMR spectroscopy

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ABSTRACT

We investigated the drug solubilization mechanism of α-glucosyl stevia (Stevia-G) which was synthesized from stevia (rebaudioside-A) by transglycosylation. ^{1}H and ^{13}C NMR peaks of Stevia-G in water were assigned by two-dimensional (2D) NMR experiments including ^{1}H-^{1}H correlation, ^{1}H-^{13}C heteronuclear multiple bond correlation, and ^{1}H-^{13}C heteronuclear multiple quantum coherence spectroscopies. The ^{1}H and ^{13}C peaks clearly showed the incorporation of two glucose units into rebaudioside-A to produce Stevia-G, supported by steviol glycoside and glucosyl residue assays. The concentration-dependent chemical shifts of Stevia-G protons correlated well with a mass-action law model, indicating the self-association of Stevia-G molecules in water. The critical micelle concentration (CMC) was 12.0 mg/mL at 37 °C. The aggregation number was 2 below the CMC and 12 above the CMC. Dynamic light scattering and 2D ^{1}H-^{1}H nuclear Overhauser effect spectroscopy (NOESY) NMR experiments demonstrated that Stevia-G self-associated into micelles of a few nanometers in size with a core-shell structure, containing a kaurane diterpenoid-based hydrophobic core and a glucose-based shell. 2D ^{1}H-^{1}H NOESY NMR measurements also revealed that a poorly water-soluble drug, naringenin, was incorporated into the hydrophobic core of the Stevia-G micelle. The Stevia-G self-assembly behavior and micellar drug inclusion capacity can achieve significant enhancement in drug solubility.

1. Introduction

More than 40% of the failures in new drug development is attributed to poor pharmaceutical properties, particularly water insolubility. The enhancement of drug solubility has been studied for many decades, and a number of techniques have been employed, e.g., the use of cyclodextrins (Higashi et al., 2009), solid dispersion systems (Dahlberg et al., 2010; Kojima et al., 2012; Sinha et al., 2010), nanoparticles (Zhang et al., 2012), and micelles (Mikhail and Allen, 2010). Recently, we have examined the feasibility of transglycosylated food additives as new pharmaceutical excipients in order to improve the dissolution and bioavailability of poorly water-soluble drugs (Tozuka et al., 2010, 2012; Uchiyama et al., 2010a, 2012; Zhang et al., 2011). The transglycosylated food additives are synthesized by applying enzymatic transglycosylation to bioactive compounds using glycosyltransferases (Kittl and Withers, 2010; Kometani, 2010; Wang and Huang, 2009). Among these transglycosylated food additives, α-glucosyl stevia (Stevia-G, Fig. 1A), which is the enzymatically transglycosylated product of stevia via α-glycosyltransferase (Varuzhan et al., 2006), is promising as a new pharmaceutical excipient. Stevia is a herb belonging to the Compositae family, estimated to comprise 150–300 species (Grashoff et al., 1972; King and Robinson, 1967). Stevioside, rebaudioside-A, rebaudioside-C, and dulcoside-A are known as the major components. Stevia, which has no significant adverse effects, has been used for 20 years as a sweetener and sugar substitute. Since Stevia-G is sweeter than stevia, it can be useful in masking the bitter taste of drugs. We previously reported that spray-dried particles of a poorly water-soluble drug (flurbiprofen and probucol) and Stevia-G showed a significant enhancement in both the dissolution and absorption of the drug without any toxic

Abbreviations: CAC, critical aggregation concentration; CMC, critical micelle concentration; COSY, correlation spectroscopy; δ_{mic}, micelle chemical shifts; δ_{mon}, monomer chemical shift; DLS, dynamic light scattering; HMBC, heteronuclear multiple bond correlation spectroscopy; HMQC, heteronuclear multiple quantum coherence spectroscopy; NaTC, sodium taurocholate; NOESY, nuclear Overhauser effect spectroscopy; NRG, naringenin; SDS, sodium dodecyl sulfate; Stevia-G, α-glucosyl stevia; TSP, 3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid, sodium salt.

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effects on Caco-2 cells (Uchiyama et al., 2010b). Interestingly, Stevia-G and surfactant formed a hybrid nanocomposite in water to improve drug dissolution (Uchiyama et al., 2012). It was demonstrated that a mixture of 0.1% sodium dodecyl sulfate (SDS) and 1% Stevia-G solution had little cytotoxicity to Caco-2 cells, whereas 0.1% SDS solution showed high toxicity.

Fluorescence investigations in the previous study suggested a drug solubilization mechanism in which Stevia-G forms micelle-like nanostructures in water and dissolves the drugs in these structures (Uchiyama et al., 2011). However, the solubilization mechanism at the molecular level was not yet understood. To understand the drug solubilization phenomenon in detail and efficiently design pharmaceutical formulations, it is necessary to clearly determine the solubilization mechanism. In recent decades, NMR has become a crucial method for chemical structure identification, and has been further applied in the conformational determination of self-assembled aggregates. For example, the static (chemical environment, degree of association, size, and shape) and the dynamic (molecular mobility, kinetics of aggregation, solubilization) properties of the aggregates are characterized by various NMR techniques (Emin et al., 2007). The proton chemical shift is sensitive to subtle changes in the local environment and used for detecting molecular association. Two-dimensional (2D) ^{1}H-^{1}H nuclear Overhauser effect spectroscopy (NOESY), based on the dipole-dipole interactions between nuclei in spatial proximity, is a powerful tool for studying the arrangement of aggregates (Denkova et al., 2009; Schedlbauer et al., 2009; Yang et al., 2009).

