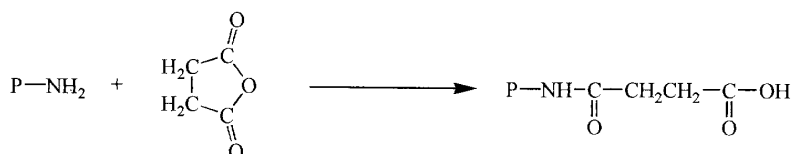


SUCCINYLATION OF CHEMICALLY MODIFIED WOOL KERATIN AND THE EFFECT ON HYGROSCOPICITY AND WATER ABSORPTION

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INTRODUCTION

Since wool is a natural protein having different reactive functional groups as well as a clothing material of excellent quality, it can be converted into a biodegradable and functional polymeric material by suitable chemical modification. Succinic anhydride generally reacts with a functional group such as an amino group (1), which can introduce a new carboxyl group into wool fibers as shown below.



Succinylation followed by additional modification with metal salts had been reported to retard felt shrinkage of wool fabric (2). Toyoda et al. reported that succinylated collagen fibers showed remarkably higher water absorbancy than intact collagen (3). Since wool contains more lysine (Lys) and arginine (Arg) residues than collagen, succinylation of wool is also expected to raise the water absorbability. In this work, intact wool and some kinds of wool keratin derivatives were succinylated and the effects on water absorption and hygroscopicity were studied for the purpose of re-utilization of waste or used wool fibers as a new functional polymeric material.

Chemical treatments such as reduction, oxidation and partial hydrolysis were made on wool fibers before succinylation to improve the reactivity with succinic anhydride. Cleavage of disulfide crosslinks by reduction or oxidation is expected to raise the accessibility of the reagent to the wool and hydrolysis increased the amount of amino groups as well as carboxyl groups. Powdery wool (Merry powder 30) and the partially hydrolyzed one were also used as another type of keratin derivatives, disulfide cross-linking of which had been ruptured.

EXPERIMENTAL

Materials

Wool and Merry powder 30

The wool fibers (New Zealand Corriedale) were degreased according to Nakamura et al. (4). Merry powder 30 obtained from wool by reduction and coagulation with alkali, was offered by Kyoisha Chemical Co. Ltd.

Chemical treatments

Partial hydrolysis

Wool fibers (20 g) had been shaken in 1N or 3N HCl solution (liquor ratio, 1:50) at 50°C for 24 h, followed by thorough washing with distilled water. Merry powder 30 was similarly hydrolyzed with 1N or 2N HCl.

Oxidation

Wool fibers were shaken in a solution containing performic acid (99% formic acid: 30% hydrogen peroxide = 1:1) at 0°C for 24 h according to the procedure previously described (5). The oxidized wool was washed with distilled water and air-dried after the solution had been removed by filtration.

Reduction

After wool fibers (5 g) had been shaken in 1M aqueous 2-mercaptoethanol solution (liquor ratio, 1:100) at 40°C for 6 h, the reduced samples were washed enough with distilled water and air-dried.

Succinylation

Intact wool fibers, Merry powder and the chemically treated samples as described above were succinylated according to the procedure of Toyoda et al (3). Succinic anhydride was added to the sample in 1/15M phosphate buffer solution at pH 8.0 (liquor ratio, 1:50) and the mixture was stirred at room temperature for 24 h. During the reaction, pH of the solution was kept at 8.0 by adding of 10N NaOH. Succinylated sample was thoroughly washed with distilled water and air-dried. The sample that set to gel by succinylation was dialyzed with water for over 72 h and lyophilized.

Measurements

Add-on

Add-on was evaluated as the amount of succinic acid in the hydrolyzed sample as follows: Succinylated sample (5~10 mg) was hydrolyzed in 6N HCl (5 mL) at 110°C for 48 h and evaporated to dryness. Amino acids in aqueous solution of the hydrolyzate were removed by passing the aqueous solution through a column of Amberlite IR120B (H⁺ form). After the eluent had been evaporated to dryness, content of succinic acid in the sample was determined by colorimetry using 2-nitrophenylhydrazine hydrochloride and dicyclohexylcarbodiimide according to the method of Momose (6).

Water absorption

Sample (0.2 g) dried at 110°C for 1 h was weighed and immersed in distilled water (5 mL) for 4 h at 30°C with shaking. After the sample in water had been centrifuged at 3,000 r.p.m. for 5 min, the supernatant was removed. Water uptake (g/g-dry sample) was evaluated from the weight of the swelling sample.

Hygroscopicity

Moisture regain of samples was measured under 22.8, 43.3, 64.9 and 85.0% R.H. using the saturated aqueous solution of CH₃COOK, K₂CO₃, NH₄NO₃ and KCl. Sorption behavior of water vapor into the samples was measured at 20°C using the sorption apparatus described before (7).

