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PURIFICATION OF XYLANASE FROM *TRICHODERMA VIRIDE* BY PRECIPITATION WITH AN ANIONIC POLYMER EUDRAGIT S 100

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SUMMARY

Endo-xylanase from *T. viride* has been purified 4.2 fold by precipitation with a commercially available enteric polymer Eudragit S-100. Electrophoretic analysis also indicated removal of contaminant proteins. The enzyme could be recovered in more than 89% yield. The binding of the enzyme to the polymer was predominantly by electrostatic interaction.

INTRODUCTION

The endo-ß-1,4-xylanases (ß -1.4- D- xylan xylanohydrolase; EC 3. 2. 1. 8) have found numerous applications such as prebleaching of pulps for paper manufacture, clarification of juices and wines, saccharification of agricultural and forestry wastes and modification of cereal flours (Coughlan and Hazelwood, 1993; Visser et al, 1992). Hence extensive efforts continue to be directed towards their purification (Dabeire-Gosselin et al., 1992; Debeire-Gosselin et al., 1992 a; Fisk and Simpson, 1993; Ghose and Mattiasson, 1993). Lately, reversibly soluble-insoluble polymers have found considerable applications in enzyme purification (Fujii and Taniguchi, 1991). These polymers precipitate from the solution by changing some parameter like temperature, pH or addition of salt or some other chemical. Eudragit, a commercially available enteric (and so obviously nontoxic) polymer, is one such polymer which has been used in affinity precipitation in a number of cases (Fujii and Taniguchi, 1991; Kamihara et al., 1993). Recently, about 9 fold purification of a D-lactate

dehydrogenase by precipitation with Eudragit S 100 (as a result of hydrophobic binding of the enzyme to the polymer) was observed in this laboratory (Guoqiang et al., in press). As hydrophobic interaction has often been used as one of the final steps in the purification of endoxylanases (Dabeire-Gosselin et al., 1992; Dabeire-Gosselin et al., 1992a; Fisk and Simpson, 1993), it was thought worthwhile to attempt precipitation of a commercially available endoxylanase from *Trichoderma viride* with Eudragit S 100. It turned out that while the enzyme did bind to the polymers, the binding was predominantly electrostatic in nature. This was exploited for precipitation and consequent purification of the enzyme.

MATERIALS AND METHODS

Materials. Eudragit S 100 was a gift from Röhm Pharma GmbH, Weiterstadt, Germany. Xylanase from *Trichoderma viride* was purchased from Fluka, Switzerland. The substrate, birch wood xylan was bought from Carl Roth Karlsruhe, Germany (Thanks are due to Leif Dahlberg for sharing this substrate). All other chemicals were of analytical grade.

Preparation of Eudragit S 100 solution. Eudragit S 100 (50 g) was dissolved under constant stirring in 800 ml of distilled water by adding dropwise 3 M NaOH solution to pH 11. After the polymer was fully solubilized, the pH of the solution was reduced to 7.0 by adding HCl, and the volume was made up to 1 litre with distilled water. The solution was stored at 4 °C for further use and diluted with appropriate buffer wherever required.

Precipitation of xylanase with Eudragit solution. Xylanase (20 mg /ml) was dissolved in acetate buffer (10 mM, pH 5.6). One ml of the enzyme solution was mixed with Eudragit solution (0.4 %, diluted with the acetate buffer). After 15 min incubation at room temperature, the polymer was precipitated at pH 4.3 by the addition of 120 μl of 0.1 M acetic acid. After 20 min, the suspension was centrifuged at 3000 xg for 15 min. The extent of enzyme binding was determined by analysing the enzyme activity in the supernatant.

Elution of the enzyme from Eudragit. The precipitate of Eudragit bound enzyme was washed with 2 ml of acetate buffer (10 mM, pH 4.3). The washed precipitate was dissolved in 2 ml of phosphate buffer (0.1 M, containing 1 M NaCl, pH 7.0). After 5 min with occasional vortexing, the polymer was precipitated by adding acetic acid (0.1 M, 400 μl) to lower the pH to 4.3. After 20 min, the suspension was centrifuged at 3000 xg for 15 min.

Determination of endo-xylanase activity. This was carried out by slightly modifying the procedure described by Bailey and Poutanen (1992). One ml of xylan (1% in 50 mM acetate buffer, pH 5.0) was diluted with 0.9 ml of the acetate buffer and preincubated at 50 °C before initiating the reaction with the addition of 0.1 ml of enzyme solution. The reaction was

stopped after 30 min by adding dinitrosalicylic acid reagent for determining reducing sugars. One enzyme unit is defined as the amount that releases 1 µmole of reducing groups per min under the above described conditions.

