Carbohydrate Research, 230 (1992) 179–183
Elsevier Science Publishers B.V., Amsterdam

Note

An assay for oligo-(1→4)→(1→4)-glucantransferase activity in the glycogen debranching enzyme system by using HPLC with a pulsed amperometric detector

Shiro Tabata [a] and Yoshiko Dohi [b]

Departments of [a] Chemistry and [b] Public Health, Nara Medical University, Kashihara, Nara 634 (Japan)

(Received September 23rd, 1991; accepted November 12th, 1991)

In mammalian tissues and yeast, glycogen is debranched by a two-component enzyme system comprising[1] (1→4)→α-D-glucan : (1→4)-α-D-glucan 4-glucosyltransferase (EC 2.4.1.25) and amylo-(1→6)-glucosidase (EC 3.2.1.33). The former enzyme transfers maltosyl and maltotriosyl segments from branched chains, thereby exposing the glucosyl moiety in the branch linkage which is then hydrolysed by the latter enzyme to release D-glucose.

The debranching activity is usually assayed by the combined action of the two enzymes[2,3]. Although the two enzyme activities appear to be associated with one protein[3,4], methods by which they can be assayed separately are necessary. Several assays have been developed for the glucosidase, using either glucosidase-specific substrates such as 6-*O*-α-D-glucosylmaltotetraose[5] and 6-*O*-α-D-glucosylcyclomalto-oligosaccharides[6], or the reversion reaction that incorporates radioactive glucosyl moieties into polysaccharide[7]. An assay for the transferase has been reported[8] that utilises the change in the spectra of the complex of amylopectin with iodine. Although this method is rapid and convenient, it is not stoichiometric.

The transferase of the yeast debranching enzyme system acts on maltopentaose to yield[9] a mixture of maltose, maltotriose, maltoheptaose, and malto-octaose, which can be fractionated by HPLC on an anion-exchange resin with a mobile phase of high pH and a pulsed amperometric detector[10].

We now report an assay for the transferase activity of the debranching enzyme system of *Saccharomyces*, which uses maltopentaose as a substrate. The method is stoichiometric, the substrate is readily available, and only μg amounts of enzyme are required.

Correspondence to: Dr. S. Tabata, Department of Chemistry, Nara Medical University, Kashihara, Nara 634, Japan.

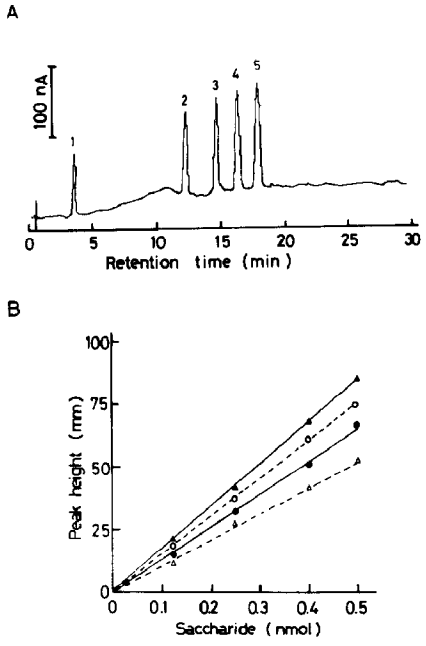

Fig. 1. A, HPLC of a mixture of D-glucose (1) and maltose to maltopentaose (2–5, 250 pmol of each) on a column of Dionex CarboPac PA-1. Chromatographic conditions: see Experimental. B, Calibration curves for glucose (△), maltose (●), maltotriose (○), and maltopentaose (▲).

Fig. 1A shows a typical fractionation by HPLC of the mixture D-glucose–maltopentaose (250 pmol of each), and Fig. 1B shows some calibration curves which were almost linear in the range 100–500 pmol.

