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PROPERTIES OF PURIFIED α-L-ARABINOFURANOSIDASE FROM *CORTICIUM ROLFSII*

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SUMMARY

1 An α-L-arabinofuranoside was purified from the culture fluid of *corticium rolfsii* IFO 4878 The process was as follows salting out by $(NH_4)_2SO_4$, gel filtration on Sephadex G-100 and G-200, heat treatment, chromatography on DEAE-Sephadex A-50, SE-Sephadex C-50 and QAE-Sephadex A-50

2 The highly purified enzyme was demonstrated to be homogeneous by ultracentrifugal analysis and disc electrophoresis, respectively

3 The activity of the purified enzyme towards phenyl α-L-arabinofuranoside and beet arabinan was found to be maximum at pH 2 5 The enzyme was also found to be stable over a wide pH range, pH 1 5 to 10, and to be unusually acid stable, even after storage at pH 2 0 and 5° for 72 h, 83% of enzymic activity remained

4 K_m values were determined to be 2 86 10^{-3} M for phenyl α-L-arabinofuranoside, 8 47 g/l for arabinan and 28 6 g/l for 1,5-arabinan The values of v_{max} were also determined to be 124 0 μmoles for phenyl α-L-arabinofuranoside, 53 0 μmoles for arabinan and 16 7 μmoles for 1,5-arabinan per min per mg of protein, respectively

An α-L-arabinofuranosidase of *Aspergillus niger* was highly purified by a method proposed by KAJI *et al* [1], and found to be active exclusively on arabinofuranoside It was recently obtained in crystalline form, and the amino acid composition and some physicochemical properties were presented in the previous paper[2]

In 1970, the authors[3] reported that *Corticium rolfsii* produces an α-L-arabino furanosidase in the culture fluid and the crude enzyme has a very low pH optimum and high acid tolerance The present communication describes the properties of highly purified α-L-arabinofuranosidase produced by *C rolfsii* IFO 4878 in respect to comparative biochemistry

C rolfsii is a plant-pathogenic, oxalate-forming fungus It was cultured by the method described in the previous paper[3] α-L-Arabinofuranosidase was purified from 5 6 l of culture filtrate by the method of salting out by $(NH_4)_2SO_4$, gel filtration on Sephadex G-100 and G-200, heat treatment, chromatography on DEAE-Sephadex A-50, SE-Sephadex C-50 and QAE-Sephadex A-50 The details of purification were

TABLE I

PURIFICATION OF α-L-ARABINOFURANOSIDASE

Steps 3 and 6 were repeated twice In Step 4, the enzyme solution was heated at 70° and pH 5 0 for 10 min Step 10 was performed as follows QAE-Sephadex A-50 was equilibrated with 0 01 M sodium phosphate buffer (pH 7 5) and packed in a column, 1 6 cm × 15 cm The enzyme solution 90 ml, containing 10 3 mg protein or 320 units of α-L-arabinofuranosidase, was poured onto the column The enzyme was eluted with a step-wise addition of 0 1, 0 2, 0 3 and 0 4 M NaCl, successively with constant 0 01 M sodium phosphate buffer (pH 7 0)

Step		*Vol (ml)*	*Total protein (g)*	*Total activity (units)*	*Yield (%)*	*Specific activity (units/mg)*	*Size of column (cm)*
1	Culture filtrate	5600	14 4	9500	100	0 66	
2	$(NH_4)_2SO_4$ (0 4–0 7 satn)	450	6 4	8600	91	1 3	
3	Sephadex G-100	1000	3 05	7000	74	2 3	5 0 × 80
4	Heat treatment	950	2 15	2830	30	1 3	
5	$(NH_4)_2SO_4$ (0 2–0 9 satn)	110	1 06	1460	15	1 4	
6	DEAE-Sephadex A-50 (I)	280	0 25	1160	12	4 6	2 5 × 30
7	SE-Sephadex C-50	160	0 025	720	7 6	29	2 5 × 30
8	DEAE-Sephadex A-50 (concentration)	42	0 015	470	4 9	31	1 6 × 10
9	Sephadex G-200	90	0 0103	320	3 4	31	2 5 × 40
10	QAE-Sephadex A-50	70	0 0092	290	3 1	32	1 6 × 15
11	DEAE-Sephadex A-50 (II)	33	0 0066	290	3 1	44	1 2 × 10

analogous to those described in the previous paper[2] except for the column chromatography on QAE-Sephadex

The results of the over all purification procedures are summarized in Table I and show the 67-fold increase in specific activity achieved Steps 7, 9 and 10 effectively removed the very small amount β-D-galactosidase which remained in the purified enzyme preparation The single zone in Fig 1 and the clear patterns in Fig 2 demonstrate the homogeneity of the purified α-L-arabinofuranosidase

