Food Hydrocolloids 26 (2012) 181–186

Contents lists available at ScienceDirect

Food Hydrocolloids

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

NMR studies of the gelation mechanism and molecular dynamics in agar solutions

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ARTICLE INFO

Article history:
Received 26 January 2011
Accepted 11 April 2011

Keywords:
Gradient NMR
Diffusion coefficient
Gel
Agar
Agarose
Dendrimer
Hydrodynamic shielding length
Mesh size

ABSTRACT

Changes in the molecular mobility of agar were measured by pulsed-field-gradient stimulated echo (PFG-STE) ^{1}H NMR at various temperatures in order to elucidate the mechanism of gelation in solutions. The echo signal intensity of agar decreased steeply and the diffusion coefficient D of agar increased near the sol-to-gel transition temperature T_{s-g}, and D decreased with further cooling. These results suggested that the polysaccharide chains in agar aggregated in bundles to form a network at around T_{s-g}. High molecular weight chains aggregated preferentially in agar, with the soluble, non-aggregated agar ("solute agar") left in the network forming loose aggregates upon further cooling. Evidence for this behavior was obtained from GPC measurements on the solute agar squeezed from the gel. These loose aggregates readily disassociated on reheating, whereas the aggregated bundles were quite thermally stable, which corresponded well with the thermal stability of the gel strength. The changes in the restrictions on molecular mobility in these solutions were evaluated from measurements of D of a dendrimer added as a probe molecule, which was sensitive to the dilution of the solute agar accompanying gelation. The hydrodynamic shielding length ξ, which was considered to represent the hydrodynamic mesh size created by the solute agar, was calculated from D of the dendrimer, shedding light on the changes in the microscopic environment during gelation.

1. Introduction

Agar is a sulfated galactan extracted from marine red algae and is widely used as a texturing agent in food and non-food applications (Nussinovitch, 1997, chap. 1). The wide use of agar is based on its unique ability to form strong gels in aqueous solutions. On cooling, the gelation of agar solutions is initiated by a coil-to-helix transition, followed by the aggregation of the helices to form a network structure, i.e., a gel. Agar is composed of agarose and agaropectin, the former consisting of neutral polysaccharides with a high gelling ability and the latter consisting of ionic polysaccharides with a low gelling ability (Nussinovitch, 1997, chap. 1). The proportion of agarose to agaropectin in agar varies depending on the seaweed source, affecting the physicochemical, mechanical and rheological properties of agar solutions.

Field-gradient NMR is a powerful tool for studying the self-diffusion of molecules in a gel matrix as well as for investigating gel structure and analyzing the mechanism of gel formation (Baldursdóttir, Kjøniksen, & Nyström, 2006; Kwak & Lafleur, 2003; Matsukawa et al., 1999; Walderhaug, Söderman, & Topgaard, 2010). The diffusion coefficient D of the diffusant decreases significantly with increasing intermolecular interaction, particularly hydrogen bonding (Matsukawa & Ando, 1996). If no direct intermolecular interaction occurs, D of the diffusant decreases because of hydrodynamic interactions associated with solvent movement (Doi & Edwards, 1986, chap. 3). In this article, the self-diffusion of solute polysaccharide chains in agar was investigated by the pulsed-field-gradient stimulated echo (PFG-STE) ^{1}H NMR method to elucidate structural changes and dynamics in agar solutions during the gelling process. To shed further light on this process, a dendrimer (a highly branched spherical molecule with a narrow molecular weight distribution) with COONa terminal groups was introduced into a 2.3 wt% agar system, and the reduction in D of the dendrimer was related to the microscopic environment in the agar solution.

2. Materials and methods

2.1. Materials

The agar (BA-10, $M_w = 3.44 \times 10^5$ g/mol) was kindly supplied by Ina Food Industry Co. Ltd. (Nagano, Japan) and used without further purification. A 5 wt% solution of poly(amidoamine) (PAMAM) dendrimer (generation 6.5, COONa terminal groups) in methanol was purchased from Sigma–Aldrich Ltd., diluted with D_2O and

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doi:10.1016/j.foodhyd.2011.04.021

concentrated by repeated nitrogen purging to evaporate the methanol.

2.2. Sample preparation

The agar powder was dispersed in D_2O with stirring at room temperature overnight, and then dissolved completely by stirring at 95 °C for 6 h. The agar solution used to measure the diffusion of the dendrimer was made by stirring the agar solution with the dendrimer solution at 80 °C for 30 min. The resulting solutions were immediately transferred into preheated 10 mm NMR tubes. Finally, 2.3 wt% agar solutions with and without 0.1 wt% dendrimer were prepared. After the measurements, the agar content was measured by drying samples at 105 °C for ca. 12 h and weighing the residues.

A 0.1 wt% agar solution was prepared for GPC measurements by dissolving agar powder at 95 °C. In order to squeeze the solute agar from the gel for GPC measurements, gels were prepared by cooling agar solutions from 60 to 25 °C at 0.1 °C/min in a temperature-programmed oven, cut into small pieces and centrifuged for 15 min at 10,500 g at 25 °C to give supernatants containing the solute agar.

2.3. Methods

2.3.1. Measurement of sol-to-gel transition temperature (T_{s-g})

The sol-to-gel transition temperature (T_{s-g}) was determined using a falling-and-rolling ball method. A small nylon-coated steel ball was placed in a test cell with the agar solution. The samples were placed in a temperature-programmed oven, which tilted from 12° to −12° alternatively every 2 min. The temperature was decreased from 60 to 25 °C at 0.1 °C/min to determine T_{s-g}, the temperature at which the ball stopped moving.

