J. Korean Soc. Food Nutr.
22(3), 340 ~ 348(1993)

한국영양식량학회지

Cross-Synergistic Interactions between *Trichoderma viride* and *Penicillium funiculosum* Cellulase

Jeong-Hwa Hong

Food Science Institute, Inje University, Kimhae 621-749, Korea

Abstract

Cross-synergistic interactions were evaluated with purified enzymes from Trichoderma viride and Penicillium funiculosum cellulase. Different synergistic patterns between enzyme components were observed. Exo-exo type synergism was found to be the most effective for degrading Avicel in all cases. Exo-endo type synergism was found to be slightly less effective. Extended hydrolysis of Avicel was carried out using mixtures of purified enzyme components with the crude cellulase from a different source. Addition of β-glucosidase from P. funiculosum cellulase to T. viride cellulase provided the great enhancement of Avicel hydrolysis. In addition, exoglucanase from T. viride cellulase was found to enhance P. funiculosum cellulase in degradation of Avicel. In conclusion, it was possible to enhance the hydrolysis of Avicel by altering the proportions of enzyme components by supplementing enzyme components from a different source. Different types of synergisms acted together to achieve maximum conversion.

Key words : synergism, cellulase, *Trichoderma viride, Penicillium funiculosum*

INTRODUCTION

Cellulosic biomass constitute a massive and annually renewable resource having much potential as a supply of fuels, chemicals for industry and feedstuffs for animals[1]. However, transformation of the polysaccharides depends upon the all-important initial step (s) of saccharification by effective cellulolytic microbes having a full complement and high level of the cellulase complex. However, the cellulase systems produced by different organisms vary in their efficiency either because of the catalytic properties of the enzymes themselves or because of the different enzyme proportions within a given system. Therefore, by supplementing the weak enzyme components of one system with a complementary enzyme component, hydrolysis of cellulose can be enhanced. This requires knowledge of the relative activities of various components within the cellulase complex and synergistic actions between them.

Synergism between exo- and endo-acting enzymes has been studied for cellulases from *Trichoderma reesei*[1-3], *T. koningii*[4], *Penicillium funiculosum*[5], and *Fusarium solani*[6,7]. In all cases, synergistic actions were observed when mixtures of endo- and exo-acting enzymes were used for cellulose hydrolysis.

Cross-synergism, in which an endocellulase from one source and an exocellulase from another catalyze hydrolysis of crystalline cellulose, has also been observed[4,8-10]. Cellobiohydrolase II (CBH II) of *P. pinophilum* acted synergistically with the cellobiohydrolases (CBH) of *T. koningii or F. solani* to solubilize Avicel. CBH II from *P. pinophilum* showed no capacity for co-operating with *T. koningii* or *F. solani* to solubilize crystalline cellulose, but CBH I did[9]. Syenergism was also observed with many different mixtures of exo- and endo-glucanases of *F. solani, T. koningii* and *P. funiculosum* in solubilizing cotton cellulose[8,11]. Coughlan et al.[10] also showed synergistic action by different combinations of endoglucanases and exocellobiohydrolases from *F. solani, Talaromyces emersonii, P. funiculosum* and *T. koningii*. However, these synergistic interaction studies have not investigated the optimum mixing ratio of endo- and exoglucanase to produce maximum cellulose hydrolysis. To understand the synergistic interaction in more quantitative terms, different mixing ratios of these enzyme components need to be tested. For this reason, this study

focused on the enhancing the limiting enzyme components by supplementing the enzyme component from the different source. By evaluating the optimum mixing ratio, different synergistic patterns were compared to understand the mode of action.

MATERIALS AND METHODS

Preparation of enzymes

Crude cellulases produced by different fungi were obtained for this research from commercial suppliers. The following crude preparations were used : *T. viride* cellulase (Spezyme, Finnsugar Biochemicals, Inc., Schaumburg, IL) ; *P. funiculosum* (Cellulase Type Ⅶ, Sigma Chemical Co., St. Louis, MO).

Impurities from the crude enzyme preparations 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.