Fig. 2 shows the HPLC profile of the products formed by the action of the transferase on maltopentaose, namely, maltose, maltotriose, maltoheptaose, and malto-octaose, the last two oligosaccharides arising by transfers of maltosyl and maltotriosyl segments, respectively, from maltopentaose to maltopentaose. The

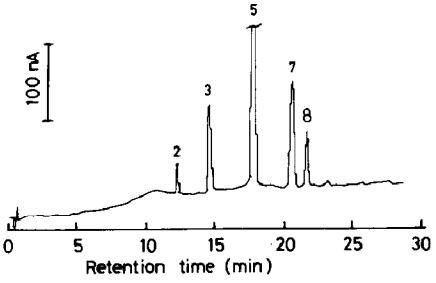

Fig. 2. HPLC of the products formed by the action of the transferase on maltopentaose. The reaction mixture (total volume, 25 μL), which contained 24 mM maltopentaose in 40 mM phosphate buffer (pH 6.5) and 1 μg of the debranching enzyme (0.0035 U), was incubated at 30° for 20 min, then boiled, and diluted with distilled water (1 mL), and an aliquot (25 μL) was used for HPLC. The numbers on the peaks indicate the dp of the malto-oligosaccharides.

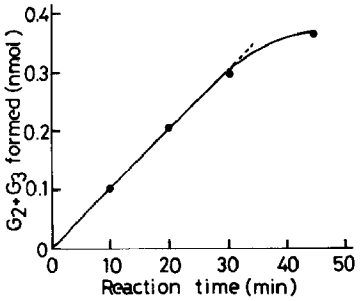

Fig. 3. Production of maltose and maltotriose from maltopentaose by the transferase as a function of time. The conditions were the same as those in Fig. 2, and samples were taken at 10-min intervals, boiled, and then subjected to HPLC. G_2+G_3 expresses the sum of the maltose and maltrotriose (nmol/25 μL).

proportions of maltotriose and maltoheptaose, and of maltose and malto-octaose were equivalent[9]. Thus, the rate of transferase action can be calculated from the sum of maltosyl and maltotriosyl units transferred, which is equal to the sum of maltose and maltotriose residues detected by HPLC.

Fig. 3 shows that the action of the enzyme as a function of time was linear only up to 30 min. It is possible that maltoheptaose and malto-octaose, produced by the action of the transferase, may be substrates for the enzyme. When > 10% of the maltopentaose had been consumed, maltotetraose and maltodecaose, arising from the product, maltoheptaose, could be detected.

Fig. 4 shows that the assay departs from linearity when > 2 μg enzyme was used, probably because the products of the transferase reaction are also substrates for the enzyme.

Fig. 5 shows the effect of varying the concentration of the substrate (maltopentaose); the apparent K_m for maltopentaose, estimated from the double reciprocal plot, was 12 mM and V_{max} was 0.5 μmol/min/mg of protein.

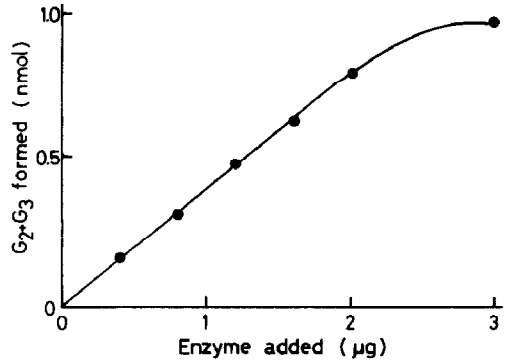

Fig. 4. Production of maltose and maltotriose from maltopentaose by the transferase as a function of enzyme concentration (see Experimental). The incubation time was 10 min. G_2+G_3 expresses the sum of the maltose and maltotriose formed (nmol/min/25 μL).

