Carbohydrate Polymers 75 (2009) 719–723

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Short communication

pH-Dependent interaction between sodium caseinate and xanthan gum

Toshiro Kobori *, Atsuko Matsumoto, Shigeru Sugiyama

Nanobiotechnology Laboratory, National Food Research Institute, National Agricultural and Food Research Organization, 2-1-12 Kan-nondai, Tsukuba 305-8642, Japan

ARTICLE INFO

Article history:
Received 13 June 2008
Received in revised form 4 September 2008
Accepted 6 October 2008
Available online 17 October 2008

Keywords:
Xanthan gum
Sodium caseinate
Rennet
Atomic force microscopy

ABSTRACT

Xanthan gum and sodium caseinate are used to improve stability and texture of food. To investigate interactions between them, the effects of pH on structure of sodium caseinate–xanthan gum complex were analyzed. HCl titration showed that the absorbance of the mixture was different from that of sodium caseinate alone throughout the acidification, and that syneresis in the mixture was delayed in acidic pH. Rennet digestion clarified that xanthan gum retarded degradation of κ-casein at pH 2.7. Atomic force microscopy revealed that xanthan gum interaction with sodium caseinate was pH-dependent. Sodium caseinate particles were individually bound with xanthan gum at pH 6.6, and a side-by-side aggregation of sodium caseinate along xanthan gum was observed at pH 4.2. The mixture formed a network composed of rod-like fibers at pH 2.7. These results indicate that hydrophobic and electrostatic interactions play a role in the complex formation at neutral and acidic pH, respectively.

1. Introduction

Food products are composed of numerous ingredients, in which physical properties, such as texture and stability, are mainly controlled by gels or foams built up with biopolymers including proteins and polysaccharides. Therefore, it is imperative to understand the underlying mechanisms that regulate the interactions between polysaccharides and proteins (Dickinson, 1995; Tolstoguzov, 1997), thereby contributing to the improvement and modification of the physical properties of food.

Due to its nutritional and functional importance, Na-caseinate is used as an ingredient in a wide range of food products. Na-caseinate is prepared from coagulated casein-micelles, which are subsequently washed and neutralized with NaOH. Na-caseinate contains four phosphoproteins, α_{s1}-, α_{s2}-, β-, and κ-caseins (Aoki, Uehara, Yonemasu, & El-Din, 1996) and is ~10 nm in diameter (Chu, Zhou, Wu, & Farrell, 1995; Pepper & Farrell, 1982). Na-caseinate particles are considerably smaller than casein micelles (50–500 nm, Fox, 2003). As observed in casein-micelles, acidification of Na-caseinate facilitates a gel formation around the isoelectric point (pI). The physical properties of acid-induced Na-caseinate gel are closely associated with its intrinsic chemical properties (Swaisgood, 1993) and extrinsic factors, including pH, temperature, and ionic strength (Casanova & Dickinson, 1998; Lee, Morr, & Ha, 1992; Lieske & Konrad, 1994). Xanthan gum is an anionic polysaccharide widely used in food products due to its specific physical (viscosity, pseudoplasticity) and chemical (water solubility, pH stability) properties. In the presence of xanthan gum, Na-caseinate forms a gel upon acidification. Na-caseinate does not show a clear phase separation at neutral pH in the presence of xanthan gum at low concentrations (~0.1%) (Nash, Pinder, Hemar, & Singh, 2002). However, an interaction between Na-caseinate and xanthan gum remains undetermined.

Atomic force microscopy (AFM) has been successfully implemented in visualizing the conformation and gelation of xanthan gum at the single molecular level (Iijima, Shinozaki, Hatakeyama, Takahashi, & Hatakeyama, 2007; Kirby, Gunning, & Morris, 1995, 1996) Although AFM can also visualize protein–polysaccharide complexes (Adams, Kroon, Williamson, Gilbert, & Morris, 2004; Kirby, MacDougall, & Morris, 2006; Morris, Gunning, Faulds, Williamson, & Svensson, 2005), to our knowledge, there is no direct evidence for xanthan gum–protein complex. In this study, we describe an interaction between xanthan gum and Na-caseinate at both neutral and acidic pH based on biochemical analyses combined with AFM.

