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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

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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).

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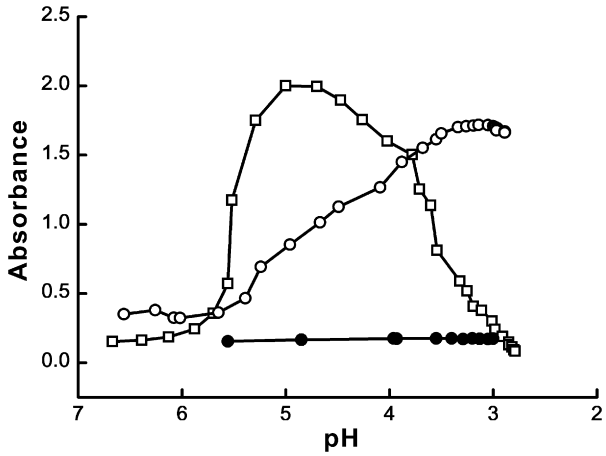

Fig. 1. Absorption profiles during HCl titration. Changes in absorption (515 nm) during HCl titration for Na-caseinate (open square), Na-caseinate–xanthan gum mixture (open circle), and xanthan gum (filled circle) were measured at room temperature.

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

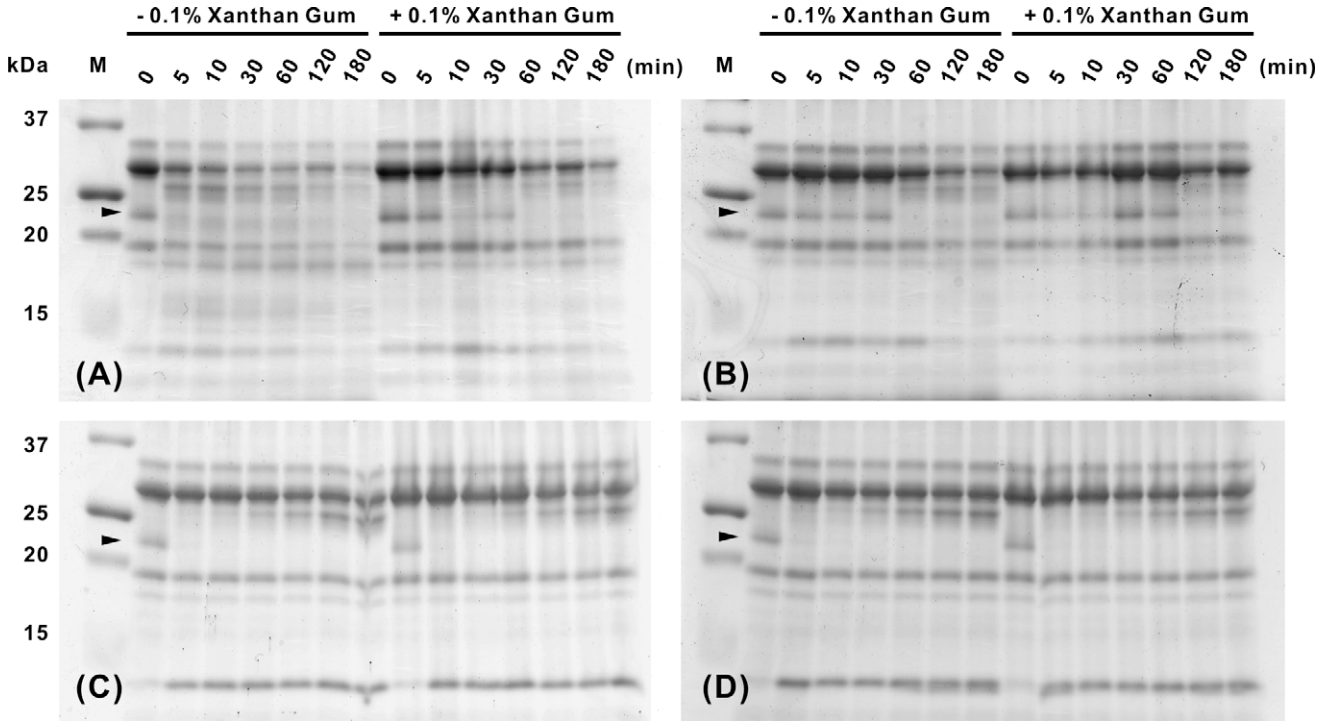

Fig. 2. Effect of incubation time on κ-casein digestion (in the presence or absence of xanthan gum) by rennet. Na-caseinate (1 mg/ml) alone or complexed with xanthan gum (1 mg/ml) were treated with rennet at 37 °C for 0, 5, 10, 30, 60, 120, 180 min at pH 2.7 (A), pH 4.2 (B), pH 5.1 (C), and pH 6.6 (D).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