Table 1. Abbreviations and add-ons of the samples used in this work

Abbr.	Keratin derivative	succinic anhydride (g/g)	Add-on (%)	Abbr.	Keratin derivative	succinic anhydride (g/g)	Add-on (%)
W	Intact wool	-	-	OW	Oxidized wool	-	-
SC-Wa	Intact wool	5.0	6.7	SC-OWa	Oxidized wool	2.0	5.7
SC-Wb	Intact wool	6.8	8.2	SC-OWb	Oxidized wool	6.8	7.5
SC-Wc	Intact wool	6.8* ¹	11.8	MP	Merry powder	-	-
1HW	Hydrolyzed wool* ²	-	-	SC-MPa	Merry powder	2.0	10.7
SC-1HW	Hydrolyzed wool* ²	6.8	9.3	SC-MPb	Merry powder	6.8	12.9
3HW	Hydrolyzed wool* ³	-	-	1HMP	Hydrolyzed Merry powder * ²	-	-
SC-3HWa	Hydrolyzed wool* ³	2.0	9.0	SC-1HMP	Hydrolyzed Merry powder * ³	2.0	11.0
SC-3HWb	Hydrolyzed wool* ³	5.0	9.0	2HMP	Hydrolyzed Merry powder * ⁵	-	-
SC-3HWc	Hydrolyzed wool* ³	6.8	11.4	SC-2HMPa	Hydrolyzed Merry powder * ⁵	6.8	12.2
SC-3HWd	Hydrolyzed wool* ³	8.0	-. ⁴	SC-2HMPPb	Hydrolyzed Merry powder * ⁵	6.8* ¹	15.9
SC-3HWe	Hydrolyzed wool* ³	6.8* ¹	14.4				
RW	Reduced wool	-	-				
SC-RW	Reduced wool	6.8	9.5				

*¹ Succinylation was repeated for twice.*² Sample was hydrolyzed in 1N HCl.*³ Sample was hydrolyzed in 3N HCl.*⁴ The product dissolved in water.*⁵ Sample was hydrolyzed in 2N HCl.

Table 2. Water absorbability of succinylated keratin derivatives

Sample	Water absorbability (g/g·dry sample)	Sample	Water absorbability (g/g·dry sample)
W	4	OW	4
SC-Wa	3	SC-Owa	8
SC-Wb	4	SC-OWb	35
SC-Wc*	12		
		MP	6
1HW	8	SC-MPa	8
SC-1HW	10	SC-MPb	15
3HW	11		
SC-3HWa	13	1HMP	3
SC-3HWb	15	SC-1HMP	11
SC-3HWc	20	2HMP	3
SC-3HWd	soluble	SC-2HMPa	19
SC-3HWe	20	SC-2HMPb*	29
RW	12		
SC-RW	20		

* Succinylation was repeated for twice.

