Enzymatic approach to analyze the effects of mercaptans on hair

CHIKARA YAMAUCHI, AKIMASA MOCHIZUKI, KEISUKE TAKAYAMA, SATOSHI SUZUKI, and ATSUSHI SAKAINO, *Higashi-Matsuyama Laboratories, Arimino Co. Ltd., 25-5 Miyako, Namekawa-machi, Hiki-gun, Saitama 355-0812, Japan, and* WATARU OKAZAKI, *Department of Life Sciences, Faculty of Life Sciences, Toyo University, 1-1-1 Izumino, Itakura-machi, Ora-gun, Gunma 374-0193, Japan.*

Synopsis

Different mercaptans were used to prepare reduced hair and permed hair samples. The reduced hair showed much higher protease degradation than the permed hair; also, the protease degradability of permed hair increased with repetitive treatments. The degradability of reduced hair was closely related to its cystine content, and that of permed hair was nearly unaffected by its cystine content. The degradability of both the reduced and permed hair was related to the reduction power of each mercaptan used on the hair and increased in the following order: CYS-treated hair < CA-treated hair < TG-treated hair.

INTRODUCTION

In a permanent wave treatment, the hair-waving technique is a two-step operation involving the breaking of disulfide bonds in hair using a mercaptan, and then regenerating these bonds using an oxidizing agent (1). To evaluate the effect of mercaptans, many studies have been conducted (2–5).

Morphologically, hair consists of a cuticle layer that envelops the fibrous hair cortex, constituting the bulk of the fiber (6). The cortex mainly consists of two types of proteins: about 60% of intermediate filament proteins and about 40% of interfibrillar-associated proteins, traditionally called microfibril (Mf) and matrix (Ma), respectively. The Mf is crystalline protein that is mainly composed of an α-helical protein with low cystine, and the Ma is an amorphous globular protein with high cystine (7,8).

Recently, some investigators examined the effect of permanent waving on hair using a biochemical method, and reported interesting findings about structural change in hair protein (7,9). We have also reported the correlation between the extent of protease degradation and the frequency of TG permanent wave treatments (10). Additionally, we showed that the degradation is closely related to the denaturation of Mf protein (10,11).

In this study we examine the protease degradability of hair samples reduced with different mercaptans. The following mercaptans were used: thioglycolic acid (TG), cysteamine (CA), and cystein (CYS). After reduction the hair samples were either blocked with iodoacetate or oxidized to reform broken disulfide bonds.

EXPERIMENTAL

MATERIALS

Fifty percent ammonium thioglycolate, 50% cysteamine hydrochloride, and *l*-cystein were employed as mercaptans, which consisted of thioglycolic acid (TG), cysteamine (CA), and cystein (CYS), respectively. The mercaptans, 28% ammonium hydroxide, and sodium bromate were of cosmetic grade, and a special reagent grade sodium dodecyl sulfate (SDS) was used without further purification. Pronase E was supplied by Sigma (St. Louis, MO) for *Streptomyces griseus*, 12.4 U/mg. All other chemicals used were of reagent grade. The untreated hair (71 μm average diameter) used was from Chinese women in their 20s who had never had chemical hair treatments. The hair samples were soaked in 1 wt% SDS aqueous solution for 10 min at 25°C, washed with water for 30 min, and air dried.

PREPARATION OF REDUCED HAIR AND PERMED HAIR

Reduced hair. Reduced hair samples were prepared by reduction and subsequent blocking steps (12). About 1.0 g of untreated hair was soaked for various lengths of time in 0.50 M mercaptan aqueous solution, as shown in Table I, using a 10:1 solution-to-hair ratio at 25°C. The hair was then removed and immediately soaked for 5 min in 1 wt% ice-cold iodoacetic acid aqueous solution, using a 100:1 solution-to-hair ratio. The hair was then treated for 45 min in 1 wt% iodoacetic acid aqueous solution (adjusted to pH 8.4 with sodium hydroxide), using a 100:1 solution-to-hair ratio at 80°C, to effect blocking of the SH groups. The hair was then washed with water for 30 min and air dried.