Separation procedures

Initial fractionations were carried out on a 5.0× 50cm column (Kontes Life Sciences Products, Vineland, NJ) containing preswollen Sephacryl S200 gel (Pharmacia Fine Chemicals, Uppsala, Sweden). Lyophilyzed cellulase (400mg solid) was suspended in 20ml sodium acetate buffer (0.05M, pH 5.0) and stirred at 4° C for 1hr. Insoluble material was removed by centrifufation at 4° C and 10,000×g for 20min. The suspernatant fraction was applied to the column and eluted with the same buffer at a flow rate of 60ml/hr. Fraction of 12ml were collected, and each fraction was analyzed for exoglucanase, xylanase, and β-glucosidase activities.

Cellulase pools from the Sephacryl S200 separation were further fractionated by chromatofocusing using Polybuffer PBE 94 exchanger (Pharmacia Fine Chemicals, Uppsala, Sweden). Dimensions of column and flow rate were 1.6×90cm and 60ml/hr, respectively. Sample (300mg) was dissolved in either 0.025 M imidazole buffer (pH 7.2) for *T. virde* cellulase or 0.025 M bis Tris buffer (pH 6.5). Elution was carried out with polybuffer 74/HCl (pH 4.0) diluted to 1/10.

Enzyme pools from the chromatofocusing separation were further fractionated by gel filtration using a TSK SW 3000 column. The amount of enzyme applied and the flow rate were 100μg and 1.0ml/min, respectively. Elution was carried out with 0.05M sodim acetate buffer(pH 5.0) containing 0.3M NaCl. Protein concentration was monitored at 280 nm. Fractions were collected by detection of peaks. Activities toward Avicel, CMC, xylan, and cellobiose were measured.

Protein determination

In most cases, protein concentration was monitored at 280nm, but interference from eluting buffer was detected in enzyme fractions from chromatofocusing. In addition, some pigments present in enzyme solutions were also absorbed at 280nm. To avoid this interference, the Bradford method was used[12].

Enzyme assay

Two different procedures were used to measure the activities toward Avicel, xylanase, CMC, and cellobiose. For purposes of purification and preliminary characterization, suspensions of 1% substrate were used. Glucose oxidase reagent (Sigma) was used for β-glucosidases. For other activity assays, the amount of reducing sugar was determined by the DNS method[13]. To 1ml of substrate, 50μl of each fraction was added. Hydrolysis was carried for 1hr (Avicel), 30 min (CMC or xylan), or 20min (cellobiose).

To determine specific activities, 2% substrate suspensions were used. To 1ml substrate, an appropriate dilution of enzyme and 0.05M sodium acetate buffer was added to give a final volume of 2 ml. Hydrolysis was carried out at pH 5.0, 50° C.

Laemmli SDS gel electrophoresis

To evaluate the purity of each enzyme obtained, SDS polyacrylamide gel electrophroesis was carried out according to Laemmli[14] in 7.5% acrylamide gel.

Synergism studies

Cellulose degradation experiments were carried out using experimental conditions similar to the exoglucanase assay. A 1% suspension was used. To evaluate limiting components, one-hour hydrolysis of

Avicel was carried out with mixtures of crude cellulase (30μg protein) and each purified enzyme component (3μg protein). To screen for synergistic interactions between components from the same source, equal amounts (3μg protein) of the components were mixed together and their hydrolytic activity toward Avicel was evaluated after 1hr. For synergism pattern studies, each pair of enzyme components was mixed together in proportions of 2 : 1 and 1 : 2, keeping the total amount of protein constant (2μg protein). All samples were filtered immediately following the hydrolysis period, and total sugar released was measured by the phenol-sulfuric acid method[15].

Calculated activity was determined as the sum of individual enzyme activities toward Avicel after a given hydrolysis time. This value was compared with the experimentally measured activity to determine the synergistic effect of enzyme mixtures.

For studies of extended hydrolysis, 0.1M sodium acetate buffer (pH 5.0) containing 100ppm sodium azide was used to suspend Avicel. The final volume of Avicel suspension used for a test was 10ml, and 0.2ml aliquots were taken at predetermined times to monitor the degree of hydrolysis. Samples were filtered and analyzed for total sugar by the phenol-sulfuric acid method[15]. For each test a fixed amount (15μg protein) of each component was added to the crude cellulase (150μg protein) for the cross-synergistic interaction study.

Synergism was determined by comparing percentage conversion at a given hydrolysis time. If any mixture showed a higher percentage conversion than crude cellulase did, that mixture was considered to have synergism.

