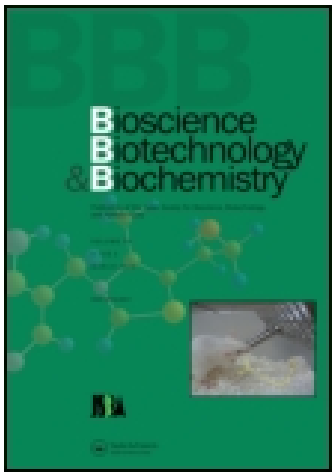

Bioscience, Biotechnology, and Biochemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tbbb20

Purification and Characterization of α-D-Mannosidase from the Seeds of Kaya, Torreya nucifera

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Published online: 12 Jun 2014.

To cite this article: Tomomi Shiroo, Kimiko Ohtani, Kyoko Huchigami, Masato Nakatani, Isao Yuasa & Akira Misaki (1996) Purification and Characterization of α-D-Mannosidase from the Seeds of Kaya, Torreya nucifera, Bioscience, Biotechnology, and Biochemistry, 60:4, 687-688, DOI: 10.1271/bbb.60.687

To link to this article: http://dx.doi.org/10.1271/bbb.60.687

Biosci. Biotech. Biochem., **60** (4), 687 688, 1996

Note

Purification and Characterization of α-D-Mannosidase from the Seeds of *Kaya, Torreya nucifera*

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Received September 21, 1995

Alpha-D-mannosidase was purified from the extract of seeds of *Kaya, Torreya nucifera*. The purified enzyme had a molecular mass of $\sim 3.6 \times 10^5$ daltons. This enzyme had an optimum pH at 4.5, and was stable at pH between 5.5 and 6.5. This enzyme appeared to be a metal enzyme containing Zn^{2+}. The enzyme hydrolyzed *p*-nitrophenyl-α-D-mannoside, methyl-α-D-mannoside, α-1→3-mannobiose, and α-1→6-mannobiose, with K_m of 0.785 mM, 0.236 M, 2.505 mM, and 0.268 mM, respectively. The hydrolysis of various α-linked mannobioses indicated that the enzyme hydrolyzes the α-mannobioses in the order of α-(1→2)> -(1→6)> -(1→3).

Key words: α-D-mannosidase; Kaya; *Torreya nucifera*; purification; characterization

Alpha-D-mannosidase is widely distributed in animal tissues,[1,2] plant seeds,[3] and microorganisms.[4] Many plant seeds contained not only α-D-mannosidase but various types of glycosidases, which must be important in germination processes, and α-D-mannosidase in mammalian cells may be important in the processing of the carbohydrate chain of the glycoproteins and glycolipids. And various types of α-D-mannosidase were purified as we isolated them from papaya seed,[5] and used as probes for the structural investigation of the oligosaccharides.

The seeds of *Torreya nucifera* are used as a source of an edible oil and as an anthelmintic for extermination of parasitic worms in Japan. In the course of investigation on the biological active components contained in the seeds of *Torreya nucifera*, we found relatively high α-D-mannosidase activity (same as papaya seed[5]) together with non-type specific hemagglutinating activity in the phosphate buffered-saline extract. This paper reports the purification and the characterization of the α-D-mannosidase from *T. nucifera*.