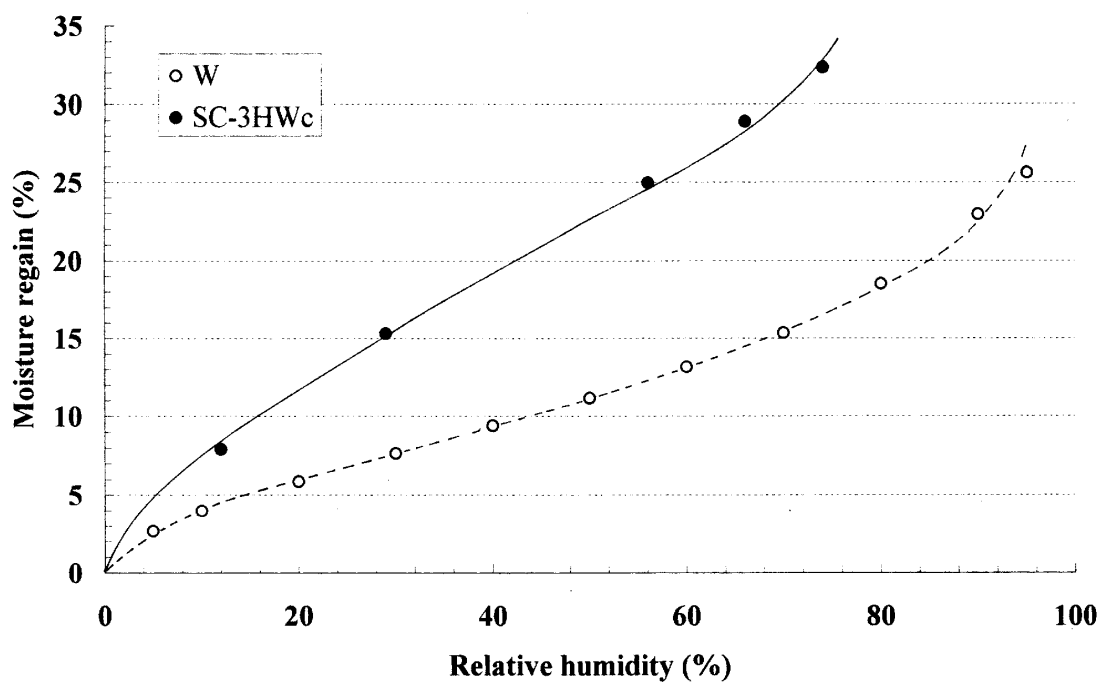


Figure 1. Sorption isotherms of wool and succinylated keratin derivative.

RESULTS AND DISCUSSION

Succinylation

Mainly amino groups of Lys residues and guanidino groups of Arg residues in the samples are thought to react with succinic anhydride under the conditions applied in this work (1). Intact, partially hydrolyzed, oxidized and reduced wool fibers and Merry powder and partially hydrolyzed one were succinylated in phosphate buffer solution at pH 8.0, where 23~92 mole of succinic anhydride was added for one equivalent of Arg and Lys residues in the samples.

Abbreviations of the keratin derivatives used in this work and the add-ons were shown in Table 1. Add-ons of the samples were increased by increase of added amount of succinic anhydride. The wool fibers partially hydrolyzed in 3N HCl became soluble in water after succinylation with 8 g/g succinic anhydride (SC-3HWd). Add-ons of succinylated intact wool were in the range of 6.7~11.8%. If amino groups of Lys and guanidino groups of Arg residues react with succinic anhydride except for the partially hydrolyzed samples, maximum add-on calculated from the contents of the amino acid residues in intact wool fibers (8) will be 8.75%. However, the add-ons of some succinylated wool samples were higher than this value. These facts suggest that succinic anhydride may have reacted with the other functional groups such as hydroxyl groups of the side chain or amino groups resulted from hydrolysis of main chain, which had occurred as a side reaction through succinylation.

Effect on water absorption

Water absorption of intact wool was scarcely changed by the first succinylation, though succinic anhydride added to the samples to a certain extent, 5~6.7%. Both add-on and water absorption were increased by repeating the succinylation. The second succinylation of partially hydrolyzed wool (SC-3HWe) also raised the add-on but the water absorption did not exceed in the value of the corresponding sample which had been succinylated first (SC-3HWd). The add-ons of oxidized or reduced wool were scarcely different from that of intact wool, while the water absorbabilities of the reduced or oxidized and succinylated samples were much higher than that of succinylated wool (Table 2).

In the case of Merry powder, water absorbability was not so raised only by hydrolysis, but remarkably increased by a combination of hydrolysis and succinylation (Figure 1). Though water absorption of all samples increased with the add-on, the relationship between these values was not always linear. This fact suggests that water absorbance is not influenced only by degree of succinylation but also by other changes in wool such as hydrolysis of the main chain, change in molecular geometry by the treatments and coupling of carboxylic groups in samples and Na⁺ from NaOH added during succinylation and so on.

Absorption of aqueous solution of different pH

Behaviors of intact wool (W), the oxidized wool (OW), Merry powder (MP) and the partially hydrolyzed Merry powder (1HMP) in absorption of different pH solutions were shown with those of the succinylated samples in Figure 2. The unsuccinylated samples showed the almost similar characteristic. Uptake in each sample was not significantly changed by pH of the solution, though that of the solution of pH 12.0 was high in all unsuccinylated samples except for intact wool.