Protein estimation. Protein concentration was determined by bicinchoninic acid method (Smith et al., 1985) using bovine serum albumin as standard.

Polyacrylamide gel electrophoresis. SDS-polyacrylamide gel with 12% gel was performed according to Hames (1986) using a BioRad Mini Protean II electrophoresis unit and standard molecular weight markers (BioRad Laboratories Ltd., Richmond, CA, USA). The gel was stained with Kenacid blue.

RESULTS AND DISCUSSION

Precipitation by polymers is often used for concentration and simultaneous separation of proteins and is a technique in which scale up is easily possible. Earlier in this laboratory, precipitation of D-lactate dehydrogenase by Eudragit S 100 has provided a successful means of separation of the enzyme from a crude mixture (Guoqiang et al., in press). In that case, the binding between the enzyme and the polymer was mostly hydrophobic in nature.
Endo-xylanase from *T. viride* is a useful enzyme which is required in large amounts for various applications (Coughlan and Hazelwood, 1993). When the binding of this enzyme to Eudragit S 100 was studied in the presence of various concentrations of ammonium sulfate, it was found that the salt in fact inhibited the binding and about 98% of the enzyme could be bound to the polymer in the absence of the salt under the condition tried (Fig. 1).

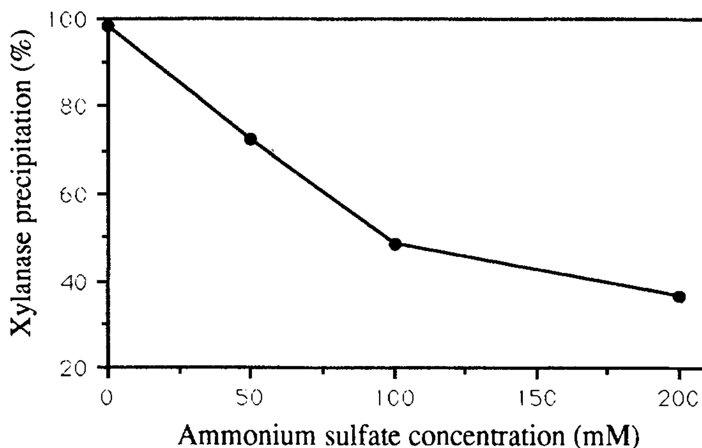

Fig. 1. Effect of ammonium sulfate concentration on the precipitation of xylanase with Eudragit S 100. The final concentration of the polymer was 0.4%.

The capacity of the polymer for binding the enzyme seemed quite good and increased with increase in the enzyme load. About 160 units could be adsorbed per mg Eudragit at the highest load of 736 U/ml which was equivalent to 87% binding (Fig. 2). The enzyme load of 259 U/ml which gave a binding of 96% was used for xylanase purification.

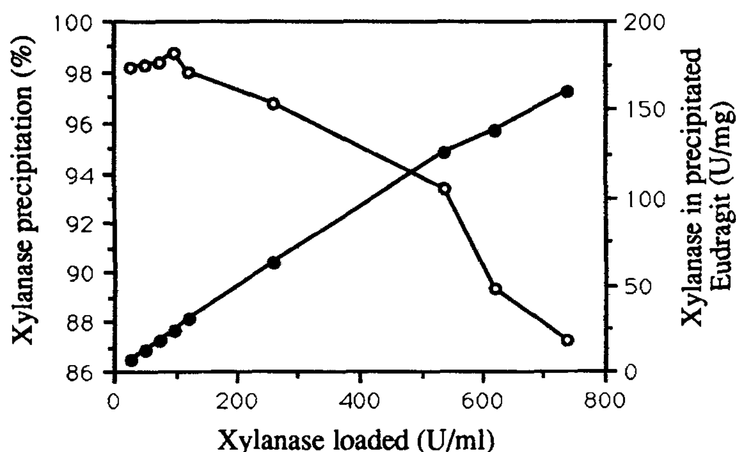

Fig. 2. Binding of xylanase to Eudragit S 100 in terms of percent enzyme precipitated (o), and the enzyme units bound per mg polymer (•). The final concentration of Eudragit was 0.4%.

The washing of the precipitate at low pH removed a small amount of the protein, and the enzyme could be dissociated from the polymer by dissolving the precipitate of the complex of

Table 1
Purification of xylanase by precipitation with Eudragit S 100

Step	Volume (ml)	Total activity (U)	Specific activity (U/mg protein)	Enzyme Yield (%)	Purification factor
Starting	4.0	1055.9	255.3	100.0	1.0
Supernatant	3.8	66.1	ND	6.2	ND
Washing	2.0	33.2	ND	3.1	ND
Elution 1	2.0	739.9	1179.7	70.1	4.6
Elution 2	2.0	145.2	781.3	13.8	3.1
Elution 3	2.0	59.0	852.6	5.6	3.3

ND: not determined

enzyme bound polymer by raising the pH to 7.0 and using 1 M sodium chloride. When the pH was lowered back to 4.3-4.5 in the presence of salt, about 70% of the bound enzyme activity could be recovered. Two more elution steps yielded another 19% enzyme activity. Thus, a total yield of about 89% with about 4.2 fold purification could be obtained (Table 1).

