Carbohydrate Polymers 82 (2010) 861–866

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

NMR structural analysis of epigallocatechin gallate loaded polysaccharide nanoparticles

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ARTICLE INFO

Article history:
Received 12 May 2010
Received in revised form 31 May 2010
Accepted 3 June 2010
Available online 11 June 2010

Keywords:
NMR
DOSY
Relaxation
Epigallocatechin gallate
Polysaccharide nanoparticles
Drug carriers

ABSTRACT

Nuclear magnetic resonance (NMR) spectroscopy has been employed for structural characterization of epigallocatechin gallate loaded maltodextrin/gum arabic nanoparticles (EGCG-MD/GA). Measurements of the nuclear relaxation times (T_1) and application of diffusion ordered spectroscopy (DOSY), obtained through pulsed field gradient (PFG) NMR experiments, have been performed to determine the structure of the epigallocatechin gallate–polysaccharide conjugates and to clarify the mechanisms of drug immobilization into the polymer matrix. The results suggest the entrapment of EGCG into the polysaccharide matrix of maltodextrin/gum arabic (MD/GA) and support the potential of these vehicles for their sustained delivery and release.

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1. Introduction

Catechins are a class of polyphenolic flavonoids predominantly found in foods and beverages, such as apples, chocolate, red wine and green tea (Hackman et al., 2008; Rice-Evans, Miller, Bolwell, Bramley, & Pridham, 1995). The main catechin species include epicatechin (EC), catechin (C), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG). Among them, EGCG is the most abundant catechin found in the green tea. The catechins possess antioxidant activity due to their ability of trapping free radicals by donation of the phenolic hydrogen atoms (Mendoza-Wilson & Glossman-Mitnik, 2006). As radical scavengers, catechins show interesting medicinal properties, including anti-inflammatory, antiviral, anti-cancer and antifungal activities (Hirasawa & Takada, 2004; Kuzuhara, Suganuma, & Fujiki, 2008; Zaveri, 2006). However, their pharmaceutical application is limited by several factors like their poor solubility, inefficient permeability, instability, first pass effect and gastrointestinal (GI) tract degradation (Mochizuki, Yamazaki, Kano, & Ikeda, 2002; Neilson et al., 2007; Zhu, Zhang, Tsang, Huang, & Chen, 1997). Therefore, there is a need to develop strategies for new medical tools for more effective protection and delivery of catechins, considering the performance and maintaining of their original physical properties.

Nanoparticles made of polysaccharides, due to their unique properties are promising carriers to deliver and protect the physiological properties of hydrophilic drugs and have been successfully applied as drug-delivery systems (Gonçalves, Pereira, & Gama, 2010; Hu et al., 2008; Liu, Jiao, Wang, Zhou, & Zhang, 2008; Vauthier & Couvreur, 2000). As natural biomaterials, polysaccharides are stable, safe, non-toxic, hydrophilic and biodegradable. In addition, polysaccharides have abundant resources in nature and low cost in their processing. Recently, polysaccharides nanoparticles based on maltodextrin and gum arabic have been reported as a delivery system for catechins (Ferreira, Rocha, & Coelho, 2007; Gomes et al., 2010).

The nanoparticle structure and properties are critical to understand and develop novel therapeutic agents. The knowledge of the nature of the intermolecular interactions between the species presented in these nanostructures is of fundamental importance in the understanding of the factors that determine their biological activity. It is therefore essential to elucidate the structure of drug-loaded nanoparticles and to clarify the mechanisms of drug immobilization in the polymer matrix.

Nuclear magnetic resonance (NMR) spectroscopy is one of the most powerful experimental methods for investigation of the structure and intermolecular interactions of multicomponent systems

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0144-8617/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2010.06.007

(Evans, 1995; Teng, 2005). The combined application of diffusion ordered NMR spectroscopy (DOSY) and measurement of the nuclear spin-lattice relaxation times (T_1) offers a chance to gain insight into the structure of intermolecular aggregates in solution (Bakhmutov, 2004; Cohen, Avram, & Frish, 2005). DOSY is nowadays a well-established technique for characterizing the structure and dynamics of complex systems. The self-diffusion coefficients and structural properties of the molecules are connected by the dependence of their self-diffusion coefficients on the molecular size, weight, shape, etc. (Brand, Cabrita, & Berger, 2005; Cohen et al., 2005; Johnson, 1999). Therefore, DOSY experimental technique has become a valuable tool for studies of molecular interactions in solution.

The aim of the present study was to investigate the structure of EGCG-loaded maltodextrin/gum arabic nanoparticles (EGCG-MD/GA) and to estimate the origin of the intermolecular interactions responsible for the EGCG–polymer particulate formation on the base of high-resolution NMR spectroscopy. The structure and intermolecular interactions of drug-loaded systems determine their capability and biological activity as drug-delivery nanomedicines.

2. Experimental part

2.1. Sample preparation

2.1.1. Preparation of unloaded and EGCG-loaded polysaccharide nanoparticles

Maltodextrin/gum arabic nanoparticles (MD/GA) were obtained by homogenization of gum arabic (GA, M_W = 250 kDa, Sigma–Aldrich Co.) and maltodextrin (MD, 1 kDa, DE 16.5–19.5, Grain Processing Corporation) dissolved in ultrapure water (Nanopure Diamond Water Purification, Barnstead Thermo Scientific, USA, resistivity = 18.2 MΩ cm) at 50–60 °C under magnetic stirring. The suspension was homogenized at a constant speed of 9500 rpm with a dispersing device IKA DI25 Basic. Further, the suspension was spray-dried in a pilot spray-dryer (designed by Niro A/S) wherein the inlet and outlet air temperature were 160 ± 5 and 60 ± 5 °C, respectively. After leaving the drying chamber, the nanoparticles were recovered using a cyclone and finally collected in a powder collector vessel.

