SHORT COMMUNICATIONS

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Properties of purified α-L-arabinofuranosidase from *Aspergillus niger*

Since the report by EHRLICH AND SCHUBERT[1], in 1928, on the enzyme that liberates arabinose from beet araban, little more has been reported on this problem. To add to our knowledge in this field, we have been studying this enzyme and have succeeded in finding a method for purifying it from *Aspergillus niger*[2]. The highly purified enzyme is an α-L-arabinofuranosidase; knowledge of its function would be important for studying arabinose-containing polysaccharides.

The enzyme was prepared from the culture fluid of *A. niger* (for details see previous paper[2]). The process is as follows: salting out by ammonium sulfate, gel filtration on Sephadex G-100, chromatography on DEAE-Sephadex, DEAE-cellulose, SE-Sephadex and hydroxylapatite. The enzyme used in the present work was confirmed to be homogeneous by ultracentrifugal analysis (Fig. 1).

A reaction mixture containing 0.5 ml of 0.025 M phenyl α-L-arabinofuranoside, 0.25 ml of 0.1 M McIlvaine buffer (pH 4.0), 0.5 ml of enzyme solution, 0.125 ml of water and 2 drops of toluene was incubated at 30°. The arabinose produced was measured by the method of SOMOGYI[4] and NELSON[3]. The linear relationship between the arabinose formed and the amount of purified enzyme could be demonstrated up to 0.72 μg of enzyme per ml of reaction medium during a 30-min incubation.

Crystalline phenyl α-L-arabinofuranoside was prepared by the method of

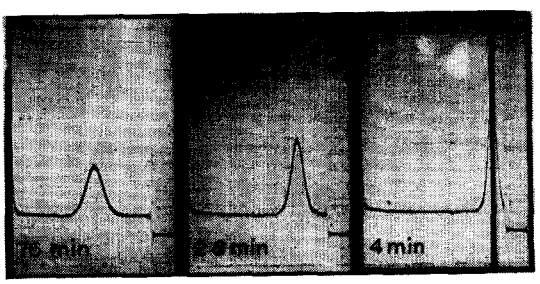
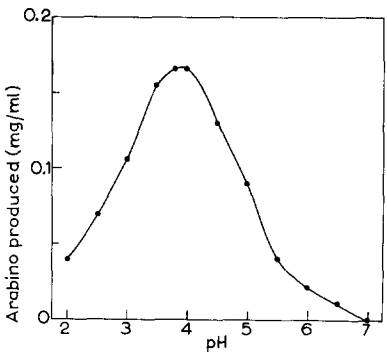


Fig. 1. Velocity sedimentation of α-L-arabinofuranosidase. Schlieren pictures were taken with a Spinco Analytical Ultracentrifuge model E at 59780 rev./min and 20°. The purified enzyme in 0.1 M acetate buffer, (pH 6.0) was analyzed at a concentration of 6 mg enzyme protein per ml.

BÖRJESON *et al.*[5]. Nitrophenyl α-L-arabinopyranoside was synthesized by the method of SNYDER AND LINK[6]. Crude beet araban was prepared by the method of HIRST AND JONES[7] and partially purified as mentioned in the previous paper[2]. Further purification was carried out by gel filtration on Sephadex G-50. The purified beet araban was 97.8% pure and had a $[\alpha]_D^{25}$ — 160.8° in water (*c*, 1.0).

A pH–activity curve was obtained as shown in Fig. 2. The activity of the enzyme towards phenyl α-L-arabinofuranoside was found to be maximum at pH 3.8 to 4.0.

Initial reactions on phenyl α-L-arabinofuranoside and beet araban were observed during a 10-min incubation. As shown in Fig. 3, the amounts of arabinose released in



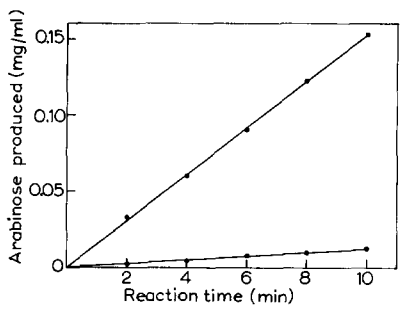


Fig. 2. Effect of pH on enzymic activity. 0.5 ml of 0.025 M phenyl α-L-arabinofuranoside, 0.25 ml of 0.1 M McIlvaine buffer, 0.5 ml of enzyme (0.5 μg of protein) and 0.125 ml of water were incubated at 30° for 10 min.

Fig. 3. Action on phenyl arabinoside and beet araban (initial reaction). 2.0 ml of 0.025 M phenyl α-L-arabinofuranoside, 1.0 ml of 0.1 M McIlvaine buffer (pH 4.0), 2.0 ml of enzyme (2.0 μg of protein) and 0.5 ml of water were incubated at 30°. 3.0 ml of 0.5% beet araban, 0.75 ml of 0.1 M McIlvaine buffer (pH 4.0) and 1.0 ml of enzyme (1.0 μg of protein) were incubated at 30°. upper line: phenyl arabinoside, under line: beet araban.

the media were assayed as a linear function of reaction time, and determined to be 42.3 μg for phenyl α-L-arabinofuranoside and 5.4 μg for araban per min per μg of enzyme, respectively. Only L-arabinose was found during the course of hydrolysis by the method of paper chromatography. Nitrophenyl α-L-arabinopyranoside was examined by enzymic action, but an increase in reducing value was not observed.

Michaelis constants were determined to be 1.10 g per l for phenyl α-L-arabinofuranoside and 0.26 g per l for araban by the method of LINEWEAVER AND BURK[8].

For studying the inhibitory effect of salts on the enzymic action, the enzyme solution, 0.25 ml (0.5 μg protein) was mixed with 0.25 ml of McIlvaine buffer (pH 4.0) and 0.25 ml of salt solution and the mixture was allowed to stand at 20° for 10 min. Then 0.5 ml of phenyl α-L-arabinofuranoside solution and 0.125 ml of water were added and the enzymic activity was assayed. As shown in Table I, the inhibitory effect of mercuric ion was clearly observed.

TABLE I

EFFECT OF METAL ION ON ENZYMIC ACTION

Salt (10^{-3} *M*)	*Relative activity* (%)
None	100.0
$MnSO_4$	97.1
$Fe_2(SO_4)_3$	73.6
$FeSO_4$	95.4
$CuSO_4$	91.2
$ZnSO_4$	96.5
Calcium acetate	95.2
Mercuric acetate	0
$CoCl_2$	90.5

The results in the present work demonstrate that the highly purified enzyme from *A. niger* is α-L-arabinofuranosidase and its optimum pH is 3.8 to 4.0. The enzyme was also active on beet araban, and L-arabinose was liberated as the enzymic product in both cases.

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