The SDS- PAGE electrophoresis of the starting protein and the purified one showed significant purification (Fig. 3). The purified enzyme in fact showed one major enzyme band and one low molecular weight impurity.

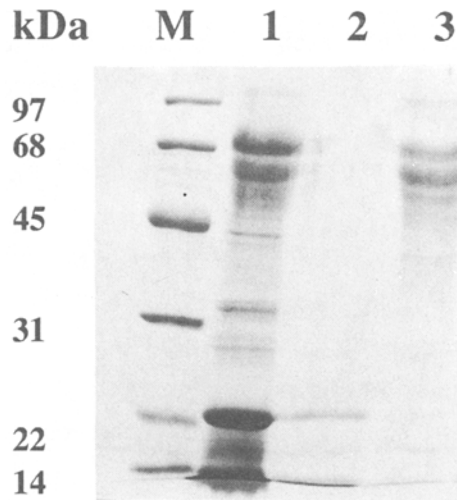

Fig. 3. SDS-PAGE (12%) pattern of xylanase sample. Lane M, marker proteins; Lane 1, commercial enzyme from Fluka; Lane 2, the enzyme eluted from precipitated Eudragit and Lane 3, the supernatant after enzyme was precipitated with Eudragit at pH 4.3.

The position of the enzyme band around 25,000 molecular weight agrees with the reported value for xylanase from *T. viride* enzyme (Wong and Saddler, 1992). In fact, the SDS-PAGE of *T. viride* enzyme is often known to yield a low molecular weight fragment (Wong and Saddler, 1992). Thus, the purified enzyme obtained in this instance may in fact be a single species.

Thus precipitation with non-toxic polymer Eudragit S 100 provides an efficient final step for purifying *T. viride* endo-xylanase.

ACKNOWLEDGEMENT

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REFERENCES

Bailey, M.J., and Poutanen, K. (1992). In: *Xylans and Xylanases*, J. Visser, G. Beldman, M.A.Kusters-van Someren, and A.G. J. Voragen, eds. pp. 171-186, Amsterdam: Elsevier.

Coughlan, M.P., and Hazelwood G.P. (1993). *Biotechnol. Appl. Biochem.* 17, 259-289.

Debeire-Gosselin, M., Loonis, M., Samain, E. and Debeire, P. (1992). In: *Xylans and Xylanases,* J. Visser, G. Beldman, M.A.Kusters-van Someren, and A.G. J. Voragen, eds. pp. 463-466, Amsterdam: Elsevier.

Debeire-Gosselin, M., Touzel, J. P., and Debeire, P. (1992a). In: *Xylans and Xylanases,* J. Visser, G. Beldman, M.A.Kusters-van Someren, and A.G. J. Voragen, eds. pp. 471-474, Amsterdam: Elsevier.

Fisk, R. S. and Simpson, C. (1993) In: *Stability and Stabilization of Enzymes,* W.J.J. van den Tweel, A. Harder, R. M. Buitelaar, eds. pp.323-328, Amsterdam: Elsevier.

Fujii, M. and Taniguchi, M. (1991). *Trends Biotechnol.* 9, 191-196.

Ghose, S., and Mattiasson, B. (1993). In: *Thermostability of Enzymes*, M.N. Gupta, ed. pp. 24-42, New Delhi: Springer Verlag.

Guoqiang, D., Kaul, R., and Mattiasson, B. (in press). *Bioseparation.*

Hames, B.D. (1986). In: *Gel Electrophoresis of Proteins- a practical Approach,* B.D. Hames and D. Rickwood, eds. pp. 1-86, Oxford: IRL press.

Kamihara, M., Kaul, R., and Mattiasson B. (1992). *Biotechnol. Bioeng.* 40, 1381-1387.

Smith, P.K., Krohn, R. I. and Hermanson, E. K. (1985). *Anal. Biochem.* 150, 76-85.

Visser, J., Beldman G., Kusters-van Someren, M.A., and Voragen, A.G. J., eds. (1992). *Xylans and Xylanases,* Amsterdam: Elsevier.

Wong, K.K.Y., and Saddler, J.N. (1992).In: *Xylans and Xylanases,* J. Visser, G. Beldman, M.A.Kusters-van Someren, and A.G. J. Voragen, eds. pp. 171-186, Amsterdam: Elsevier.