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Note

Surface structure of chitosan and hybrid chitosan-amylose films—restoration of the antibacterial properties of chitosan in the amylose film

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Abstract—The surface structure of films prepared by casting aqueous solutions of mixtures of water soluble chitosan (WSC) and amylose as well as a fully deacetylated chitosan was studied. Zeta potential measurements indicated that the surface of WSC and fully deacetylated chitosan films is positively charged but very weakly, whereas, a film of amylose blended with WSC exhibited an obvious positive charge. X-ray photoelectron spectra of these films suggest that less amino groups are exposed on the surface of WSC and fully deacetylated chitosan films, whereas, more amino groups are exposed on the surface of a WSC film blended with amylose. A sheet structure in which free amino groups are less exposed on the surface of the film of WSC or fully deacetylated chitosan is proposed. This accounts for the loss of antibacterial activity of chitosan on the WSC film surface. When blended with amylose, the morphology of the film may be disrupted, resulting in strong antibacterial properties.

Keywords: Surface structure; Chitosan–amylose film; Zeta potential; X-ray photoelectron spectroscopy; Antibacterial property

Chitosan, a linear polysaccharide composed of randomly distributed (1→4)-linked 2-amino-2-deoxy-β-D-glucose and 2-acetamido-2-deoxy-β-D-glucose units, is among the most promising biomaterials in the world, not only because of its abundance but also due to its various properties. Chitosan is known for its antibacterial properties. However, recent results from our group[1] revealed that a film prepared by casting an aqueous solution of water soluble chitosan (abbreviated as WSC), which is approximately 50% N-acetylated, did not show any antibacterial activity, although WSC showed a strong activity in aqueous solution, as measured by the turbidity method involving *Escherichia coli*.[2] Comparatively, an amylose film containing a low concentration (2.5% w/w or more) of WSC showed a strong antibacterial activity. Since the antibacterial properties of chitosan are considered to be due to the primary amino groups on the molecule,[3] this difference in antibacterial action between WSC and WSC–amylose blend films has to be explained from a morphological point of view. The amino groups may be exposed on the surface of the blended film, but they might be hidden in a WSC film. In order to elucidate the surface structures of such films, zeta potential and X-ray photoelectron spectroscopy measurements were

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performed on the film surface of amylose, WSC, a mixture thereof and a fully deacetylated chitosan film.

Zeta potential measurements have been used to characterize the surface structures of weak ionic charged membranes. Matsumoto et al. have measured the potential of membranes of polyethylene glycol (PEG) derivatives with pendant ionizable groups, and found that a basic type membrane showed positive potential, whereas, not only acid type membrane but also unmodified (neutral PEG) membrane exhibited negative potential.[4]

Present zeta potential measurements indicated that the surfaces of water soluble chitosan films (degree of N-deacetylation, DDA, 44.1%) and fully deacetylated chitosan (DDA 100%) had very small positive potential (Table 1), whereas amylose films containing WSC showed larger positive potential, even though these contained only a small amount of WSC. These findings may suggest that the free amino groups of glucosamine residues are less exposed on the surface of WSC films, resulting in the loss of antibacterial properties[1] and that more amino groups are exposed on the surface of amylose films containing WSC with strong antibacterial properties.[1] Amylose films showed a negative potential as expected for neutral polymer films.[4]

In order to confirm the exposure of amino groups on the surface of the films of WSC (or fully deacetylated chitosan) and WSC–amylose mixtures, we examined X-ray photoelectron spectra (XPS) of WSC–amylose films. As shown in Figure 1 left, the amylose film (a) gave a photoelectron spectrum at a binding energy around 287 eV, reflecting the presence of carbon atoms (C_{1s}) on this polysaccharide. The amylose film containing 2.5% WSC (b) showed, in addition to a C_{1s} peak around 287 eV, a small peak around 402 eV due to N_{1s} of amino and acetamido groups of the WSC molecule. This peak is small because of the low concentration

