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〈Research Paper〉

Effect of Earthworm Protease on Dyeing Properties of Protein Fibers

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Abstract— An earthworm protease, Lumbricus rubellus, was used to improve the dyeing properties of protein fibers such as wool and silk. The optimal condition for the activity of the earthworm protease was about 40°C at pH 7. The wool and silk were treated with the protease extracted from an earthworm and the K/S values of the dyed wool and silk were measured using a spectrophotometer in order to compare the dye uptake. The protease treatment enhanced the dyeing properties of protein fibers without severe changes in mechanical properties. The surface appearances of protease-treated fibers were observed by microscopy analysis.

Keywords: *earthworm, lumbricus Rubellus, dyeing property, wool, silk*

1. Introduction

For the past few decades, various kinds of dyeing and textile processing technologies have been developed and the processes have performed effectively. However, these technologies are not necessarily ideal from the points of view of energy consumption, environmental impacts and safety in working circumstances. In recent years, utilization of enzyme has gained widespread acceptance for textile processing to solve these problems. The applications of such technologies are usually performed prior or subsequent to dyeing and finishing processes[1-3].

Earthworms secrete serine proteases that degrade a wide variety of proteins including fibroin, collagen, and elastin. The lyophilized powder of the earthworms have long been used for antipyretic and diuretic purposes in Chinese medicine under the name "Jiryu". Recently, the therapeutic effects of the earthworm fibrinolytic enzymes have been reported[4-6].

The scalar structure of the wool fiber cuticle is the major component responsible for the tendency of wool goods to undergo felting shrinkage. During laundering, wool fibers experience small movements in the direction of the fiber root, as a consequence of the difference in coefficient of friction of fibers in the "with-scale" or "against-scale" directions. This progressive entanglement mechanism ultimately results in felting. One route to reduce wool felting is to degrade the scales in the wool fiber surface, so that the scales are softened or removed, for example, proteases[7].

Based on these concepts, many investigations for the wool modification using enzymes have been carried out. Wool treatment with mesophilic proteases leads to a reduced felting tendency and an increased dyeing affinity. However, the new technology for the wool modification using enzymes has not been established as effective process to replace the conventional process[1,8].

The industrial process takes advantage of the different chemical and physical properties of the two silk components, fibroin and sericin. While

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the former is water-insoluble owing to its highly oriented and crystalline fibrous structure, the latter is readily solubilized by boiling aqueous solutions containing soap, alkali, synthetic detergents, or organic acids. Two strands of fibroin are embedded in the sericin matrix. The inherent luster and touch of silk appears after the sericin is removed by the degumming process. In recent years, various studies have dealt with the removal of sericin by using proteolytic enzymes[9,10].

In this study, protease was extracted from earthworm and applied to the protein fibers such as silk and wool. The dyeing properties, surface properties, and tensile strength of the protease treated fibers were investigated.

2. Experimental

2.1. Materials

Earthworms(*Lumbricus rubellus*) were purchased and used in this study.

Hammarsten-casein was purchased from Merck Inc. The silk and wool fabrics(KS K 0905) were used for protease treatment and dyeing throughout the study. All the chemicals used in this study were laboratory-reagent grade.

Sumifix Supra Navy 3GF(C.I. Reactive Blue 222) and Itofix Navy CTR(C. I. Reactive Blue 171) used for the dyeing of wool and silk, were kindly supplied by Sumitomo.

2.2. Preparation of culture supernatant

The earthworms were broken up and homogenized in 0.1M citric acid-Na_2HPO_4 buffer solution of pH 7.0 and then filtered. The filtrate was separated using a centrifugal separator (8,500rpm) and used as the culture supernatant to investigate the characteristics of protease and their effects on wool and silk samples.

2.3. Determination of protease activity

Protease activity was determined by measuring the degree of casein degradation by H. Onishi method. The protease activity in a broth culture was determined by spectrophotometry(optical density at 590 nm), and total protein content was determined by a Bradford assay[11], after the removal of cells by centrifugation. One milliliter of a substrate solution(0.6% casein in 0.1M citric acid-Na_2HPO_4 buffer solution, pH 7.0) was mixed with 0.2ml of the culture supernatant, which had been diluted to appropriate levels with a coenzyme solution-(Na_2CO_3-Na_2HPO_4, pH 10), and the samples were incubated at 65°C for 30 minutes. 15%(w/v) Trichloroacetic acid solution was added to the samples to stop the reaction, and the samples were again incubated at 37°C for 15 minutes. The protease in the samples was precipitated by adding 2.5 ml of a Na_2CO_3 solution(0.55 M) with 0.5 ml of a phenol reagent, diluted three times with H_2O, in order to determine one unit of protease. One protease unit was determined by optical density at 660 nm, and was defined as the amount of protease necessary to produce 1 μg of tyrosine from casein.

