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Activation and stabilization of 10 starch-degrading enzymes by Triton X-100, polyethylene glycols, and polyvinyl alcohols

Seung-Heon Yoon, John F. Robyt *

Laboratory of Carbohydrate Chemistry and Enzymology, Department of Biochemistry, Biophysics, and Molecular Biology, 4252 Molecular Biology Bldg., Iowa State University, Ames, IA 50011, USA

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Abstract

A dilute solution (0.01–0.1 unit/mL) of porcine pancreatic α-amylase (PPA) at pH 6.5, 24 °C, and 1 mM $CaCl_2$ was found to lose 98% of its activity on standing for 2 h. Addition of 0.02% (w/v) Triton X-100 gave 41% activation and stabilization for 3 h. Because Triton X-100 has a polyethylene glycol side chain, seven polyethylene glycols (PEGs), ranging in MWs of 400–8000 Da and two polyvinyl alcohols (PVAs) of MWs of 10,000 and 50,000 Da were tested as activators and stabilizers of 10 starch-degrading enzymes: PPA, human salivary α-amylase (HSA), *Aspergillus oryzae* α-amylase (AOA), *Bacillus amyloliquefaciens* α-amylase (BAA), *Bacillus licheniformis* α-amylase (BLA), *Aspergillus niger* glucoamylase (GA), barley β-amylase (β-A), *Pseudomonas amylodermosa* isoamylase (IA), *Bacillus acidopullulyticus* pullulanase (PUL), and *Bacillus macerans* cyclomaltodextrin glucanyltransferase (CGTase). Although nearly all of the additives gave activation and stabilization of the 10 enzymes, there was one specific additive and concentration for each enzyme that gave a maximum degree of activation: 0.02% (w/v) PEG 1500 Da gave 77% activation for β-A; 0.04% PEG 1500 Da gave 70% for PPA; 0.04% PEG 2000 Da gave 58% for IA; 0.04% PEG 1500 Da gave 53% for AOA; 0.04% PVA 50,000 Da gave 48% for GA; 0.04% PEG 4500 Da gave 46% for BLA; 0.02% Triton X-100 gave 45% for HSA; 0.04% PEG 1000 Da gave 42% for BAA; 0.02% Triton X-100 gave 27% for PUL; and 0.02% PEG 1500 Da gave 20% for CGTase. The mechanism of activation and stabilization is postulated to be the binding of the additives to the enzyme-proteins to give a single tertiary structure that results in a maximum enzyme activity, whereas without the additive the enzymes have several tertiary structures in equilibrium, each with a specific activity that results in an average lower activity.

Keywords: α-Amylases; β-Amylase; Glucoamylase; Isoamylase; Pullulanase; Cyclomaltodextrin glucanyltransferase; Enzyme activation; Enzyme stabilization; Triton X-100; Polyethylene glycols; Polyvinyl alcohols

1. Introduction

One of the frustrating things that occurs when working with or studying enzymes is the loss of activity of a dilute solution on standing and/or during reaction. We recently found that a dilute solution of porcine pancreatic α-amylase (PPA) under optimum conditions of pH 6.5, 24 °C, and 1 mM $CaCl_2$ lost 98% of its activity on standing for a 2 h period. An oblique reference was found where 0.02% of the nonionic detergent, Triton X-100, stabilized sweet potato β-amylase [1].

On addition of 0.02% (w/v) Triton X-100 to a freshly prepared solution of PPA, it was found that the enzyme was stabilized, but much to our surprise, it was also activated to the extent of 41%. An examination of the structure of Triton X-100 indicated it had a polyethylene glycol chain of 10–11 monomer units attached to the benzene ring. We then studied the effects of seven polyethylene glycols (PEGs) of average molecular weights of 400–8000 Da and two polyvinyl alcohols (PVAs) of average molecular weights of 10,000 and 50,000 Da and found that mostly all of them gave activation and stabilization of PPA but there was one specific additive, PEG 1500 Da that gave a maximum degree of activation of 54% at 0.02% and 70% at 0.04% (w/v).

Because there are technological applications and theoretical interests in the action of different starch-degrading enzymes [2], the effects of Triton X-100, PEGs, and PVAs were examined on 10 different enzymes (five α-amylases,

* Corresponding author. Tel.: +1 515 294 1964; fax: +1 515 294 0453.
E-mail address: jrobyt@iastate.edu (J.F. Robyt).

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β-amylase, glucoamylase, isoamylase, pullulanase, and cyclomaltodextrin glucanyltransferase) from different biological sources that act differently and produce different kinds of products from starch. It was found that primarily all of the additives gave activation and stabilization, but that there was one specific additive for each enzyme that gave a maximum degree of activation that ranged from 20 to 77% for concentrations of 0.02 and 0.04% (w/v).

2. Experimental

2.1. Materials

Soluble amylose was prepared by first allowing the amylose fraction of potato starch to retrograde; 1-butanol was added to the supernatant of the retrograded amylose, and the 1-butanol precipitate was a soluble amylose fraction that was dehydrated by treating with anhydrous acetone 10 times and one treated with anhydrous ethanol, followed by drying under vacuum for 15 h at 40 °C. Cyclomaltohexaose was obtained from Ensuiko Sugar Refining Co. (Yokohama, Japan).

