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Short communication

Isolation of starch branching enzyme I from potato using γ-cyclodextrin affinity chromatography

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Abstract

This study presents a novel, fast and easy method to isolate starch branching enzyme I (EC 2.4.1.18, SBE-I) from potato (*Solanum tuberosum* L. cv. 'Dianella') by γ-cyclodextrin (γ-CD) affinity chromatography of the supernatant obtained after polyethylene glycol 6000 precipitation of the crude homogenate. SBE-I was specifically eluted by competition with free γ-CD. The resulting protein fraction was homogeneous, as analysed by sodium dodecyl sulphate–polyacrylamide gel electrophoresis and contained no contaminating hydrolytic activities, as monitored by activity staining using zymograms and specific assays for α- and β-amylase. The overall purification was 296-fold and the yield was 38%. © 1998 Elsevier Science B.V.

Keywords: Potato; Affinity adsorbents; Starch branching enzymes; Enzymes

1. Introduction

Columns prepared with agarose gel beads substituted with cyclic 1,4-α-glucans with six glucose units (α-cyclodextrin, α-CD) or seven glucose units (β-cyclodextrin, β-CD) have been used as affinity media in the purification of a range of starch-metabolising enzymes, i.e. α- and β-amylases [1–3] and starch synthases [4].

Cyclodextrins do not serve as substrates or are very poor substrates for most starch-metabolising enzymes. However, strong binding of cyclodextrins may occur either in the active site [5] or at specific starch binding domains [6,7]. Binding to the active sites of amylases results in competitive inhibition of this group of enzymes [3,8,9].

Using conventional chromatography methods, starch branching enzyme I (EC 2.4.1.18, SBE-I) was purified from several sources, e.g. potato [10–12], rice [13], sorghum [14], castor bean [15], maize [16], pea [17] and wheat [18].

In this report, a novel affinity chromatography method is presented to isolate SBE-I from a potato tuber crude extract using a column consisting of agarose beads substituted with a cyclodextrin containing eight glucose units (γ-cyclodextrin, γ-CD).

2. Experimental

2.1. Plant material and chemicals

Tubers of potato (*Solanum tuberosum* L. cv. 'Dianella') were obtained from four-month-old green

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house-grown plants. Chemicals were from Sigma (St. Louis, MO, USA) and of highest purity available unless otherwise stated.

2.2. Preparation of the γ-cyclodextrin column

γ-Cyclodextrin was coupled to the Mini-Leak High matrix (divinyl sulphone activated agarose, Kem-En-Tech, Copenhagen, Denmark) according to the method described by Vretblad [1].

2.3. Analytical procedures

Protein was determined using the Bio-Rad protein assay reagent (Bio-Rad, Hercules, CA, USA). Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed according to [19] and zymograms with amylopectin included were performed as described in Ref. [20].

The activity of SBE-I was measured using the iodine–amylose assay, according to the method described in reference [10]. The activities of α- and β-amylases were measured using the ''Betamyl'' (*p*-nitrophenylmaltopentaoside) and ''Ceralpha'' (benzylidene-blocked *p*-nitrophenylmaltoheptaoside) substrates (Megazyme, Sydney, Australia), respectively, as in Ref. [3].

3. Results and discussion

Freshly harvested potato tubers (250 g) were homogenised in a fruit juicer. The homogenate was immediately mixed with dry Tris base, ethylenediaminetetraacetic acid (EDTA), dithiothreitol (DTT), benzamidine and poly(ethylene glycol) (PEG 6000) in a glass beaker to give final concentrations of 50 m*M* Tris–HCl, 2 m*M* EDTA, 5 m*M* DTT, 1 m*M* benzamidine, 10% PEG 6000 and a final pH of 7.2 to 7.8. The homogenate was left for 10 min on ice with continuous stirring and was subsequently centrifuged at 30 000 *g* for 15 min. In this step, the major fraction of the starch synthases and amylases precipitates [10]. The resulting supernatant was collected and applied to a γ-CD column (24×1.6 cm; flow-rate, 2 ml min^{-1}). The column was washed with 200 ml of 50 m*M* Tris–HCl, pH 7.5, 100 m*M* NaCl, 5 m*M* DTT, and 2 m*M* EDTA, to remove loosely bound protein, including the remaining α- and β-amylases.

