Powderization of Wool Keratin by Alkali Hydrolysis in Higher Alcohol/Water Binary Systems

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(Received January 7, 2008; CL-080011; E-mail: nonoy@yz.yamagata-u.ac.jp)

We successfully powderized keratin fibers under mild conditions. Wool keratin was hydrolyzed in a binary system, consisting of a higher alcohol phase and an alkali aqueous solution phase.

Keratin is a protein, and a component of human hair, wool, feathers, and nails. In recent years, some composite materials for traumatotherapy, cell scaffolds, and drug delivery have been prepared using keratin powder and films.[1] It is difficult to powderize keratin fibers, because keratin protein contains cysteine cross-links. Keratin powder prepared by traditional alkali hydrolysis methods under heating are degraded in the preparation procedures.[2] In recent studies, this powderization has been achieved by some reduction methods,[3] enzyme hydrolysis methods,[4] and mechanical grinding methods.[5] However, complicated processes or toxic substances are used in these methods. We found that wool keratin easily powderized by alkali hydrolysis in a higher alcohol/water binary system. The wool fibers dispersed in the higher alcohol phase are hydrolyzed by the dissolved alkaline component. The alcohol improves the swelling ability of the keratin fibers.[6] In this study, we show the advantage of mild binary phase method over the traditional hydrolysis method. The effects of the molecular structure and the amount of the alcohol component on grinding efficiency are also studied to determine optimum conditions for powderization.

Wool fibers were sonicated in a binary system consisting of an octanol phase and a NaOH aqueous solution phase (60:40, wt/wt) for 25 min at 298 K and agitated for 5 min, as shown in Figure 1.[7,8] The aqueous solution phase was separated from the higher alcohol phase in the sonication process and dispersed in the octanol phase as spherical droplets in the agitation process.

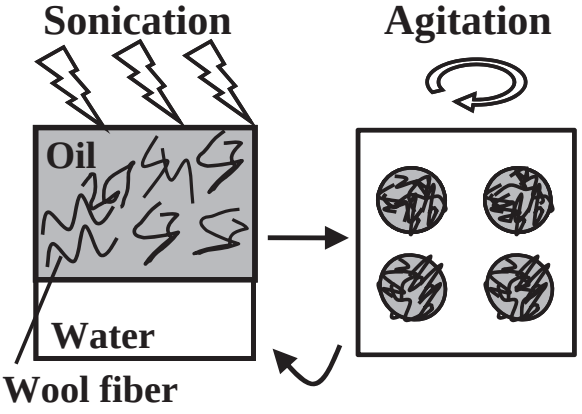

Figure 1. A schematic illustration of powderization of wool keratin by the binary phase method.

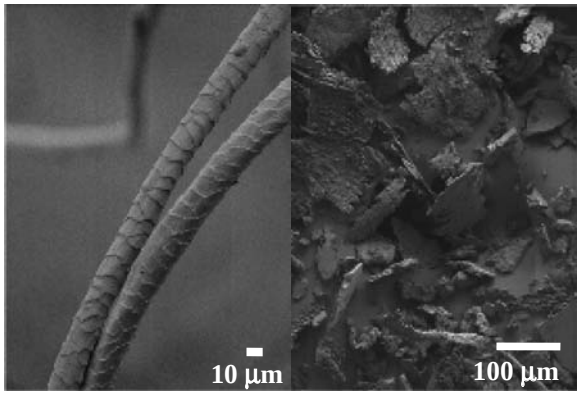

Figure 2. SEM images of wool fibers (a) and keratin powder (b).

A change in wool fiber morphology is shown in Figure 2. The wool fibers covered with cuticle scales transformed into plate-shaped particles 59 μm in diameter.[9] The size of the keratin powder is larger than that of the wool fiber, because the precipitated keratin powder aggregates, when the dispersed wool is neutralized by HCl aqueous solution. The dispersed wool prepared by the binary phase method does not have a smell, whereas that prepared by a traditional hydrolysis method possesses an irritating smell due to volatile sulfur components. Elemental analysis and IR spectra showed that keratin degradation was inhibited in the binary phase method.[9] All characteristic IR absorption bands of the particles prepared by the binary phase method are approximately consistent with the bands of the wool fibers, whereas the N–H deformation band of the particles prepared by the traditional hydrolysis method is 33 cm^{-1} higher than that of the wool fibers. In the particles prepared by the binary phase method, the sulfur component is 2% lower compared with that of the wool fibers. On the other hand, in the particles prepared by the traditional hydrolysis method, the components of sulfur, nitrogen, and carbon are more than 2, 6, and 8% higher compared with those from the wool fibers. These results imply that the keratin powder prepared by the binary phase method exhibit loss of the cysteine cross-links, but is not degraded seriously.

