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STUDIES ON DEXTRANASE

PURIFICATION OF DEXTRANASE FROM *PENICILLIUM FUNICULOSUM* AND ITS ENZYMATIC PROPERTIES

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SUMMARY

Dextranase (α-1,6-glucan 6-glucanohydrolase, EC 3.2.1.11) from *Penicillium funiculosum* was purified by Bio Gel P-60 gel filtration, CM-cellulose and DEAE-cellulose chromatography. The enzyme preparation was further fractionated into two fractions, dextranase I (pI 3.98) and dextranase II (pI 4.19), by isoelectrofocusing. The purified dextranases I and II were both electrophoretically homogeneous.

A molecular weight of 44 000 for both enzymes was obtained by gel filtration. The enzymes were most active at pH 6.0 and stable over a pH range from 5.0 to 7.5 at 37 °C for 60 min. They were activated by Co^{2+}, Mn^{2+} and Cu^{2+}, and inactivated by Ag^{+}, Hg^{2+}, *N*-bromosuccinimide and iodine.

Dextranase II hydrolyzed preferentially a series of dextrans with α-1,6 linkages.

INTRODUCTION

The presence of dextranase in filamentous and defective fungus was reported firstly by Ingelman[1]. It was then found to be present in many molds[2–8], bacteria[9], kidney, liver, small intestine and spleen[11,12], and its properties were examined by many investigators. In their reports, however, crude or partially purified enzyme was employed for investigations. Only Fukumoto and co-workers purified dextranase from *Penicillium luteum*[2], *Aspergillus carneus*[6] and *Chetomiam grafile*[7] as a single substance on disc electrophoretic pattern and reported their enzymatic properties.

The authors have obtained and purified the extracellular dextranase from *P. funiculosum*, in order to examine its enzymatic properties. In this paper, some properties of the purified enzyme are described.

MATERIALS AND METHODS

Enzyme

The crude enzyme, supplied by Amano Pharm. Co. Itd, was the ethanol fraction of a culture filtrate of *P. funiculosum.*

Substrate and reagents

Dextran, molecular weight 50 000, was a gift from Meito Sangiyo Co. Bio Gel P-60 was made by Bio-Rad Laboratories. CM-cellulose and DEAE-cellulose were products of Pharmacia Co. Myoglobin (Calbiochem.), bovin serum albumin (Miles Laboratories) and egg albumin (Neutritional Biochem. Co.) were used as the standards for the determination of dextranase molecular weight by gel filtration. Other chemicals used were of the reagent grade.

Assay of enzyme activity

Dextranase activity was assayed as follows. 2 ml of 2% dextran solution (in 100 mM phosphate buffer, pH 6.0) were preincubated for 5 min at 37 °C and then incubated with 1 ml of the enzyme solution for 30 min. After incubation, the reaction was stopped by the addition of 3 ml of Sumner reagent[13] and the mixture was heated for 10 min in boiling water. Then the cooled mixture at room temperature was diluted to 25 ml with deionized water and the absorbance at 575 nm was determined. One unit of dextranase activity was defined as that amount of enzyme which will produce reducing sugars in an amount equivalent to 1 μmole of glucose per min. Specific activity was calculated as the activity per absorbance unit (A) at 280 nm. Protein concentration was determined spectrophotometrically by measuring absorption at 280 nm.

Isoelectrofocusing and disc electrophoresis

Isoelectrofocusing was carried out as described by Vesterberg and Svensson[14]. Disc electrophoresis was performed by the procedure of Davis[15] and Nagai[16] with apparatus from M. S. Instrument Co.

RESULTS

Purification of dextranase

The purification procedures are summarized in Table I. The crude enzyme powder was subjected to water extraction and ammonium sulfate fractionation, and then fractionated with Bio Gel P-60 equilibrated with 50 mM acetate buffer (pH 5.0). At this step the enzyme was purified about 20-fold. The enzyme was then passed through a column of CM-cellulose equilibrated with 10 mM acetate buffer (pH 5.0) and adsorbed on a column of DEAE-cellulose equilibrated with 10 mM phosphate buffer (pH 6.0). The adsorbed enzyme was eluted by a linear gradient system. By these procedures, dextranase was purified about 30-fold from the crude enzyme, but it consisted of two parts on disc electrophoresis, as shown in Fig. 1c. The active fraction obtained by DEAE-cellulose chromatography was subjected to isoelectrofocusing. As presented in Fig. 2, two peaks with dextranase activity, pI 3.98 (dextranase I) and pI 4.19 (dextranase II), were obtained. Both preparations were electrophoretical-

