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Short communication

Visualizing surface active hydrocolloids by atomic force microscopy

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Abstract

Gum arabic from *Acacia senegal* and water-soluble soybean polysaccharide extracted from soybean cotyledons have been visualized using atomic force microscopy (AFM). The images of gum arabic have revealed predominantly linear structures that are considered to represent the arabinogalactan fraction constituting the major fraction in the gum. In contrast, soybean polysaccharide appears as highly branched structures with linear branches of ca. 20–80 nm in length. This observation is consistent with the previously proposed structural model hypothesizing the presence of long neutral sugar side-chains bound to the backbone of rhamnogalacturonan. AFM has provided the first direct evidence for the difference in branching structures in these hydrocolloids widely utilized in the food industry as effective emulsifiers and stabilizers in oil-in-water emulsions.

Keywords: Gum arabic; Soybean polysaccharide; Atomic force microscopy; Emulsifier; Surface activity

1. Introduction

Gum arabic is one of rare hydrocolloids widely used as an emulsifier in the food industry. Not a single polymer species can represent the entire range of chemical components in the gum but three macromolecular fractions have been identified (Islam, Phillips, Sljivo, Snowden, & Williams, 1997; Randall, Phillips, & Williams, 1988, 1989; Williams, Phillips, & Stephen, 1990). The major fraction constituting 70–90% of the total is arabinogalactan, while the second major fraction constituting ca. 10%, arabinogalactan–protein complex, is largely responsible for the emulsifying capability of the gum. Hydrophobic amino acid residues in the protein component are considered to adsorb onto oil droplets at the oil–water interface and anchor hydrophilic polysaccharide chains extending into the water phase. This polysaccharide layer provides an excellent emulsion stability through steric hindrance against aggregation among emulsion droplets. The third fraction representing ca. 1% of the total gum is glycoprotein that should play a certain role in both emulsification and stabilization, albeit to a limited extent due to its low content.

Recently, a novel polysaccharide-based emulsifier has been developed as a byproduct from the manufacturing process of soy protein isolate (SPI) and designated as soybean soluble polysaccharide (SSPS) (Nakamura, Furuta, Kato, Maeda, & Nagamatsu, 2003; Nakamura, Yoshida, Maeda, Furuta, & Corredig, 2004). Similar to the case of gum arabic, this hydrocolloid is considered to contain polysaccharide–protein conjugates, the major polysaccharide fraction of which has been proposed to possess a rhamnogalacturonan backbone with neutral sugar side-chains of 1→4 linked β-galactan, or 1→3 or 1→5 linked α-arabinan (Nakamura, Furuta, Maeda, Nagamatsu, & Yoshimoto, 2001; Nakamura, Furuta, Maeda, Takao, & Nagamatsu, 2002a,b). An intriguing feature of this structural model is the high degree of polymerization of the galactan side-chain that has been estimated to be ca. 43–47 on average (Nakamura et al., 2002b). If this is indeed the case, the molecule should occupy relatively compact space in an aqueous solution compared to an ordinary linear polysaccharide with an identical molecular weight, which may indicate potentialities of realizing novel functionalities using this polysaccharide.

Further structural details in these polysaccharides are yet to be understood. Selective enzymatic degradation analysis

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seems to be a sound approach for determining chemical structures in branched polysaccharides. However, structural heterogeneity among individual molecules is inherent in biopolymers and it tends to be averaged in such chemical analyses. Individual hydrocolloid molecules can be directly visualized using microscopy methods, among which atomic force microscopy (AFM) is particularly of use for visualizing raw biopolymers without making their replicas or staining polymers (Ikeda & Morris, 2002; Ikeda, Morris, & Nishinari, 2001; Ikeda et al., 2004a; Ikeda, Nitta, Temsiripong, Pongsawatmanit, & Nishinari, 2004b; Ikeda & Shishido, 2005; Morris, Kirby, & Gunning, 1999). In this paper, we describe our procedure for AFM imaging of gum arabic and soybean polysaccharide to compliment previously reported results based on chemical and spectroscopic structural analyses.

