Rapid Communication

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**CONFORMATION OF FRAGMENTS FROM DEGRADATION
OF WOOL-Cu(II) COMPLEX BY HYDROGEN PEROXIDE***By Kazuhiko Fukatsu and Mariko Isa*(Faculty of Living Science, Kumamoto Women's University,
Kengun-machi, Kumamoto 862, Japan)**ABSTRACT**

The fragments from the degradation of wool-Cu(II) complex by hydrogen peroxide have a partly ordered structure (α -helix) in water. As the pH increases, the partly ordered structure transforms into a random coil. Further, the fragments undergo random coil- α -helix transition in ethanol-water mixtures with increasing ethanol component. These behaviors are similar to those of charged polyamino acids, in spite of that the fragments of wool undergo a less sharp transition in comparison with charged polyamino acids.

1. INTRODUCTION

Three distinct fragments of protein were isolated from α -keratin; the low-sulphur fragment, the high-sulphur fragment and the high-glycine-tyrosine fragment¹⁾. These fragments were examined by means of the X-ray diffraction, infrared absorption and circular dichroism methods, and were shown to have ordered structures such as α -helix or β -conformation in solution and in solid state.

Inagaki and coworkers^{2,3)} investigated the conformations of a high-sulphur and a high-glycine fragments from wool keratin and found that these tend to form partly β -conformation in the films cast from formic acid solution. Crewther and coworker⁴⁾ showed that the fractions obtained by partial chymotryptic digestion of S-carboxymethyl derivatives of the low-sulphur fragment have an α -helix content of about 83% in borate solution.

It is well known⁵⁾ that wool-Cu(II) complex is easily obtained by a reaction of wool with copper(II) ion. With a view to obtaining functional protein metal complexes we examined the complexation of wool keratin and found that Cu(II) complex of wool can be degraded by hydrogen peroxide (H_2O_2) more readily than that of silk fibroin. We consider it to be interesting to clarify the conformation of these degraded fragments and

the composition of polypeptide species contained in them. The present communication deals with a circular dichroism (CD) study on conformations of these fragments of wool-Cu(II) complex in water or in ethanol-water mixtures.

2. EXPERIMENTAL

Wool keratin fiber was employed in the form of woven fabric cloth made up from Merino 64's quality wool. All other chemicals were of reagent grade and were used without further purification.

The sample fragments were prepared from wool-Cu(II) complex as follows: Sample of wool fiber (10g) was added in 150 ml aliquot of 2.7×10^{-2} M $Cu(NO_3)_2$ aqueous solution, and the flask was shaken to facilitate the wetting-out of the fiber, followed by the addition of 400 ml of 0.25 N NaOH aqueous solution to make wool-Cu(II) complex. Then three 15 ml portions of 30% H_2O_2 aqueous solution were added at two hours intervals. The reaction mixture thus prepared was allowed to be shaken at room temperature until the wool fiber was no longer detachable. After the reaction had completed, conc. HCl was added to adjust the pH of reaction mixture at about 6. The mixture was filtered and the filtrate was then dialyzed against distilled water; the resultant turbid solution was centrifuged to remove insoluble materials.

The pH of the solution was 6.54. The concentration of Cu(II) ion was 2.24×10^{-5} M, being negligibly low. The concentration of sample fragments was 4.18 g/l. Assuming that the amino acid composition was not changed by the degradation, its apparent molar concentration of mean residue was estimated as 3.3×10^{-2} M/l.

CD measurements of the sample fragments solutions were obtained on a Jasco J-40 spectropolarimeter in the region of 200–260 nm at room temperature. The CD spectra were expressed in the term of molar ellipticity using the apparent molar concentration of the solution.

3. RESULTS

Figure 1 shows the CD spectra of sample fragments in aqueous solution at various pH. The fragments in water exhibit a CD spectrum characteristic of a helix with a double minimum at 222 and 206 nm, though the residue ellipticities values $[\theta]$ at both wavelength are small (Figure 1, curve a) and decrease with rising the pH (Figure 1, curve b, c), suggesting that the fragments tested contain partially ordered structure (α -helix), which undergoes a transition from the α -helix to the random coil. This fact is possibly due to the electrostatic repulsion between involved anionic functional groups in an analogy with poly-L-glutamic acid⁶⁾,

Fig. 1. CD spectra of the fragments of wool-Cu(II) complex degraded with H_2O_2 obtained in water at different pH. Curve a, pH=5.25; curve b, 7.85; curve c, 10.40.

because the dissociation of the charged side chains (glutamyl and aspartyl residues) in the system will be promoted as the pH increases.

It is well known^{7,8)} that the addition of alcohol to an aqueous solution of polypeptides enhances the helix stability. Pispisa and coworker⁹⁾ reported on the basis of some experimental evidences that the role of organic solvent in solution appears more complicated than that of strengthening the intramolecular hydrogen bonds within the polymer. A similar result was obtained in this investigation. Figure 2 shows the CD spectra of the sample fragments in ethanol-water mixtures. As the ethanol ratio increases, the α -helix content induced, as indicated by the change of CD spectra (Figure 2, curves b~g), but the transition shown by the fragments of wool was not so sharp as that for the formation of fully charged α -helical structures. In the insert of Figure 2, the values of

Fig. 2. CD spectral changes of the fragments of wool-Cu(II) complex degraded with H_2O_2 as a function of ethanol concentration (by vol.).

Curve a, 0% ethanol (pH=6.54); curve b, 10%; curve c, 20%; curve d, 30%; curve e, 50%; curve f, 70%; curve g, 90%.

All curves were recorded immediately after preparation of solutions.

Insert: Variation of ellipticity at 222 nm as a function of solvent composition in terms of % ethanol.

$[\theta]$ at 222 nm gradually changed with increasing ethanol component.

The present results indicate that the fragments from the degradation of wool-Cu(II) complex by H_2O_2 have a conformational transition in water and ethanol-water mixtures in a similar manner as those found for charged polyamino acids.

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羊毛-銅(II)錯体の過酸化水素分解による フラグメントの二次構造

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羊毛-銅(II)錯体を過酸化水素で分解して得られたフラグメントの水溶液中及びアルコール-水混合溶媒中の二次構造について、円偏光二色性を用いて検討した。水溶液中で、一部 α -ヘリックス構造が形成され、pHの増加とともに減少した。又、アルコール-水混合溶媒中で、アルコール組成の増加にともなって、 α -ヘリックス

構造の増加が観測された。これら一連の転移は荷電ポリアミノ酸(ポリ-L-グルタミン酸, ポリ-L-リジン, など)に観測されたように側鎖解離基にもとづくものと考えられる。しかし、転移は荷電ポリアミノ酸に較べて、緩慢である。