RESULTS AND DISCUSSION

Separation and purification of cellulases

Cellulase contained impurities which was larger than 200kd in molecular weight and these should be removed before the purification by chromatofocusing. Therefore, large scale gel filtration was employed. Upon gel filtration with Sepharcry S200, cellulase pools were collected for chromatofocusing. *T. viride* cellulase was further fractionated into 10 enzyme components by chromatofocusing. Specific activities toward various substrates and isoelectric point for each enzyme pool are listed in Table 1. For simplicity, *T. viride*, xylanase, and endoglucanase, exoglucanase were abbreviated to T, xyl, and endo, exo, repectively.

For this work, the ratio of Avicelase to CMCase activity was used to designate an enzyme as exo or endo, with all activities measured in terms of reducing sugar productions. If the activity ratio was greater than 0.1, it was considered to be exoglucanase. For lower ratios, it was designated endoglucanase. Six endoglucanses, two exoglucanases, and two xylanses were separated. Texo-2, in particular, contained β-glucosidase activity.

The *P. funiculosum* cellulase from gel filteration was also further fractionated into 11 enzyme comp-

Table 1. Characteristics of purified enzyme components from *T. viride* cellulase

Designation[1]	pI	Specific activity (μmole glucose or xylose/min/mg protein)				Avicelase/CMCase
		CMC	Avicel	Xylan	Cellobiose	
Txyl-1	6.8	0	0	66	0	-
Txyl-2	6.6	0	0	195	0	-
Tendo-1	6.5	136	0	0	0	-
Tendo-2	6.43	10	0	0	0	-
Tendo-3	6.2	99	4.64	0	0	0.047
Texo-1	5.83	50	5.45	0	0	0.109
Tendo-4	5.5	249	8.68	194	0	0.035
Tendo-5	5.32	139	7.53	124	0	0.054
Tendo-6	4.90	88	0	0	0	-
Texo-2	4.00	33	12.10	25	3.0	0.367

[1] Designation stands for the following : Txyl, xylanase from *T. viride* ; Tendo, endoglucanase from *T. viride* ; Texo, exoglucanase from *T. viride*

onents by chromatofocusing. Activities toward various substrates and isoelectric point of each enzyme are listed in Table 2. Abbrebiations were the same as in Table 1. In this case, P and βg were abbreviated forms of *Penicillium funiculosum* and β-glucosidase, repectively. Four endoglucanases, two exoglucanase, and two xylanases, two β-glucosidases were separated. Pβg-2 showed strong β-glucosidase activity.

A Preliminary study was carried out to determine the possiblity of cross-synergism. Eleven enzyme components were selected and their purities were evaluated by SDS-PAGE. Among selected enzymes, impure enzymes were further purified by a second gel filteration step using a TSK SW 3000 HPLC column. Activities toward various substrates and isoelectric point of each enzyme are listed in Table 3. Pendo-3 was resolved into Pendo-3a and Pβg-3. β-glucosidase and endoglucanase activity were increased to 7-fold and 8-fold, repectively. In addition, Pexo-1 was separated into Pexo-1a and Pexo-1b. Only Pexo-1b showed strong activity toward xylan. Endoglucanase activity of Tendo-3a was also increased considerably.

Molecular weights were determined by SDS-PAGE for enzymes selected for cross-synergism study (Table 4). Molecular weights were varied from 45Kd to 65Kd. This result explains the difficulty of purification of these enzyme components by gel filtration using conventional column chromatography.

Cross-synergistic interactions

Since hydrolysis of Avicel can be enhanced by

Table 2. Characteristics of purified enzyme components from *P. funiculosum* cellulase

Designation[1]	pI	Specific activity (μmole glucose or xylose/min/mg protein)				Avicelase/CMCase
		CMC	Avicel	Xylan	Cellobiose	
Pxyl-1	6.40	22	0	127	0	-
Pxyl-2	5.89	41	0	274	0	-
Pendo-1	5.71	71	0	0	0	-
Pxyl-3	5.52	19	0	250	0	-
Pendo-2	5.10	9	0	25	0	-
Pexo-1	4.97	5	5.25	52	0	1.05
Pβg-1	4.63	20	0	0	170	-
Pendo-3	4.45	32	7.72	0	14	0.24
Pexo-2	4.28	2	1.14	0	0	0.57
Pβg-2	4.16	8	0	0	57	-
Pendo-4	4.00	24	0	0	0	-