2.4. Protease treatment of silk and wool fibers

The supernatant of the broth culture, which was diluted with the coenzyme solution, was prepared for the treatment of the silk and wool fabrics. A fresh liquid medium and a heat-treated broth culture were also prepared as a control. Forty milliliters of the prepared protease-containing broth samples, along with the control, was applied to the silk and wool fabrics(15cm × 5 cm) in 50-ml conical tubes. They were then incubated at 37°C with shaking at 130 rpm.

2.5. Microscopic analysis

Single fibers of silk and wool fabrics, which were treated with activated protease and inactivated protease(control), were observed using an optical microscope of 400x magnification and a SEM(Scanning electron microscope, Akasi Alpha 25A) of 1500x magnification in order to examine surface properties[12].

2.6. Tensile strength test

Tensile strength test of the fabric was performed using a tensile strength tester(Instron,

model no.4468) (KS K0520)[12]. Protease-treated fabrics, along with inactivated protease treated fabrics(controls), were tested five times by the Ravelled Strip method(elongation speed : 30 cm/min), and the means were generated statistically.

2.7. Dyeing method

C. I. Reactive Blue 171 and C.I. Reactive Blue 222 were used for the dyeing of silk and wool, respectively. The fabric samples were dyed in a laboratory dyeing machine(DL-6000, Daelim starlet, Korea) at a liquor ratio of 50:1. Dyeings were carried out at the temperature of 60°C for 30 minutes with the dye concentration of 2%owf. The dyed fabrics were washed and dried after dyeing[13,14].

The dyeing properties of the dyes on the silk and wool fabrics were investigated by measuring K/S values of dyed samples[15], defined as

$$K/S = \frac{(1-R)^2}{2R} \quad (1)$$

where, K: absorption coefficient of dyed samples
S: scattering coefficient of dyed samples
R: spectral reflectance

The K/S values were measured using spectrophotometer(Color-Eye 3100, Macbeth, USA).

3. Results and Discussion

3.1. Activity of protease

The activity of earthworm protease was optimized at pH 7.0 and it was dramatically decreased at alkaline pH of over 9.0. In the case of temperature, the activity of earthworm protease was found to be optimal in the condition of 40℃ at pH 7. This result is similar to the previous report of Nakajima[16].

Earthworm protease is a serine protease, which belongs to endopeptidase. This class comprises two distinct families. The chymotrypsin family, which includes the mammalian enzymes such as chymotrypsin, trypsin, elastase or kallikrein and the substilisin family, which include the bacterial enzymes such as subtilisin.

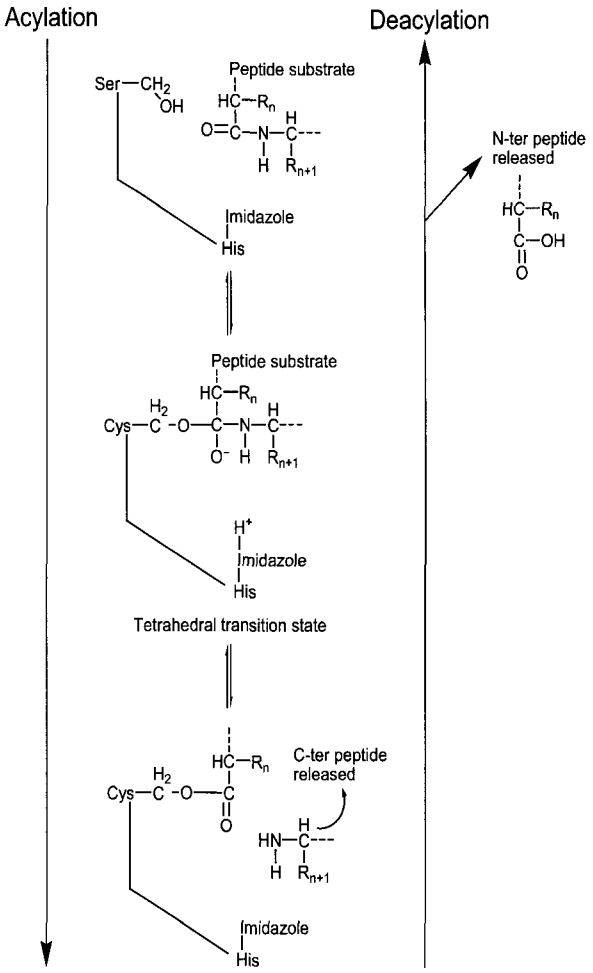

Fig. 1. Catalytic mechanism of serine proteinases (Ser: Serine, His: Histidine).

