Note

Hydrolysis of α-1, 6-Glucosidic Linkages by α-Amylases

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In our previous papers,1~3) we have reported that the α-amylase from Thermoactinomyces vulgaris R-47 (abbreviated to TVA) attacks α-1,6-glucosidic linkages in isopanose (6-α-maltosylglucose, IP3) and some other α-1,4-; 1,6-glucooligosaccharides. At the same time, the hydrolysis of α-1,6-links by this amylase was proved to be catalyzed by the same active site as that for hydrolysis of α-1,4-links. Further to elucidate this property, we have intended to examine whether this α-amylase and other α-amylases cleave 6'2-α-maltosylmaltose (B2), 4G-α-glucosylsucrose (G2F), and 6'2-α-glucosylmaltotriose (IMM) as well as isopanose.

TVA, porcine pancreatic α-amylase (PPA), and Pseudomonas stutzeri maltotetraose-forming α-amylase (EC 3.2.1.60, G4A) were prepared as described previously.1~5) Streptomyces hygroscopicus SF1084 α-amylase (SHA) kindly supplied by Meiji Seika Co., Ltd. was purified by the method of Hidaka et al.6) Bacillus saccharifying α-amylase (BSA), Streptococcus bovis α-amylase (SBA),7) and Taka-amylase A (TAA) were generous gifts from Prof. T. Yamamoto, E. Mizokami, and Sankyo Co., Ltd., respectively. Bacillus liquefying α-amylase (BLA) was purchased from Seikagaku Kogyo Co., Ltd., and sweet potato β-amylase (EC 3.2.1.2, β-A) purchased from Sigma was purified as described by Vretblad8) before use. These enzymes were either electrophoretically homogeneous or crystalline preparations.

To measure the enzyme activity toward starch, each enzyme was dissolved in an appropriate buffer, i.e., 50 mM acetate buffer (pH 5.0) for TVA and β-A, 50 mM acetate buffer (pH 5.5) for SHA, SBA, BSA and TAA, 50 mM acetate buffer (pH 6.0) for BLA, 20 mM glycerophosphate buffer (pH 6.9) containing 7 mM NaCl for PPA, and 20 mM Tris-HCl buffer (pH 8.0) for G4A. All buffers contained 5 mM CaCl2 (1 mM CaCl2 for PPA). Each 50 μl of the enzyme solution was added to 200 μl of 0.5% soluble starch solution, and the mixture was incubated at 40°C for TVA, SHA, SBA and TAA or 30°C for BSA, BLA, PPA, β-A and G4A for 5 min. The amount of reducing sugar liberated was measured by Nelson-Somogyi method.9) One unit of each enzyme was defined as the amount which liberated reducing sugar equivalent to 1 μmol of glucose.
per min.

IP₃ was prepared as described previously. B₄ was prepared from maltose through reversion by Bacillus pullulanase (EC 3.2.1.41) obtained from Novo Industri Japan Co., Ltd., and purified by gel filtration with Toyopearl HW40S. Purified B₄ was hydrolyzed by isopullulanase (EC 3.2.1.57) to produce IP₃ and glucose as well as hydrolyzed by Klebsiella pneumoniae pullulanase to produce maltose. IMM and G₂F were gifts from Dr. S. Okada and Dr. S. Kitahata of the Osaka Municipal Technical Research Institute.

Each enzyme solution 50 µl was added to 200 µl of 0.5% oligoglucan dissolved in the same buffer as used in the enzyme assay, and allowed to act at 30°C or 40°C as indicated for the enzyme assay. Aliquots of the digest were taken after appropriate reaction periods, and the products formed were analyzed by paper chromatography or paper electrophoresis as described elsewhere.