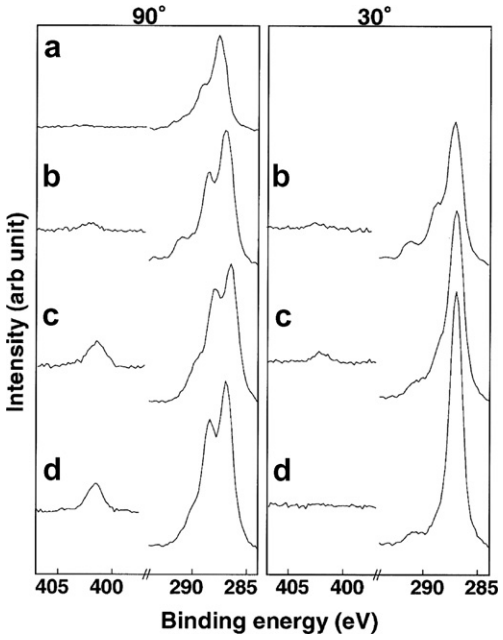

Figure 1. Narrow scan X-ray photoelectron spectra of (a) amylose, (b) water soluble chitosan (WSC)–amylose blend (WSC content 2.5%), (c) WSC, and (d) fully deacetylated chitosan films. Left: photoelectron spectra emitted perpendicular (90°) to the surface of the sample films, and right, at an angle of 30° to the film surface. Spectra showing peaks around 402 eV correspond to nitrogen 1s and those around 287 eV to carbon 1s electrons.

(2.5%) of WSC in the amylose film. In contrast, WSC (c) and fully deacetylated chitosan (d) films showed clear N_{1s} peaks around 402 eV. The ratio of the peak area of N_{1s} and C_{1s}, N/C, roughly reflects the ratio of the concentration of these atoms in the WSC–amylose blend film. Table 2 shows the peak area ratios observed for amylose films blended with various amounts of WSC and those of 100% WSC as well as fully deacetylated chitosan films. The ratio of photoelectrons emitted perpendicularly (90°) to the film surface increased with the WSC concentration in the film. However, this increase in N/C is not directly proportional to the WSC concentration. If the N/C ratio would be directly proportional to the WSC concentration, the ratio divided by the WSC concentration should not change with the WSC concentration. However, as shown in Table 2, the (N/C)/WSC concentration decreased drastically with the WSC concentration. These facts suggest that the concentration of amino groups in the vicinity of the surface of

Table 1. Zeta potential of WSC–amylose blend and fully deacetylated chitosan films

	WSC concentration (%)				Fully deacetylated chitosan	ESA
	5	10	50	100		
Zeta potential (mV)	21.57	29.78	23.30	0.88	0.47	−39.68

Table 2. Ratio of (nitrogen 1s/carbon 1s) areas for blended films of amylose and chitosan

Photoelectron emitted at	WSC concentration in the blend film (%)					Fully deacetylated chitosan
	2.5	5	10	50	100	
N/C at 90°	0.038	0.049	0.053	0.085	0.117	0.117
(N/C)/WSC concn	0.0196	0.0096	0.0053	0.0017	0.0012	0.0012
N/C at 30°	0.039	0.052	0.068	0.042	0.032	0.004

WSC–amylose blend films did not increase in proportion to the WSC concentration.

This tendency appeared more clearly when spectra were measured by tilting (60°) the sample holder of the apparatus, in other words, for photoelectron spectra emitted at an angle of 30° to the film surface. By this tilting, we were able to obtain spectra emitted from half the thickness (sin 30°) of the film surface to spectra emitted perpendicular to the film surface. It is known that a photoelectron is generated by X-ray irradiation of a material and that, due to attenuation of the photoelectron energy passing through the film, only electrons generated at a depth of 0.5–3 nm from the material surface—depending on the material—are emitted. On the film of WSC or fully deacetylated chitosan, a relatively very small (N/C)/WSC concentration was observed for the perpendicular emission of electrons (Table 2). In particular, the N_{1s} area and (N/C) of these films became smaller at an angle of 30° to the film surface (Fig. 1 and Table 2). These facts indicate that the WSC or pure chitosan film surface has less amino groups exposed, although a quantitative assessment of surface/volume anisotropy is not possible with the present results. This is consistent with the results obtained for zeta potential measurements, which indicate that the surface of these films show a small potential (Table 1).