[1] Designation stands for the following : Pxyl, xylanse from *P. funiculosum* ; Pendo, endoglucanase from *P. funiculosum* ; Pexo, exoglucanse from *P. funiculosum*, Pβg, β-glucosidase from *P. funiculosum*

Table 3. Summary of purified enzymes on TSK SW 3000 molecular sieve chromatography

Designation[1]	Specific activity (μmole reducing sugar/min/mg)				Avicelase/CMCase
	CMC	Avicel	Cellobiose	Xylan	
Pendo-2a	32.1	0	0	36.1	-
Pexo-1a	18.2	6.3	0	0	0.35
Pexo-1b	18.3	7.8	0	51.1	0.42
Pβg-1a	26.1	0	177	0	-
Pβg-3	0	0	98	0	-
Pendo-3a	250.4	4.5	0	0	0.018
Pβg-2a	15.1	0	22.9	0	-
Tendo-3a	478.5	1.2	0	0	0.003
Texo-2a	45.0	9.0	0	0	0.200

[1] Designation stands for the following : Pendo, endoglucanse from *P. funiculosum* ; Pexo, exoglucanase from *P. funiculosum* ; Pβg, β-glucosidase from *P. funiculosum* ; Tendo, endoglucanse from *T. viride* ; Texo, exoglucanase from *T. viride*

Table 4. Molecular weight of purified enzyme components

Enzyme[1]	Molecular weight
Pendo-2a	47,000
Pexo-1a	56,000
Pexo-1b	45,000
Pβg-1a	60,000
Pendo-3a	56,000
Pβg-2a	63,000
Tendo-1	52,000
Tendo-3a	47,000
Texo-1	65,000
Tendo-4	57,000
Texo-2a	62,000

[1] Refer to Table 3

Table 5. Effect of *T. viride* enzyme components on *P. funiculosum* cellulase

Mixture[1]	Total sugar (μg/ml)	Difference (μg/ml)
Pf only	1080	0
Pf + Txyl-1	1084	4
Pf + Txyl-2	1078	−2
Pf + Tendo-1	1165	85
Pf + Tendo-2	1081	1
Pf + Tendo-3	1110	30
Pf + Texo-1	1239	159
Pf + Tendo-4	1100	20
Pf + Tendo-5	1100	20
Pf + Tendo-6	1100	20
Pf + Texo-2	1239	159

[1] Pf stands for *P. funiculosum* cellulase
For other abbreviatins, refer to Table 1

increasing the proportions of specific components of a crude enzyme complex, it may also be possible to achieve similar effects by mixing an enzyme component from one source with crude cellulase from another. Results from Avicel hydrolysis studies using mixtures of purified enzyme components from *T. viride* cellulase with crude *P. funiculosum* cellulase showed that Texo-1 and Texo-2 significantly increased overall hydrolysis with a somewhat smaller increase produced by Tendo-1 (Table 5). For mixures of *P. funiculosum* components with *T. viride* cellulase, only Pβg-1 and Pβg-2 produced a significant increase in hydrolysis of Avicel (Table 6). Knowing that *T. viride* cellulase systems are β-glucosidase-deficient, it seems reasonable that β-glucosidase activity contributed to this enhanced hydrolysis.

Cross-synergism studies were then carried out using mixtures of Tendo-1, Texo-1, or Texo-2 and purified *P. funiculosum* enzyme components (3μg protein each) ; in addition, either Pβg-1 or Pβg-2 was mixed with *T. viride* enzyme components purified from chromatofocusing to evaluate synergistic actions. Results for mixtures showing enhanced activity toward Avicel are shown in Fig. 1.

Table 6. Effect of *P. funiculosum* enzyme component on *P. funiculosum* enzyme component on *T. viride* cellulase

Mixture[1]	Total sugar (μg/ml)	Difference (μg/ml)
Tv only	1080	0
Tv + Pxyl-1	1081	1
Tv + Pxyl-2	1080	0
Tv + Pendo-1	1077	−3
Tv + Pxyl-3	1080	0
Tv + Pendo-2	1100	20
Tv + Pexo-1	1124	44
Tv + Pβg-1	1151	171
Tv + Pendo-3	1100	20
Tv + Pexo-2	1082	2
Tv + Pβg-2	1147	67
Tv + Pendo-4	1082	2

