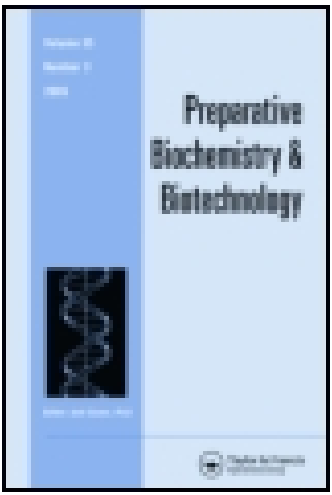

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A Simple Preparative Method for the Isolation of Amylose and Amylopectin from Potato Starch

Yasmin Y. Talib [a], Meena S. Karve [a], Shobhana V. Bhide [a] & N. R. Kale [a]
[a] Department of Chemistry, Division of Biochemistry, University of Poona, Pune, 411 007, India
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A SIMPLE PREPARATIVE METHOD FOR THE ISOLATION OF AMYLOSE AND AMYLOPECTIN FROM POTATO STARCH

Yasmin Y. Talib, Meena S. Karve, Shobhana V. Bhide and N.R. Kale†

Department of Chemistry, Division of Biochemistry,

University of Poona, Pune 411 007 (India).

ABSTRACT

The defatted starch was dispersed in NaOH (1 M) and neutralized with HCl (1 M). The amylose 1-butanol complex is adsorbed on defatted cellulose powder in the solvent system containing acetate buffer (pH 4.8,0.1 M) + urea (2 M) + 1-butanol (8.5 %,v/v). The complex adsorbed on cellulose powder is separated by centrifugation (2418 g). The sediment is washed with the solvent system-I to obtain the intermediate fraction. The adsorbed amylose is eluted with urea (2 M) in acetate buffer (pH 4.8, 0.1 M). The amylose, intermediate fraction and amylopectin were precipitated with ethanol, washed free of urea and air dried. They were characterized by determining their blue value and β-amylolysis limit.

INTRODUCTION

In plants, starch is deposited in the form of starch granules. It is a homopolymer of D-glucose consisting of a heterogeneous mixture of linear (amylose) and branched (amylopectin) components. Amylose consists of linear chains of D-glucopyranose units linked through α-(1→4) bonds and is heterogeneous with respect to molecular weight (1.5×10^5 - 2.4×10^6). Amylopectin contains short linear chains of α-(1→4) linked D-glucopyranose residues, which are interlinked mainly by α-(1→6) linkages to form a highly branched structure. Amylopectin is heterogeneous with respect to degree of branching and molecular weight (1.0×10^6 - 6×10^7). There is some evidence for the presence of a material (5-10 %) in starch granules which is intermediate between amylose and amylopectin. This fraction is characterized as amylose with α-(1→6) linkages which are resistant to β-amylase but hydrolyzed by pullulanase.

The most commonly used method for the isolation of amylose is the slow cooling of a hot aqueous solution of dispersed starch, saturated with 1-butanol or 1-pentanol as described by Schoch[2]. It gives a microcrystalline precipitate of amylose-1-butanol complex or amylose-1-pentanol complex which is sedimented by high speed centrifugation (17400×g). Further it has to be recrystallized several times to obtain pure amylose. Repeatedly recrystallized amylose from aqueous 10 % 1-butanol contains trace quantities of amylopectin[3]. Amylopectin obtained from the supernatant contains trace quantities of amylose which are extremely difficult to remove. Taki[4] has studied the efficacy of different amylose precipitants for the fractionation of amylose and amylopectin and shown the presence of amylopectin in amylose fraction and amylose in amylopectin fraction.

Patil et al[5] have reported the use of defatted cellulose for the removal of trace quantities of amylose from amylopectin fractions in urea (2 M) ethanol (35 %, v/v) system. Here we describe a simple preparative method based on the 1-butanol induced adsorption of amylose on cellulose powder.

MATERIALS AND METHODS

Potato starch was prepared according to the method described by Schoch[6].

Defatting of starch granules and cellulose powder : The dry starch granules (100 g) were defatted by repeated extraction with chloroform:methanol (2:1, 300 ml) at 80°. Cellulose powder - 123 (Selecta D-3354, Dassel, W.Germany) was defatted in the same manner.

Dispersion of starch granules : The defatted starch granules (20.0 g) were dispersed by treatment with sodium hydroxide (500 ml, 1.0 M) at room temperature (26-28°) with slow stirring in the atmosphere of nitrogen. It was neutralized to pH 7.0 with HCl (500 ml, 1.0 M). The neutralized solution was filtered through a fritted glass filter funnel (G-2) under suction. The freshly prepared solution was used for fractionation.

Solvent system I : Acetate buffer (pH 4.8, 0.1 M) + 1-butanol (8.5 %, v/v) + urea (2 M).