A reaction mixture containing 1 0 ml of 25 mM phenyl α-L-arabinofuranoside, 0 25 ml of 0 1 M HCl–sodium citrate buffer (pH 2 5), 1 0 ml of enzyme solution, 0 25 ml of deionized water, and 2 drops of toluene was incubated at 30° for 30 min The reaction was stopped by the addition of 1 ml of 0 05 M Na_2CO_3 to 1 ml of reaction mixture The arabinose released by the action of enzyme was determined by the method of NELSON AND SOMOGYI[4–6] One unit of α-L-arabinofuranosidase is that amount of enzyme which liberates 1 μmole of aldehyde per min under the above conditions The actions on arabinan and 1,5-arabinan were tested in a reaction mixture containing 1 0 ml of 0 5% substrate (pH 2 5), 0 25 ml of 0 1 M HCl–sodium citrate buffer (pH 2 5), 1 0 ml of enzyme solution, 0 25 ml of deionized water and 2 drops of toluene

Phenyl α-L-arabinofuranoside was prepared according to the method reported by BORJESON *et al*[7] Purified beet arabinan and 1,5-arabinan were prepared as described in the previous paper[8]

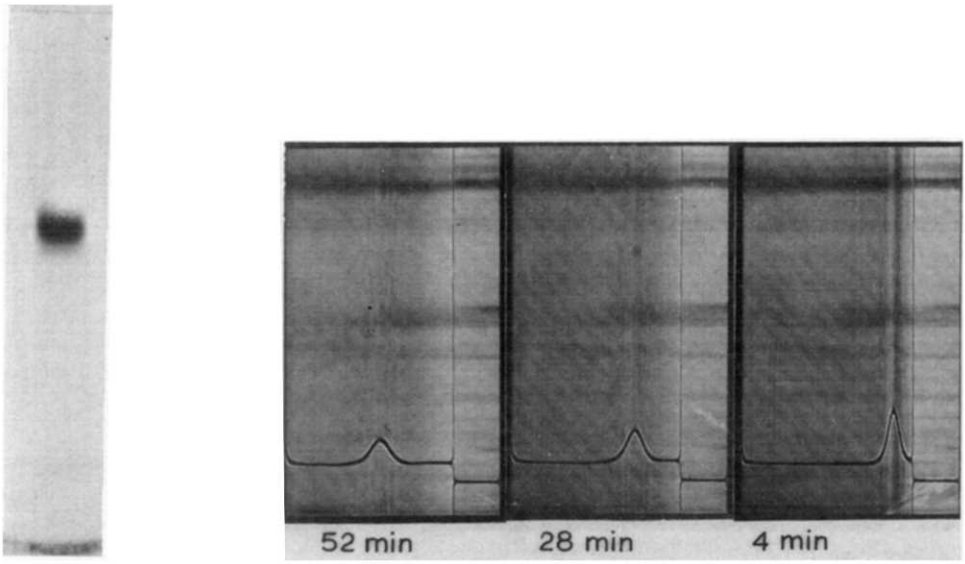


Fig 1 Disc electrophoresis of α-L-arabinofuranosidase on polyacrylamide gel The purified enzyme solution containing 91 μg of protein was layered on a column of 7% polyacrylamide gel and allowed to migrate in Tris–glycine buffer (pH 8 3) for 90 min Protein was stained with Amido black 10B

Fig 2 Velocity sedimentation of α-L-arabinofuranosidase Schlieren pictures were taken at 59 780 rev /min and 20° The purified enzyme in 0 1 M sodium acetate buffer (pH 6 0) was analyzed at a concentration of 6 4 mg enzyme protein per ml

The amount of reducing sugar produced is a linear function of enzyme concentration up to 2 0 μg protein per ml of reaction medium under the conditions mentioned above

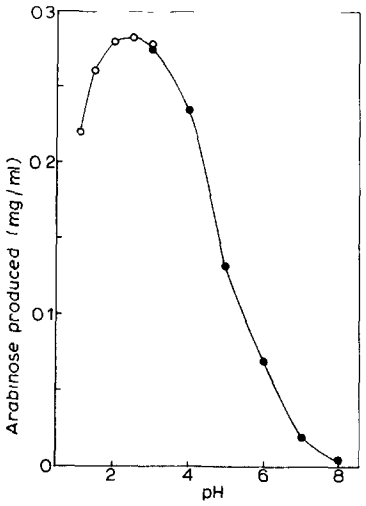
Effect of pH on activity of enzyme

The effect of pH on the activity of purified enzyme is shown in Fig 3 The optimum pH value is 2 5 and the high activity appears in the range of pH 1 5 to 3 0 The purified α-L-arabinofuranosidase is still active at the very low pH of 1 1 The pH curve was the same when 0 1 M HCl–KCl buffer was used in place of 0 1 M HCl–sodium citrate buffer In order to examine the influence of Cl^- on enzymic activity, the activity was assayed in the reaction mixture containing various concentrations of NaCl or KCl The result shows that Cl^- does not activate the action of α-L-arabinofuranosidase

The optimum pH was also pH 2 5 when beet arabinan was used as substrate in place of phenyl α-L-arabinofuranoside

