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Coil-to-helix transition of gellan in dilute solutions is a two-step process

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ABSTRACT

Thermodynamics of coil-to-helix transition of natural polysaccharide - gellan was studied in dilute solutions in 130 mM NaCL in concentration range 0.01–1.5% using SETARAM C80 3D Calvet calorimeter operated in a passive scanning mode at low cooling rate (0.04 K/min at the transition temperature). It was shown that the negative values of the total enthalpy of the coil-to-helix transition substantially increased in a step-wise fashion if the concentration of the solution decreased below the threshold of coil overlapping in accordance with the results of dynamic light scattering. The shape of thermograms depended on the concentration of solutions. While in semi-dilute solutions with gellan concentration above 0.25% only one exothermic peak was indicated in the cooling mode, in dilute solutions two distinct peaks of the two-step transition: first - endothermic and second - exothermic were clearly observed. The endothermic and exothermic peaks overlapped for semi-dilute solutions of gellan and became progressively separated as the concentration diminished. The reasons, which underlie the two-step nature of coil-to-helix transition of gellan are discussed based on the origin of molecular interactions involved.

1. Introduction

Gellan is a commercially available industrial extracellular bacterial polysaccharide synthesized by *Sphingomonas elodea* (formerly *Pseudomonas elodea*) (Kang, Veeder, Mirrasoul, Kaneko, & Cottrell, 1982; Pollock, 1993). It is commercially used as a gelling agent in food, pharmacy, medicine, cosmetics and related applications (Gibson, & Sanderson, 1997, Osmałek, Froelich, & Tasarek, 2014, Prajapati, Jani, Zala, & Khutliwala, 2013; Sanderson, 1990). Gellan has an anionic linear chain which comprises tetrasaccharide repeat units of two residues of b-D-glucose, one of b-D-glucuronate, and one of a-L-rhamnose (Jansson and Lindberg, 1983; Milas, Shi, & Rinaudo, 1990). As biosynthesized, gellan repeat unit has glyceryl and acetyl substituents in one of the glucose units (Kuo, Mort, & Dell, 1986). However, in commercial products they are typically removed during the hot alkali treatment of the fermentation broth. Like other industrial gelling agents such as xanthan, guar, agarose, etc., gellan can be readily dissolved in hot water and undergoes gelation upon cooling of the solution, which is in fact a target property for its application. Apart from its undisputable value for the industrial applicability the process of gelation is a very interesting phenomenon from the fundamental point of view in respect to the basic principles of behavior of native polysaccharides in their water solutions. The basic features of gellan gelation were extensively studied and are presented in several profound reviews (Morris, Nishinari, & Rinaudo, 2012; Nishinari, 1996; Rinaudo & Milas, 2000; Valli & Clark, 2010).

It was shown that the essence of the gelation phenomenon of gellan is the coil-to-helix transition of its macromolecular chains (Morris et al., 2012; Upstill, Atkins, & Atwool, 1986). At elevated temperature the coil conformation of gellan macromolecule is thermodynamically favorable. Upon cooling it, however, converts to the conformation of a double 3-fold left-handed helix with a pitch of 5.64 nm (Chandrasekaran, Millane, Arnott, & Atkins, 1988;; Morris et al., 2012). The temperature of this transition is around 40 °C and it is affected by a number of factors like salt concentration, pH, presence of other polysaccharides and organic additives (Morris et al., 2012). The coil-to-helix transition in gellan solution provided a consequent effect on a variety of measurable properties, which were extensively studied (Morris et al., 2012). In the present

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study we focus on the thermodynamic aspects of this transition, which are presented by the differential scanning calorimetry (DSC).

In several studies (Mazen, Milas, & Rinaudo, 1999; Miyoshi and Nishinari, 1999; Miyoshi, Takaya, & Nishinari, 1994; Miyoshi, Takaya, & Nishinari, 1995; Miyoshi, Takaya, & Nishinari, 1996; Ogawa, Takahashi, Yajima, & Nishinari, 2006) it was established that the coil-to-helix transition upon cooling of gellan solution provides an exothermic peak at DSC thermogram. The peak shifts to high temperatures if salt is added to the solution (Miyoshi et al., 1995, Miyoshi and Nishinari, 1999). The enthalpy of the transition was found to be close to −10 J/g in the 1% solution (Miyoshi and Nishinari, 1999). It was clearly shown that while there is a single exothermic peak on DCS thermograms of the transition in cooling experiments there can be one or two endothermic peaks on DSC plots in heating experiments depending on the concentration of gellan solution (Morris et al., 2012; Miyoshi and Nishinari, 1999). In the concentration range 1–3% of gellan only one endothermic peak was indicated at the same temperature as the coil-to-helix transition took place upon cooling. At higher concentration, however, the second endothermic peak at higher temperature was also observed. The temperature interval between the peaks increased as the concentration grew up. These two peaks were attributed to the melting of the solitary helixes of gellan and to the melting of the aggregated helixes, which formed the true gel (Morris et al., 2012).