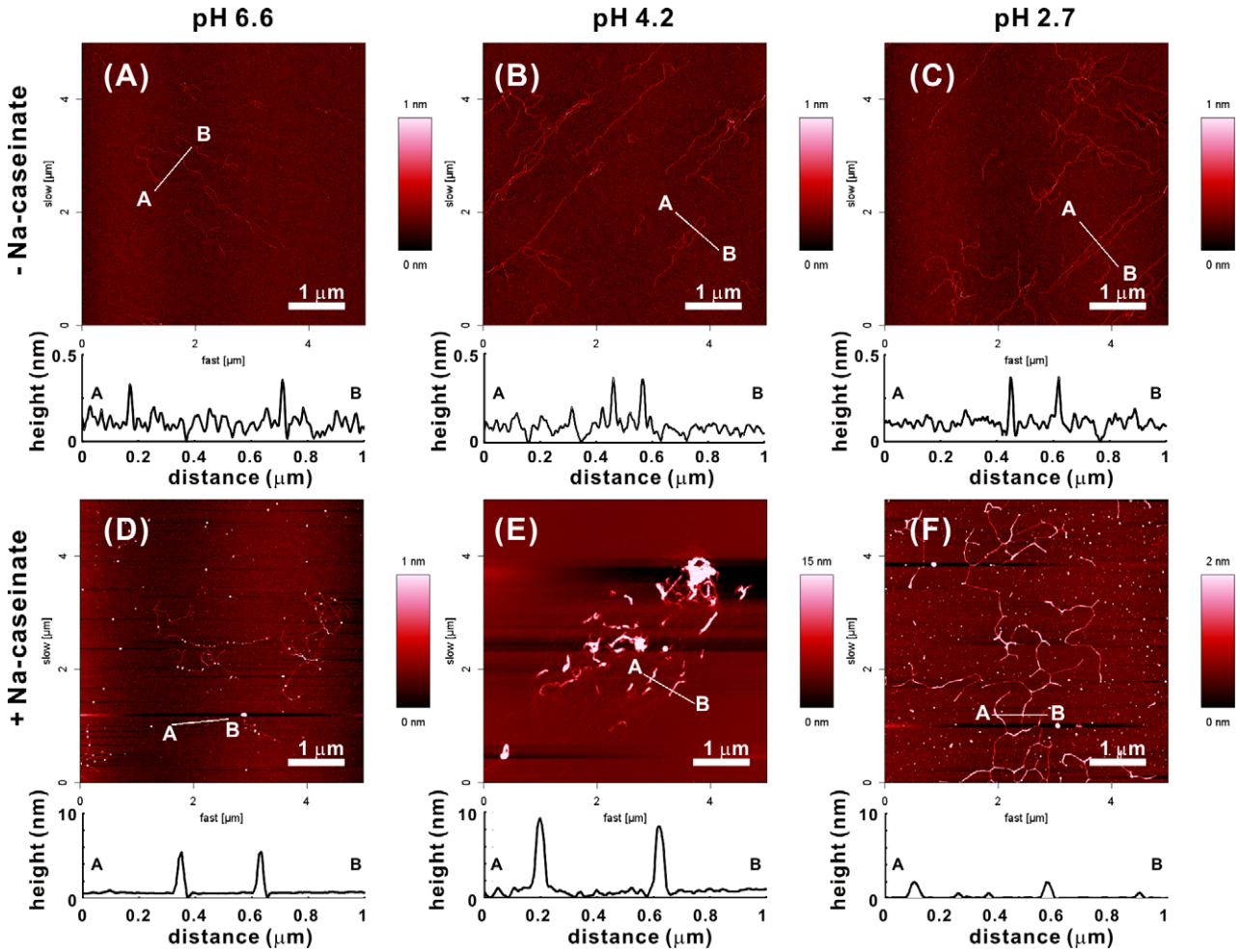

Fig. 3. Effect of pH and Na-caseinate on the structure of xanthan gum. The structure of xanthan gum was imaged using AFM at pH 6.6 (A), pH 4.2 (B) and pH 2.7 (C). The structure of xanthan gum in the presence of Na-caseinate was imaged using AFM at pH 6.6 (D), pH 4.2 (E) and pH 2.7 (F). Scale bars indicate 1 μm. Height scales are 1 nm for (A)–(D), 15 nm for (E), and 2 nm (F). A section profile along the A–B line is shown in a panel below each AFM image.