Hydrolysis without serious degradation is realized by adding higher alcohols, especially octanol. Figure 3 shows the effects of the alkyl chain length and the composition of the alcohols on the state of the treated wool keratin. The keratin particles or fibers are observed in four different states, i.e., powdery state, coexistence state, transparent state, and fiberous state. In the powdery state, the keratin fibers were completely powderized. This state was achieved when the higher alcohol phase with a C8–C12 alkyl chain was separated from the water phase. In the coexis-

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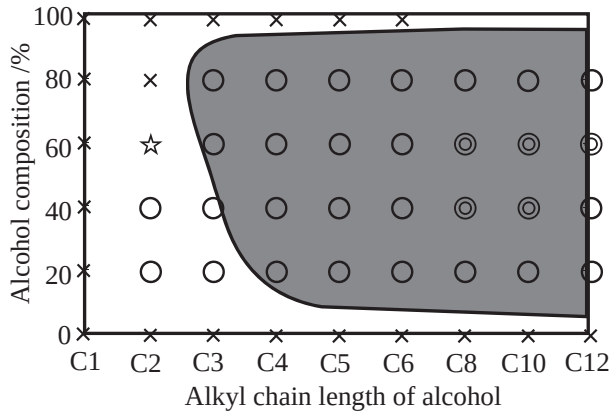

Figure 3. Effects of the alkyl chain length and the composition of the alcohols on the state of the treated wool keratin. The keratin particles are obtained in four different states, i.e. powdery state (◎), coexistence state (○), transparent state (☆), and fiber state (×). The grey region is in the binary systems consisting of a higher alcohol phase and a water phase.

tence state, both powder particles and fibers were mixed in the precipitates. The transparent state was the clear single phase in which finely broken particles were dispersed in the medium. This state was observed when the medium contained 60% ethanol. When the hydrolysis occurred in the alkali aqueous solution or alcohol, the wool fibers were not powderized at all, and remained in the fiberous condition. These results show that a binary system consisting of a higher alcohol phase and an alkali aqueous solution phase is suitable to powderize wool fibers.

Why are the keratin fibers powderized completely in the binary system? We suppose that the higher alcohol improves the swelling ability of the wool fibers.[6] Higher alcohol osmoses into the wool fibers through the cuticle–cuticle intercellular region, and enhances the mobility of the keratin molecules. The addition of higher alcohol inhibits the degradation of the keratin protein. According to elemental analysis and IR spectra, the composition of carbon, hydrogen, and nitrogen in the keratin powder is almost the same as that of the wool fibers. The mechanism of inhibition by the higher alcohol is not clear at present. The effects of the higher alcohol on the conformation of the keratin protein and cell membrane complexes should be studied to show the inhibition mechanism.

This work was supported by Grant-in-Aid for Young Scientists (Start-up) No. 19810001 from the Ministry of Education, Culture, Sports, Science and Technology, Japan. The authors would like to thank Dr. Mitsuhiro Kimata for the particle size distribution measurement.

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7 Polwarth wool 24–26 μm in diameter were obtained as a keratin source. Reagent grade organic solvents, NaOH and HCl, were purchased from Kanto Chemical Co., Ltd.
8 As a pre-treatment, 0.1 g of wool fibers were cleaned using a commercial detergent and ethanol, and were immersed in water at 50 °C for 5 min. In the binary phase method, the treated wool fibers were immersed in 100 mL of aqueous solution containing 4 g of NaOH and alcohol. This mixture was sonicated for 25 min at 298 K, and agitated with a magnetic stirrer for 5 min. The sonication and agitation procedures were repeated twice. Then, the mixture was neutralized with HCl to obtain a wool powder precipitate. The precipitate was dialyzed using a cellulose tube (molecular mass cut-off: 12000–14000 Da, Nihon Medical Science Co., Ltd.) for one week. The water in the outer phase was changed twice a day during the dialysis. After centrifugation at 15000 rpm for 15 min, the precipitate was cleaned with acetone, and dried to powder. This hydrolysis procedure was studied with 20, 40, 60, 80, and 100% alcohol composition in the medium. In traditional hydrolysis methods, the wool fibers were immersed in 100 mL of the aqueous solutions containing 4 g of NaOH. The mixture was agitated with a magnetic stirrer for 1 h. After-treatment procedures were similar to those in the binary phase method.
9 Anal. Found: (wool fibers) C, 46.32; H, 7.027; N, 14.47; S, 2.68%, (the keratin powder prepared by the binary method) C, 47.21; H, 7.19; N, 14.18; S, 0.62%, (the keratin powder prepared by the traditional hydrolysis method) C, 54.53; H, 8.36; N, 8.38; S, 0.54%. IR($/cm^{-1}$): (wool fiber) 3294 (NH), 2922 (CH), 2852, 1647 (C=O), 1509 (NH), 1458, 1398; (the keratin powder prepared by the binary method) 3294 (NH), 2926 (CH), 2854, 1653 (C=O), 1509 (NH), 1456, 1397; (the keratin powder prepared by the traditional hydrolysis method) 3285 (NH), 2917 (CH), 2850, 1650 (C=O), 1542 (NH), 1470, 1404. Particle size was measured by the Shimadzu SALD-7100 laser scattering particle distribution analyzer with the powder dispersed in water.

Published on the web (Advance View) February 16, 2008; doi:10.1246/cl.2008.338