2. Materials and methods

Gum arabic (Vistop D-2144) from *Acacia senegal* and water-soluble soybean polysaccharide (SM-699) extracted from defatted soybean cotyledons were supplied by San-Ei-Gen F.F.I., Ltd (Toyonaka, Japan) in the form of spray-dried powders. The weight average molecular weight (M_w) and the number average molecular weight (M_n) of gum arabic were $M_w = 585{,}000$ and $M_n = 441{,}000$, respectively ($M_w/M_n = 1.3$), and those of soybean polysaccharide were $M_w = 800{,}000$ and $M_n = 487{,}000$, respectively ($M_w/M_n = 1.6$), according to the supplier's specification based on static light scattering analyses. Other chemicals were of reagent grade quality.

Gum arabic and soybean polysaccharide were dispersed into distilled water to be ca. 1 mg/mL and moderately stirred using a magnetic stirrer overnight at room temperature. These solutions were then diluted to 1–5 μg/mL using distilled water and a 10 mM aqueous solution of a non-ionic surfactant Tween 20 (Kanto Chemical Co., Inc., Tokyo, Japan). Aliquots (2 μL) of the diluted samples were immediately deposited onto freshly cleaved mica surfaces, air-dried for 10–20 min, and imaged by AFM in air. AFM imaging was performed at room temperature using an alternating current (ac) cyclic contact mode of a multimode imaging unit (SPA-400, Seiko Instruments Inc., Chiba, Japan) controlled by a probe station (SPI3800N, Seiko Instruments Inc., Chiba, Japan). Beam-shaped Si cantilevers with a quoted spring constant of 12 N/m were excited at a frequency proximate to a resonant frequency of 136 kHz and the sample surface was scanned with the probe at a scanning frequency of 0.5–1 Hz.

3. Results and discussion

AFM imaging of biopolymer is normally conducted in air or under a liquid in order to avoid excessive dehydration. A typical sample preparation procedure consists of spreading of a dilute (1–10 μg/mL) polymer solution onto a freshly cleaved mica surface and successive air-drying under ambient pressure, temperature, and humidity. Under these conditions, the mica surface is believed to retain a thin (<1 nm) layer of water (Balnois et al., 2000). Fig. 1(a) shows a topographical AFM image of gum arabic deposited from a 1 μg/mL aqueous solution. Only spherical lumps can be seen in the figure, the diameter and height of the lumps ranging from 40 to 140 nm and 1 to 3 nm, respectively. In literatures, polysaccharide molecules prepared using procedures equivalent to the present study have revealed branched or unbranched chain- or rod-like structures with heights in the order of angstroms: the only exception we are aware is the case of barley β-glucan that appears to be spherical particles in certain conditions (Morgan, Roberts, Tendler, Davies, & Williams, 1999). The heights of the spherical structures in Fig. 1(a) are much taller than that of a single polysaccharide chain, suggesting that inter- and/or intra-molecular aggregation is involved. When deposition was made at a slightly higher polymer concentration (5 μg/mL), irregularly shaped large structures were formed together with smaller particles (Fig. 1(b) and (c)). These results demonstrate that the spherical lumps in Fig. 1(a) presumably represent the shape of solution droplets formed during air-drying of sample solutions deposited on mica. Such abnormal behavior does not seem to be surprising if the exceptional surface activity and high water-solubility of gum arabic are taken into account.

Since the arabinogalactan in gum arabic contains negatively chargeable glucuronic acid (Randall et al., 1989), we have tried to electrostatically fix the molecule onto the mica surface by modifying freshly cleaved mica surfaces with positively charged 3-aminopropyltriethoxysilane (Lyubchenko et al., 1992). We have also tried to retain polymer conformation in solution by adding 50–70% of glycerol before deposition (Stokke & Brant, 1990). However, the best and reproducible results were obtained only when a non-ionic surfactant Tween 20 was added to sample solutions (without glycerol) before deposition onto unmodified mica surfaces in order to prevent aggregation of polymer chains during drying. Fig. 2 shows an AFM image of gum arabic deposited onto a freshly cleaved mica surface from a 1 μg/mL aqueous solution containing 2 mM Tween 20. Individual polymer chains, the measured heights of which are reasonably uniform (0.6–0.8 nm), are clearly seen, confirming that adding this level of Tween 20 are sufficient to prevent lateral aggregation between polymer chains. Additionally, judging from the uniformity of the heights, these structures are considered to represent the major fraction of arabinogalactan. Evaluated values of the contour length range from 90 to 270 nm, which seems to be reasonable in comparison with the previously reported values of the radius of gyration and the hydrodynamic radius that are typically in the order of 10 nm (Islam et al., 1997). Nevertheless, these chains appear to be relatively

