J Food Sci. Nutr.
Vol 1, No. 1, p. 117~120(1996)

Enhanced Hydrolysis of Cellulose by Combination of *Humicola insolens* Cellulase with Immobilized Glucose Isomerase

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Abstract

To enhance cellulose hydrolysis *Humicola insolens* cellulase(HIC) was employed with immobilized glucose isomerase(IGI). Optimum pH and temperature for HIC were 6.5 and 55°C, respectively, whereas those for IGI were 7.0 and 60°C, respectively. Optimum loading size of IGI was 200mg(130 units) with 15units of HIC. Reaction conditions were determined to be as follows: 55°C, pH 6.5, HIC 15 units and IGI 130 units. After 24h hydrolysis, more than 65% of avicel was converted to glucose and fructose; in contrast, the conversion ratio of control was 40%.

Key words: *Humicola insolens*, cellulase, glucose isomerase, immobilization

INTRODUCTION

Rapidly dwindling energy reserves forced researchers to explore alternatives to conventional fossil fuels. One such alternative is ethanol which can be obtained by the conversion of renewable cellulosic biomass to glucose syrups and fermentation of the latter to ethanol (1). The first step is referred to as saccharification and carried out by cellulases.

In cellulase complex, three types of enzymes act together in saccharification of cellulose to glucose : exo-glucanases(cellobiohydrolases and exo-glucohydrolases), endo-glucanases, and β-glucosidase(2-7). These enzymes act in a synergistic manner and yield glucose and cellobiose as major reaction products. Unfortunately, cellobiose inhibits both cellobiohydrolase and endoglucanase, and if it builds up during saccharification, it retards the conversion of cellulose to glucose(8,9). The inhibition can be relieved either by the addition of supplementary β-glucosidase(10) or by employing β-glucosidase mutant with resistance to end-product inhibition(1). Alternatively, this problem can be overcome by removing glucose from the reaction mixture as soon as it is formed. This may be achieved either by subsequent conversion of glucose to ethanol in a single step(11,12) or by removing glucose using ultrafiltration(13).

In contrast, Woodward and Arnold(14) suggested that high rates of cellobiose hydrolysis catalyzed by β-glucosidase may be prolonged by converting the reaction product glucose to fructose using a suitable preparation of glucose isomerase. The enzyme glucose isomerase(D-xylose ketol-isomerase EC 5.3.1.5) catalyzes the reversible isomerization of D-glucose to D-fructose. This enzyme is now used in the food- processing industry to produce a sugar mixture as sweet as sucrose(15).

However, major problem of this approach lies in the difference in optimum reaction conditions between cellulases and glucose isomerase. Most cellulases exerted high activities in the pH ranges between 4.0 and 6.0, whereas glucose isomerases were between pH 7.5 and 9.0 (16). To compromise this difference in optimum conditions, a commercial cellulase preparation of *Humicola insolens* was employed for this work and an extended hydrolysis of cellulose was carried out with cellulase and glucose isomerase to evaluate the possibility.

MATERIALS AND METHODS

Materials

Commercial cellulase from *Humicola insolens*(SP 227, Novo laboratories, Inc.) was dispersed in 10 volumes of 1% NaCl and stirred for 1h at 3°C followed by

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centrifugation at 10,000×g for 20min. Impurities from the supernatant were removed by diafiltration (M.W. cut-off, 10,000 dalton). The crude enzyme solution was flushed with 4 volumes of deionized water, and this step was repeated three times. The retentate was then lyophilyzed.

Immobilized glucose isomerase(Sweetzyme Q) was obtained through Miles Laboratories, Inc.(U.S.A). Sweetzyme Q is immobilized in a way the enzyme protein and other cell components are converted into an insoluble matrix by crosslinking with glutaraldehyde. The crosslinked cell mass is made into pellets by extrudation in an axial extruder.

Effect of pH

Effects of pH on the activities of *Humicola insolens* cellulase(HIC) and immobilized glucose isomerase(IGI) were measured in 0.1M buffer solutions(0.1M sodium acetate buffer, pH 3.0~5.5; sodium phosphate buffer, pH 6.0~7.5; ammonia water-ammonium chloride buffer, pH 7.5~8.9). These buffers contained 0.1M $MgSO_4$ and 0.001M $CoCl_2$. For cellulase activity assay, avicel PH101(Fluka) was suspended in an appropriate buffer to give final concentration of 1.0%(w/v). To 2.0 ml of avicel suspension, 20mg of *Humicola insolens* cellulase (15 units) was added. Hydrolysis was carried out at 50°C for 1h. After centrifuging the reaction mixture at 10,000×g for 20min : reducing sugar in the supernatant was measured by the DNS method(17). One unit of cellulase activity corresponds to the amount of enzyme that produce 1μmole reducing sugar per minute.