The general 3Dstructure is different in the two families but they have the same active site geometry and then catalysis proceeds via the same mechanism. The serine proteinases exhibit different substrate specificities, which are related to amino acid substitutions in the various enzyme subsites interacting with the substrate residues. The first step in the catalysis is the formation of an acyl enzyme intermediate between the substrate and the essential Serine(Fig. 1)[17].Formation of this covalent intermediate proceeds through a negatively charged tetrahedral transition state intermediate and then the peptide bond is cleaved. During the second step or deacylation, the acyl-enzyme intermediate is hydrolyzed by a water molecule to release the peptide and to restore the Ser-hydroxyl of the enzyme. The deacylation, which also involves the formation of a tetrahedral transition

state intermediate, proceeds through the reverse reaction pathway of acylation. A water molecule is the attacking nucleophile instead of the Ser residue.

The His residue provides a general base and accept the OH group of the reactive serine. Fig. 1 shows a schematic representation of the catalytic mechanism of cysteine proteinases.

3.2. Microscopic analysis of the silk and wool fibers

The silk and wool fabrics were treated with the protease extracted from earthworm, and were observed by microscop. It is known that raw wool contains 25-70% by mass of impurities, which consist of wool grease, perspiration products-(suint), dirt and vegetable matter such as burrs and seeds. Fig. 2 shows the wool fiber treated with activated protease(Fig. 2b) was cleaner than that with inactivated protease(Fig. 2a). It shows that the protease remove the impurities and scales from the wool. The results are consistent with the previous reports[18,19].

The similar trends were also observed in the case of silk. The protease-treated sample(Fig. 3b) was cleaner than the control(Fig. 3a). However, the thickness of samples treated with activated protease diminished compared with that of inactivated protease treated samples, which demonstrates that the residual sericin or fibroin of silk fiber was partially degraded by protease treatment.

3.3. Tensile strength

In order to investigate the effects of the protease treatment on the physical properties of the fabric, tensile strength was measured. The components of the wool fiber are made up of the protein keratin.

The preferred configuration of the keratin molecule is the helices joined by disulfide bonds, which is relaxed form of the molecule and is known as α-keratin. The molecules can be changed into β-keratin by hot water, steam, alkali, or by elongation due to the cleavage of hydrogen bonds parallel to the axes and the disulfide linkage. Also, when tension on the fiber is removed, the molecules return to the α form, and the fiber returns

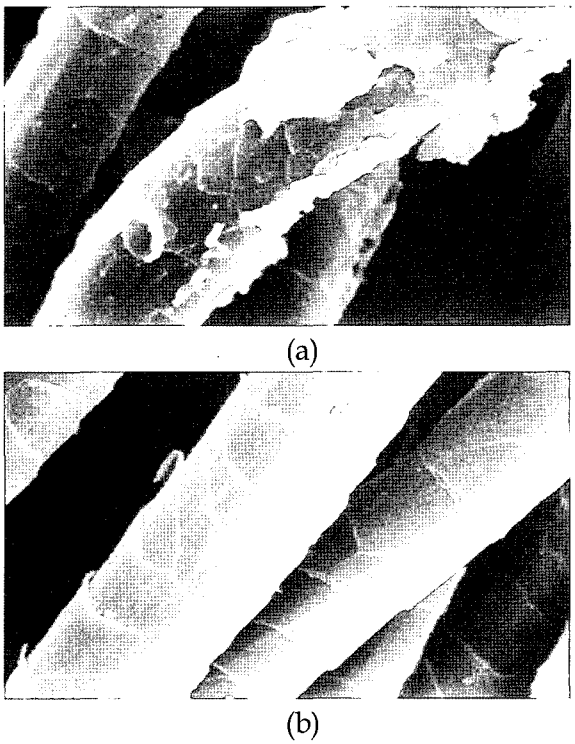

Fig. 2. Microscopic photos of wool fibers (a) SEM of wool fibers treated with inactivated earthworm protease for 48h (b) SEM of wool fibers treated with activated earthworm protease for 48h.

