Suitability of Isoamylase and Pullulanase M1 from Megazyme International Ireland for Starch Fine Structure Research

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Starch, the main form of stored energy in green plants, is composed principally of amylose and amylopectin. Amylose, which constitutes about 15-35% in most plants, is essentially made up linear chains of α-(1-4)-linked glucose units. The confirmation of amylopectin as the major and highly branched component of starch by Meyer and co-workers in 1940 initiated research into how the chains of amylopectin are organized. With recent developments of methods and instrumentation for starch research, unravelling new insights into key structural features of starches, especially their amylopectin structure, has become possible. Furthermore, the availability of starch debranching enzymes such as Isoamylase and Pullulanase, coupled with high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD), has enabled researchers to study unique structural characteristics of amylopectin with increased resolution (Annor et al, 2014; Bertoft et al 2008; Koizumi et al 1991; Wong and Jane 1995; Hizukuri 1985). For starch structural research, Pullulanase M1 (*Klebsiella planticola*) is normally used instead of Pullulanase M2 (*Bacillus sp.*), as Pullulanase M2 is known to attack both α-(1-4) and α-(1-6) linkages in starch. It is however very important that these debranching enzymes are pure and devoid of any other starch hydrolysing enzyme activity. Megazyme International Ireland, a global leader in the development, manufacture and supply of analytical reagents, enzymes and assay kits for the food, feed, dairy and wine industries is at the forefront in the production of pure starch debranching enzymes for starch structural research. With its unique enzyme purification methods, the company has made available pure starch debranching enzymes for researches involved in starch fine structural research. This information sheet shows the suitability of Isoamylase and Pullulanase M1 produced by Megazyme International Ireland for starch structural research.

Enzymes

Pullulanase M1 *(Klebsiella planticola)*
Product code: E-PULKP
Lot number: 130103b
Stabilized in 3.2 M ammonium sulphate and is electrophoretically homogeneous.
Specific activity: 34 International Units of activity /mg

Isoamylase (*Pseudomonas sp.*)
Product code: E-ISAMY
Lot number: 130104b
Stabilized in 3.2 M ammonium sulphate and is electrophoretically homogeneous
Specific activity: 280 International Units of activity /mg

Methods

Waxy maize starch (2.0 mg) was dissolved in 90% DMSO (50 µL) with gentle stirring overnight. The solution was diluted by adding warm water (400 µL) (80 °C) after which 0.01 M sodium acetate buffer (50 µL) (pH, 5.5) was added. Isoamylase (1 µL, 1000 U/mL) and pullulanase M1 (1 µL, 700 U/mL) were added to the mixture, which then was stirred (~ 20h) at room temperature (~22°C). After debranching, the enzymes were inactivated by heating the reaction tubes in a boiling water bath for 5 min., the volume adjusted to obtain a final concentration of 1 mg/mL, the sample filtered through a 0.22 µm nylon filter. The filtered

sample (25 µL) was injected into the Dionex ICS 5000^{+} DC HPAEC system (Dionex Corporation, Sunnyvale, CA, USA) equipped with a pulsed amperometric detector (PAD), CarboPac PA-100 ion-exchange column (4 x 250 mm) and a similar guard column (4 x 50 mm). The samples were then eluted with a flow rate of 1 mL/min. The eluents used were A (150 mM sodium hydroxide) and B (150 mM sodium hydroxide containing 500 mM sodium acetate). An elution gradient was made by mixing eluent B into eluent A as follows: 0–9 min, 15–36% B; 9–18 min, 36–45% B; 18–100 min, 45–100% B; 100–112 min, 100–15% B; 112–130 min, 15% B was used. The system was stabilized by elution at 15% B for 60 min between runs. The areas under the chromatograms were corrected to carbohydrate concentration using the method of Koch et al (1998).

Discussion

In starch fine structure research, it is important pure enzymes are used, as small differences observed in the unit and internal chain profiles of starch significantly translates into large functional differences (Bertoft et al 2016). Figures 1a and 1b shows the chromatograms of the unit chain profile of waxy maize starch debranched with isoamylase and pullulanase M1 from Megazyme International Ireland. The chromatograms depict the typical profile of debranched amylopectin with degree of polymerization (DP) 6 as the first major peak. What is interesting about these chromatograms is the virtual absence of any peak before DP 6, indicating the absence of any starch hydrolysing enzymes in the isoamylase and pullulanase enzymes produced by the company. The presence of any starch hydrolysing enzymes especially glucoamylase in starch debranching enzymes normally results in unwanted peak before DP 6, which may significantly affect the chain profile of the debranched starch especially the internal chain profile. With a robust quality assurance program, Megazyme International Ireland produces Isoamylase and Pullulanase M1 that has relative areas of all the peaks before DP 6 and that of the glucose peak of less than 0.4 and 0.04% respectively. Due to the unparalleled purity of the starch debranching enzymes from the company, its starch debranching enzymes are used in starch structural research around the world and their use reported in numerous starch structural studies published in literature (Bertoft et al 2016; Annor et al 2014; Kalinga et al 2014; Gayin et al 2016 etc.)

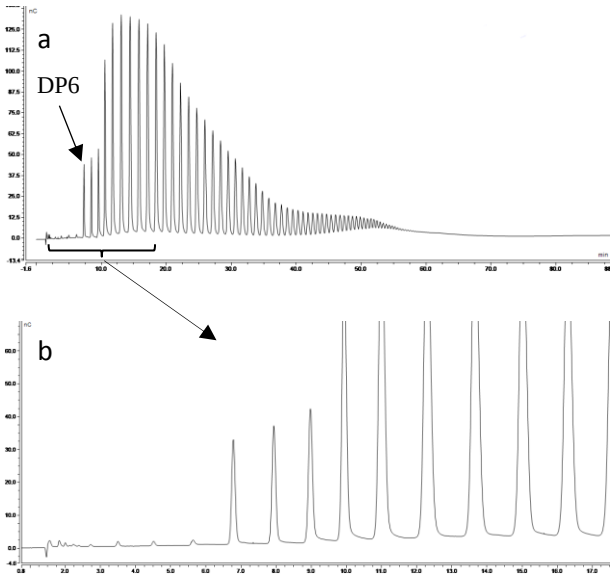

Fig. 1: Chromatogram of waxy maize starch debranched with Isoamylase and pullulanase M1

Summary

Isoamylase (*Pseudomonas sp.)* with product code: **E-ISAMY** and Pullulanase M1 (*Klebsiella planticola*) with product code: **E-PULKP** are the best on the market for starch fine structural research.

These enzymes can be ordered online at **https://www.megazyme.com**

Barry McCleary, CEO
Megazyme International Ireland

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