Glucose isomerase activity was determined in a system containing 0.1M buffer containing 0.275M D-glucose(18). The activity of the immobilized glucose isomerase is defined, according to the international recommendations, so that one enzyme unit is the amount of enzyme which catalyzes the conversion of glucose to fructose, with an initial velocity corresponding to one micromole per minute under standard conditions. The standard conditions are 65°C, pH 8.5.

Effect of temperature

Temperature ranges of 35~80°C were used at the interval of 5°C. pH of substrate solution was selected from the result of pH effect on the enzyme activities. Reaction time and procedures were the same as those of pH effect assay.

Effect of GI loading size on cellulose hydrolysis

To determine the optimum ratio of enzymes, immobilized glucose isomerase to *Humicola insolens* cellulase, various amounts of IGI and fixed amount of HIC were added to 2% avicel suspension at optimum pH and temperature. After incubating for 24hr, reducing sugar released was measured by the DNS method.

Extended hydrolysis of cellulose

To evaluate the effect of IGI on cellulose hydrolysis by *H. insolens* cellulase, extended hydrolysis of cellulose was carried out at 50°C. The reaction mixture(50 ml) was composed of avicel 1.0g, HIC 15 units, IGI 130 units, and 0.1M sodium phosphate buffer, pH 7.5. Sampling was carried out at the predetermined time. Reducing sugar released was determined by the DNS method.

RESULTS AND DISCUSSION

Effect of pH

The effect of pH on IGI and HIC were shown in Fig. 1. pH optima for IGI and HIC were pH 7.0 and 6.5, respectively. HIC showed considerable loss of activity at pH lower than 5.0 or higher than 7.5. Unlike other fungal cellulases, HIC had rather high optimum pH. In the case of cellulases from *H. insolens* YH8, optimum pH was pH 5.3 and enzyme was quite stable in between pH 3 and 10(19). However, cellulase SP227 showed high activity toward avicel up to pH 7.0. IGI maintained high activity in between pH 5.5 and 8.0 with the highest at pH 7.0. For further study, pH 6.5 was selected because cellulose should be disintegrated substantially for β-glucosidase to react. Thereby subsequent action of glucose isomerase could be carried out.

Effect of temperature

Temperature profile showed a similar pattern to the pH profile(Fig. 2). Activity of HIC sharply declined at 45°C or lower and at 65°C or higher with optimum temperature of 55°C. This was rather different from that of *H insolens* YH-8 (14). They found optimum temperature to be 50°C. However, they also observed the considerable loss of activity at 65°C or higher.

In contrast, cellulase from *H. grisea var. thermaidea* YH-78 showed the similar thermostability(20); that

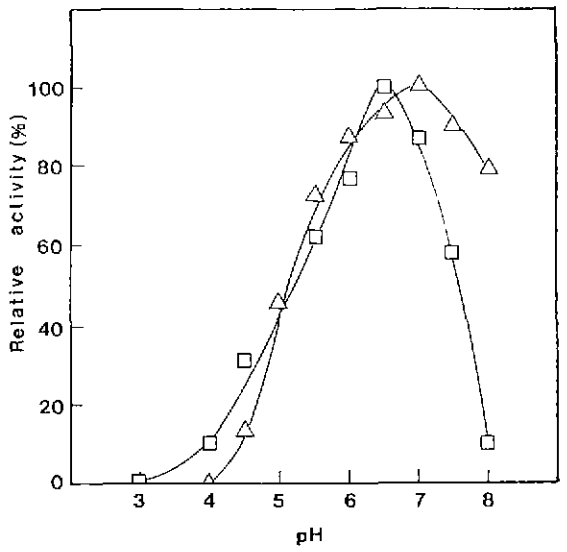

Fig. 1. Effect of pH on the activities of *H. insolens* cellulase and glucose isomerase.
△: IGI, □: HIC