2.3.2. NMR measurements

Self-diffusion coefficient measurements for polymers were carried out on a Bruker Avance II 400WB spectrometer operating at 400.13 MHz for protons, using the PFG-STE NMR pulse sequence (Fig. 1). The interval τ_2 between the first two 90° pulses and the interval τ_1 between the last two 90° pulses were set at 2.12 ms and 7.85 ms, respectively. The 90° pulse length for ^{1}H was 16 μs with a relaxation delay of 5 s, and 32 accumulations of 8192 data points each were collected. The temperature was controlled using a variable temperature unit (Bruker BVT-3200) and was continuously monitored with an optical fiber thermometer (Takaoka Electric Manufacturing Co., Tokyo, Japan) placed in the sample tube. The temperature was reduced from 60 to 25 °C at 0.1 °C/min, held at 25 °C for 15 h and then increased to 60 °C at the same rate. The self-diffusion coefficients were calculated from the decrease in peak intensity with increasing field gradient (Johnson, 1999; Stejskal & Tanner, 1965). The attenuation of peak intensity in the spin-echo spectra was expressed as follows:

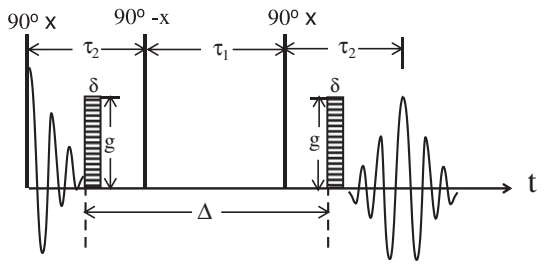

Fig. 1. PFG-STE pulse sequence.

$$I(g) = I(0)\exp\left[-\gamma^2 g^2 D\delta^2(\Delta - \delta/3)\right] \quad (1)$$

where $I(g)$ and $I(0)$ represent the echo intensities at $t = 2\tau_2 + \tau_1$ with and without the field gradient, respectively, and γ is the gyromagnetic ratio of ^{1}H. The gradient pulse length δ and the interval Δ between the two gradient pulses were 1 ms and 10 ms, respectively. The gradient field strength g varied from 2.0 to 8.0 T/m. Chemical shifts (relative to DSS) were referenced to HDO at 4.766 ppm at 25 °C.

2.3.3. GPC measurements

The samples were filtered using a 0.45 μm filter just before GPC (HLC-8120 GPC, Tosoh Co. Ltd., Japan) measurements. Aqueous 0.1 M NaCl was used as the carrier solvent at a flow rate of 1 ml/min. The inlet and column temperatures used for GPC measurements on different samples are listed in Table 1. GPC calibrations were performed using the Pullulan standard P-82 (Shodex, Japan), with both the inlet and column temperature set at 35 °C.

2.3.4. Determination of the dendrimer diffusion coefficient in dilute D_2O solution

Measurements of the dendrimer diffusion coefficient in dilute D_2O solution ($D_{dend,0}$) at 24.3, 32.0, 35.1 and 40.3 °C were carried out by varying the diffusion time in order to correct for convection effects (Matsukawa, Sagae, & Mogi, 2009). The diffusion time dependence of $D_{dend,0}$ at 24.3 °C was small and the correction for convection effects was successfully performed using the linear relation between $D_{dend,0}$ and the diffusion time. On the other hand, $D_{dend,0}$ at 32.0, 35.1 and 40.3 °C increased greatly with increasing diffusion time, with considerable variation. Therefore, the hydrodynamic radius R_H of the dendrimer was calculated from $D_{dend,0}$ at 24.3 °C using the Stokes–Einstein equation:

$$R_H = k_B T/(6\pi\eta_s D_{dend,0}) \quad (2)$$

where k_B, T, and η_s are the Boltzmann constant, the absolute temperature and the viscosity of the solvent, respectively. Because R_H of the dendrimer was taken to be constant, the values of $D_{dend,0}$ at various temperatures were calculated as:

$$D_{dend,0} = k_B T/(6\pi\eta_s R_H) \quad (3)$$

3. Results and discussion

3.1. Molecular mobility of agar chains

Fig. 2 shows an example of stacked PFG-STE ^{1}H NMR spectra for a 2.3 wt% agar solution at various temperatures. Peaks due to HDO were completely eliminated due to its faster diffusion, leaving only agar signals remaining. A marked decrease in the intensity of the agar peaks could be observed during cooling and a slight increase during heating. The intensity of the agar peaks at chemical shifts from 3.0 to 5.8 ppm was analyzed. An example of a plot of $I_{agar}(g)$ at 50 °C against $\gamma^2 g^2 \delta^2(\Delta - \delta/3)$ is shown in Fig. 3, which was obtained

Table 1
Experimental conditions for GPC measurements on different samples.

Samples	Temperature/°C			
	Squeezing	Setting	Inlet	Column
(a). Prepared by dissolving agar powder in water	–	95	60	60
(b). Squeezed from 2.3 wt% agar gel	25	r.t.	30	30
(c). Squeezed from 2.3 wt% agar gel and heated at 95 °C for 1 h	25	95	60	60