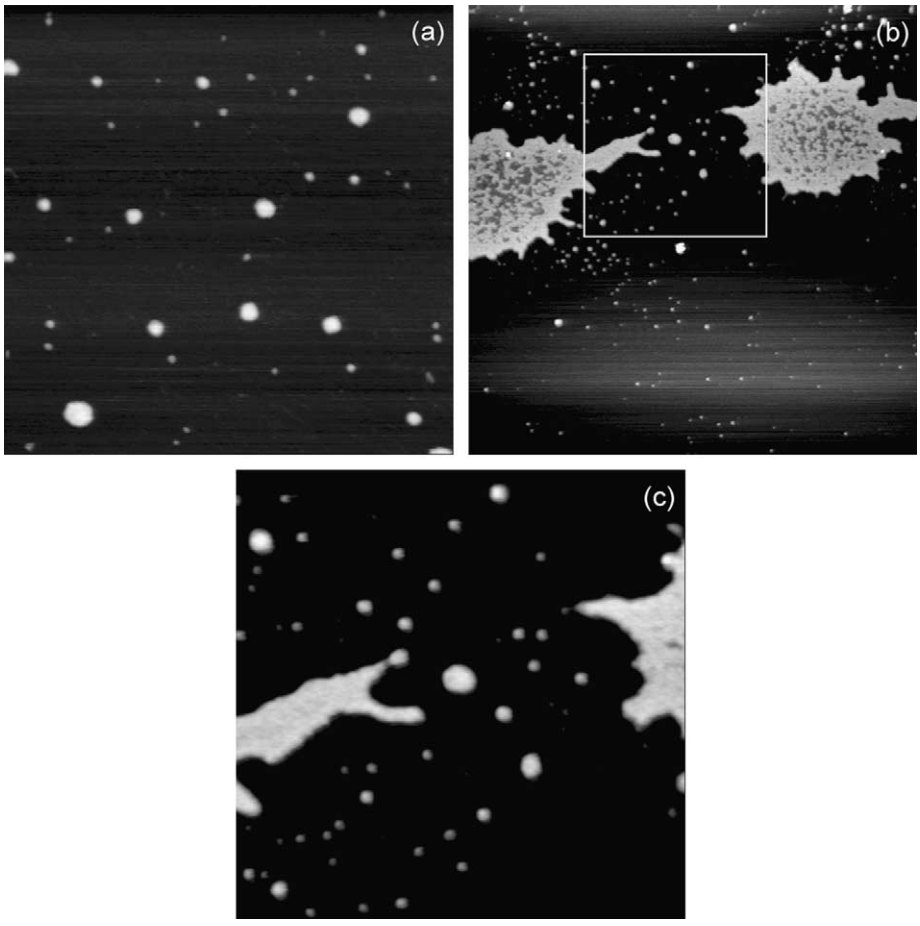

Fig. 1. (a) Image of gum arabic deposited from a 1 μg/mL solution (image size 2 μm×2 μm). (b) Image of gum arabic deposited from a 5 μg/mL solution (image size 5 μm×5 μm). (c) Zoomed-in view (image size 2 μm×2 μm) of the area highlighted in image (b).

short in comparison with the estimated molecular weight (i.e. M_n=441,000), suggesting that only a population of short chains can be visualized using the present method and/or that the chain length per unit mass is relatively short due to highly branched structures. Short side-chains such as the disaccharide side-chain of xyloglucan and the trisaccharide side-chain of xanthan have turned out to be invisible in an AFM image at the present level of magnification (Camesano & Wilkinson, 2001; Ikeda et al., 2004a) although, at the highest, AFM is capable of resolving even sub-molecular structures such as twists of double-stranded helices of DNA and polysaccharide (Morris et al., 1999). Only a small population (∼10%) of visualized molecules had branches longer than ca. 20 nm in this study.

The present sample preparation procedure was successfully applied to imaging of a novel polysaccharide-based emulsifier manufactured from defatted soybean (Fig. 3). This polysaccharide appears as structures resembling *star* or *comb polymer*, the overall dimension of which is typically smaller than 100 nm. The length of long branches ranges approximately from 20 to 80 nm. These results are consistent with the previously proposed structural model consisting of a rhamnogalacturonan backbone with long galactan or arabinan side-chains, the estimated degree of polymerization of which is as large as 47 (Nakamura et al., 2001, 2002a,b). In the present study, a small number of multi-branched polymers are evenly flattened on the mica surface, an example of such a polymer being evident in

Fig. 2. Image of gum arabic deposited from a 1 μg/mL solution containing 2 mM Tween 20 (image size 1.5 μm×1.5 μm).