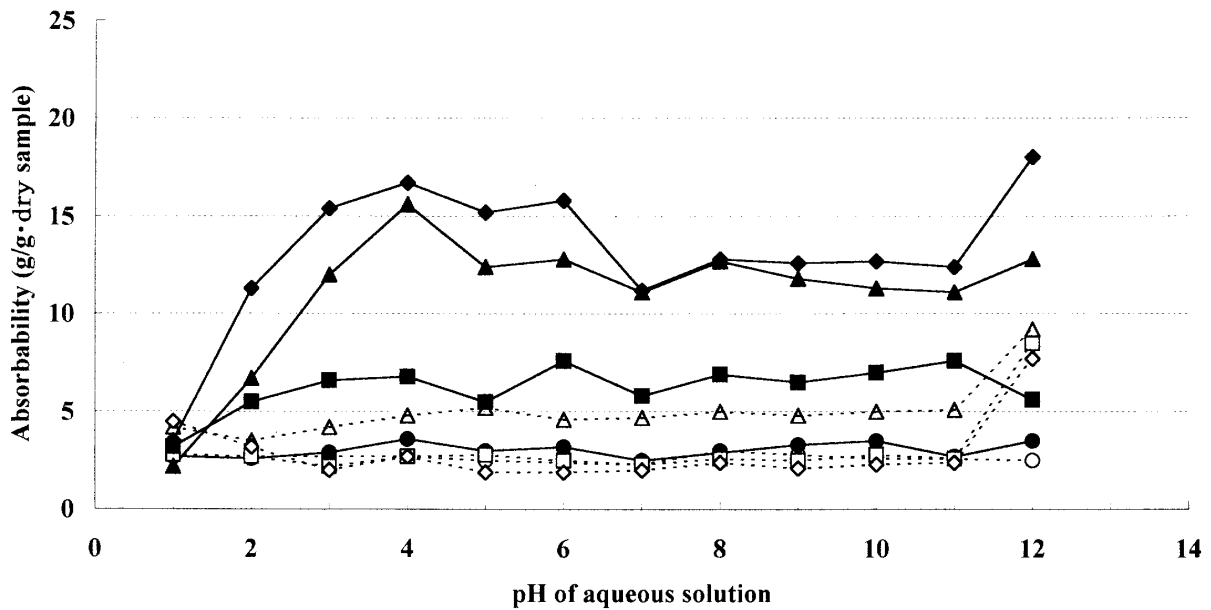


Figure 2. Absorption of aqueous solution of different pH

○··· W ●··· SC-Wa □··· OW ■··· SC-OW △··· MP ▲··· SC-MP ◇··· 1HMP ◆··· SC-1HMP

No remarkable difference in absorption of different pH solution was recognized between intact wool and succinylated one with low add-on. On the other hand, the behavior of oxidized wool sample in the absorption changed after succinylation.

Absorption of MP and 1HMP was increased by succinylation in general, which significantly changed depending on pH. The succinylated OW, MP and 1HMP, in which disulfide cross-linking were ruptured, showed almost similar features. The uptakes of the solution at pH 12.0 were significantly different between the tested samples, while the uptakes of the solution at pH 1.0 were close with each other. This fact indicates that acidic functional groups such as a carboxyl group can not dissociate at pH 1.0, but can dissociate at pH 12.0 and the amount of dissociating functional groups of the samples relate with the amount of water molecule absorbed in the samples.

Effect on hygroscopicity and behavior of moisture sorption

The chemical treatments made before succinylation showed poor effect to raise the hygroscopicities of the samples (Table 3). By succinylation the hygroscopicities were increased especially under the circumstance of high humidity.

Table 3. Moisture regain of succinylated keratin derivatives

Sample	Relative humidity (%)				Sample	Relative humidity (%)			
	22.8	43.3	64.9	85.0		22.8	43.3	64.9	85.0
W	6.8	10.0	12.9	17.7	OW	6.2	8.9	11.5	17.1
SC-Wa	7.9	11.9	16.8	24.2	SC-OW	8.2	11.2	14.6	20.4
SC-Wc	8.5	11.6	16.1	24.1	RW	5.2	10.1	10.4	15.3
3HW	5.7	10.6	11.2	16.6	SC-RW	4.8	10.5	11.5	20.1
SC-3HWc	6.5	11.2	12.2	21.2	MP	7.4	10.6	13.5	19.9
					SC-MPb	7.4	11.3	15.9	29.3

The behavior of moisture sorption of SC-3HWc was compared with that of intact wool (figure 2). Moisture regain of intact wool fibers in circumstances of different humidities was the average of the values reported by Speakman and Cooper (9), Bull (10) and Rowen and Blaine (11). The B. E. T.'s linear plots could be represented by good linear relationship for both samples at relative humidities less than ca, 50%. From the slope and intercept of the plots the values of V_m (maximum volume of adsorbed water in monolayer fashion per one gram of dry material) and C (adsorptive energy factor) were respectively determined as 0.118 and 13.19, both of which were higher than the corresponding values of intact wool (V_m : 0.064, C : 12.17). Isotherms calculated from the B.E.T.'s multilayer adsorption model by fixing the values of V_m and C determined above, and by varying the number of layers (i) from 2 to 5 were also compared with observed isotherms. The maximum number of layers can be found as 5 to 7 for intact wool fiber. On the other hand, the value of SC-3HWc seems to be over 6, though the observed isotherm was not completely in accord with the calculated isotherm.

CONCLUSION

Water absorbability of wool fibers was increased by succinylation and those of wool fibers and powdery wool were more remarkably increased by combinations of succinylation and the other chemical treatments such as partial hydrolysis, reduction or oxidation. Though the combination of these chemical treatments and succinylation

gave less effect on the hygroscopicity than the water absorption, V_m and C values from the water sorption isotherms of partial hydrolyzed and succinylated wool were significantly higher than the corresponding values of intact wool.

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