In the present study, the chemical structure of Stevia-G, its detailed conformation in water, and the specific drug solubilization mechanism were investigated by NMR spectroscopy. The Stevia-G, which was the transglycosylation product of rebaudioside-A, had a purity over 98%. Rebaudioside-A (Fig. 1A) has the basic skeleton of a kaurane diterpenoid with three glucose moieties attached to the C-13 hydroxyl and one glucose moiety in the form of an ester at C-19 (Steinmetz and Lin, 2009). 1D ^{1}H and ^{13}C NMR and 2D ^{1}H-^{1}H correlation spectroscopy (COSY), ^{1}H-^{13}C heteronuclear multiple bond correlation spectroscopy (HMBC), and ^{1}H-^{13}C heteronuclear multiple quantum coherence spectroscopy (HMQC) NMR experiments were employed to identify the chemical structure of Stevia-G. The self-assembly process of Stevia-G in water was assessed by observing the chemical shift changes of stevia protons depending on the concentration. 2D ^{1}H-^{1}H NOESY NMR experiments were performed to evaluate the spatial structure of Stevia-G in water and the localization of a model drug, naringenin (NRG, Fig. 1B).

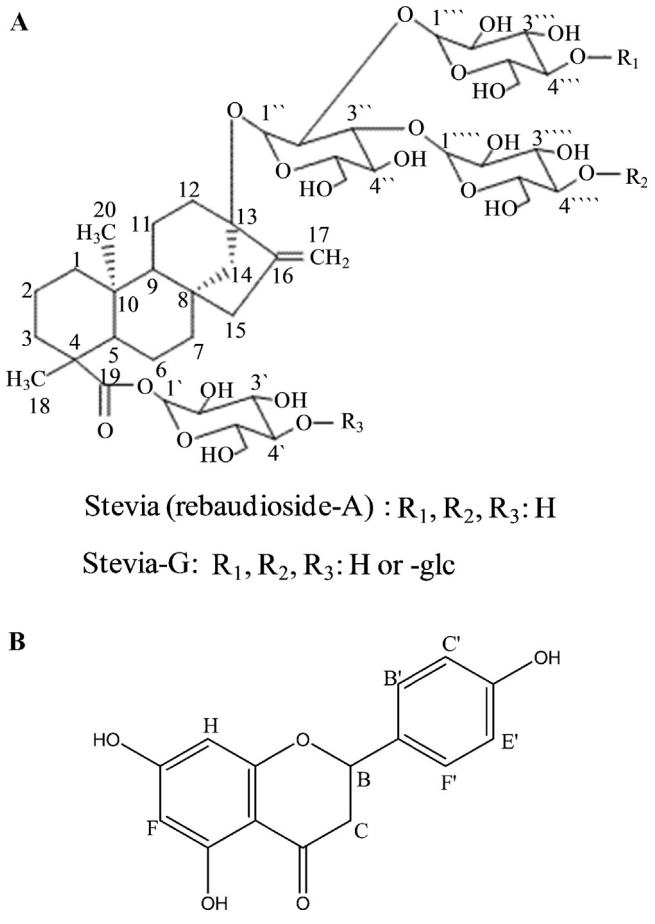

Fig. 1. Chemical structures and atom numberings of (A) α-glucosyl stevia (Stevia-G) and (B) naringenin (NRG).

2. Materials and methods

2.1. Materials

Stevia-G, synthesized from rebaudioside-A in high purity (over 98%), was a kind gift from the Toyo Sugar Refining Co., Ltd. (Tokyo, Japan). NRG was purchased from Tokyo Kasei Co., Ltd. (Tokyo, Japan) and used without further purification. Commercial deuterium oxide (D_2O, 99.9%, Aldrich, St. Louis, MO, USA) was used as received. All other chemicals and solvents were of reagent grade. The chemical structures and atom numbering of Stevia-G and NRG are shown in Fig. 1.

2.2. Preparation of spray-dried powders

Powders of NRG-loaded Stevia-G were prepared using a spray-drying method. To prepare samples by this method, NRG (500 mg) and Stevia-G (5 g) were dissolved in an ethanol/water solution (8:2 v/v). This solution was fed to a spray dryer (GS31; Yamato, Tokyo, Japan) at a rate of 10 mL/min, and sprayed into the chamber from a nozzle with a diameter of 406 μm at a pressure of 0.13 MPa. The inlet and outlet temperatures of the drying chamber were maintained at 120 and 70 °C, respectively. All spray-dried powders were dried in a desiccator with blue silica gel under reduced pressure for 1 day before their physicochemical properties were tested.

2.3. Assay of steviol glycosides and glucosyl residues in Stevia-G

The determination analysis followed those prescribed by Japan's Specifications and Standards for Food Additives (8th Edition). Stevia-G (~1.0 g) was dissolved in water (50 mL). The solution was transferred into a resin column (2.5 cm diameter) packed with acrylic acid ester resin (25 mL, XAD-7, Organo Co., Ltd., Japan). The solution was drained from the column at a rate of less than 3 mL/min, and then the column was washed with water (250 mL) to remove the unreacted glucoses. The Stevia-G adsorbed on the column was eluted using 50% (v/v) ethanol (250 mL) at a flow rate below 3 mL/min. The eluted solution was evaporated to remove ethanol; then, glucoamylase was added in the solution to fully react. The obtained solution was assayed by HPLC to determine the content of steviol glycosides. The glucose content in the solution was determined using a glucose assay kit (Wako Pure Chemical Industries, Ltd., Japan).

2.4. Particle size analysis

The volumetric particle size distribution was determined by a dynamic light scattering (DLS) method using a Microtrac UPA® (Nikkiso Co., Ltd., Japan). The mean particle size was the average