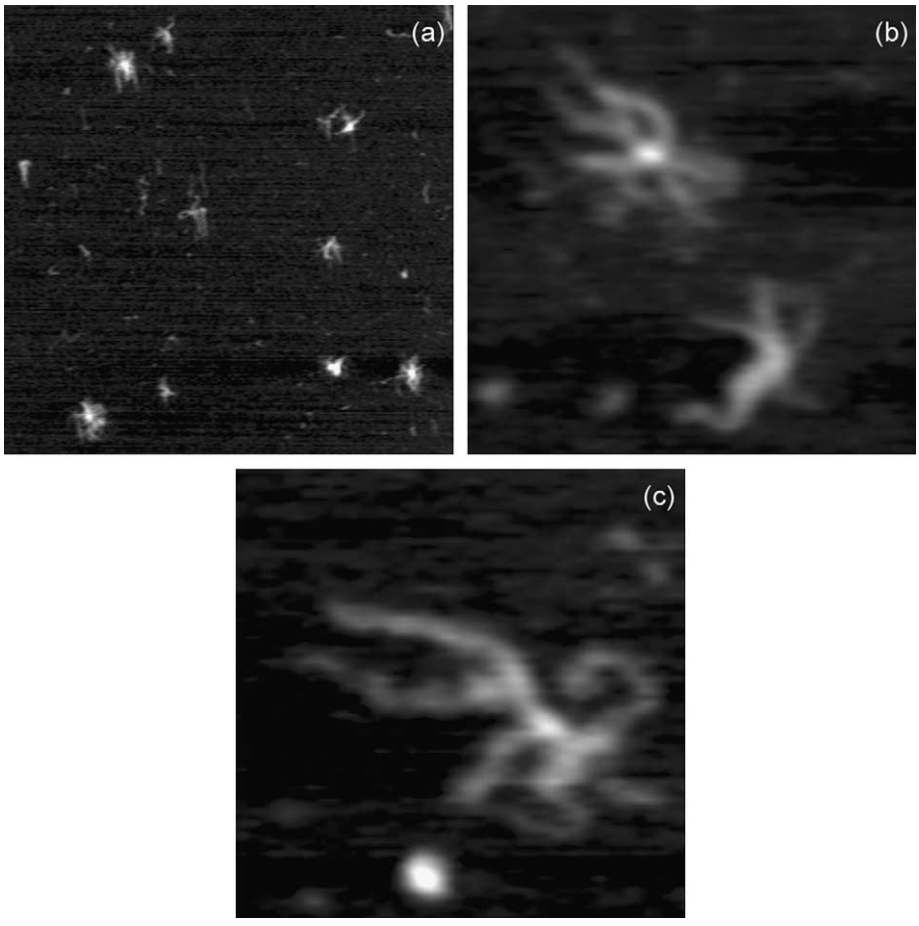

Fig. 3. Image of soybean polysaccharide deposited from a 1 μg/mL solution containing 2 mM Tween 20. Image size: (a) 1 μm×1 μm; (b) 200 nm×200 nm; (c) 150 nm×150 nm.

Fig. 3(c). The uniform height at the branching points in the figure confirms that the structure does not represent overlapping multiple chains but a single branched polymer. The presence of multiple long side-chains is a unique feature of this polysaccharide. While we cannot identify chemical species in each branch at this point, further AFM studies using chemically modified probes and labeling techniques are anticipated to provide more insights into structural details.

4. Conclusions

AFM is confirmed to be capable of directly probing differences in branching structures between gum arabic and soybean polysaccharide. Such structural information is uniquely obtainable using AFM and is also essential for understanding polysaccharide functionalities. The present procedure enables reliable AFM imaging of these surface-active hydrocolloids on a routine basis. Investigation on the whole range of macromolecular components, especially that on polysaccharide–protein complexes that are largely responsible for their emulsifying capabilities and also for mechanisms for stabilizing oil-in-water emulsions, is needed to fully understand structure–function relationships of hydrocolloid emulsifiers.

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