[1] Tv stands for *T. viride* cellulase
For other abbreviations, refer to Table 2

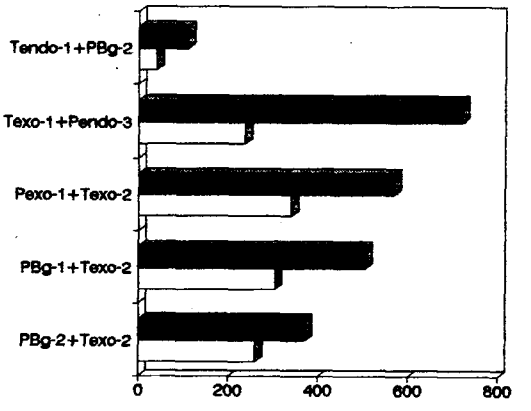

Fig. 1. Cross-synergism between *T. viride* and *P. funiculosum* enzyme components.
Enzyme component of 3μg was mixed together and Avicel was hydrolyzed at 50°C for 1hr ; ■, measured ; □, calculated

Tendo-1 acted synergistically only with Pβg-2, indicating this synergism might be an endo-endo type. Texo-1 cooperated effectively with Pendo-3 giving the largest increase in activity for these combinations. Texo-2 showed synergistic interactions with Pexo-1, Pβg-1, and Pβg-2. Pendo-3, which was expected to cooperate with Texo-2, did not show any synergistic effect with Texo-2.