Permed hair. Permed hair samples were prepared by reduction and subsequent oxidation steps (10). About 1.0 g of untreated hair was soaked for various lengths of time in mercaptan aqueous solutions, as shown in Table I, using a 10:1 solution-to-hair ratio at 25°C. The hair was immediately soaked for 15 min in an oxidizing agent, as shown in Table I,

Table I
Permanent Waving Solutions

	Mercaptan solutions			
	TG	CA	CYS	Oxidizing agent
Ammonium thioglycolate	0.50 M	—	—	—
Cysteamine hydrochloride	—	0.50 M	—	—
L-Cystein	—	—	0.50 M	—
Ammonium hydroxide	pH 8.6	pH 8.6	pH 8.6	—
Sodium bromate	—	—	—	0.4 M
Phosphoric acid	—	—	—	pH 4.0
Deionized water	to 1000 ml	to 1000 ml	to 1000 ml	to 1000 ml

using a 10:1 solution-to-hair ratio at 25°C. The hair was not rinsed before neutralization, since this was found to cause reoxidation. Then the hair was washed with water for 30 min and air dried. This process was repeated to prepare damaged hair samples, with a reduction time fixed at 15 min.

Hair samples were hydrolyzed in 6 N HCl at 105°C for 24 hr in a nitrogen atmosphere. After membrane filtration of the solution, the acid and water were removed on a rotary evaporator at 45°C, and phenylthiocarbamyl (PTC) derivatization of the hydrolysates was performed using the method of Bennett and Solomon (13).

The PTC derivatives were analyzed on a Jasco HPLC system consisting of a PU-980 pump, a DG-980-50 degasser, an LG-980-02 gradient unit, a CO-965 column oven, and a MD-910 multi-wavelength detector set at 230 nm. The system was delivered in an isocratic and subsequent gradient mode to an Inertsil ODS-2 column (100 mm × 4.6 mm i.d., GL Science Inc.), as described in a previous article (14). The degree of cystine (disulfide bonds) reduction of hair was calculated as follows:

$$R(\%) = [(R_1 - R_0)/R_1] \times 100$$

where R is the percentage of cystine reduction, and R_1 and R_0 are the amounts of cystine in untreated hair and reduced or permed hair, respectively. The value represents an average of the result of triplicate experiments.

PROTEASE TREATMENT

Hair samples were cut to 2.0-cm length, and about 100 mg of the hair sample was incubated in 2.0 ml of Tris-HCl buffer (20 mM, pH 8.0) containing 0.05 wt% Pronase E, or without addition of Pronase E (control), at 37°C for 100 hr. After centrifugation of this solution at 9,000 rpm for 5 min, the supernatant was discarded. The residual hair was washed with water, and after centrifugation of this solution, at 9,000 rpm for 5 min, the supernatant was discarded. This process was repeated three times. After drying, the residue was weighed and the degree of degradation was calculated as follows:

$$P(\%) = [(P_1 - P_0)/P_1] \times 100$$

where P is the percentage of the extent of degradation, and P_1 and P_0 are the weights of hair before and after Pronase E treatment, respectively. The value represents an average of the result of triplicate experiments.

WATER RETENTION

Water retention was measured using a method similar to that of Kohara et al. (15). Hair samples were immersed in deionized water for 4 hr at 25°C, then centriguged at 3000 rpm

for 5 min and weighed (W_1). After drying for 3 hr at 105°C, the hairs were weighed (W_0) and the degree of water retention calculated as follows:

$$W(\%) = [(W_1 - W_0)/W_0] \times 100$$

SCANNING ELECTRON MICROSCOPY (SEM)

Hair samples were cut at their edges with scissors, and part of these samples was treated with the protease described above. The hair samples before and after Pronase E treatments were mounted on stainless steel stubs and sputtered with gold (10 mA, 5 min). The morphology of the hair was examined using a Jeol JSM-5200 scanning electron microscope (accelerating voltage: 15 kV; magnification × 1000).