Of the α-amylases tested, TVA, BSA, SBA, TAA and PPA hydrolyzed IP₃. TVA produced only glucose and maltose from IP₃ as shown in Fig. 1(A), indicating that only α-1,6-link in IP₃ was attacked. The other four α-amylase formed isomaltose together with glucose and maltose as shown in Fig. 1(B) for BSA, indicating that either α-1,4- or α-1,6-link in IP₃ was hydrolyzed. SHA, BLA, G₂A and β-A did not act on IP₃.

TVA produced only maltose from B₄. BSA, SBA, PPA and TAA produced glucose, maltose, and a trisaccharide tentatively named B₃. SBA gave a lot of maltose and a small amount of B₃. The B₃ produced by each α-amylase was the same, and had the same mobility as panose (6'-α-glucosylmaltose) in paper chromatography and electrophoresis (not shown). Moreover the B₃ was degraded by isopullulanase to form glucose and isomaltose. Thus, the trisaccharide B₃ was identified as panose. IMM was hydrolyzed by TVA completely into glucose and panose in 24 hr (Fig. 2). All other α-amylases did not act on this oligoglucan.

TVA and SHA hydrolyzed G₂F into fructose and maltose. BSA, SBA, TAA and PPA gave fructose, glucose and maltose (Fig. 3). Results showed that TVA and SHA split only α-maltosidic linkages in G₂F while the other four α-amylases split α-maltosidic or α-1,4-glucosidic linkage in

![Fig. 2. Paper Chromatogram Showing the Action of TVA on IMM.](image)

IMM, 6'-α-glucosylmaltotriose; P₃, panose; IM, isomaltose; G₁, G₂, G₃, etc., glucose, maltose, maltotriose, etc.

1. IMM + isopullulanase (after 24 hr of reaction); 2. IMM + TVA (after 24 hr of reaction).

![Fig. 3. Paper Chromatograms showing Actions of α-Amylases on G₂F.](image)

A, action of TVA; B, action of BSA.

GF, sucrose; F, fructose; G₂F, 4⁵'-α-glucosylsucrose. See legend under Fig. 1 for other symbols.
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Fig. 4. Action Pattern of TVA.

○, glucose residue; △, fructose residue; —, α-1,4-glucosidic linkage; →, α-1,6-glucosidic linkage; ↑, point of hydrolysis. Areas surrounded by dashes indicate α-maltosyl residues.

BLA, G4A and β-A did not act on G2F.

The results described above indicated that α-1,6-glucosidic linkages in some oligoglucans can be cleaved by some α-amylases. Also these results were supported by the reports of Okada et al.14,15 Thus, the ability to hydrolyze α-1,6-linkages was proved not to be confined to TVA, but to be extended also to several other α-amylases. The ability was the highest for TVA, because it formed only glucose and maltose from IP3, and only maltose from B4 (α2-α-maltosylmaltose). Other α-amylases showed variable activities toward α-1,4- and α-1,6-links, giving products which were formed by the cleavage of either α-1,4- or α-1,6-bonds.

The action pattern of TVA in splitting α-1,6-links in IP3 and B4 as well as α-1,2-link in G3,F led us to consider that this α-amylase would recognize and combine α-maltosyl residues in these substrates, and split the α-1,6- and α-1,2-link as well as α-1,4-link at the reducing end side of α-maltosyl residues. The hydrolysis of IMM into glucose and panose by TVA can be also explained by assuming the tendency of this amylase to combine with an α-maltosyl residue and to split its reducing end side linkage. This action pattern also coincides with the hydrolysis of pullulan into panose by TVA as shown in Fig. 4.

The characteristic of combining with α-maltosyl residues mentioned above is also recognized in several other α-amylases, i.e., BSA, SBA, SHA, TAA and PPA, but to a lesser extent. Thus, these amylases split either α-1,6- or α-1,4-glucosidic linkages in IP3 and B4, and either α-glucosidic or α-maltosidic linkages in G2,F. They did not act on IMM. This result seems reasonable, as this tetrasaccharide is known to be the smallest dextrin produced by many α-amylases from amylopectin and glycogen.

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REFERENCES