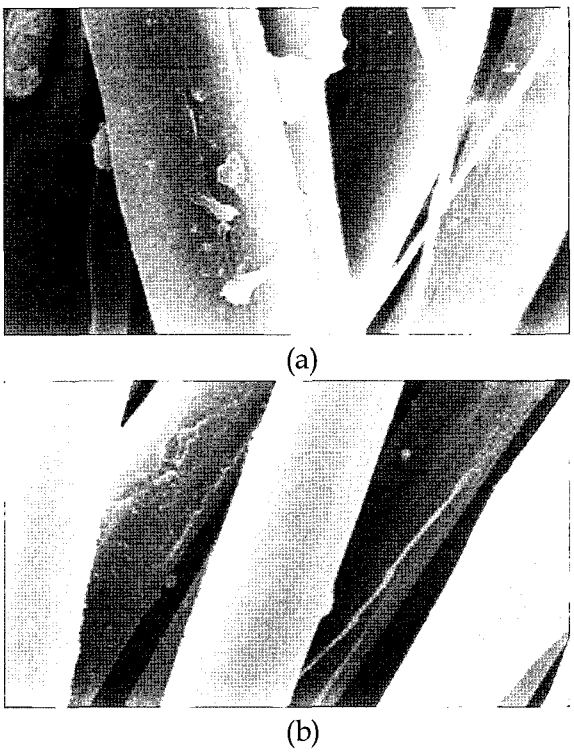

Fig. 3. Microscopic photos of silk fibers (a) SEM of silk fibers treated with inactivated earthworm protease for 48h (b) SEM of silk fibers treated with activated earthworm protease for 48h.

to its original shape and dimensions. Therefore, treatment with hot water, steam, or alkali can change the tensile strength of the wool. However, as shown in Fig. 4, the results of the tensile strength analysis did not show considerable differences, after 48 hours of the protease treatment, between the treated wool samples and the control samples treated with the heat-inactivated protease. Therefore, it is considered that the protease produced by earthworm was able to remove the scales of the wool fabrics, but it did not change the internal or chemical structure of the wool.

It has been established that the fibroin comprising the majority of silk yarn is composed of polypeptide chains, which themselves are composed of 17 different amino acids. The majority of these amino acids, however, are glycine and alanine, which possess shorter side chains than others, therefore, hydrogen bonds can be more easily formed between their polypeptide chains. However, >C=O and - NH- in the polypeptide chains of silk fiber contribute to the structural stability of silk against various stress conditions. The tensile strength difference between the protease treated silk samples and the control samples were negligibly small as shown in Fig. 5 Consequently, this result demonstrated that the protease partially degraded the residual sericin or fibroin of silk, but it did not alter their tensile properties dramatically.

3.4. Dyeing properties of protease treated wool and silk fibers

The fabrics which were treated with protease at intervals of 3, 9, 24, and 48 hours were dyed, washed, and dried. In order to compare the dyeing properties of protease treated fabrics, K/S values were measured using spectrophotometer interfaced with a personal computer.

Fig. 6 shows that the K/S values of protease-treated wool fabrics gradually increased as protease treatment time increase. It is considered that the increase in dye uptake is due to the protease-induced degradation of the scales and impurities, especially the serine residues, in the wool. This result was also verified by scanning electron microscopy as shown in Fig. 2. It is well known that the cuticle cells, or scales, constitute the outermost surface of the wool fiber and are responsible for

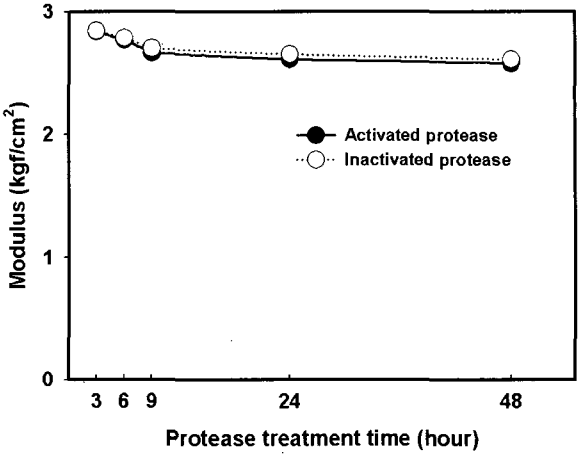

Fig. 4. Modulus of wool fibers treated with active protease and inactivated protease (kgf/cm^2)