2. Materials and methods

2.1. Materials

Na-caseinate and xanthan gum were purchased from Sigma Aldrich (MO, USA). The molecular weight of this commercial xanthan gum has been reported to be a few million (Khouryieh, Herald, Aramouni, Bean, & Alavi, 2007; Sato, Norisuye, & Fujita, 1984). Twenty-five milligram per milliliter Na-caseinate solution was prepared according to the method previously described (Semo, Kesselman, Danino, & Livney, 2006). Xanthan gum was dissolved in Milli-Q water at a final concentration of 5 mg/ml, and was completely dispersed by gentle rotation for 16 h at room temperature.

* Corresponding author. Tel.: +81 29 838 8054; fax: +81 29 838 7319.
E-mail address: tkobo@affrc.go.jp (T. Kobori).

0144-8617/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2008.10.008

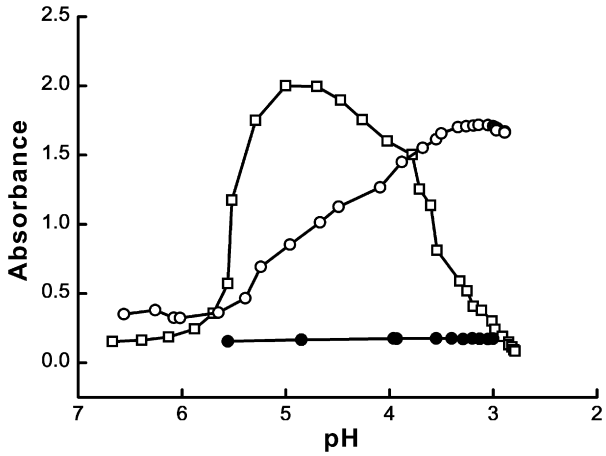

Fig. 1. Absorption profiles during HCl titration. Changes in absorption (515 nm) during HCl titration for Na-caseinate (open square), Na-caseinate–xanthan gum mixture (open circle), and xanthan gum (filled circle) were measured at room temperature.

2.2. Absorbance measurement of Na-caseinate

The absorbance and pH of 1 mg/ml Na-caseinate with or without 1 mg/ml xanthan gum were measured as previously described (Ye, Flanagan, & Singh, 2006) with some modifications. Twenty milliliter of each sample was titrated by repeated additions of 0.2 ml of 0.01 N HCl, and the pH value was measured 10 min after every addition. Absorbance was also measured at 515 nm using UV-1600 (Shimadzu Corporation, Japan).

2.3. Degradation of Na-caseinate by rennet

One milligram per milliliter Na-caseinate with or without 1 mg/ml xanthan gum was resuspended in McIlvaine's buffers (Diem & Leutner, 1970) at various pH values (2.7, 4.2, 5.1, and 6.6) The samples were incubated at 37 °C for 60 min, and digested with 33 ng/μl rennet (MP biomedicals, CA, USA) at 37 °C for 0, 5, 10, 30, 60, 120, and 180 min. Rennet digestion was terminated by heating the samples. The samples were run on SDS–PAGE.

2.4. Atomic force microscopy of xanthan gum

One milligram per milliliter xanthan gum, with or without 1 mg/ml Na-caseinate, was prepared at pH 2.7, 4.2 and 6.6. After incubation at 37 °C for 60 min, the samples were diluted with Milli-Q water to provide 1 μg/ml xanthan gum. Freshly cleaved mica was treated with 10 mM $MgCl_2$ and 5 μl of each sample was incubated on the mica for 5 min at room temperature. The sample droplet was blown away and dried by air. The samples were subjected to AFM in air using NanoWizard (JPK instruments, Germany). AFM was operated in the intermittent contact mode. A silicon cantilever, OMCL-AC160TS-C2 (Olympus Corporation, Japan), was used for imaging. The captured images were flattened prior to analyses with the software equipped with the AFM.