TABLE I

PURIFICATION PROCEDURE OF DEXTRANASE

	Total activity (units)	*Specific activity (units/A unit)*
Original powder		
↓ extracted with water and fractionated with ammonium sulfate (0.3–0.6 satn) and dissolved in water	3984	43
Solution	2643	125
↓ fractionated with Bio Gel P-60		
Effluent	1468	954
↓ dialyzed against water and passed CM-cellulose		
Effluent	1358	964
↓ adsorbed on DEAE-cellulose and eluted by a linear gradient system of NaCl (0–0.5 M)		
Effluent	904	1242
dialyzed against water and lyophilized		
Isoelectrofocusing		
Dextranase I	—	1230
Dextranase II	—	1260

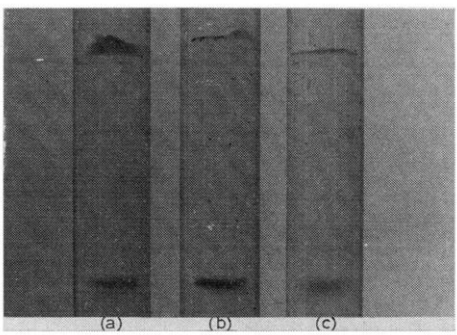

Fig. 1. Disc electrophoretic patterns of purified dextranase I and II. About 30 μg of purified dextranase were subjected to electrophoresis at pH 9.4. A current of 2 mA per tube was supplied for 80 min at 3 °C. Staining was carried out using amidoschwarz 10B. (a) dextranase I, (b) dextranase II, (c) dextranases I and II.

ly homogeneous, as shown in the Figs 1a and 1b. The total recovery of activity was about 24%.

Optimum pH and dextranase stability

Maximum activity of dextranases was observed at pH 6.0 under standard conditions. For the examination of the pH stability, dextranase was incubated at various pH values at 37 °C for 60 min and the residual activity was determined. The enzymes were stable over the pH range 5.0–7.5. Thermal stability was also examined at pH 6.0 for 30 min. Under these conditions, both dextranases were stable at temperatures up to 40 °C, but were rapidly inactivated above 40 °C. At 50 °C, a 70% loss of activity was noted.

Effect of some metal salts and chemicals on the enzyme activity

As shown in Table II, both enzymes were found to be remarkably inactivated

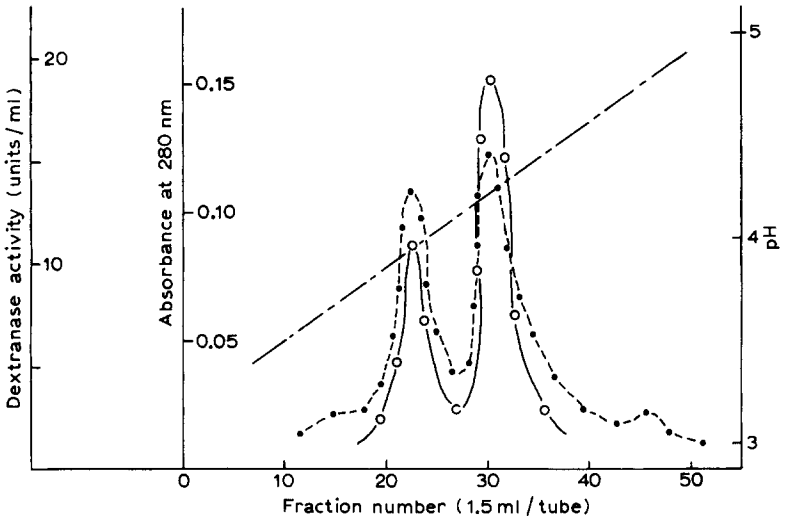

Fig. 2. Isoelectrofocusing pattern of dextranase. A dextranase preparation of about 10 mg, purified by DEAE-cellulose chromatography, was used. A carrier ampholyte was selected to give a pH range from 3 to 5 and used at 1% concentration. Electrofocusing was performed for 48 h with a potential of 700 V at 4 °C. The column volume was 110 ml. ●---●, protein; ○—○, dextranase activity.

TABLE II

EFFECT OF METAL SALTS ON DEXTRANASES I AND II

1 ml of dextranase solution was incubated with the same volume of 2 mM salt solution (pH 6.0) at 37 °C. After 30 min, the mixture was diluted 100-fold with water and the residual activity was determined.

