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Improved method for crystallization of sweet potato β-amylase

Sweet potato containing abundant β-amylase (α-1,4-glucan maltohydrolase, EC 3 2 1 2) was first purified to a crystalline state by BALLS *et al* [1] in 1948 Since then some modified procedures have been reported[2–4] A simple method is desired for obtaining the pure enzyme which is useful in the determination of starch and glycogen structures The procedure reported in this communication required only about 6 h to obtain the crystalline enzyme (Table I)

Step 1 Extraction Sweet potatoes (1 7 kg) were washed, and after the skin and cambium were removed, they were ground in a home juicer (Toshiba, JC-200N) The cambium was removed because it contained a yellow substance perhaps included in a final preparation Unless otherwise specified, all the followed operations were conducted in an ice bath at 0°, and centrifugation was performed at 10 000 $\times$ *g* at 0° The juice obtained was centrifuged for 7 min to remove starch and some insoluble mater als (crude extract)

Step 2 Acid treatment The crude extract (220 ml) was slowly acidified to pH 3 6 with 1 M HCl to inactivate α-amylase After standing 10 min, the coagulated mass was centrifuged off Immediately after the 5-min centrifugation, the pH of the resulting supernatant was restored to 4 8 with 3% aq NH_4OH This operation was conducted rapidly to avoid inactivation of the enzyme

Step 3 First acetone fractionation To the above solution (210 ml), ice-cold acetone was gradually added until 47% (v/v) The precipitate formed was collected

TABLE I

PURIFICATION OF β-AMYLASE

β-Amylase was assayed as follows 0 1 ml of the enzyme solution (0 05 M acetate buffer, pH 4 8) was added to 0 1 ml of the test mixture containing 2% soluble starch in 0 05 M acetate buffer (pH 4 8) After a 10-min incubation at 37°, the reaction was stopped by adding 2 ml of the diluted Somogyi's reagent (1 1) The solution was heated for 10 min in a boiling-water bath After cooling, 1 ml of Nelson's reagent was added [6] The contents were taken to 10 ml with water The developed color was read in a Klett–Summerson photoelectric colorimeter with the filter No 54 using maltose as a standard Acid phosphatase activity was assayed colorimetrically as described by BERGMEYER[7] using *p*-nitrophenyl phosphate as a substrate in 0 05 M acetate buffer (pH 4 8) A unit of the activity was defined as the liberation of μmole of *p*-nitrophenol per min Protein was determined by the method of LOWRY *et al* [8] using bovine serum albumin as standard

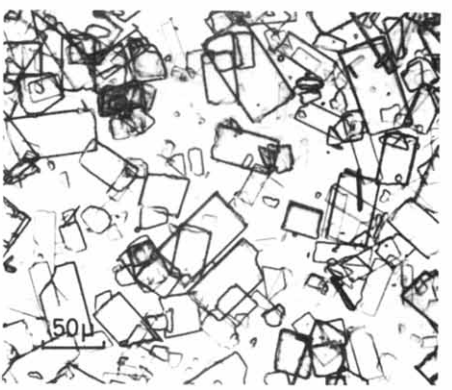
Purification steps	*Vol (ml)*	*Protein (mg/ml)*	*Specific activity (μmoles maltose per min per mg)*	*Recovery (%)*	*Acid phosphatase activity / β-amylase activity (× 10⁻⁴)*
1 Crude extract	220	19 3	117	100	62 8
2 Acid treatment	210	8 5	218	80 2	
3 First acetone ppt	20	12 6	1170	59 3	
4 $(NH_4)_2SO_4$ ppt	12	15 2	1360	49 8	
5 Second acetone ppt	8	18 7	1550	46 8	
6 Third acetone ppt (crystal suspension)	2 9	40 0	1520	35 5	2 24
7 Washed crystal suspension	2 9	20 8	1580	19 2	0 27

immediately by centrifuging for 5 min and was suspended in about 20 ml of distilled water The insoluble residue was centrifuged off