EGCG-loaded nanoparticles (EGCG-MD/GA) were prepared by the same methodology, but in this case, EGCG was added to the MD/GA suspension in a ratio of 5 wt.% before the homogenization. The constant speed of homogenization and inlet and outlet air temperature of spray-drying were kept the same.

2.1.2. Preparation of physical mixtures

Physical mixtures of free EGCG with MD/GA nanoparticles (EGCG + MD/GA) and free EGCG with MD and GA (EGCG + MD + GA) were prepared by mixing the components in the same proportion employed in the preparation of EGCG-MD/GA particles. Each of the mixtures (20 mg) was dissolved in 600 μL of D_2O under magnetic stirring.

2.2. NMR spectroscopy

All NMR experiments have been recorded on a Bruker Avance III 400 spectrometer, operating at 400.15 MHz for protons, equipped with pulse gradient units, capable of producing magnetic field pulsed gradients in the z-direction of 50 G/cm. The spectra have been acquired in D_2O solution at 30 °C in 5 mm tubes. Sodium trimethylsilyl-[2,2,3,3-d4]-propionate (TSP) has been used as an internal standard for both the chemical shift and the diffusion measurements. Unloaded (MD/GA) and EGCG-loaded nanoparticles (EGCG-MD/GA), physical mixture of EGCG and unloaded nanoparticles (EGCG + MD/GA) and physical mixtures of EGCG, MD and GA (EGCG + MD + GA) were prepared for NMR measurements as described above (see Section 2.1). With the exception of EGCG, in all cases 20 mg of solute, 600 μL D_2O and 20 μL 0.05 mM solution of TSP in D_2O were used. NMR spectra of EGCG were recorded in D_2O at concentration of 10 mg/mL. All NMR measurements have been done with standard Bruker pulse sequences.

Two-dimensional $^1H/^1H$ correlation spectra (COSY) and gradient-selected $^1H/^{13}C$ heteronuclear single quantum coherence (HSQC) spectra were recorded using the standard Bruker software. 2D COSY spectra were acquired with a multiple quantum filter, gradient pulses for selection, a gradient ratio of 16:12:40 and a relaxation delay of 1.5 s. A total of 2048 data points in F2 and 512 data points in F1 over a spectral width of 6500 Hz were collected. 2D $^1H/^{13}C$ HSQC experiments via double inept transfer, using sensitivity improvement and decoupling during the acquisition were carried out with a spectral width of ca. 6000 Hz for ^1H and 28,000 Hz for ^{13}C, a relaxation delay of 1.5 s, FT size 2 K × 256 W.

The two-dimensional nuclear Overhauser effect spectroscopy (NOESY) experiments were acquired in phase-sensitive mode with gradient pulses in the mixing time, using the standard pulse sequences with optimized mixing time of 400 ms. Generally, 64 scans and 512 F1 slices were obtained and the spectral width in both dimensions was 6500 Hz.

The spin-lattice relaxation rates were measured using the inversion-recovery pulse sequence, ($180°–\tau–90°$). Thirty-two τ increments were used for the experiments, with values between 0.01 and 13.0 s and relaxation delay of 13 s. Relaxation times were calculated by exponential regression analysis of recovery curves of longitudinal magnetization components.

The DOSY experiments were performed using the bipolar longitudinal eddy current delay (BPPLED – Bipolar Pulsed Field Gradient Longitudinal Eddy Delay) pulse sequence (Wu, Chen, & Johnson, 1995). The experimental conditions (amount of the solute and the solvent, temperature, air flow, sample rotation) for all DOSY experiments were kept constant. Before all NMR experiments, the temperature was equilibrated and maintained at 30 °C, as measured using the spectrometer thermocouple system. The measurements were carried out in D_2O and the solutions were prepared at constant concentration of 20 mg/0.60 mL. The diffusion coefficient of TSP (6.68×10^{-10} m^2 s^{-1}, calculated standard deviation of 3.1×10^{-3}) obtained from ^1H DOSY experiments was used as an internal reference for the diffusion measurements. The spectra were recorded in 5 mm NMR tubes with an air flow of 535 L/h. Typically, in each experiment a number of 32 spectra of 16K data points and 64 scans were collected, with values for the duration of the magnetic field pulse gradients (δ) of 4 or/and 5 ms, diffusion times (Δ) of 160–200 ms and an eddy current delay set to 5 ms. The pulse gradient (g) was incremented from 2 to 95% of the maximum gradient strength in a linear ramp. The spectra were first processed in the F2 dimension by standard Fourier transform and after baseline correction the diffusion dimension was processed with the Bruker Topspin software package (version 2.1). The diffusion coefficients are calculated by exponential fitting of the data belonging to individual columns of the 2D matrix. The diffusion coefficients (D) were obtained by measuring the signal intensity at more than one place in the spectra. Between 5 and 10 different measurements were done for the determination of each diffusion coefficient. The TSP was used as an internal diffusion reference for the DOSY measurements and all diffusion coefficients are given as a ratio D/D_{TSP} according procedure published before (Cabrita & Berger, 2001) and average values have been calculated.

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