RESULTS AND DISCUSSION

EFFECT OF REDUCTION TIME ON HAIR

First, reduced hair and permed hair samples were prepared using different reduction times; then the extent of degradation of the hair with protease was measured. As shown in Figure 1, the degradation extent of each mercaptan-reduced type of hair increased with increasing reduction time. It appears that the efficacy of protease increased due to the cleavage of disulfide bonds (cystine) in hair. Furthermore, in the entire treatment time, the degradation extent of CYS-reduced hair was the smallest of all the reduced hairs, that of CA-reduced hair was much higher, and that of TG-reduced hair was the greatest. The differences in the extent of degradation may due to the different extents of cystine cleavage in the hair.

For permed hair, the degradation extent of each hair also increased with increasing reduction time (Figure 2). The difference in the extent of degradation between the permed hair was smaller than that of reduced hair. It appears that the oxidation treatment of permed hair affects the protease degradation. In the oxidation treatment of the permanent waving
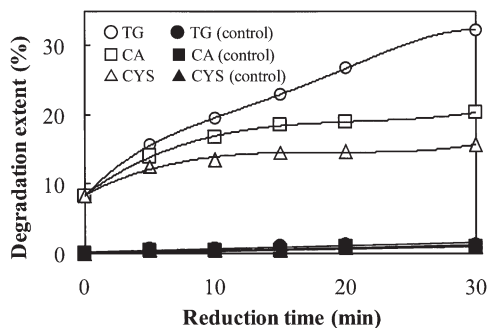

Figure 1. Correlation between the degradation extent and the reduction time of reduced hair. The reduction time of the reduced hair is 5, 10, 15, 20, and 30 min.
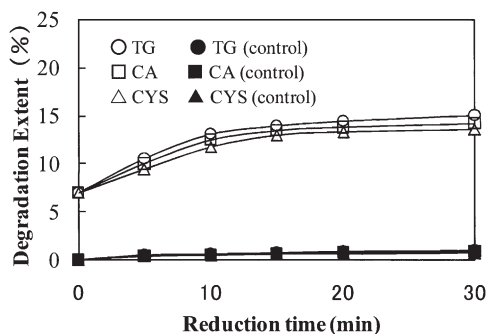
Figure 2. Correlation between the degradation extent and the reduction time of permed hair. The reduction time of the permed hair is 5, 10, 15, 20, and 30 min. The oxidation time was fixed at 15 min.

process, cystine is regenerated and the protein structure becomes more closely packed. For this reason, it appears that the protease attack is less effective, and so the permed hairs showed a small difference in degradability between different mercaptan treatments. Yet these results also indicate that a structural change also occurs with permanent waving treatment that cannot be reversed by oxidation treatment.

EFFECT OF CYSTINE CONTENT OF HAIR

In order to compare the cystine content of hair with different mercaptan treatments, we measured cystine reduction as a function of reduction time. As shown in Figure 3, the cystine reduction no longer increased after 30 min of reduction in each mercaptan treatment. This finding indicates that the reduction in each mercaptan treatment is almost finished at around 30 min, at which time the penetration of mercaptans may be finished (3,4). Furthermore, in the entire reduction time, the cystine reduction of CYS-treated hair was smallest for all hairs, that of CA-treated hair was much higher, and that of TG-treated hair was greatest. Especially in the case of TG-reduced hair, about 90% of cystine was cleaved after about 30 min of reduction.
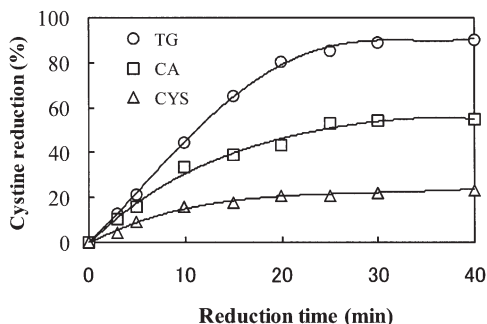

Figure 3. The time course of cystine reduction of hair with different mercaptans. Cystine reduction % (parentheses show the reduction time): TG reduction: 0(0), 13.1(3), 21.5(5), 44.7(10), 65.0(15), 80.4(20), 85.0(25), 89.0(30), 91(40). CA reduction: 10.3(3), 15.8(5), 33.7(10), 39.0(15), 43.0(20), 53.0(25), 54.3(30), 55.0(40). CYS reduction: 4.5(3), 8.9(5), 15.8(10), 18.0(15), 20.6(20), 21.0(25), 22.0(30), 23.4(40).

