Molten Urea as a Novel Dissolving Solvent for Wool and Other Animal Proteins

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Abstract: The function of molten urea as the solvent for wool and other animal proteins were investigated. Molten urea at 150 °C could dissolve wool without addition of water or any other chemicals. Also, it could dissolve other animal proteins such as hair, feather and silk. When the concentration of wool in molten urea was up to about 10 %, the recovery of water soluble and water insoluble keratins were determined by ¹³C-CP/MAS NMR spectroscopy. It was found that the water soluble keratin consists of 61 % β -sheet and 39 % α -helix, and the water insoluble keratin almost consists of β -sheet. The result of nitrogen content assay by elementary analyzer revealed that 9.2 % (w/w) urea per water soluble keratin might form chemical bonds with the keratin.

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1. Introduction

Typical cellulosic biomass, such as wood, cotton and linen etc. are abundant in nature. There are several substances can be used as efficient solvents for cellulose. This fact illustrates an advantage of using wet spinning method to regenerate yarn (viscose, cupra rayon or tencel fiber) from wood cellulose (pulp) or cotton linter [1].

Nevertheless, the effective utilization of natural polymers from animal biomass is a little. Animal biomasses are mainly composed of proteins [1]. Silk protein is composed of “fibroin” and “sericin”. Wool, hair and feather protein are “keratin”, which are epidermal proteins. The fiber which composed of fibroin or keratin are difficult to dissolve in solvent without their hydrolysis. In particular, keratin has a high content of cysteine containing intra- and inter-molecular disulfide bonds. Under this situation, there are many investigations to dissolve silk [2], wool [3-5], hair [6] and feather [7]. The methods of dissolution of wool proteins are mainly classified into oxidative and reductive methods. The oxidative method, disulfide and peptide bonds are simultaneously disrupted, which induce hydrolysis. Wool keratin is converted to keratose, known as a soluble derivative of keratin, by a cleavage of disulfide bonds [8]. The keratose is different from keratin on the reactivity of sulfur. Thus, the principal to dissolve wool is the reductive method, which dissolves wool with 8 M urea

solution, reducing agent and surfactant at high temperature or ultrasonication [3-5]. Other ways to dissolve wool are Cu-oxam metal complex system ($[\text{Cu}(\text{NH}_3)(\text{OH})_2]$) with reductant [9] or ionic liquid method like 1-butyl-3-methyl imidazolium (BMIM^+Cl^-) [10]. These solvents need long treatment time and are not environmentally-friendly solutions.

Our laboratory found out that molten urea could dissolve wool and other animal proteins [11]. This molten urea method was different from oxidative and reductive methods. The molten urea method is assessed to be an environmentally-friendly, because the dissolving solvent is used only urea without other chemicals, and the used urea can be reused for agricultural fertilizer.

This report deals with the dissolution behavior of proteins in molten urea using the chemical analysis.

2. Experimental Procedure

2.1 Materials and analytical instruments

Wool yarn (2/60) purchased from NIHON KEORI Co., Ltd. Hair was supplied from a 10-years old boy. The feather was collected from chicken at the experimental field in Gifu University. The silk was supplied from Shinano Kenshi Co., Ltd. The urea (melting point 132.7 °C) was purchased from NAKALAI TESQUE, INC.

2.2 Dissolution of protein materials to molten urea

After 5 g of urea were melted at 140-180 °C, 0.1 g of animal epidermic protein materials such as wool, hair, feather and silk were added. The solubilities were observed by visual examination and recorded the dissolving time when the solution became transparently brown. Then, 5 ml of water were added to each solution. After cooling, the solution was dialyzed against distilled water using a cellulose bag which molecular weight cut off 14 kDa. During dialysis, the urea was removed and results in the water soluble and the precipitated fractions. After filtration, the precipitated fraction was obtained as the water insoluble protein, while the water soluble fraction was freeze dried as the water soluble protein.

2.3 Production of water soluble keratin

To produce water soluble keratin, 10 g of urea were melted in an oil bath at 150 °C. Then, 1 g of wool fiber each 10 mm in length was added to the molten urea. After 20 min, the fragmentary wool was dissolved and became transparently brown. Five ml of water were then added to the solution. Subsequently, water soluble keratin powder and insoluble keratin solid were prepared accordingly to the method described previously.

For the reductive method, water soluble keratin was prepared from mixture of 8 M urea, 3% SDS and 3% $N_2S_2O_5$ at 100 °C for 30 min [12].

2.4 ^{13}C -CP/MAS NMR analysis

The solid state ^{13}C -CP/MAS NMR spectra of crude wool, water soluble keratin and water insoluble keratin were determined by JEOL JNM- ECA500 spectrometer with NM-93030 CPM probe. Samples were packed into a cylindrical rotor made of zirconia and spun at frequency of 1.2 kHz. The contact time and the repetition time were 2 ms and 5 s, respectively.

2.5 SDS-PAGE and nitrogen content assay

The molecular weight of water soluble keratin was determined by 10 % SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). The nitrogen content was measured by elementary analyzer (Yanaco CHN corder MT-6).

3. Results and discussion

3.1 Dissolution of protein materials in molten urea

Wool, hair and feather (0.1 g) were dissolved in molten urea at 150 °C for 10-20 min. Then, the water soluble protein (32-53 mg) and the water insoluble protein (10-51 mg) were obtained. While, the dissolution

Table 1 Dissolution conditions of animal proteins in molten urea, and the regeneration with water.