Effect of pH on stability of enzyme

Stability of purified α-L-arabinofuranosidase at various pH values and 5° for 72 h was examined using 0 1 M HCl–sodium citrate buffer for pH 1 1 to 3 0, 0 1 M sodium citrate–0 2 M sodium phosphate buffer for pH 3 0 to 8 0, 0 2 M Tris–0 1 M HCl buffer for pH 8 0 to 9 0 and 0 05 M Na_2CO_3–0 1 M $NaHCO_3$ buffer for pH 9 0 to 11 0 After incubation, the enzyme activity was then determined by the method



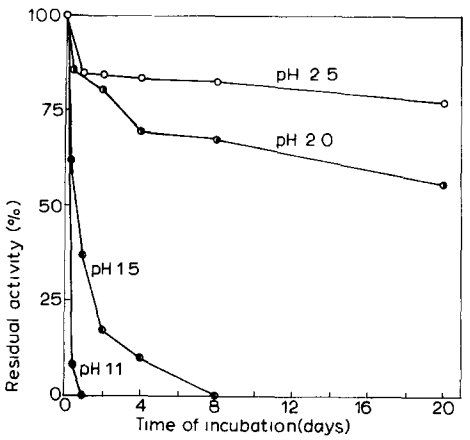


Fig. 3. Effect of pH on activity of α-L-arabinofuranosidase. A reaction mixture containing 1.0 ml of 2.5 mM phenyl α-L-arabinofuranoside, 0.25 ml of buffer, 1.0 ml of enzyme solution (3 μg of protein) and 0.25 ml of deionized water was incubated at 30° for 30 min. 0.1 M HCl–sodium citrate buffer was used for pH 1.1 to 3.0, 0.1 M sodium citrate–0.2 M sodium phosphate buffer for pH 3.0 to 8.0.

Fig. 4. Effect of pH on stability of α-L-arabinofuranosidase. 2.5 ml of enzyme solution (62.5 μg of protein) and 0.1 M HCl–sodium citrate buffer were used for acid stability. Each mixture was allowed to stand at 30°. The procedure was described in the text. Residual activity is represented as per cent of untreated enzyme solution.

mentioned above. The results show that purified α-L-arabinofuranosidase is relatively stable over a wide pH range, 1.5 to 10.0. Even after the enzyme solution was stored at pH 2.0 and 5° for 72 h, 83% of activity remained and at pH 1.1, 47% of activity still remained.

Stability of the purified enzyme at low pH values and 30° for 5 h to 20 days was also examined using 0.1 M HCl–sodium citrate buffer. The procedure was the same as described above and the temperature of incubation was adjusted to 30° in place of the low temperature of 5°. As shown in Fig. 4, the purified enzyme was inactivated after 24 h of incubation at pH 1.1, but 56% or 77% of enzyme activity remained after 20 days of incubation at pH 2.0 and 2.5, respectively.

These results evidently demonstrate the unusual stability at low pH which is characteristic of α-L-arabinofuranosidase produced by *C. rolfsii*.

Action of α-L-arabinofuranosidase on various arabinosides

The purified enzyme was confirmed to be able to liberate L-arabinose from beet arabinan, 1,5-arabinan, arabinoxylan and phenyl α-L-arabinofuranoside, respectively. The enzymic product was paper chromatographically demonstrated to be only L-arabinose. As already described above, the highly purified enzyme was also confirmed to be inactive on *p*-nitrophenyl β-D-galactopyranoside. This substrate specificity is the same as that of α-L-arabinofuranosidase from *A. niger*[1,2].

Michaelis constants were determined to be 8.47 g/l for beet arabinan, 28.6 g/l for 1,5-arabinan and 2.86 10^{-3} M for phenyl α-L-arabinofuranoside, respectively. The

values of v_{max} were also calculated to be 53 0 μmoles for beet arabinan, 16 7 μmoles for 1,5-arabinan and 124 0 μmoles for phenyl α-L-arabinofuranoside per min per mg of protein These constants were determined by the method of LINEWEAVER AND BURK[9]

The results show that α-L-arabinofuranosidase from *C rolfsii* is active exclusively on furanosides of arabinosides, as already reported in the previous paper on α-L-arabinofuranosidase from *A niger*[1,2]

The highly purified enzyme was found to be active at very low pH values, 1 1 to 3 0, and to have an optimum for catalytic activity at pH 2 5 The purified enzyme was also demonstrated to be acid tolerable at pH 1 1 to 2 5 and 5° for 72 h, or at pH 2 0 to 2 5 and 30° for 20 days According to these remarkable results, it is concluded that the high enzymic activity at very low pH values and the unusual tolerance to hydrogen ions are characteristics of α-L-arabinofuranosidase from *C rolfsii* Endopolygalacturonase[10] and β-D-galactosidase[11] produced by the same fungus were found to be highly active at pH 1 5 to 2 5 and acid tolerable These enzymes, including α-L-arabinofuranosidase, might be called acid glycosidase or acid glycanase because of their remarkable properties

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