Gumprecht *et al.* (16) reported much lower cystine reduction than shown in our data, on the order of only 20% in spite of similar conditions. The difference in cystine reduction is believed to be due to the solution-to-hair ratio. It has been known that in cystine reduction, larger concentrations of mercaptan than cystine in hair should drive a reaction to completion (17). Gumprecht *et al.* used a simulated actual hair-waving method; they used a solution-to-hair ratio of about 1:1, while we used a ratio of 10:1. This is the reason why our data show the much higher cystine reduction.

Cystine reduction is considered to be one of the most important factors in protease degradation. We compared the relationship between the extent of degradation and cystine reduction for the reduced hair and permed hair (Figure 4). The reduced hair was used with the different reduction time described above. The permed hair was used with repetitive treatment, because the cystine reduction of hair permed only once was remarkably low. In the reduced hair, all points were plotted on the same curvature in spite of different mercaptan reductions. The degradation appears to be affected by cystine content. In permed hair, it was found that there was a correlation between the extent of degradation and cystine reduction. The cystine reduction of permed hair was remarkably small even with repetitive treatment. This finding indicates that the extent of degradation of permed hair is nearly unaffected by its cystine content.

EFFECT OF WATER RETENTION OF HAIR

Protease degradation of hair occurs in aqueous media. Therefore, protease degradation is considered to be affected by water retention of hair (14). We measured the water retention of reduced and permed hair. The relationship between the extent of degradation and water retention of the reduced hair is shown in Figure 5. In CA- and CYS-reduced hair, the data were plotted in the same linear relationship, and in TG-reduced hair, the data were plotted on a curve. The behavior shows that the TG-reduced hair is more swellable than
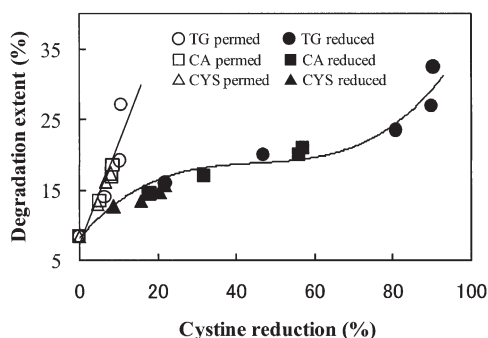
Figure 4. Correlation between degradation extent and percentage of cystine reduction in reduced and permed hair. The reduction time in reduced hair is 5, 10, 15, 20, and 30 min. The repetitive time of permed hair is 1, 3, and 5 times. The degradation extent (%) of reduced hair (parentheses show the reduction time): TG-reduced hair: 8.4(0), 16.0(5), 20.0(10), 23.5(15), 27.0(20), 32.4(30). CA-reduced hair: 14.5(5), 17.1(10), 19.8(15), 21.2(30). CYS-reduced hair: 12.6(5), 13.5(10), 14.7(15), 15.6(30). The degradation extent (%) of permed hair (parentheses show the repetitive time): TG-permed hair: 8.4(0), 14.0(1), 19.2(3), 27.5(5). CA-permed hair: 13.5(1), 16.9(3), 18.5(5). CYS-permed hair: 13.0(1), 16.2(3), 17.3(5).
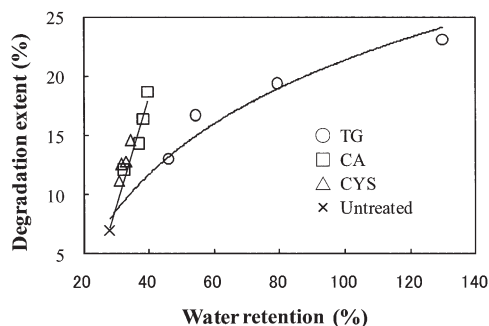
Figure 5. Correlation between the degradation extent and the water retention of hair reduced with TG, CA, and CYS. The reduction time of the reduced hair is 3, 5, 10, and 15 min. The hair source is the same as in Figure 4.

other reduced hairs. It appears that the distance between proteins is extended due to the cleavage of much of the cystine in TG-reduced hair. Otherwise it was probably due to the effect of the cleavage of cystine existing in a different environment (18).