Crude pullulan was obtained from Archer Daniel Midland (Clinton, IA, USA). It was purified by dissolving it in water (20 mg/mL), followed by precipitation with 2 vol. of ethanol. The pullulan was reduced by dissolving 2 g in 90 mL of 80 °C water, removal of insoluble material by centrifugation, addition of 10 mL of pyridine and the pH adjusted to 8.80 by the addition of 1N NaOH; 100 mg of $NaBH_4$ was added and the reduction reaction was allowed to go 1 h at 70 °C. The solution was cooled to ∼20 °C and the reduced pullulan was precipitated by adding 2 vol. of anhydrous ethanol, which was kept at 4 °C for 15 h. The precipitated-reduced pullulan was dehydrated, using the same methods described for soluble amylose above.

Triton X-100 was obtained from Sigma Chem. Co. (St. Louis, MO, USA). Polyethylene glycols (PEGs) 400–8000 Da and polyvinyl alcohols (PVAs), 10,000 and 50,000 Da, were obtained from Aldrich Chem. Co. (Milwaukee, WI, USA).

2.2. Enzymes

Aspergillus oryzae α-amylase (AOA) [EC 3.2.1.1], *Bacillus licheniformis* α-amylase (BLA), *Bacillus acidopullulyticus* pullulanase (PUL) [EC 3.2.1.41], and *Pseudomonas amylodermosa* isoamylase (IA) [EC 3.2.1.68] were obtained from Sigma Chem. Co. (St. Louis, MO, USA). Porcine pancreatic α-amylase (PPA) and *Bacillus amyloliquefaciens* α-amylase (BAA) were obtained from Boehringer Mannheim (Indianapolis, IN, USA). Human salivary α-amylase (HSA) was crystallized in the laboratory, using the method of Fischer and Stein [3]. *Bacillus macerans* cyclomaltodextrin glucanyltransferase (CGTase) [EC 2.4.1.19] was obtained by growing *Bacillus macerans* ATCC 8517 on a starch/wheat bran medium, centrifuging, and purifying the enzyme from the culture supernatant, using a modification of the method of Kobayashi et al. [4], as previously described [5].

3. Methods

3.1. Measurement of α-amylase activity

Soluble amylose (1.0% (w/v) 1.5 mL) in 25 mM imidazolium–HCl buffer (pH 6.5), containing 1.0 mM $CaCl_2$, was used as the substrate solution for PPA, BAA, BLA, and HSA, and was preincubated at 37 °C for 10 min. The same substrate solution (1.50 mL) but in 20 mM pyridinium acetate buffer (pH 5.5), was preincubated at 37 °C for 10 min and used for AOA. The α-amylase reactions were started by the addition of 10–20 μL of AOA (0.63 U/mL), BAA (0.85 U/mL), HSA (1.0 U/mL), and PPA (1.4 U/mL), with or without the additives of Triton X-100, PEGs or PVAs to the enzyme solutions or to the substrate solutions. Samples of 150 μL were taken every 5 min for 25 min and the reaction was stopped by adding 300 μL of 0.05 M NaOH giving a pH of 12.5. The reducing value was measured by the microcopper-bicinchoninate method, using maltose as a standard [6]. One unit (U) of α-amylase was defined as the hydrolysis of 1 μmol of α-1 → 4 glycosidic linkages/min at a specified pH and 37 °C.

3.2. Measurement of β-amylase activity

Waxy maize starch (1.0% (w/v) 1.50 mL) in 25 mM imidazolium–HCl buffer (pH 6.0) was used as the substrate solution. It was preincubated at 37 °C for 10 min and the reaction was started by adding 10 μL of β-amylase (1.1 U/mL), with or without additives. The reducing value was determined by the microcopper-bicinchoninate method, using maltose as a standard [6]. One unit of β-amylase was defined as the formation of 1 μmol of maltose/min at pH 6.0 and 37 °C.

3.3. Measurement of isoamylase activity

Waxy maize starch (1.0% (w/v) 1.50 mL) in 20 mM pyridinium acetate buffer (pH 5.0) was used as the substrate solution and was preincubated at 37 °C for 10 min. The reaction was started by adding 10 μL of isoamylase (1.2 U/mL), with or without additives; 150 μL samples were taken every 5 min for 25 min, and the reaction was stopped by adding 300 μL of 0.05 M NaOH, giving pH 12.5. The amount of hydrolysis was determined by measuring the reducing value by the microcopper-bicinchoninate method, using maltose as a standard [6]. One unit of isoamylase was defined as the hydrolysis of 1 μmol of α-1 → 6 glycosidic linkages/min at pH 5.0 and 37 °C.

3.4. Measurement of glucoamylase activity

Waxy maize starch (1.50 mL, 1.0%, w/v) in 20 mM pyridinium acetate buffer (pH 5.0) was used as the substrate solution and was preincubated at 37 °C for 10 min; 10 μL of glucoamylase (1.0 U/mL), with or without additives was added to start the reaction at pH 5.0 and 37 °C; 200 μL samples were taken every 5 min for 25 min, and the reaction was