In general, it means that the helix-to-coil transition (melting of helixes) of gellan is affected by the spatial restrictions. Meanwhile, it looks reasonable that the spatial restrictions due to the overlapping of coils might as well influence the formation of double helixes of gellan in the coil-to-helix transition upon cooling. The idea to clarify the limits of such restrictions originated the objective of the present study. We intended to perform DSC measurements at the conditions providing gradual diminishing of spatial restrictions for the coil-to-helix transition of gellan. Apparently, it required the measurements in gellan solutions with progressive dilution below the concentration around 1% which is up to date the lowest concentration reported in DSC studies. It is shown below that the dilution of gellan solution strongly affects the total value of the enthalpy and reveals two separate steps of coil-to-helix transition.

2. Experimental section

2.1. Materials

Gellan was purchased from Sigma-Aldrich under the brand name Phytagel™ (Sigma-Aldrich, St. Louis, USA). Gellan solutions were prepared as described elsewhere (Tanaka & Nishinary, 2007). Gellan powder was vigorously stirred in 130 mM NaCl in distilled water at 90 °C until visual homogeneity was achieved. Then obtained solutions were hot filtered through 10 μm Pyrex glass filter and thermostated for 4 h at 90 °C before measurements.

2.2. Methods

Differential scanning calorimetry (DCS) measurements were performed in the passive scanning mode, which was developed earlier (Vshivkov and Safronov 1997) for the observation of a coil – globule phase transition in polystyrene solutions at low concentration and low cooling rate. We elaborated SETARAM 3D C80 microcalorimeter with 15 cm^3 cells. In this case we gained both from the high sensitivity of the 3D calorimeter sensor and from the extended capacity of the cell, which allowed to load up to 12 g of gellan solution compared to maximum 1 g of it in the commercial SETARAM μDSC3 evo. However, certain limitation arose as well. Due to the comparatively large size of a thermostat and the consequently high thermal inertia SETARAM C80 microcalorimeter is not well suited for the temperature scanning in a linear mode regime. This mode implies low thermal inertia of the cell to maintain the stability of a controlled linear baseline. To overcome this limitation we used the regime which consisted of the four consequent steps: 1) linear heating from room temperature up to 90 °C at 1 K/min, 2) isothermal equilibration at 90 °C for 1.5 h, 3) cooling down to 20 °C temperature at 1 K/min, 4) isothermal step for 20 h. In fact, in the third and in the fourth steps the heater of the thermostat was effectively turned off and the calorimeter slowly cooled down to the room temperature in a passive mode. The total duration of these steps took approximately 24 h. The third and the fourth steps of the DSC regime were scheduled for the nighttime to insure the stability of the external conditions of passive scanning mode. During the passive scanning both the temperature and the baseline signal exponentially decreased. Due to the exponential decrease of the temperature the scanning rate was not a constant but a diminishing value. Meanwhile, it was very low: 0.1 K/min at the beginning of the scanning at 90 °C and 0.04 K/min at temperatures around the coil-to-helix transition. Such a low scanning rate insured the equilibrium conditions for the transition.

Another limitation of the passive scanning was that the room temperature was the lowest achievable temperature in the experiment. As the coil-to-helix transition of gellan in pure water takes place near the room temperature (Morris et al., 2012), its observation under such a limitation becomes uncertain. Therefore the study was performed for the solutions of gellan in 130 mM NaCl. It was reported in literature (Miyoshi et al., 1995;; Miyoshi and Nishinari, 1999;; Morris et al., 2012) that the temperature of the transition increases at the presence of simple electrolytes. At 130 mM NaCl it was reported between 40 °C and 50 °C (Morris et al., 2012). It made a necessary deviation from room temperature to perform reliable measurements.

During the experiments the weighted amount of gellan solution was placed in a 15 cm^3 stainless steel working cell of a C80 calorimeter and was tightly closed. The reference cell contained the equal amount of distilled water. The cells were weighted before and after the experiment to control the absence of vapor leakage during temperature steps.

Dynamic light scattering (DLS) of gellan solutions at 60 °C was performed using Brookhaven ZetaPlus analyzer. Stock gellan solution (1%) for the measurements was prepared as described in the Materials section. Then the hot solution was diluted to the diminishing concentrations. The resulted solutions were not further filtered through fine (100 nm) Wattman filters but placed in optical cuvettes and stored for 20 h at 60 °C for the sedimentation of dust particles. Then they were placed in the analyzer and thermally equilibrated at 60 °C before the measurements.

3. Results and discussion

According to the objective of the study DSC thermograms were taken for gellan solutions with the diminishing concentration to see the effect of spatial limitations upon the enthalpy of coil-to-helix transition. In all cases we used solutions of gellan in 130 mM NaCl.

Fig. 1 presents the DSC thermogram (cooling) for 1% solution of gellan in 130 mM NaCl obtained in the passive scanning mode. The temperature of the calorimeter decreased as a smooth exponent. The exothermic peak of coil-to-helix transition is clearly indicated at the DSC plot. The baseline for the transition was interpolated as a double exponential decay function fitted to the parts of the thermogram before and after the transition. The baseline is shown beneath the peak as a dashed line. In the same fashion DSC thermograms were obtained for the series of diminishing concentrations of gellan solution starting from 1.5% down to 0.01%. All these