Pexo-1 and its counterpart Texo-2 acted in differ-

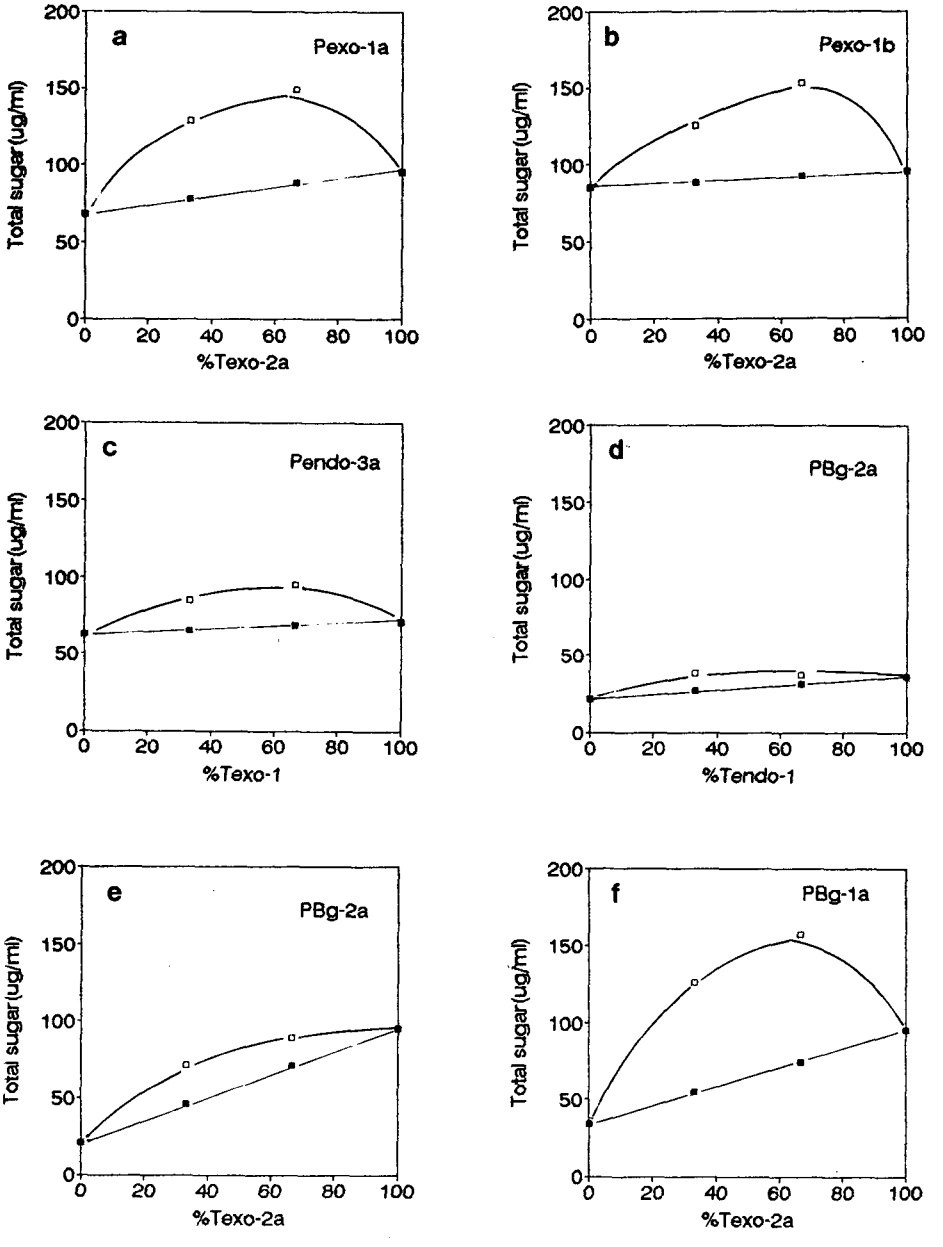

Fig. 2. Cross-synergism between enzyme components of different sources.

■, calculated ; □, measured.

a, Texo-2a and Pexo-1a ; b, Texo-2a and Pexo-1b ; c, Texo-1 and Pendo-3a ; d, Tendo-1 and Pβg-2a ; e, Texo-2a and Pβg-2a ; f, Texo-2a and Pβg-1a (*total protein of 2μg was mixed together at proportions of 0 : 3, 1 : 2, and 2 : 1, 3 : 0*)

ent modes. Pexo-1 showed synergism only with Texo-2, whereas Texo-2 acted synergistically with Pexo-1, Pβg-1, and Pβg-2. This explains why addition of Texo-2 to *P. funiculosum* cellulase enhanced Avicel hydrolysis effectively.

Both Pβg-1 and Pβg-2 acted synergistically only with Texo-2. Since both Pβg-1 and Pβg-2 contained low endo-activity, this synergistic effect seems

mainly due to β-glucosidase activity, but cooperative effects of low endo-activity with exoglucanase cannot be ruled out.

Cross-synergistic interactions were further investigated by varying the ratios of components in a given mixture. To minimize the effect of contaminating enzyme activities, only electrophoretically homogenous enzyme components were used. Exo-exo type synergism was observed for mixtures of Texo-2a with Pexo-1a or Pexo-1b (Fig. 2a and b). These mixtures had similar patterns, with maximum activity toward Avicel occurring at a proportion of 2 : 1 between Texo-2a and either Pexo-1 component. This pattern resembles the CBH I-CBH II interaction of *T. viride* cellulase reported by Henrissat[3]. Their results indicated that CBH I and CBH II show significant synergism with an optimum ration of 1 : 4.

Exo-endo type synergism was observed with mixtures of Texo-1 and Pendo-3a. The optimum mixing ratio for Texo-1 and Pendo-3a was 2 : 1, indicating only a small amount of Pendo-3a was needed to achieve the maximum effect (Fig. 2c). However, the magnitude of synergistic effect was quite low compared to exo-exo type synergism. when Pβg-2a was mixed with Tendo-1 (Fig. 2d) the result was similar to that displayed by the mixture of Texo-2a with Pβg-2a (Fig. 2e). Optimum mixing ratio was 1 : 2 (Tendo-1 : Pβg-2a) ; however, the overall effect from this mixture was not any greater than that obtained with Tendo-1 alone. β-Glucosidase (Pβg-1a) significantly enhanced the hydrolytic activity of exoglucanase Texo-2a toward Avicel (Fig. 2f). Since a 2 : 1 mixture (Texo-2a : Pβg-1a) showed the highest increase, only a small amount of Pβg-1a was needed to achieve maximum Avicel hydrolysis. In the case of Texo-2a and Pβg-2a interaction(Fig. 2e), the pattern of synergism was different. Knowing that Pβg-2a also has relatively high β-glucosidase activity, this different pattern may be due to lower endoglucanase activity of Pβg-2a than Pβg-1a.

