Partial Purification and Characterization of Alpha-Glucosidase from *Pseudomonas fluorescens* W

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**Abstract.** The α-glucosidase (α-D-glucoside glucohydrolase, EC 3.2.1.20) of *Pseudomonas fluorescens* W was partially purified by (NH₄)₂SO₄ fractionation, Sephadex G-200 and DEAE-cellulose column chromatography. The enzyme showed great specificity for maltose hydrolysis, with very little action against polymeric forms. Sucrose, isomaltose, α-methylglucoside, and maltobionic acid were not hydrolyzed. Turanose was a strong competitive inhibitor, and glucose a weaker one. Tris (2-amino-2-hydroxymethylpropan-1:3-diol) inhibited enzyme activity significantly only at alkaline pH. Mercuric, cupric, and silver cations strongly inhibited, and EDTA (ethylenediaminetetraacetate) weakly inhibited the enzyme. The isolated enzyme was rather unstable even at 4°C, and was destroyed by freezing and lyophilization. Inositol and albumin had a slightly protective effect. Sulphydryl-binding reagents strongly inhibited the enzyme.

**Key words:** *Pseudomonas fluorescens* W — Maltose hydrolysis — α-Glucosidase—Partial purification.

The maltose splitting enzyme α-glucosidase has been found in a variety of organisms including yeasts (Halvorson and Elias, 1958; Phillips, 1959; Khan and Eaton, 1967), filamentous fungi (Yamasaki et al., 1973), plants (Meyer and Bourillon, 1973) and animal tissue (Lieberman and Ito, 1957; Palmer, 1971b; Brown et al., 1972). While many bacterial species are reported to use maltose as a carbon source very few reports have appeared concerning α-glucosidase in bacteria (French and Knapp, 1950; Sugimoto et al., 1974). Since several other mechanisms of maltose cleavage have been reported in bacteria (Fitting and Scherp, 1952), α-glucosidase may not be as common as supposed.

*Pseudomonas fluorescens* W has been shown to use maltose exclusively by hydrolyzing it to glucose via an inducible α-glucosidase (α-D-glucoside glucohydrolase, EC 3.2.1.20) located entirely intracellularly (Guffanti and Corpe, 1975). In the present work the specificity and some other properties of the enzyme recovered in a partially purified state were compared to the description of α-glucosidases from other sources.

**MATERIALS AND METHODS**

**Organism, Growth Media, and Culture Conditions.** The bacterium studied in this work was isolated from soil by T. L. Whiteside and identified as *Pseudomonas fluorescens* (Whiteside and Corpe, 1969). The organism, designated as strain W, was routinely transferred every two weeks on slants of peptone-glucose-yeast extract (PGYE) agar (Whiteside and Corpe, 1969). Cultures were stored at 4°C between transfers.

The defined medium used contained the following ingredients in g/l deionized water: (NH₄)₂SO₄, 2.0; KH₂PO₄, 5.3; Na₂HPO₄, 8.66; MgSO₄·7H₂O, 0.2; NaCl, 0.01; FeSO₄·7H₂O, 0.01; MnSO₄·H₂O, 0.01. p-A-methionine, 30 μg/ml was supplied as the only required growth factor.

**Isolation and Partial Purification of α-Glucosidase.** One of the ammonium-salts-methionine medium supplemented with 10 mM maltose in a 91 serum bottle was stoppered with cotton and autoclaved. The cooled medium was inoculated with 20 ml of a log phase culture grown aerobically in the same medium. A sterile glass capillary tube was inserted into the bottle and sterile air was bubbled in at a rate of about 100 bubbles per minute. Incubation was at 30°C.

A log phase culture was chilled in a cold room (4°C) and centrifuged at that temperature at 12000 g for 15 min. All subsequent steps were carried out in the cold. Cells were washed and resuspended in about 100 ml of 0.1 M potassium phosphate buffer, pH 7.0, and disrupted in a sonic oscillator (MSE Ultrasonic Disintegrator, Instrumentation Assoc., N.Y.C., 60 watts), maintaining the temperature at 4—6°C in an ice bath. The broken cell suspension was centrifuged at 27000 g for 15 min and the crude extract...
various concentrations of p-nitrophenol.

The protein in the cell extract was fractionated with ammonium sulfate. 40\% of saturation produced a substantial precipitate that had some protein but only 5\% of the total \(\alpha\)-glucosidase activity. It was discarded. Ammonium sulfate was added to 60\% saturation and the precipitate removed by centrifugation as before. The 60\% saturation fraction contained about 80\% of the \(\alpha\)-glucosidase activity. The rest of the enzyme was distributed through the several other ammonium sulfate fractions (70–100\%); routinely, only the 60\% fractions was recovered for study.