	Temperature	Time	Water soluble protein	Water insoluble protein
	(°C)	(min)	(%)	(%)
Wool	150	20	53	10
Hair	150	20	32	51
Feather	150	10	50	15
Silk	180	50	8	60

of silk (0.1 g) was required a higher temperature (180 °C) and a longer time (50 min). And also the productivity of water soluble protein from silk was lower than the other materials tested. (Table 1)

The secondary structure of silk is an example of the beta pleated sheet. In this structure, individual protein chains are aligned side-by-side with every other protein chain aligned in an opposite direction. The protein chains are held together by intermolecular hydrogen bonding, that is hydrogen bonding between amide groups of two separate chains [13]. This is reason why silk is required a higher temperature and a longer time for the dissolving. In contrast, urea cleaves the intra- and inter-molecular hydrogen bond of keratin polymer easier than silk fibroin.

3.2 Production of water soluble keratin

By pouring water into molten urea dissolved wool, the water soluble and insoluble keratins were obtained. The yields of water soluble and insoluble keratins were 53 and 10 %, respectively. The molecular weight of water soluble keratin was estimated to be about 75 kDa by SDS-PAGE (Fig.1). Previously, the molecular weight of keratin which prepared from the reductive method was 52-69 kDa [14]. The difference molecular weight of the water soluble keratin and the reductive keratin were indicated that the dissolvent mechanism of the novel molten urea method is different from the reductive mechanism.

Fig. 1 SDS-PAGE of water soluble keratin.

Fig. 2 ^{13}C -CP/MAS NMR spectra of (a) crude wool, (b) water soluble keratin and (c) water insoluble keratin.

3.3 Secondary structure of water soluble and insoluble keratins

Secondary structures of proteins were determined by the difference of chemical shifts of the carbonyl carbons in ^{13}C -CP/MAS NMR. The chemical shift of carbonyl peak was assigned to be 175.8 ± 0.8 ppm and 170.9 ± 1.2 ppm which correspond to α -helix and β -sheet, respectively, and the intensities are proportional to the contents [15].

The NMR spectra were shown in Fig. 2. The peak positions at 25, 55, 130, 175 ppm were assigned to $\text{CH}_2\text{-C}$, -N/S-O , C=C and C=O , respectively [16].

The peaks of NMR spectra at 165-185 ppm were shown in Fig. 3. Two peaks of crude wool were detected at 172 and 176 ppm which corresponded to protein of β -sheet and α -helix, respectively (Fig. 3a). The water soluble keratin prepared from molten urea method, two peaks were also found at 173 ppm and 176 ppm which corresponded to the mixture of 61 % β -sheet and 39 % α -helix. For the water insoluble keratin prepared from the molten urea method, only one peak was found at 173 ppm. This indicated that the water insoluble keratin consists of only β -sheet. The native wool fiber consists of intermediate filaments (microfibrils) composed of low-sulfur proteins which are embedded in a nonfilamentous matrix. The nonfilamentous matrix usually contains two classes of proteins; one is a high-sulfur protein and other is a HGT (high Glycine-Tyrosine fractions) [17]. The secondary structures of low-, high-sulfur proteins are

Fig. 3 ^{13}C -CP/MAS NMR spectra observed at 165-185 ppm. Dotted line and smooth line represent NMR spectra and divided peaks by Lorentz function, respectively. (a) crude wool, (b) water soluble keratin and (c) water insoluble keratin.

contain α -helix and β -sheet, respectively. While, HGT protein contains random coil structure and a bit of β -sheet [16, 18]. Therefore, the results suggest that the water soluble keratin was the mixture of low-, high-sulfur proteins and HGT, and water insoluble keratin was high sulfur protein and HGT.

3.4 Urea bonding to the water soluble keratin

The nitrogen content of crude wool and prepared keratins were determined by elementary analyzer as shown in Table 2. The increased nitrogen content was only found in the water soluble keratin which was prepared by the molten urea method. They were expected that some urea molecules might bond to the water soluble keratin. The ratio of chemically bonded urea with the water soluble keratin was calculated. The result revealed that 9.2 % (w/w) urea per water soluble keratin might form chemical

Table 2 Nitrogen content (N %) in the three types of keratins and crude wool.

Samples	N %
Water soluble keratin	19.69
Water insoluble keratin	14.74
Water soluble keratin prepared by reductive method	15.12
Crude wool	15.39

bonds with the keratin.

Conclusion

It was found that molten urea could dissolve wool, hair and feather at 150 °C for 10-20 min, and silk at 180 °C for 50 min. For wool dissolution, the water soluble keratin and insoluble keratin could be obtained. The molecular weight of water soluble keratin was about 75 kDa. The water soluble keratin was found to consist of 61 % β -sheet and 39 % α -helix, and the water insoluble keratin consisted of only β -sheet. The result of nitrogen content revealed that 9.2 % (w/w) urea per water soluble keratin may form chemical bonds with the keratin.

The dissolution mechanism of wool in molten urea was proposed. The ammonia molecules which were decomposed from urea by high temperature may intrude into the crystal region of keratin and then the hydrogen bonds of keratin were disrupted by the ammonia molecules. However, the mechanism of the wool dissolution will be investigated.

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