In permed hair, the relationship between the extent of degradation and water retention is shown in Figure 6. It was found that there was a linear correlation between the extent of degradation and water retention. Notice that the water retention and protease degradability increased with repetitive treatment. In hair permed both three and six times, the degradability and water retention of hair increased in the following order: CYS-treated hair < CA-treated hair < TG-treated hair.

SCANNING ELECTRON MICROSCOPY (SEM)

Figure 7 shows typical surface morphology of hair permed three times after protease treatment. The lateral area of TG-permed hair (a) shows that the major part of the cuticle layers was degraded. The degradation was found to affect the inner part of the hair due to a pleated, irregular, and porous cross-section in contrast to other hairs.
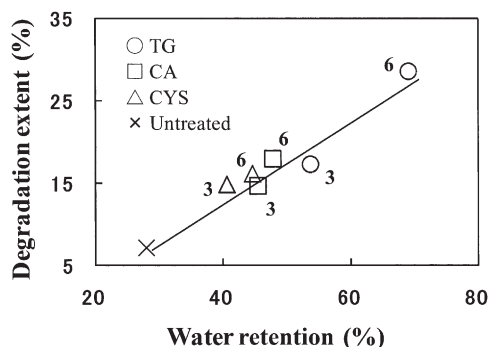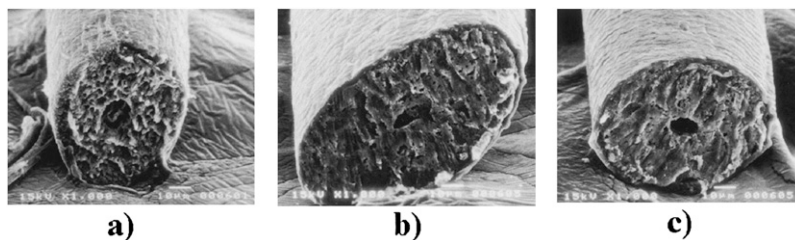

Figure 6. Correlation between the degradation extent and the water retention of hair repetitively permed with TG, CA, and CYS. Numbers 3 and 6 correspond to the number of repetitive times. The hair source is the same as in Figure 4.

Figure 7. SEM photographs of hair after pronase E treatment. (a) TG-permed hair, 3 times. (b) CA-permed hair, 3 times. (c) CYS-permed hair, 3 times. The extent of protease degradation is (a) 19.2%, (b) 16.9%, and (c) 16.2%.

The lateral areas of CA-permed hair (b) and CYS-permed hair (c) exhibited relatively clear cuticle edges, suggesting that the protease degradation of the cuticle layer is not very much advanced. The degradation of CA-permed hair was a little greater than that of CYS-permed hair. Since the cross sections of CA- and CYS-permed hair were relatively plainer than that of TG-permed hair, the degradation was found to be not very much advanced in the inner part of the hair. As a result, it was found that the surface morphology of hair after protease treatment reflects its degradability.

CONCLUSIONS

The protease degradability of reduced hair and permed hair was related to the reduction power of the mercaptans used on the hair. The degradability increased in the following order: CYS-treated hair < CA-treated hair < TG-treated hair.

In reduced hair, it appears that accessibility may be increased due to the cleavage of disulfide bonds. Therefore, reduced hair became swellable and enzymatic attack was found to increase. As a result, the degradability of reduced hair progresses further. In permed hair, though the cleavage of disulfide bonds was remarkably small even with repetitive treatment, accessibility is relatively high. Thus the swellability and degradability of repetitively permed hair appears to progress. Protease degradation is a useful method for evaluating damaged permanent-waved hair. The method is simple, and hair damage can be assessed both visually and through gravimetric analysis.

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