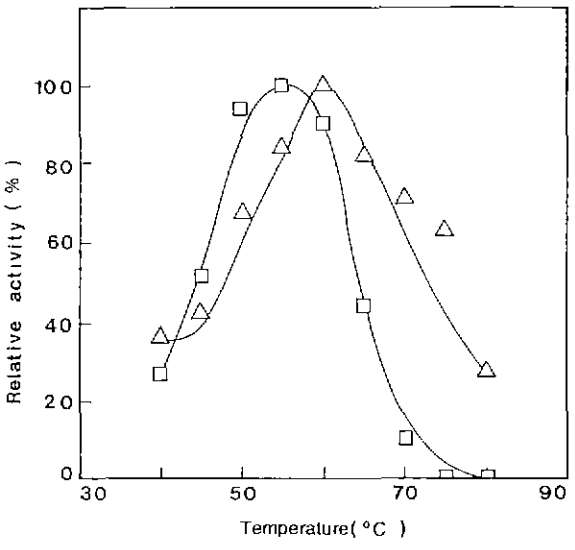

Fig. 2. Effect of temperature on the activities of *H. insolens* cellulase and glucose isomerase.
△. IGI. □: HIC

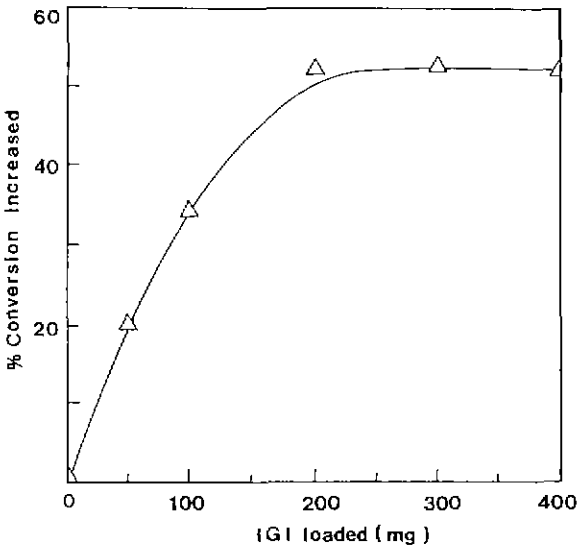

Fig. 3. Effect of immobilized glucose isomerase loading size on hydrolysis of avicel.

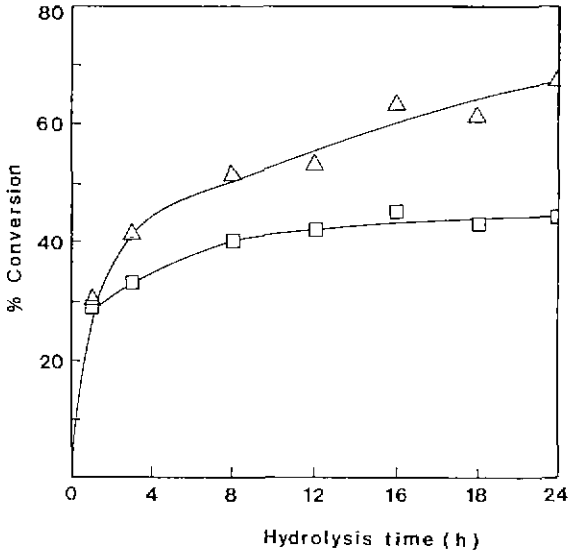

Fig. 4. Extended hydrolysis of avicel by *H. insolens* cellulase either with or without addition of glucose isomerase.
△: HIC+IGI, □: HIC

that is, optimum temperature around 60°C. As compared to HIC, optimum temperature of IGI shifted to 60°C; however, optimum temperature range was rather narrow. At 45°C the activity toward avicel decreased to 44%, whereas IGI was considerabley stable at high temperatures, especially over 65°C. IGI maintained relative activity of 31% at 80°C; at the same temperature, HIC lost its activity completely. Since this study was focused on the enhanced hydrolysis of cellulose, cellulase activity should be emphasized over IGI. For this reason, 55°C was selected to be the reaction temperature for further study.

Optimum loading size of immobilized glucose isomerase

To facilitate the contact of cellulose hydrolysis pro-

duct with IGI, the proper amount of IGI should be determined. If reaction mixture contained a large amount of the solid other than cellulose, cellulase diffusion would be limited, resulting in poor hydrolysis. As amount of IGI increased, conversion of cellulose to glucose was linearly increased at the low loading size (Fig. 3). However, the conversion ratio remained practically constant over 200mg IGI. Thus, IGI of 200mg was selected to be optimum loading size of IGI for further study.

Extended hydrolysis of avicel

Hydrolysis of cellulose by HIC was undertaken with or without the addition of IGI(Fig. 4). Without IGI, hydrolysis of avicel virtually stopped after 8hr; in contrast, with IGI, avicel was steadily converted to glucose. After 24hrs hydrolysis more than 65% of avicel was converted to glucose and fructose as compared to 40% of control. This indicated that HIC with IGI acted cooperatively on avicel hydrolysis by relieving product inhibition of cellulose. Further research should be pursued using cellulase with strong activity at high pH and temperatures.

