EFFECT OF RELATIVE HYDROPHILICITY ON ENZYMATIC ACTIVITY OF MODIFIED CELLULASE

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Introduction

In our previous work^{2, 3)}, characteristics of modified cellulase with synthetic copolymer of polyoxyalkylene (POA) derivative and maleic anhydride (MA) were reported. The modified cellulase showed high stability against environmental conditions of cellulosic hydrolysis²⁾ and showed effective adsorption control onto substrate, thus enhancing the hydrolytic reaction³⁾.

In this study, the effects of relative hydrophilicity of modifier on the modification reactivity with cellulase, the initial enzymatic activity and the long-term saccharification ability were investigated.

Experimental

Modifier: The copolymers of POA derivative and MA (Nippon Oil & Fats Co., Japan) used as modifiers are listed in **Table 1**. The alkylene oxide (AO) chain consists of ethylene oxide (EO) and propylene oxide (PO). The weight ratio of total EO to total AO, denoted by *EO/AO*, is varied from 20 to 100 %. The relative hydrophilicity of the modifier is expected to increase with increasing

EO/AO.

Cellulase: Crude enzyme TP-60 from *Trichoderma reesei* (Meiji Seika Ltd., Japan) was used as cellulolytic enzyme. Cellulose hydrolysis activity was represented by filter paper activity (FPase activity). The enzyme was assayed as reported by Mandels *et al.*⁴⁾ with FP-5C (Toyo Roshi Ltd., Japan) as substrate. The enzyme had a specific FPase activity of 6.3×10^{-3} kat/kg. The activity was defined by the moles of reducing sugar produced per kg of enzyme per second.

Cellulase modification: The modification reaction was carried out at 4°C in 0.15 mol/dm³ borate buffer of pH 8.5. 1000 mg of native cellulase was put into 100 cm³ of the buffer solution and the modifier was added stepwise at one-hour intervals with constant stirring. The quantity of modifier added in each step corresponded to half of the weight of cellulase in the mixture. This is because the condensation reaction between MA groups of the copolymer and amino groups of the cellulase competes with the ring-opening hydrolysis of MA groups. The degree of modification (*DM*)²⁾ could be changed by varying the addition times of the modifier, which corresponds to varying the weight ratio (*WR*) of the modifier

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Table 1. Types of synthetic copolymers used as modifier

modifier	EO/AO [%]	<i>n</i>	<i>k</i>	MW*
AKM1510	100	33	10	16,000
AHM1510	65	31	10	16,000
AEM1510	40	28	10	16,000
ADM1510	30	27	10	16,000
ACM1510	20	26	10	16,000

* MW values are approximate values

Fig. 1 Relationship between *DM* and weight ratio of copolymer to cellulase

to cellulase.

Saccharification reaction: The saccharification reaction was carried out with gentle shaking at 50°C in 0.2 mol/dm³ acetate buffer of pH 5.2 with addition of 0.03 % sodium azide. Filter paper FP-5C was used as cellulosic substrate. The initial concentration of the substrate was 4.1 % (w/v) and that of enzyme was 0.10 g/l.

Assay procedures: The FPase activity, the *DM* of modified cellulase and the reducing sugar produced by hydrolyzing cellulose were determined by the methods described previously^{1, 4, 5}.

Results and Discussion

Modification reactivity: The relationship between weight ratio (*WR*) and modification degree (*DM*) is shown in **Fig. 1**. In all types of the modifier, the modification reaction proceeded readily and *DM* increased with increase in *WR*. Comparing the values of *DM* at the same *WR*, the more hydrophilic copolymer showed higher *DM* value.

Initial activity: The relative initial activity was defined as

Fig. 2 Relationship between relative FPase activity and *DM* **Fig. 3** Reducing sugar production of native cellulase and modified cellulase with time

the ratio of the activity of modified cellulase to that of native cellulase, as determined by hydrolyzing the substrate for 60 min at 50°C³). As shown in **Fig. 2**, the relative FPase activity is influenced by the modifier and the *DM* value.

There was no significant difference in the initial activity between modifiers other than ACM. The modified cellulases under 50 % degree of modification showed higher initial activities than did native cellulase.

In the case of the most hydrophobic modifier (ACM) used in this study, the modified cellulase showed the lowest activity. Modified cellulase over 20 % degree of modification, however, showed approximately 90 % of native activity.

Reducing sugar conversion: Experimental examples of the time course of reducing sugar production by enzymatic hydrolysis are shown in **Fig. 3**. Modified cellulase with AKM and AEM were used in this experiment. These two modified cellulases had 58 % and 15 % of modification degree and 1.00 and 1.05 relative initial activity, respectively.

In the case of native cellulase, the hydrolysis rate decreased with time, and the hydrolysis almost stopped after 80 h.

Fig. 4 Relationship between relative conversion after 60h saccharification and *DM*

The modified cellulases showed hydrolysis rates similar to that of native cellulase in the initial period of saccharification. After 10 h, however, the hydrolysis rates of all modified cellulases were always higher than that of native cellulase and the hydrolysis still proceeded after 80 h.

Comparing the reducing sugar production after 100 h, modified cellulase was superior to native cellulase by a factor of 1.28 to 1.38. This factor is higher than the factor of initial activities.

To evaluate long-term saccharification ability, the relationships between *DM* and relative conversion after 60 h hydrolysis are shown in **Fig. 4**. The relative conversion was defined as the ratio of conversion by modified

cellulase to that by native cellulase at the same reaction time.

The relative conversion after 60 h of modified cellulase were superior to that of native cellulase. As shown in **Fig. 2**, the initial activities of modified cellulases are not always superior to that of native cellulase. In our previous work^{2,3)} it was reported that cellulase modification with copolymer enhanced thermostability and effectively controlled strong adsorption of cellulase onto the substrate. The result shown in **Fig. 4** can be explained by the additive effect of these two factors.

The study described above reveals that approximately 30 % degree of modification with hydrophilic copolymers, such as AKM, AHM and AEM, is effective in hydrolyzing cellulosic substrate for a long period.

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Literature Cited

- 1) Habeeb, A.F.S.A.: *Anal. Biochem.*, **14**, 328-336 (1966)
- 2) Kajiuchi, T. and J.W. Park: *J. Chem. Eng. Japan.*, **25**, 202-206 (1992)
- 3) Kajiuchi, T. and J.W. Park: *J. Chem. Eng. Japan.*, **26**, 28-33 (1993)
- 4) Mandels, M., R. Andreotti and C. Roche: *Biotechnol. Bioeng. Symp.*, **6**, 21-33 (1976)
- 5) Miller, G.L.: *Anal. Chem.*, **31**, 426-428 (1959)