It is well known that in a film of a linear high molecular weight polymer such as WSC, fully deacetylated chitosan or amylose, prepared by casting a polymer solution, the direction of the polymer chain is oriented parallel to the film surface. A sheet structure may be proposed where amino groups are less exposed such as in the crystal structure of hydrated chitosan in which all of the amino groups of a chitosan molecule are present in the direction parallel to the sheet, not perpendicular.[5] Thus, less amino groups would be exposed on the film surface, which is consistent with the zeta potential and X-ray photoelectron spectrum measurements, resulting in the loss of antibacterial activity of the chitosan block on the WSC film.

Based on gas permeability measurements, mechanical properties, and the antibiotic properties of films of amylose and WSC–amylose mixtures, amylose and WSC molecules appear to have good miscibility.[1] The drastic decrease in (N/C)/WSC concentration with an increase in WSC concentration (Table 2) suggests that only a few WSC molecules can form a complex with a large number of amylose molecules. This speculation is supported by zeta potential measurements since the zeta potential did not change with an increase in WSC concentration up to 50% (Table 1). Although no morphological model for such WSC–amylose complex has been available up to now, one can anticipate that due to the complex formation the sheet structure of chitosan is broken down resulting in enhanced exposure of amino groups and concomitant restoration of the antibacterial activity of the chitosan molecule.

1. Experimental

1.1. Materials

Water soluble chitin (degree of N-deacetylation: 44.1% and viscosity average molecular weight: 1.40×10^6)[1] was supplied by Yaizu Suisankagaku Industry Co., Ltd, Yaizu, Japan. This sample was prepared from chitin by solid-state N-deacetylation, a heterogeneous reaction, indicating a block-wise distribution of 2-amino-2-deoxy-β-D-glucose (chitosan block) and 2-acetamido-2-deoxy-β-D-glucose (chitin block) residues on each molecule. We refer to this material as 'water soluble chitosan' (abbreviated as WSC) since chitosan having a higher degree of N-deacetylation (DDA) is not soluble in water. Antibacterial activity occurs on the free amino groups of the deacetylated part of the chain and not the acetylated one. It should be noted that water soluble chitosan is not dissolved directly in water. When an aqueous acid solution of WSC is neutralized by the addition of aqueous alkali, it is still soluble, which is the reason as to why the sample is called as 'water soluble'. A chitosan powder (M_w: 3×10^5) fully N-deacetylated, that is, 100% DDA, was supplied by Katokichi Co. Ltd, Kannonji, Kagawa, Japan.

An enzymatically synthesized amylose (abbreviated as ESA) of higher molecular weight (M_w: 1.4×10^6 and $M_w/M_n = 1.10$) supplied by the Sanwa Cornstarch Co. Ltd, Nara, Japan, was used for X-ray photoelectron spectra (XPS) as in our previous antibacterial measurements.[1] A lower molecular weight amylose (M_w: 2.9×10^5 and $M_w/M_n = 1.08$) prepared by the method reported[6–8] was used for zeta potential measurements since ESA films of higher molecular weight were too swollen to permit a zeta potential measurement. This amylose aqueous solution easily forms a gel and the aging (retrogradation) of the gel then proceeds.[9] Thus, this amylose film was much less swollen and could be used for the zeta potential measurements.