3.2. The mixture appeared to undergo syneresis at around pH 3. Absorbance of xanthan gum did not change as a function of pH. Absorbance for xanthan gum at pH 7 was not determined because the pH of xanthan gum suspended in Milli-Q was 5.6. However the absorbance of xanthan gum in McIlvaine's buffer (pH 6.6) was 0.16, which was similar to that in the Milli-Q-suspended xanthan gum at pH 5.6. Assuming that the absorbance of xanthan gum would remain near 0.16 at neutral pH, the absorbance of the mixture at a pH between 7 and 6 would be higher than the sum of individual absorbances for Na-caseinate and xanthan gum. Two explanations would account for these observations: (i) Na-caseinate forms a complex with xanthan gum between pH 7 and pH 6; (ii) Na-caseinate solubility decreases due to depletion flocculation. These possibilities would be true for the mixture even below pH 6.

3.2. Effect of pH on Na-caseinate degradation by rennet with or without xanthan gum

Rennet breaks a covalent bond between Phe105 and Met106 in κ-casein on the surface of Na-caseinate. To examine interactions between xanthan gum and Na-caseinate, Na-caseinate was degraded by rennet, over a pH range from 2.7 to 6.6, with or without xanthan gum (Fig. 2). At pH 2.7, complete degradation of κ-casein by rennet was observed in the absence of xanthan gum (Fig. 2A); whereas κ-casein persisted after incubation with rennet for 30 min in the presence of xanthan gum. At pH 4.2, rennet-mediated degradation of κ-casein was also prolonged in the mixture compared to that of Na-caseinate alone (Fig. 2B). Conversely, at pH 5.1 and pH 6.6, κ-casein was degraded by rennet in 5 min regardless of xanthan gum. Degradation patterns at pH 5.1 and 6.6 suggest that increase of viscosity by xanthan gum does not affect rennet activity. Additionally, a comparison of the degradation patterns at pH 2.7 and pH 6.6 shows that, in the absence of xanthan gum, rennet digested κ-casein within 5 min. However, in the presence of xanthan gum at pH 6.6, Na-caseinate was digested faster than at pH 2.7. These indicate that Na-caseinate would be associated with xanthan gum under acidic conditions below pH 4.2.

3.3. A comparison of AFM images of xanthan gum with or without Na-caseinate at pH 6.6, pH 4.2 and pH 2.7

The rennet digestion could not be determined whether the xanthan gum-induced increase in absorbance at a neutral pH range was due to interaction between Na-caseinate and xanthan gum or decreased Na-caseinate solubility (Fig. 1). To clarify effects of pH on the mixture, AFM was used to directly visualize xanthan gum structures with or without Na-caseinate over a pH range of 6.6–2.7 (Fig. 3). AFM imaging in liquid greatly improves image qualities in some cases. Indeed, xanthan gum can be routinely observed with AFM under alcohol such as butanol (Kirby et al., 1995,

1996) and isopropanol (Capron, Alexandre, & Muller, 1998). These alcohols are a precipitant for xanthan gum, and therefore, it fixes xanthan gum on substrate during imaging. However, reliable liquid-imaging of xanthan gum bound with the other material has not been established. On the other hand, alcohols could cause denaturation of Na-caseinate. Indeed, xanthan gum can be also imaged in air (Gunning, Kirby, & Morris, 1996; Iijima et al., 2007). Thus, imaging in air would be suitable for revealing a physical association of Na-caseinate with xanthan gum as is the case with DNA-protein interactions. In this study, therefore, Na-caseinate-xanthan gum mixture was directly imaged with AFM in air.

In fact, xanthan gum in the absence of Na-caseinate were indistinguishable at pH 6.6, 4.2 and 2.7 (Fig. 3A–C). These images show that no condensation effect caused by drying droplet was observed. Section profiles of xanthan gum in these images show that the fibers were ~0.3 nm high (Fig. 3A–C).

