Cellulase Treatment of Cellulosic Fabrics
—Inhibitory Effect of Ionic Dyes in Treating Solution—

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Abstract: Inhibitory effects of Congo Red and Orange II, which were present in treatment solution, on the cellulase catalytic reaction for viscose rayon substrate was investigated. Congo Red showed much more remarkable inhibitory action for the cellulase catalytic reaction than Orange II did. Visible absorption of Congo Red shifted appreciably to longer wavelength in the presence of cellulase in the solution, indicating that there may be specific interactions between the dye and the enzyme, whereas slight red shift, in contrast, was observed with Orange II. The inhibitory action of Congo Red in the treatment solution was interpreted in terms of the formation of the dye-cellulase complex in the solution, whose enzymatic activity was less than that of free cellulase.

1. Introduction

Cellulase treatments of cellulosic fabrics have been a focus of interest to produce the fabrics with soft and smooth surface and brightened colors [1-4]. It is empirically well known that the cellulase catalytic reaction rate is affected appreciably by chemicals existing both in the treatment bath and on the substrate, as well as by the system conditions such as pH and temperature. Reproducibility of the treatment process is therefore relatively poor compared to other chemical or mechanical textile processes.

In the previous papers [5, 6], we have examined the inhibitory effect of dyes and surfactants toward cellulase catalytic reaction, and we have found that ionic dyes and ionic surfactants inhibited the cellulase catalytic reaction remarkably, whereas non-ionic compounds did not. From the results we assumed that ionic dyes and surfactants may interact electrostatically with cellulase enzyme on the substrate or in the treatment solution.

In this paper, the inhibitory effects of ionic dyes such as direct and acid dye, which are present in treatment solution, on the cellulase catalytic reaction for viscose rayon substrate have been investigated for the further study for the mechanisms of the inhibitory action of ionic coexisting substances on the cellulase catalytic reaction.

2. Experimental

Cellulase (from Trichoderma viride, 1600 FDUN/mg) was obtained from Nagase Biochemicals Ltd., Kyoto, Japan. The dyes used were Congo Red (C. I. Direct Red 28) and Orange II (C. I. Acid Orange 7). They were obtained as reagent grade from Nacalai Tesque and used without further purification. Fiber substrates were viscose rayon filament yarn (75 d) and were washed in ethanol for 10 min. and subsequently in distilled water at boiling point for 1 h prior to the experiments. The procedures for cellulase treatment and weight loss measurement were previously published [5]. The conditions for the cellulase treatment were pH 5, 50 °C, bath ratio 40:1, and enzyme concentration 3g/L.

3. Results and Discussion

In Fig.1, the weight loss of the rayon filament by cellulase treatment in the presence of Congo Red in the treatment solution is plotted against treating time. The rate of weight loss of rayon sample by the cellulase catalytic reaction decreases with increasing the concentration of Congo Red in the treatment solution. The dye, whose concentration is more than 5 mmol/L, freezes the activity of the enzyme completely.

In the previous paper [5], we have investigated the
weight loss of dyed cellulosic substrate by cellulase catalytic reaction and have found that direct dye (C. I. Direct Black 22) on the cotton substrate inhibited the cellulase catalytic reaction appreciably. We assumed from the results that the mechanism of the inhibition by the direct dye on cellulase catalytic reaction may be attributed to an obstruction for an approach of cellulase enzyme toward the $\beta$-1, 4 glycoside bonds of cellulose chain. Prior to our work, Mori et al. [7] have also obtained similar results, and interpreted the phenomena in terms of a blocking effect by direct dye for the hydrolysis of the substrate catalyzed by cellulase enzyme. In the present case of Fig.1, since the dye (Congo Red) was added in the treating solution, the rayon substrate absorbed little amount of the dye at the initial treating process. Nevertheless, it was clear that the cellulase catalytic reaction was remarkably inhibited by the dye. The mechanism of the inhibition seems to be different from that of blocking or obstruction by the dye for an approach of cellulase enzyme on the substrate. The alternative interpretation for the inhibitory phenomena in this case must be necessary.

Fig.2 shows the effect of Orange II in the treatment solution on the rate of cellulase catalytic reaction. It is noteworthy that the inhibition of Orange II toward the cellulase catalytic reaction is much less than that of the corresponding concentrations of Congo Red. Orange II has approximately a half in molecular weight and in molecular size to that of Congo Red, and has a

Table 1 Apparent Rate of Weight Loss (%/h) of Rayon Fiber by Cellulase Catalytic Reaction in the Presence of Ionic Dyes in Treatment Solution

<table>
<thead>
<tr>
<th>Conc. of Dye (mmol/dm$^3$)</th>
<th>Rate of weight loss (%/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Congo Red</td>
</tr>
<tr>
<td>0</td>
<td>2.44</td>
</tr>
<tr>
<td>0.5</td>
<td>1.21</td>
</tr>
<tr>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
<td>3.5</td>
<td>-</td>
</tr>
<tr>
<td>5.0</td>
<td>0.02</td>
</tr>
<tr>
<td>10</td>
<td>-0</td>
</tr>
</tbody>
</table>
Concentration of dye (mmol/dm³)

Fig. 3 Relative rate of cellulase catalytic reaction in the presence of ionic dyes. ●, Congo Red; ○, Orange II.

Concn. of Cellulase (g/dm³)

Fig. 4 Visible absorption maximum of Congo Red and Orange II in aqueous cellulase solution. ●, Congo Red; ○, Orange II.

In Fig. 4 the shift of visible absorption maximum of Congo Red and Orange II in aqueous cellulase solution is shown. As can be seen from Fig. 4, the absorption maximum of Congo Red shifts appreciably with the presence of cellulase in the solution, whereas Orange II does not show remarkable shift. The remarkable red shift of Congo Red in the cellulase solution may be attributed to a formation of dye-enzyme complex. The inhibitory effect of Congo Red on cellulase catalytic reaction toward rayon hydrolysis as shown in Fig. 1 would therefore be attributed the complex formation between Congo Red and cellulase enzyme in the treatment solution. We assumed that conformation or structure of active site of cellulase would be changed by dye binding, and hence the activity of the dye bound cellulase would be remarkably reduced. The detail structure of the dye-cellulase complex has not been clear at present. Further investigations such as binding experiments of the dye to cellulase and conformational study of the dye-cellulase complex etc. would be necessary.

We can conclude from the present results that the inhibition of dyes to the cellulase catalytic reaction may be interpreted in two distinguishable manners, one of which is the block or the obstruction by the dye, as mentioned in the previous paper (5), on the cellulosic substrate for the approach of the cellulase enzyme to the β-1, 4 glycoside bonds of the cellulose chain, and others of which is the formation of a dye-enzyme complex in the treating solution, the enzyme of which is less active (or completely inactive) than free enzyme.

References