REFERENCES

1. Cuskey, S. M., Schamhart, D. H. J., Chase, T., Montenecourt, B. S. and Eveleigh, D. E. : Screening for β-glucosidase mutants of *Trichoderma reesei* with resistance to end-product inhibition. *Dev. Ind. Microbiol.*, **21**, 471(1980)
2. Henrissat, B. : Synergism of cellulases from *T. reesei* in the degradation of cellulose. *Bio-Technol.*, **3**, 722(1985)
3. Wood, T. M. and McCrae, S. I : Purification and properties of a cellobilhydrolase from *Penicillium pinophilum*. *Carboh. Res.*, **148**, 331(1986)
4. Chanzy, H., Henrissat, B., Vuong, R. and Schuelein, M. : The action of 1,4-β-glucan cellobiohydrolase on Valonia cellulose microcrystals : an electron microscopy study. *FEBS Lett.*, **153**, 113(1983)
5. Nummi, M., Niku-Paavola, M.-L., Lappalainen, A., Enari, T.-M. and Raunio, V. : Cellobiohydrolase from *T. reesei*. *Biochem. J.*, **215**, 677(1983)
6. Funaguma, T., Tsuji, H. and Hara, A : Purification and some properties of cellulose 1,4-β-cellobiosidase from a strain of *Penicillium* sp. *J. Ferm. Technol.*, **64**, 77(1986)
7. Gong, C. S., Ladisch, M. R. and Tsao, G. T. : Biosynthesis, purification and mode of action of cellulases of *T. reesei*. *Adv. Chem. Ser.*, **181**, 261(1979)
8. Halliwell, G. and Vincent, R. : Control of saccharification of cellulose by indevidual and cumulative activities of cellulolytic components. In "*Bioconversion of cellulosic substances into energy, chimicals and microbial protein*" Ghose, T. K.(ed.), Symp. Proc., IIT, New Delhi, p.413(1978)
9. Sinitsyn, A. P. and Klesov, A. A. : Comparative role of exo-1,4,-glucosidase and cellobiase in the enzymatic hydrolysis of cellulose. *Biokhimiya.*, **46**, 202(1981)
10. Sternberg, D., Vijayakumar, P. and Reese, E. T. : β-Glucosidase : microbial production and effect on enzymatic hydrolysis of cellulose. *Can J. Microbiol.*, **23**, 139(1977)
11. Meyers, S. G. : Ethanol fermentation during enzymatic hydrolysis of cellulose. *AIChE Symp. Ser.*, **74**, 79(1978)
12. Takaki, M., Abe, S., Suzuki, S., Emert, G. H. and Yata, N. : A method for the production of alchol directly from cellulose using cellulase and yeast. In "*Bioconversion of cellulosic substances into energy, chemical and microbial protein*" Ghose, T. K.(ed.), Symp. Proc., ITT, New Delhi, p.551(1978)
13. Herr, D. : Conversion of cellulose to glucose with cellulase *Trichoderma viride* ITCC-1433. *Biotechnol. Bioeng.*, **22**, 1601(1980)
14. Woodward, J. and Arnold, S. : The inhibition of β-glucosidase activity in *Trichoderma reesei* C30 cellulase by derivatives and isomers of glucose. *Biotech. Bioeng.*, **23**, 1553(1981)
15. Geyer, J. U. : Glucose isomerase, new aspects for the starch and food industry. *Die Starke*, **26**, 225(1974)
16. Ahn, B. Y. and Byun, S. M. : Studies on whole cell immobilized glucose isomerase. *Korean J. Food Sci. Technol.*, **11**, 192(1979)
17. Miller, G. L. : Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.*, **31**, 426(1959)
18. Huitron, C and Limon-Lason, J. : Immobilization of glucose isomerase to ion-exchange materials. *Biotech. Bioeng.*, **20**, 1377(1978)
19. Hayashida, S. and Yoshioka, H. : Production and purification of thermostable cellulases from *Humicola insolens* YH-8. *Agric. Biol. Chem.*, **44**, 1721(1980)
20. Yoshioka, H. and Anraku, S. and Hayashida, S. : Production and purification of a novel type of CMCase from *Humicola grisea* var. *thermoidea* YH-78. *Agric. Biol Chem.*, **46**, 75(1982)

(Received May 16, 1996)