Fig. 3D shows that Na-caseinate preferentially interacted with xanthan gum at pH 6.6. The height of Na-caseinate on xanthan gum was ~5 nm, which is similar to the diameter of Na-caseinate in neutral pH (Chu et al., 1995; Pepper & Farrell, 1982). Na-caseinate particles were not closely distributed, but rather associated with xanthan gum as distinct entities. Considered that xanthan gum and Na-caseinate are negatively charged at pH 6.6, a possible reason for the complex formation is that hydrophobic interactions would play a dominant role in binding Na-caseinate to xanthan gum. Indeed, hydrophobic interactions of Na-caseinate at neutral pH are found in a complex with ionic or nonionic fluorescent probes (Gatti, Risso, & Pires, 1995; Gatti, Risso, & Zerpa, 1998; Risso, Gatti, Zerpa, & Perez, 2000). The main chain of xanthan gum is primarily involved in hydrophobic interactions with ethyl-decanoate (Jouquand, Aguni, Malhiac, & Grisel, 2008). The continued activity of rennet at pH 6.6, regardless of xanthan gum (Fig. 2), suggests that the negatively-charged C-terminus of κ-casein does not participate in the complex formation. Therefore, these results suggest that hydrophobic interactions between Na-caseinate and xanthan gum would be weakly attractive due to the electrostatic repulsion.

The phase separation of casein-xanthan gum mixture depends upon a type of casein used (Hemar, Tamehana, Munro, & Singh, 2001). Skim milk and whole protein isolate promote the phase separation upon addition of xanthan gum at neutral pH, whereas, sodium caseinate mixed with xanthan gum does not show a clear phase separation at neutral pH. This loss of segregation in Na-caseinate–xanthan gum mixture is in good agreement with our observation that Na-caseinate was bound with xanthan gum fibers (Fig. 3D). The size of Na-caseinate is ~10 nm, which is much smaller than casein micelles in milk. This suggests that κ-casein distribution in Na-caseinate, which is responsible for the negative charge on the surface, would be also smaller than that of casein micelles, and instead, that hydrophobic region on the surface area in Na-caseinate would be relatively larger than that of casein micelles. Therefore, these also indicate that Na-caseinate would hydrophobically bind with xanthan gum at neutral pH.

Fig. 3E shows Na-caseinate aggregation in the presence of xanthan gum at pH 4.2, in accordance with the higher absorbance of the mixture at pH 4.2 (Fig. 1). Interestingly, Na-caseinate particles were not randomly associated with each other, but aggregated side-by-side along xanthan gum fibers. The height of the resultant fibers was ~10 nm (Fig. 3E), which was higher than Na-caseinate–xanthan gum complex at pH 6.6 (Fig. 3D). This indicates a three dimensional arrangement of Na-caseinate along or around xanthan gum. Condensation effect of the sample droplet may be also a factor of the thicker fiber-like structure in Na-caseinate–xanthan gum mixture at pH 4.2. However, the thicker fiber-like structure was not found in the specimens at pH 6.6 and 2.7, although those were prepared similarly as the specimen at pH 4.2. Although individual xanthan gum fibers were close to each other with some entanglement in the presence of Na-caseinate at pH 4.2, AFM imaging in air clarifies that more concentrated sample of xanthan gum (10 μg/mL), in the presence or absence of an additional annealing procedure, does not also show the thicker fiber-like structure (Gunning et al., 1996; Iijima et al., 2007). These suggest that the fiber-like structure at pH 4.2 would result from a physical association of xanthan gum with Na-caseinate.

The Na-caseinate–xanthan gum mixture showed syneresis at pH 2.7. The sample for AFM was prepared by diluting the complete suspension of the phase-separated mixture. Fig. 3F shows a continuous network of xanthan gum formed with Na-caseinate at pH 2.7. Unlike xanthan gum alone at pH 2.7 as well as Na-caseinate–xanthan gum complexes at pH 6.6 and pH 4.2 (Fig. 2C–E), the fibers were straightly extended and apparently rigid. The network was composed of two different fibers with heights of 1 and 2 nm (Fig. 3F), which differed from the thick fibers observed at pH 4.2 (Fig. 3E).