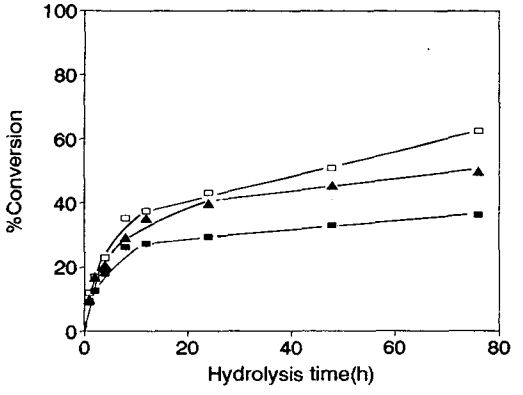

Fig. 3. Extended hydrolysis of Avicel by cross-synergistic interaction of *T. viride* cellulase with purified enzymes from *P. funiculolsum* cellulase.
■, *T. viride* cellulase alone ; □, *T. viride* cellulase + Pβg-1 ; ▲, *T. viride* cellulase + Pβg-2.
150μg of crude cellulase was mixed with 15μg of enzyme component and Avicel was hydrolyzed at 50° C

Cross-synergistic interaction during extended hydrolysis

Results from extended Avicel hydrolysis studies using *P. funiculosum* cellulase with added components from other sources showed a substantial increase in yield with Texo-2 and a slight increase with Texo-1 (Fig. 3). Tendo-1 did not enhance yields from the *P. funiculosum* enzyme, although it did enhance the hydrolytic activity during the one-hour studies. Apparently, these endoglucanases attack amorphous regions of Avicel and as these are depleted, the hydrolysis rate declines.

If enhenced hydrolysis by β-glucosidase is ignored, it is most likely the exoglucanase that limits the extended hydrolysis of Avicel by the *P. funiculosum* cellulase. From the previous cross-synergism study, Pexo-1 and Texo-2 were found to act with an exo-exo type of synergism and Texo-1 and Pendo-3a acted in an exo-endo type. Apparently, combined effects of these types of synergisms enhanced Avicel hydrolysis in these cases. Enzyme components which did not contain exo-activity apparently failed to enhance the Avicel hydrolysis, indicating their ability to act synergistically with other components is limited to the first few hours of hydrolysis.

In the case of the *T. viride* cellulase, addition of β-glucosidase activity from *P. funiculosum* cellulase increased the total conversion of Avicel to about twice the amount achieved by the *T. viride* cellulase alone (Fig. 4). This result again confirmed that *T. vi-*

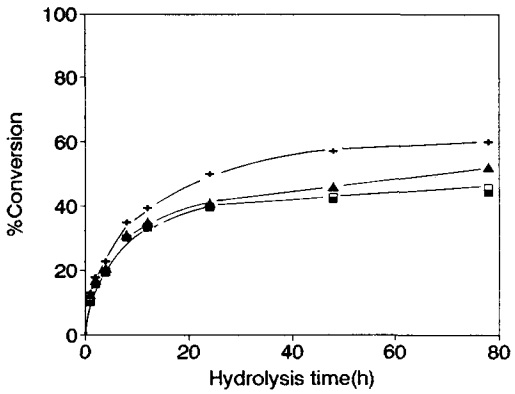

Fig. 4. Extended hydrolysis of Avicel by cross-synergistic interaction of *P. funiculosum* cellulase with purified enzymes from *T. viride* cellulase.
■, *P. funiculosum* cellulase alone ; □, *P. funiculosum* cellulase + Tendo − 1 ; ▲, *P. funiculosum* cellulase + Texo − 1 ; +, *P. funiculosum* cellulase + Texo −2. 150μg of crude cellulase was mixed with 15μg of enzyme component and Avicel was hydrolyzed at 50° C

vity and product inhibition by cellobiose adversely affected the hydrolysis of Avicel.

CONCLUSIONS

Cellulases are not as efficient for degrading cellulose as we would like. Cellulases are composed of several enzyme components which act synergistically to degrade cellulose. The hypothesis of this study is that the extent of cellulose hydrolysis is limited because amounts of some components of a given system are too low to act synergistically with other components. To improve these imperfect enzyme systems, proportions of enzyme components which comprise the complex system have to be altered the weak activity or by adding crude cellulase from another source.