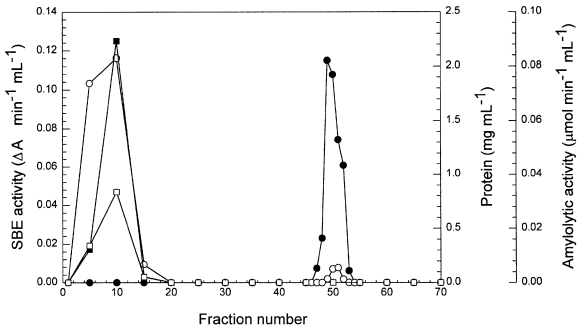

Fig. 1. γ-Cyclodextrin affinity chromatography of the potato starch branching enzyme. Protein was eluted with 5 m*M* γ-cyclodextrin, beginning at fraction forty. Protein, ○; starch branching enzyme, ●; α-amylase, ■; β-amylase, □.

Specific elution of SBE-I was accomplished with 5 m*M* γ-CD in 50 ml of 50 m*M* Tris–HCl, pH 7.5, 5 m*M* DTT and 2 m*M* EDTA (flow-rate, 2 ml min^{-1}; 2 ml fractions). The elution profile (Fig. 1) reveals a single narrow peak containing SBE-I activity that coelutes with a protein peak. All fractions were analysed for SBE-I activity, α- and β-amylase activity and protein content. SBE-I elutes as a single band of M_r 100 000 (Fig. 2), which is in accordance with a previous study [10]. As can be seen from the chromatography elution profile (Fig. 1), no other amylolytic or hydrolytic activities are present in the final enzyme preparation. Amylases and SBE-I may also be separated by affinity electrophoresis on amylopectin gels and their position can be monitored by iodine staining [21]. Isolated potato SBE-I

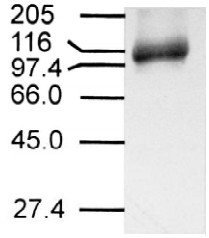

Fig. 2. SDS–PAGE of isolated potato starch branching enzyme. The location of the molecular mass markers (M_rs of 205 000, 116 000, 97 400, 66 000, 45 000, 27 400; indicated $\times 10^{-3}$) are indicated on the left side. Isolated potato starch branching enzyme (0.2 μg) was applied to each lane of the gel.

Fig. 3. Zymogram of potato starch branching enzyme. Lane 1, crude extract; 2, supernatant after PEG precipitation; 3, isolated protein from γ-cyclodextrin column (fractions 47–53). Equal amounts of protein (0.2 μg) were applied to each lane of the gel.

produces a single clear band (Fig. 3, lane 3) on such zymograms at the expected position relative to amylases. Extracts of anti-sense potato plants devoid of SBE-I activity do not give rise to this band [22].

The precipitation step resulted in a 3.6-fold purification, whereas the overall purification of SBE-I was 296-fold. The final yield was 38% (Table 1). The capacity of the γ-CD column was determined by the addition of 0.1 mg of pure SBE-I [10] to 0.5 ml of swollen affinity resin. The amount of unbound protein was measured using the protein determination method described above. The capacity using this method was 0.019 mg of SBE-I per ml of affinity resin.

The results from this study show that potato SBE-I binds very strongly to a γ-CD matrix, whereas amylases or other hydrolytic enzymes do not. To date, no structural data are available to explain the different binding capacity of γ-CD to amylases and SBE-I. However, as the strong binding of γ-CD to SBE-I occurs in competition with amylose and amylopectin substrates [9], significant differences in the architecture of the active site of amylases and SBE-I are indicated. The observation that none of the amylases binds to the γ-CD column provides a rapid and efficient method for purifying SBE-I from potato tubers to homogeneity without contaminating hydrolytic activities, as required in detailed mechanistic and kinetic studies of SBE-I.

Large quantities of SBE-I, as needed for e.g. crystallisation experiments, are also easily obtained. Likewise, the procedure should be useful for the isolation of other soluble isoforms of starch branching enzyme with different substrate specificities, e.g. SBE-II [18,23], using affinity chromatography.

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Table 1
Isolation of potato starch branching enzyme using a γ-cyclodextrin column

Fraction	Protein (mg)	Total activity (ΔA, min^{-1})	Specific activity (ΔA, min^{-1} mg^{-1})	Yield (%)	Purification (fold)
Crude extract	260.0	4.15	0.016	100	1
PEG precipitate	46.0	2.61	0.057	63	3.6
γ-CD eluate	0.332	1.58	4.75	38	296

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