4. Conclusions

Interactions between xanthan gum and Na-caseinate were studied as a function of pH. Xanthan gum affected the absorption profile of Na-caseinate during the HCl titration, and prevented the complete denaturation of Na-caseinate around pH 3. The pattern of rennet-mediated degradation of Na-caseinate, with or without xanthan gum, revealed an interaction between Na-caseinate and xanthan gum at pH 2.7, but not at pH 5.1 and pH 6.6. Based on these results and AFM imaging, we propose a model for an interaction between Na-caseinate and xanthan gum (Fig. 4): (A) xanthan gum is weakly associated with Na-caseinate at neutral pH through hydrophobic interactions; (B) hydrophobic interactions facilitate a side-by-side aggregation of Na-caseinate along xanthan gum around pI of Na-caseinate; (C) electrostatic attractions promotes a network of xanthan fibers with Na-caseinate at acidic pH.

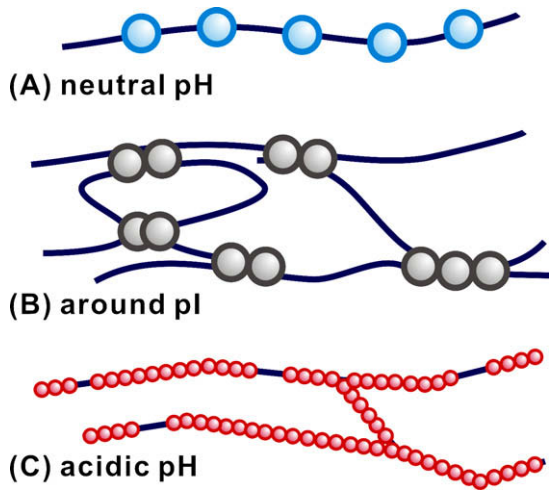

Fig. 4. A schematic representation of the pH-dependent interaction between Na-caseinate and xanthan gum. (A) At neutral pH, negatively–charged Na-caseinate (cyan circle) is loosely associated with xanthan gum fibers (blue line) by means of hydrophobic interactions. (B) Na-caseinate (gray circle) also binds to xanthan gum at pH 4.2, but due to the loss of charge, it causes a side-by-side aggregation along or around xanthan gum and mediates intra-fiber interactions. (C) Na-caseinate is partially denatured far below its pI, and becomes positively charged (red circle). These positively charged proteins are strongly associated with xanthan gum by electrostatic attraction. Colours of Na-caseinate in this figure stand for electrostatic charges of the surface (i.e. cyan, negative charge; gray, neutral charge; red, positive charge). Smaller size of the "circle" for Na-caseinate at pH 2.7 indicates acid-denatured casein proteins.

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References

Adams, E. L., Kroon, P. A., Williamson, G., Gilbert, H. J., & Morris, V. J. (2004). Inactivated enzymes as probes of the structure of arabinoxylans as observed by atomic force microscopy. *Carbohydrate Research, 339*(3), 579–590.

Aoki, T., Uehara, T., Yonemasu, A., & El-Din, M. Z. (1996). Response surface analyses of the effects of calcium and phosphate on the formation and properties of casein micelles in artificial micelle systems. *Journal of Agricultural and Food Chemistry, 44*(5), 1230–1234.

Capron, I., Alexandre, S., & Muller, G. (1998). An atomic force microscopy study of the molecular organisation of xanthan. *Polymer, 39*(23), 5725–5730.

Casanova, H., & Dickinson, E. (1998). Influence of protein interfacial composition on salt stability of mixed casein emulsions. *Journal of Agricultural and Food Chemistry, 46*(1), 72–76.

Chu, B., Zhou, Z., Wu, G., & Farrell, H. M. Jr, (1995). Laser light scattering of model casein solutions: Effects of high temperature. *Journal of Colloid and Interface Science, 170*(1), 102–112.

Dickinson, E. (1995). Emulsion stabilization by polysaccharide and protein–polysaccharide complexes. In A. M. Stephen (Ed.), *Food Polysaccharides and Their Applications* (pp. 501–515). New York: Marcel Dekker.

Diem, K., & Leutner, C. (1970). *Documenta geigy scientific tables 7th edition*. Basle: J.R. Geigy. pp. 280.

Fox, P. F. (2003). Milk proteins: General and historical aspects. In P. F. Fox & P. L. McSweeney (Eds.), *Advanced dairy chemistry-1 proteins part A* (pp. 1–48). New York: Kluwer Academic/Plenum Publishers.

Gatti, C. A., Risso, P. A. H., & Pires, M. S. (1995). Spectrofluorometric study on surface hydrophobicity of bovine casein micelles in Suspension and during enzymic coagulation. *Journal of Agricultural and Food Chemistry, 43*(9), 2339–2344.