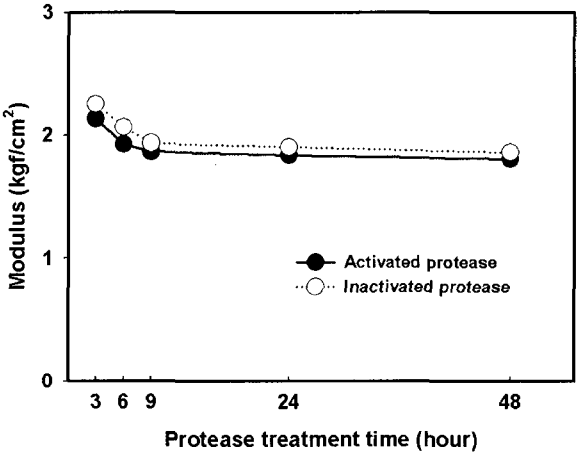

Fig. 5. Modulus of silk fibers treated with active protease and inactivated protease (kgf/cm^2)

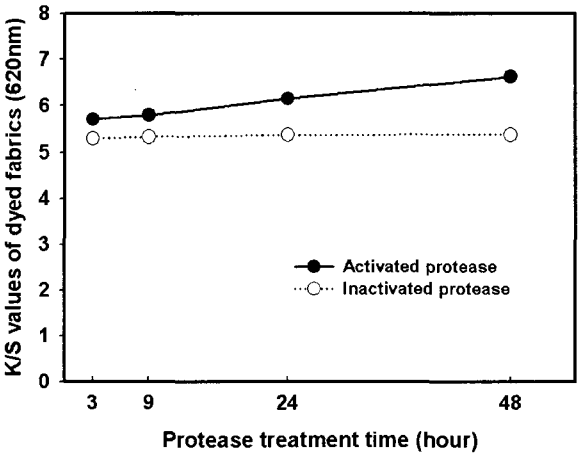

Fig. 6. Dyeing properties of wool fibers at various protease treatment time.

important properties such as wettability, tactile properties and felting behavior. Therefore, the result shows that the removing of impurities and scales by protease treatment can enhance the dye accessibility to the wool fibers via CMC(cell membrane complex). However, the earthworm-protease did not degrade the serine residues in the wool fabrics enough to change their tensile strength. But, the protease improved the dyeing properties of the wool fabrics.

In the case of silk, the similar trends were observed as shown in Fig. 7. The reaction partners are, on the side of the silk fiber, mainly the terminal amino groups of the lysine, especially when dyeing is carried out in the neutral to weakly alkaline region. In the relatively stronger alkaline region the phenol group of the tyrosine side chain may also react with the dye. Therefore, it appears that the improvement of dye uptake by the protease hydrolysis is due to the amine residues in the silk, related to the interaction between the dyes and the silk.

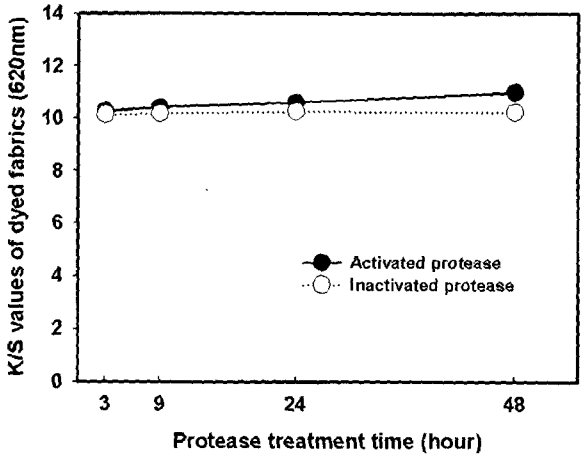

Fig. 7. Dyeing properties of silk fibers at various protease treatment time.

4. Conclusions

An earthworm produced protease, Lumbricus rubellus, was used to improve the dyeing properties of protein fibers. The optimal condition for the production of the protease by earthworms was about the temperature of 40°C at pH 7.

The activated protease removed the impurities and scales from the wool, which was verified by microscopic examination. Also, the removal of impurities and scales by protease treatment increased the dye uptake due to the improvement of dye accessibility to the wool fibers via cell membrane complex. However, the tensile strength did not show considerable differences for 48 hours of the protease treatment, between the treated wool samples and the control samples treated with the heat-inactivated protease. Therefore, it is considered that the protease did not degrade the serine residues in the wool fiber enough to decrease remarkably its tensile strength, although the protease did enhance the dyeing properties of wool.

The similar trends were also observed in the case of silk fiber. Protease treatment enhanced the dye uptake due to the increase of dyeing sites(e.g. amine residues) by the protein hydrolysis. The thickness of active protease treated samples was decreased compared with that of inactivated protease treated samples, which demonstrates that the residual sericin or fibroin of silk was partially removed by protease treatment. However, the tensile strength difference between the protease treated wool samples and the control samples were negligibly small, which indicates that the protease removed residual sericin or fibroin of silk without severe deterioration in their physical properties.

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