Endo-exo, exo-exo, and endo-endo type synergisms were observed during hydrolysis Avicel. Endo-endo type seemed to be the least effective. One component of enzyme could act synergistically with several different enzyme components. Texo-2a seemed to be the most effective enzyme components in this regard. This indicates that interactions among three or more enzyme components should be investigated. In this study, only one component was added to the crude cellulose hydrolysis. For further research, combinations of two or more components should be mixed with the crude cellulase to enhance hydrolysis of cellulose. Even for the synergism study, various mixing ratios with three or more pure enzyme components have to be tried to determine the type of synergism. One way to do this is the evaluation of various mixing ratios of two enzyme components such as β−glucosidase component. By doing so, more detailed information about synergistic actions can be obtained.

ACKNOWLEDGEMENT

This paper was supported by NON DIRECTED RESEARCH FUND, Korea Research Foundation, 1991.

REFERENCES

1. Halliwell, G. and Griffin, M. : The nature and mode of action of the cellulolytic component C1 of *T. kiningii* on native cellulose. *Biochem. J.*, **135,** 587(1973)
2. 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)
3. Henrissat, B. : Synergism of cellulases from *T. reesei* in the degradation of cellulose. *Bio-Technol.*, **3**(8), 722 (1985)
4. Wood, T. M. : Properties and mode of action of cellulases. *Biotechnol. Bioeng. Symp.*, **5,** 111(1975)
5. Wood,T. M. and McCrae, S. I. : Purification and some properties of a 1,4-β-D-glucan glucohydrolase associated with the cellulase from the fungus *P. funiculosum. Carboh. Res.*, **110,** 291(1982)
6. Wood, T. M. : The cellulase of *F. solani* purification and specificity of the β-(1-4)-glucanase and the β-D-glucosidase components. *Biochem. J.*, **121,** 353 (1971)
7. Mishra, C., Vaidya, M., Rao, M. and Deshpande, V. : Purification and properties of two exo-cellobiohydrolases for a cellulolytic culture of *Fusarium lini. Enz. Microb. Technol.*, **5,** 430(1983)
8. Wood, T. M. and McCrae, S. I. : Synergism between enzymes involved in the solubilization of native cellulose. *Adv. Chem. Ser.*, **181,** 181(1983)
9. Wood, T. M. and McCrae, S. I. : Purification and properties of a cellobiohydrolase from *Penicillium pinophilum. Carboh. Res.*, **148,** 331(1986)
10. Coughlan, M. P., Moloney, A. P., McCrae, S. I. and Wood, T. M. : Cross-synergistic interactions between *Talaromyces emersonii, Fusarium solani, P. funiculosum,* and *T. koningii. Biochem. Soc. Trans.*, **15,** 263(1987)
11. Wood, T. M., McCrae, S. I. and McFarlane, C. C. :

The isolation, purification, and properties of cellobiohydrolase component of *P. funiculosum* cellulase. *Biochem. J.*, **189**, 51(1980)
12. Bradford, M. M. : A rapid sensitive method for quantitation of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248(1976)
13. Miller, G. L. : Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.*, **31**(3), 426(1959)
14. Laemmli, U. K. : Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680(1970)
15. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. : Colorimetric methods for determination of sugars and related substances. *Anal. Chem.*, **28**(3), 350(1956)

(Received May 25, 1993)

*Trichoderma viride*와 *Penicillium funiculosum* Cellulase 성분효소 간의 상승작용에 관한 연구

홍 정 화
인제대학교 식품과학연구소

요 약

*Penicillium funiculosum*과 *Trichoderma viride* cellulase의 정제효소들을 사용하여 cross-synergism을 조사하였다. Exo-exo형 상승작용이 Avicel을 분해하는데 가장 효과적이었으며 exo-endo형은 다소 효과가 떨어졌다. 정제효소성분과 이종의 조효소를 혼합하여 70시간 이상 Avicel을 가수분해하며 생성된 total sugar를 측정한 결과, *P. funiculosum* cellulase에서 분리정제한 β-glucosidase 성분효소는 *T. viride* cellulase와 상승작용을 크게 나타내며 Avicel을 가수분해하였다. 또한, *T. viride* cellulase에서 분리정제한 exoglucanase 성분효소도 *P. funiculosum* cellulase의 가수분해능을 크게 향상시켰다. 이와 같은 결과로 미루어 볼 때, 이종의 cellulase로 부터 부족한 성분효소를 보충시켜 효소성분비율을 변화시킴으로써 Avicel의 가수분해도를 향상시킬 수 있을 것으로 사료된다. 이때 최대한 높은 가수분해를 얻기 위하여 여러 형식의 상승작용이 같이 이루어져야 할 것이다.