Gatti, C. A., Risso, P. H., & Zerpa, S. M. (1998). Study of the inhibitory effect of hydrophobic fluorescent markers on the enzymic coagulation of bovine casein micelles: action of TNS. *Food Hydrocolloids, 12*(4), 393–400.

Gunning, A. P., Kirby, A. R., & Morris, V. J. (1996). Imaging xanthan gum in air by ac "tapping" mode atomic force microscopy. *Ultramicroscopy, 63*(1), 1–3.

Hemar, Y., Tamehana, M., Munro, P. A., & Singh, H. (2001). Viscosity, microstructure and phase behavior of aqueous mixtures of commercial milk protein products and xanthan gum. *Food Hydrocolloids, 15*(4-6), 565–574.

Iijima, M., Shinozaki, M., Hatakeyama, T., Takahashi, M., & Hatakeyama, H. (2007). AFM studies on gelation mechanism of xanthan gum hydrogels. *Carbohydrate Polymers, 68*(4), 701–707.

Jouquand, C., Aguni, Y., Malhiac, C., & Grisel, M. (2008). Influence of chemical composition of polysaccharides on aroma retention. *Food Hydrocolloids, 22*(6), 1097–1104.

Khouryieh, H. A., Herald, T. J., Aramouni, F., Bean, S., & Alavi, S. (2007). Influence of deacetylation on the rheological properties of xanthanguar interactions in dilute aqueous solutions. *Journal of Food Science, 72*(3), C173–C181.

Kirby, A. R., Gunning, A. P., & Morris, V. J. (1995). Imaging xanthan gum by atomic force microscopy. *Carbohydrate Research, 267*(1), 161–166.

Kirby, A. R., Gunning, A. P., & Morris, V. J. (1996). Imaging polysaccharides by atomic force microscopy. *Biopolymers, 38*(3), 355–366.

Kirby, A. R., MacDougall, A. J., & Morris, V. J. (2006). Sugar beet pectin–protein complexes. *Food Biophysics, 1*(1), 51–56.

Lee, S. Y., Morr, C. V., & Ha, E. Y. W. (1992). Structural and functional properties of caseinate and whey protein isolate as affected by temperature and pH. *Journal of Food Science, 57*(5), 1210–1229.

Lieske, B., & Konrad, G. (1994). Thermal modification of sodium-caseinate. 1 Influence of temperature and pH on selected physicochemical and functional properties. *Milchwissenschaft, 49*(1), 16–20.

Morris, V. J., Gunning, A. P., Faulds, C. B., Williamson, G., & Svensson, B. (2005). AFM images of complexes between amylose and *Aspergillus niger* glucoamylase mutants, native and mutant starch binding domains: A model for the action of glucoamylase. *Starch/Stärke, 57*(1), 1–7.

Nash, W., Pinder, D. N., Hemar, Y., & Singh, H. (2002). Dynamic light scattering investigation of sodium caseinate and xanthan mixtures. *International Journal of Biological Macromolecules, 30*(5), 269–271.

Pepper, L., & Farrell, H. M. (1982). Interactions leading to formation of casein submicelles. *Journal of Dairy Science, 65*(12), 2259–2266.

Risso, P. H., Gatti, C. A., Zerpa, S. M., & Perez, G. R. (2000). Comparative study of the action of anionic and non-ionic hydrophobic fluorescent markers on the enzymic coagulation of heated bovine casein micelles. *Food Hydrocolloids, 14*(2), 179–185.

Sato, T., Norisuye, T., & Fujita, H. (1984). Double-stranded helix of xanthan in dilute-solution – evidence from light-scattering. *Polymer Journal, 16*(4), 341–350.

Semo, E., Kesselman, E., Danino, D., & Livney, Y. D. (2006). Casein micelle as a natural nano-capsular vehicle for nutraceuticals. *Food Hydrocolloids, 21*(5-6), 936–942.

Swaisgood, H. E. (1993). Review and update of casein chemistry. *Journal of Dairy Science, 76*(10), 3054–3061.

Tolstoguzov, V. B. (1997). Protein–polysaccharide interactions. In S. Damodaran & A. Paraf (Eds.), *Food proteins and their applications* (pp. 171–198). New York: Marcel Dekker.

Ye, A., Flanagan, J., & Singh, H. (2006). Formation of stable nanoparticles via electrostatic complexation between sodium caseinate and gum Arabic. *Biopolymers, 82*(2), 121–133.