Metal salt	*Residual activity (%)*	
	Dextranase I	*Dextranase II*
$CaCl_2$	89.8	73.5
$FeCl_3$	90.2	79.2
$HgCl_2$	0	0
$AgNO_3$	27.2	32.3
$MnCl_2$	139.1	137.6
$CoCl_2$	142.2	142.3
$CuSO_4$	121.3	120.4

by Ag^+ and Hg^{2+}. On the other hand, Co^{2+}, Mn^{2+} and Cu^{2+} were found to activate the enzymes; in particular, Co^{2+} gave 140% of relative activity. The effect of some chemicals on the enzyme activity was also examined under the same conditions described in Table II. Both enzymes were found to be remarkably inactivated by *N*-bromosuccinimide and to some extent by iodine. Partial inactivation was observed by EDTA and iodoacetic acid. H_2O_2 did not show any effect on the enzyme activity.

Molecular weight determination

The molecular weight of each dextranase was determined by gel filtration with

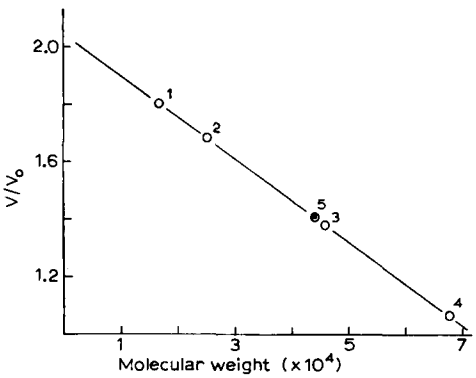

Fig. 3. Determination of the molecular weight of dextranase by gel filtration on Bio Gel P-60. A column (2 cm × 47 cm) of Bio Gel P-60 was equilibrated with 50 mM phosphate buffer (pH 7.0). (1) Myoglobin (mol. wt 18 400), (2) α-chymotrypsin (mol. wt 20 000), (3) egg albumin (mol. wt 45 000), (4) bovine serum albumin (mol. wt 69 000), (5) dextranases I and II.

Bio Gel P-60, as summarized in Fig. 3. The value of 44 000 for each enzyme was obtained by comparison with proteins having published molecular weights.

Substrate specificity of dextranase

To investigate the substrate specificity of dextranase II, the rate of hydrolysis of polysaccharides with various glucosidic linkages by the enzyme was determined. Results are presented in Table III. The dextranase hydrolyzed preferentially a series of dextrans with α-1,6 linkages. However, DEAE-Sephadex and CM-Sephadex, as derivatives of dextran, were not hydrolyzed. Polysaccharides with α-1,3, β-1,3, α-1,4 or β-1,4 linkages were also not decomposed.

TABLE III

SUBSTRATE SPECIFICITY OF DEXTRANASE II

The same volume of purified dextranase II solution and 2% of substrate were incubated at pH 6.0 and 37 °C. After incubation, the reducing sugars produced were determined and relative activity was calculated.

Substrate	*Main linkage*	*Activity (%)*
Glycogen	α-1,4	0
Starch	α-1,4	0
Amylose	α-1,4	0
Amylopectin	α-1,4	0
Cellulose	β-1,4	0
Xylan	α-1,3	0
Lichenin	β-1,3	0
Dextran	α-1,6	100
Sephadex G-25	α-1,6	4.5
Sephadex G-50	α-1,6	20.2
Sephadex G-75	α-1,6	45.5
Sephadex G-200	α-1,6	64.0
DEAE-Sephadex	α-1,6	0
CM-Sephadex	α-1,6	0

DISCUSSION

The possibility that the two protein bands on the disc electrophoretic pattern might be the subunits was of particular interest. Therefore the enzyme was subjected to DEAE-cellulose chromatography with a linear gradient elution system of guanidine·HCl (0–0.4 M), or gel filtration with Bio Gel P-60 equilibrated by 50 mM acetate buffer (pH 5.0) which contained sodium lauryl sulfate at 1% concentration. These two protein bands, however, were not fractionated by these procedures; also they both had a dextranase activity. These observations negate the fact that these two protein bands might be the subunits.

A dextranase from molds, in general, was most active at a semiacidic pH range[5,6]. The dextranase from *P. luteum*, for example, was described to be stable over a pH range from 3.5 to 7.5 at temperatures below 50 °C, and was most active at pH 5.0 (ref. 5). The dextranase from *P. funiculosum* was found to differ in pH and thermal stability from *P. luteum* dextranase. Also the dextranase from *P. luteum* was not affected by Co^{2+}, Mn^{2+} and Cu^{2+}, whereas the enzymes from *P. funiculosum* were activated by these metal ions and inactivated by Ag^{+}.

Dextranase II only hydrolyzed sugar with α-1,6 linkages, but did not hydrolyze CM- or DEAE-Sephadex, although these contain α-1,6 linkages. It is concluded that these facts are due to an ionic or steric effect caused by the carboxymethyl or diethylaminoethyl groups of the substrate.

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