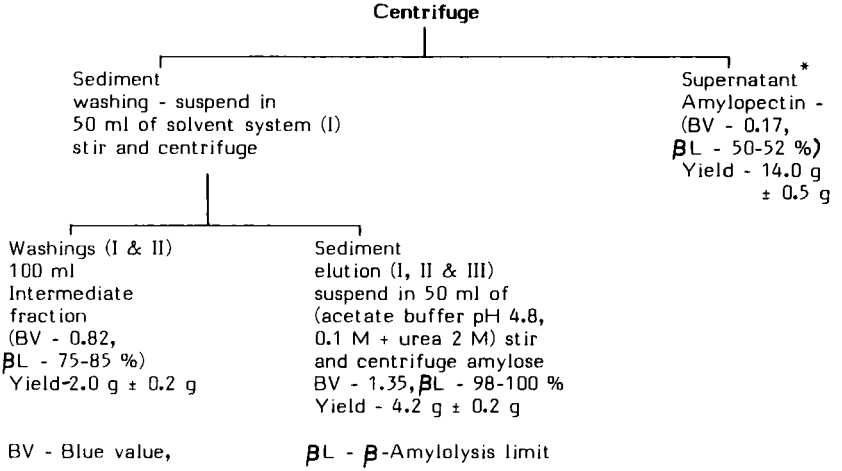
Solvent system II : Acetate buffer (pH 4.8, 0.2 M) + 1-butanol (8.5 %, v/v) + urea (4 M).

Characterization of amylose and amylopectin : The blue value was determined according to the method of McCready and Hassid[7]. The β-amylolysis limit was determined by using purified β-amylase[8,9] (EC 3.2.1.2) 100 units/mg and liberated maltose was estimated by Nelson's method[10].

Regeneration of cellulose : Cellulose was washed free of urea with distilled water. It was further treated with 0.1M NaOH and kept under stirring for 3-4 hours. It was washed free of alkali and could be used 4-5 times.

PROCEDURE

Dispersed starch solution (2 % w/v, 1 litre) + cellulose powder (40 g) suspended in 1 litre (acetate buffer pH 4.8, 0.2 M + urea 4 M) + 1-butanol (170 ml). The mixture is allowed to equilibrate at 30° for 16-18 hours with mild stirring.



BV - Blue value, βL - β-Amylolysis limit

Centrifugation was done at room temperature (26-28°). The polysaccharide from the supernatant, washings and elution fraction was precipitated by adding equal volume of ethanol (95 %).

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* If the BV and βL are high this indicates the presence of amylose which can be removed by adding cellulose in small lots of 10 g each.

RESULTS AND DISCUSSION

The amylose content of the naturally occurring starches is 15-25 %. Amylose is known to form a helical complex with variety of complexing agents such as ethanol, 1-butanol, 1-pentanol, cyclohexanol, thymol, SDS etc. Our earlier

studies have shown that amylose has no affinity for cellulose but it is the amylose-1-butanol complex which is adsorbed with high affinity in solvent system-I. When 1-butanol is added amylose forms a helical complex with it. The complexing agent keeps amylose in soluble form (V form) and prevents retrogradation. We have also used urea (2 M) in the system to prevent the aggregation of complexed amylose chains.

For the preparation of starch components, it is essential to observe mild conditions during the fractionation of starch. Amylose is more susceptible to degradation than amylopectin. Dispersion of starch granules by autoclaving or heating in presence of air can result in partial degradation of amylose[11-13]. The degradation can be minimized if dispersion of starch granules is conducted in an oxygen free atmosphere. Baum and Gilbert[11] have shown that amylose can be preferentially solubilized by treating intact potato starch granules with 0.5 M NaOH solution at 15°, for half an hour in an oxygen free atmosphere. We have used NaOH (1 M) for dispersion of starch granules and dispersion was carried out in an atmosphere of nitrogen at room temperature.

Amylose and amylopectin prepared by this method were characterized by filter paper[14] as well as column chromatographic method (manuscript in preparation). The amylose thus prepared is free from amylopectin and does not require repeated crystallization.

Our preliminary experiments showed that $\sim$ 8-10 g of amylose can be adsorbed on 100 g of defatted cellulose. (Amylose is reversibly adsorbed on defatted cellulose). For 20 g of starch we have used 40 g of defatted cellulose which is sufficiently in excess to adsorb all the amylose.

One of the most important features of this method is that it eliminates high speed centrifugation (17,400 x g). The amylose 1-butanol complex adsorbed on cellulose can be rapidly sedimented (2418 x g). We have standardized this procedure for the isolation of starch components from 20.0 g of defatted potato starch on a laboratory scale. It can be easily scaled up for isolating amylose and amylopectin on a large scale. The cellulose powder can be regenerated and ethanol can be recovered.

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