In order to make the experimental system as simple as possible, we prepared all films in a salt free state. This also eliminates the effect of salts on zeta potential measurements on a film surface. The methods of film preparation were reported in our previous paper.[1] Briefly, a 1% aqueous amylose soln (pH 6.5 at 20 °C) was prepared by dissolving amylose powder in hot water (80 °C). A 1% salt free aq WSC soln (pH 6.80 at 20 °C) was obtained by dialysis of an aq WSC soln that included NaCl, produced by the neutralization of a dilute hydrochloric acid solution of WSC with NaOH.

Each film having a thickness of 40–50 μm was prepared by casting the amylose, WSC, or their mixed solns at 60 °C as reported previously.[1] A film of fully deacetylated chitosan (thickness: 50 μm) was obtained by casting a 0.1 M aq AcOH soln of fully deacetylated chitosan (*c* 1%) on a Kapton (polyimide) film. The resulting acidic chitosan film was neutralized with 1 M aq NaOH followed by sufficient washing with water and drying.

1.2. Methods

Zeta potential measurements were carried out using an electrophoretic light scattering spectrophotometer ELS-8000HW (Otsuka Electronics Co., Ltd, Osaka, Japan).[10] To measure electrophoretic mobility, the cell for a solid-plate sample and polystyrene latex particles (pH 7) with an average diameter of 520 nm as standard monitoring particles were used. Each planar sample plate was fixed in the open side of the cell. The size of the sample plate has optimally a surface of 200×400 mm^2 and a thickness of 1–2 mm. An electric field was supplied at 80 V between two Pt-electrodes mounted at both ends of the cell.

X-ray photoelectron spectroscopy (XPS) experiments were carried out using a JPS-9010MC apparatus (JEOL Ltd, Tokyo, Japan) equipped with a Mg Kα (1253.6 eV) X-ray source generated at 7 kV and 30 mA. Samples were placed in a vacuum chamber ($<10^{-6}$ Pa) with photoelectron collection at an angle of 90° (perpendicular) to the sample film surface at room temperature. Electrons emitted at an angle of 30° to the film surface were also collected by tilting the sample. XPS narrow scan spectra were measured around 287 eV for carbon 1s and around 402 eV for nitrogen 1s.

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References

1. Suzuki, S.; Shimahashi, K.; Takahara, J.; Sunako, M.; Takaha, T.; Ogawa, K.; Kitamura, S. *Biomacromolecules* **2005**, *6*, 3238–3242.
2. Shimahashi, K.; Ogawa, K.; Kitamura, S. Unpublished data.
3. Lim, S.; Hudson, S. M. *J. Macromol. Sci. Polym. Rev.* **2003**, *C43*, 223–269.
4. Matsumoto, H.; Koyama, Y.; Tanioka, A. *Langmuir* **2002**, *18*, 3698–3703.
5. Okuyama, K.; Noguchi, K.; Miyazawa, R.; Yui, T.; Ogawa, K. *Macromolecules* **1997**, *30*, 5849–5855.
6. Kitamura, S.; Yunokawa, H.; Mitsuie, S.; Kuge, T. *Polym. J.* **1982**, *14*, 93–99.
7. Nakanishi, Y.; Norisuye, T.; Teramoto, A.; Kitamura, S. *Macromolecules* **1993**, *26*, 4220–4225.
8. Kitamura, S.; Kobayashi, K.; Tanahashi, H.; Ozaki, T.; Kuge, T. *DENPUN KAGAKU (J. Jpn. Soc. Starch Sci.)* **1989**, *36*, 257–264, in Japanese.
9. Kitamura, S. Starch polymers, natural and synthetic. In *The Polymeric Materials Encyclopedia, Synthesis, Properties and Applications*; Salamone, J. C., Ed.; CRC Press: Boca Raton, 1996; Vol. 10, pp 7915–7922.
10. Hozumi, A.; Sugimura, H.; Yokogawa, Y.; Kameyama, T.; Takai, O. *Colloids Surf., A* **2001**, *182*, 257–261.