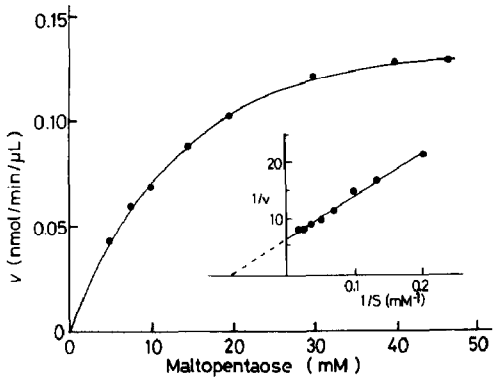

Fig. 5. Effect of the concentration of maltopentaose on transferase activity. The reaction mixture, which contained 20 μL of the substrate (final concentration indicated in the Figure) and 5 μL of the solution of the enzyme (1.5 μg), was incubated at 30° for 10 min. The HPLC conditions are described in the Experimental. Velocity is expressed as the sum of the maltose and maltotriose (nmol/min/μL of enzyme).

Maltopentaose was used as the substrate because it is available commercially in a purified form, and maltose and maltotriose arising from the transferase reaction cannot be donor substrates.

EXPERIMENTAL

HPLC.—A Dionex BioLC gradient pump and model PAD-II detector were used, with a Dionex Eluent Degas Module to sparge and pressurise the mobile phase with He. The eluents were *A*, 150 mM NaOH; *B*, 150 mM NaOH containing M NaOAc.

The gradient used for the fractionation of mixtures of oligosaccharides was 3 min with eluent *A* followed by a linear increase of eluent *B* up to 50% at 30 min on a column (4 × 250 mm) of Dionex CarboPac PA-1 at 1 mL/min at ambient temperature, with detection by a pulsed amperometric detector (PAD-II) with a gold working electrode and triple-pulsed amperometry. The following pulse potentials and durations were used: $E_1 = 0.1$ V (300 ms), $E_2 = 0.6$ (120), $E_3 = -0.8$ (300).

Enzyme assay.—To 30 mM maltopentaose in 50 mM phosphate buffer (20 μL, pH 6.5) in an Eppendorf tube at 30° were added 5 μL of a solution containing 0.3–3 μg (001–0.01 U) of enzyme. The mixture was kept at 30° for 10 min, then heated in a boiling water bath for 30 s, and diluted 10- to 100-fold with distilled water; an aliquot (10–25 μL, 10 nmol of the substrate) was used for HPLC.

Enzyme and sugars.—The debranching enzyme was highly purified by the method described[9] and the specific activity, determined from the glucose released from glycogen phosphorylase limit dextrin, was 3.5 μmol/min/mg of protein. The malto-oligosaccharides were purchased from Nacali (Kyoto, Japan).

REFERENCES

1 E.Y.C. Lee and W.J. Whelan, in P.D. Boyer (Ed.), *The Enzymes*, 3rd edn., Vol. 5, Academic Press, New York, 1971, pp. 191–234.
2 J. Larner, B. Illingworth, G.T. Cori, and C.F. Cori, *J. Biol. Chem.*, 199 (1952) 641–651.
3 T.E. Nelson, E. Kolb, and J. Larner, *Biochemistry*, 8 (1969) 1419–1428.
4 E.Y.C. Lee, J.H. Carter, L.D. Nielsen, and E.H. Fischer, *Biochemistry*, 9 (1970) 2347–2355.
5 D.H. Brown and B.I. Brown, *Methods Enzymol.*, 8 (1966) 515–524.
6 P.M. Taylor and W.J. Whelan, *Arch. Biochem. Biophys.*, 113 (1966) 500–502.
7 T.E. Nelson and J. Larner, *Anal. Biochem.*, 33 (1970) 87–101.
8 T.E. Nelson, D.H. Palmer, and J. Larner, *Biochim. Biophys. Acta*, 212 (1970) 269–280.
9 S. Tabata and T. Ide, *Carbohydr. Res.*, 176 (1988) 245–251.
10 K. Koizumi, Y. Kubota, T. Tanimoto, and Y. Okada, *J. Chromatogr.*, 464 (1989) 365–373.