T. nucifera seeds (1 kg), harvested in Osaka Pref., were homogenized and extracted with 10 mM phosphate buffer, pH 6.5. After fractionation with 30% ammonium sulfate saturation, this fraction was put onto a DEAE-Sephadex A-50 column (5.0 × 24.0 cm). Stepwise elution was done with 10 mM PBS containing 0.15 M to 0.3 M NaCl. The fraction eluted with the buffer containing 0.3 M NaCl was put onto a CM-Sephadex C-50 column (5.0 × 15 cm), which was previously equilibrated with 10 mM acetate buffer at pH 5.5. Enzyme activity in the non-adsorbed fraction was put on a Toyopearl HW65S column (2.5 × 96.0 cm), which was eluted with 10 mM PBS containing 0.3 M NaCl, as shown in Fig. Hemagglutinin had similar behavior to the α-D-mannosidase on these column chromatographies. The enzyme fraction was purified by rechromatography on the same column. One unit of the enzyme activity was defined as the amount of the enzyme that released one μmol of *p*-nitrophenol per minute at 37°C. Each step of the purification is summarized in Table I, indicating that the α-D-mannosidase was purified 172.0 fold with 2.8% of the yield. The relatively low yield and the low specific activity of the purified enzyme may partly be due to contamination with a lipid, which gave undesirable turbidity to the crude extract.

The molecular mass of the α-D-mannosidase was estimated to be $\sim 3.6 \times 10^5$ daltons by Toyopearl HW65S gel chromatography. SDS–PAGE showed that it was a monomer. The molecular weight of this enzyme was higher than that of Jack bean (M.W. 1.9×10^5),[3] *Phaseolus vulgaris* (M.W. 1.9×10^5),[6] papaya seed (1.6×10^5),[5] and soybean (M.W. $1.7–1.8 \times 10^5$)[7]. Although the α-D-mannosidase from Japanese quail oviduct[8] appeared to have a

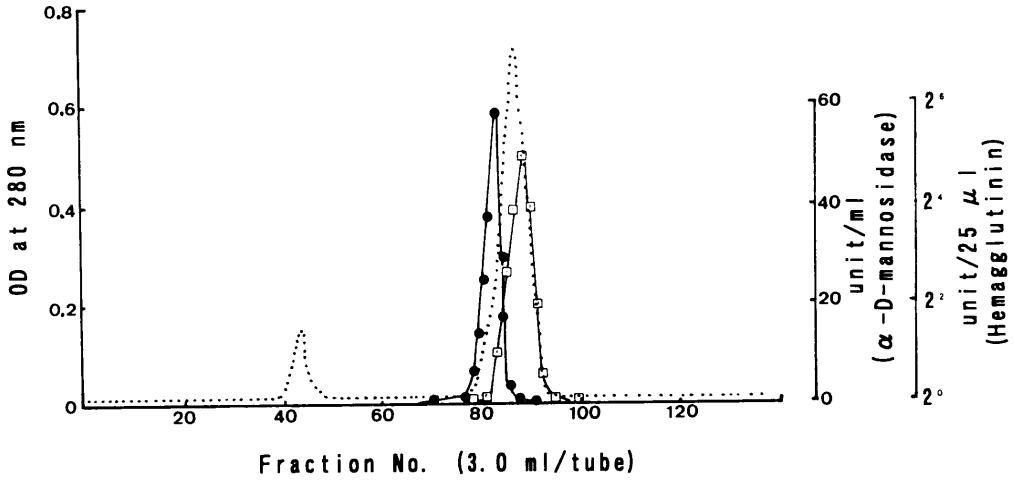

Fig. Elution Profile of α-D-Mannosidase from *Kaya* on Toyopearl HW65S (2.5 × 96 cm) Column.

Elution was done with 10 mM phosphate buffer containing 0.3 M NaCl at pH 6.5. Each fraction was 3.0 ml. ------, O.D. at 280 nm; ●, α-D-mannosidase activity (unit/ml); □, hemagglutinating activity (unit/25 μl).