The 60\% ammonium sulfate fraction was resuspended in 5 ml buffer and desalted by passage through a Sephadex G-200 column (2 cm by 25 cm). Fractions of 5 ml were eluted from the column and the peak of \(\alpha\)-glucosidase activity was pooled and chromatographed on a DEAE-cellulose column (1.5 cm by 15 cm) equilibrated to pH 7.0. The column was eluted with 50 ml of the buffer alone followed by 50 ml of each of the following solutions of NaCl in buffer: 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0\%. Five ml fractions were collected and assayed for \(\alpha\)-glucosidase and protein. Those fractions with the highest specific activity were pooled and concentrated by precipitation with 65\% \((\text{NH}_4)_2\text{SO}_4\) saturation, dialyzed against 0.1 M phosphate buffer, pH 7.0, and characterized as to enzymatic activity.

**Assay for \(\alpha\)-Glucosidase Activity.** Alpha-glucosidase activity was assayed with para-nitrophenyl-\(\alpha\)-glucoside (PNPG) as the substrate using a variation of the procedure of Han and Sinivasan (1969) for \(\beta\)-galactosidase. The reaction mixture contained enzyme in 2.5 ml of 0.1 M phosphate buffer, pH 7.0. After equilibration for 5 min in a 30\(^\circ\)C water bath, 0.5 ml of 5 mM PNPG was added and the reaction was allowed to proceed for a suitable length of time. The reaction was stopped by the addition of 2 ml of 1 M Na$_2$CO$_3$. The yellow p-nitrophenol released was read at 400 nm.

Protein was stained on the gels with 0.25\% Coomassie blue in 15\% trichloroacetic acid and 25\% methanol for 20 min at room temperature. The gels were destained with 7% acetic acid.

**Sources of Chemical and Chromatographic Media.** Isomaltose, melezitose, alpha-phenylglucoside, p-chloromercuribenzoic acid, turanose, raffinose, Sephadex G-200-120, and paranitrophenyl-alpha-D-glucopyranoside were all purchased from Sigma Chemical Co. (St. Louis, Mo.). Maltobionic acid, Azo dell, and maltotriose were all products of Calbiochem (San Diego, Calif.). Special enzyme grade, ultra pure, \((\text{NH}_4)_2\text{SO}_4\), was obtained from Schwarz/Mann Research Laboratories (Orangeburg, N.Y.). Diethylaminoethyl cellulose, new fibrous DE 23, was a Whatman product (H. Reeve Angel Co., Clifton, N.J.). Others were best available reagent grade chemicals obtained from Fisher Scientific Co. (Springfield, N.J.).

**RESULTS**

**Purification**

The growth of logarithmic phase cells of *Pseudomonas fluorescens* W was carried out in a completely defined medium which afforded good yields of the inducible \(\alpha\)-glucosidase. The enzyme was isolated from extracts of cells broken by sonication and partially purified in reasonably good yield (Table 1). Recovery in even better yield was hindered, presumably, because of the enzyme's instability in a semipurified form at 4\(^\circ\)C, as will be described below. The enzyme was recovered, along with much non-specific protein, from Sephadex G-200 as a broad peak (Fig. 1). Approximately 90\% of the total protein put on was eluted from Sephadex. The enzyme was eluted from DEAE-cellulose as a sharp peak (Fig. 2), and gel electrophoresis of the fractions under the peak showed a few minor protein bands present along with the band with \(\alpha\)-glucosidase activity. About 80\% of the total protein put on was eluted from DEAE, but only one peak of enzyme activity was found in spite of a careful search for more, even in small amount.

**Substrate Specificity**

Several glucosides were tested as substrates for the partially purified \(\alpha\)-glucosidase by measuring the amount of glucose released (Table 2). Only maltose was hydrolyzed to any significant extent. It is perhaps worth noting that the enzyme cleaved maltotriose only poorly, thus showing that it prefers non-polymerized \(\alpha\)-glucosides. Since it did not degrade \(\alpha\)-methylglucoside and degraded \(\alpha\)-phenylglucoside weakly, its substrate specificity must be quite stringent. Neither \(\alpha\)-nitrophenyl-\(\beta\)-D-glactoside nor \(\alpha\)-nitrophenyl-\(\beta\)-D-glucoside was hydrolyzed, showing the absence of beta-galactosidase or beta-glucosidase activities.