3. Results and discussion

3.1. HCl titration of Na-caseinate–xanthan gum mixture

Absorbance of 1 mg/ml Na-caseinate, with or without 1 mg/ml xanthan gum, during HCl titration was monitored at 515 nm (Fig. 1). Absorbance for Na-caseinate alone was pH-dependent. The absorbance was constant in the range from pH 7 to pH 6, and increased abruptly when the pH approached its pI. Further titration dropped the absorbance due to syneresis and denaturation. This is explained by a change in net charge of Na-caseinate as a function of pH (Ye et al., 2006). The Na-caseinate–xanthan gum mixture exhibited a profile distinct from that of Na-caseinate. The absorbance gradually increased at around pH 6, peaking at pH

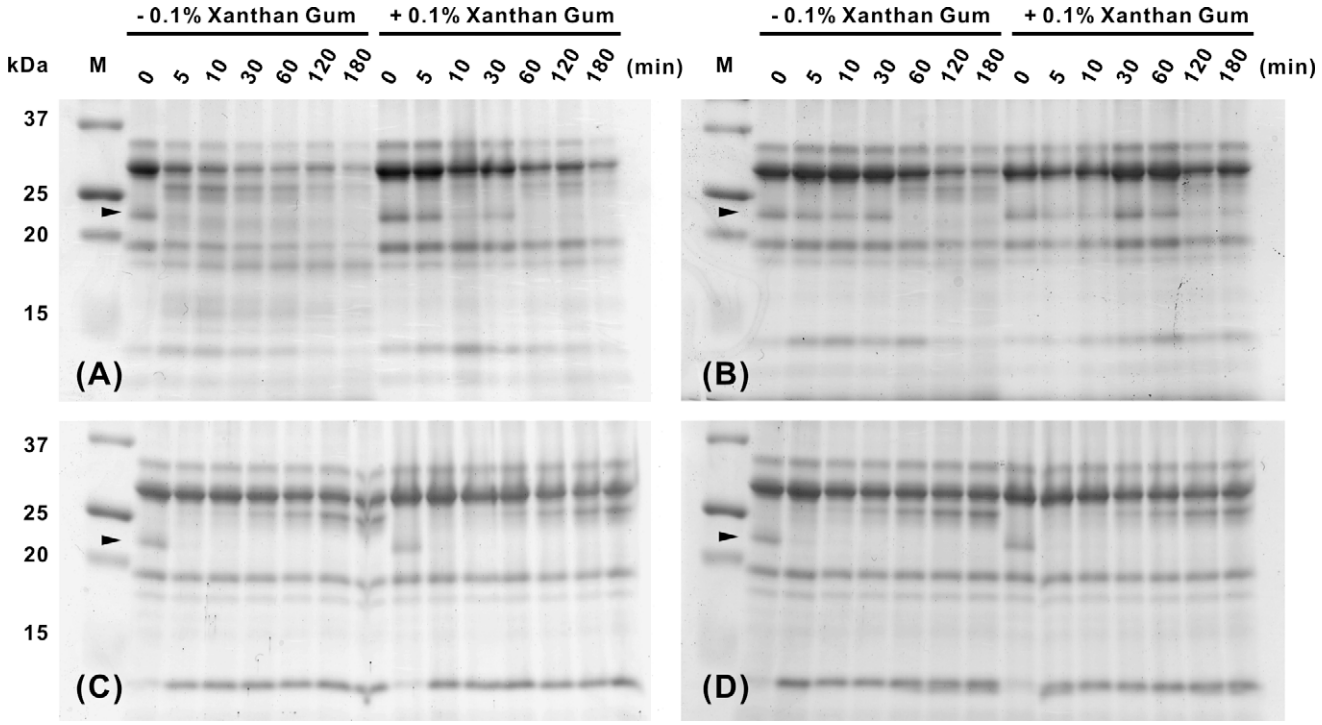

Fig. 2. Effect of incubation time on κ-casein digestion (in the presence or absence of xanthan gum) by rennet. Na-caseinate (1 mg/ml) alone or complexed with xanthan gum (1 mg/ml) were treated with rennet at 37 °C for 0, 5, 10, 30, 60, 120, 180 min at pH 2.7 (A), pH 4.2 (B), pH 5.1 (C), and pH 6.6 (D). The samples were analyzed by SDS–PAGE and CBB staining. Arrowheads indicate a band corresponding to κ-casein.