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Table I. Summary of the Purification of α-Mannosidase from the Seeds of *Kaya*

	Total protein (mg)	Total activity (unit)	Specific activity (unit/mg)	Purification (fold)	Yield (%)
Crude extract	45,067.0	359.6	0.008	1.0	100.0
0–30% fraction by $(NH_4)_2SO_4$ fraction	4,457.0	80.9	0.018	2.3	22.5
Fraction by DEAE-Sephadex A-50	1,469.0	58.1	0.039	4.9	16.1
Fraction by CM-Sephadex C-50	740.0	29.7	0.040	5.0	8.3
Toyopearl HW65S 1st	31.8	22.9	0.719	90.0	6.4
Toyopearl HW65S 2nd	6.3	8.6	1.370	172.0	2.4

Table II. Substrate Specificity of α-D-Mannosidase from the Seeds of *Kaya*

Substrate	ρ-NO_2-α-Man[a]	Me-α-D-Man[b]	α-1→3Man_2[c]	α-1→6-Man_2[d]
K_m (M)	7.85×10^{-4}	2.3×10^{-1}	2.505×10^{-3}	2.68×10^{-4}
V_{max} (μmol/mg/min)	3.32×10^{-2}	2.79×10^{-1}	1.10×10^{-2}	3.60×10^{-4}

[a] ρ-Nitrophenyl-α-D-mannoside. [b] Methyl-α-D-mannoside. [c] α 1→3 Linked mannobiose. [d] α 1→6 Linked mannobiose.

similar molecular weight, M.W. 3.3×10^5, it consists of subunits with molecular weight of 1×10^5, 6.5×10^5, and 5×10^4. Then, this α-D-mannosidase was characteristic in having a large molecular weight with monomeric configuration. The carbohydrate contents were 24.9%. The composition of amino acid, analyzed by a L-8500 high speed amino acid analyzer (Hitachi Co.), showed that this enzyme was containing relative high proportions of aspartic acid and glutamic acid, and low proportions of sulfur-containing amino acids, cystein and methionine. This enzyme had its optimum temperature at 60°C and was stable till 50°C. The optimum pH of the enzyme activity was pH 4.5, and enzyme activity was stable at pH between 5.5 and 6.5. The enzyme was completely inactivated in the presence of 5 mM Ag^+ or Fe^{2+} ions. Although the α-D-mannosidase activity was completely lost upon incubation with 25 mM EDTA at 30°C for 48 h, the addition of 5 mM Zn^{2+} afforded complete recovery of the enzyme activity, confirming that Zn^{2+} must be essential. These properties are similar to those of α-D-mannosidases of papaya seed,[5] Jack bean,[3] and *Phaseolus vulgaris*.[6]

The substrate specificity was analyzed as follows: when methyl-α-D-mannoside was used as a substrate, the liberated mannose was measured by the Somogyi–Nelson method,[9,10] and when mannobioses were used as substrates (*c* 50 μg/ml), the liberated mannose was measured by High Performance Anion Exchange Chromatography, Dionex Bio LC Model 4500i System, with a column of CarboPac PA1. The K_m and V_{max} are summarized in Table II. The α-D-mannosidase of *Kaya* seed had low affinity toward methyl-α-D-mannoside, but the V_{max} was high. Moreover this enzyme especially had a high affinity for *p*-nitrophenyl-α-D-mannoside, the K_m of which was approximately 0.8 mM, compared with the α-D-mannosidases from other origins, *i.e.*, Jack bean,[3] soybean,[7] *Phaseolus vulgaris*,[6] *Medicago sativa*,[11] and *Carica papaya*,[5] the K_ms of which were 2.4, 1.6, 1.6, 2.3, and 5.6 mM. The hydrolysis rates of three kinds of α-linked mannobioses, *i.e.*, α-(1→2), -(1→3), and -(1→6), was measured. We found that this enzyme hydrolyzed these α-D-mannobioses in the order of α-(1→2)- > -(1→6)- > -(1→3)-mannobiose. (The hydrolysis after 24 h incubation was 28.0, 20.3, and 8.5%, respectively.)

This α-D-mannosidase is also expected, like those from other origins, to provide a useful probe for the biochemical studies of α-D-mannosides containing in glycoconjugates. However, it was noted that the final yield of the enzyme was very low. For use of this enzyme, yield must be required. Then the improvement of its purification is now investigated.

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