Step 4 $(NH_4)_2SO_4$ fractionation The saturated $(NH_4)_2SO_4$ solution (saturated at 4°) was added with stirring to 20 ml of the above supernatant until 47 5% saturation After standing 10 min, the precipitate was collected by centrifuging for 5 min The supernatant was completely removed because $(NH_4)_2SO_4$ interferes with the crystallization of β-amylase in the following step The precipitate was suspended in 10 ml of distilled water Insoluble residues were discarded after centrifugation

Step 5 Second acetone fractionation To 12 ml of the former solution, cold acetone was added slowly to make 45% (v/v) before centrifuging immediately for 5 min The precipitate was dissolved in 7 ml of distilled water If insoluble materials remained, they were removed by centrifugation

Step 6 Third acetone fractionation and crystallization Cold acetone was added slowly to the 8 ml of the solution from Step 5 to give 40% (v/v) The resulting precipitate was immediately collected by centrifuging for 5 min and was dissolved in 2 5 ml of distilled water at room temperature The solution should contain as high as 40 mg protein per ml The tube containing the protein solution was immersed in an ice bath After standing several minutes, β-amylase crystallized in plate shape (Fig 1), if cold acetone was slowly added to the solution from Step 5 until 40% (v/v), β-amylase crystallized in rod shape (Fig 2)



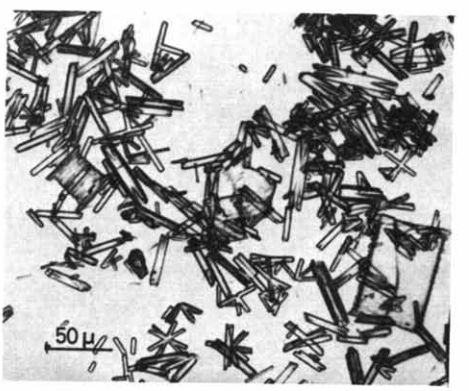


Fig 1 Crystalline β-amylase of sweet potato (crystallized from pure water)

Fig 2 Crystalline β-amylase of sweet potato (crystallized from aq acetone)

Step 7 Wash with cold water The enzyme crystallized from an aqueous solution was separated from the mother liquor by centrifugation at 3000 × *g* for 5 min It was washed by centrifugation in 2 5 ml of ice-cold distilled water The washed crystals were resuspended in 2 5 ml of cold distilled water

β-Amylase was also readily crystallized from $(NH_4)_2SO_4$ solution (pH 3 7) The specific activity of the enzyme crystallized from $(NH_4)_2SO_4$ solution was equal to that of the washed crystals

A specific extinction coefficient $E^{1\%}_{1\,cm}$ (280 mμ) was 17 7 by dry weight, and the N content of protein was 16 5% The specific activity of the washed crystals was 1600 μmoles/min per mg dry weight, corresponding to 150 4 units/mg dry weight calculated

by BALLS' unit at 37° When actually assayed by the method of BALLS at 37°, however, specific activity of the washed crystals was 234 units/mg dry weight The cause of the lower activity obtained by this assay method rather than by that of BALLS is being investigated When the activity was assayed with 0 02% Triton X-100, no difference was observed between both methods

The crystals were readily soluble in salt solutions but less soluble in pure water The crystalline β-amylase suspension could be stored at 4° for 3 months without appreciable loss of activity The crystalline enzyme was homogeneous using the criterion of disc gel electrophoresis (210 μg protein in gels at pH 7 5 and 8 3, stained by 1% Amido black in 7% acetic acid)

α-Amylase activity was assayed by reacting 20 8 μg of the pure enzyme on 4 mg of potato amylopectin in 2 ml of 0 05 M acetate buffer (pH 4 8) at 37° for 4 h. 30 min after the onset of incubation, the hydrolysis reached 56%, and no hydrolysis occurred in the prolonged incubation Therefore, the enzyme preparation was free from α-amylase The contaminant acid phosphatase activity (Table I) was as little as the other preparation[3]

THOMA *et al* [5] reported that the pure β-amylase, crystallized twice from $(NH_4)_2SO_4$ solution, included the colored material having an absorption peak at about 350 mμ The present preparation was free from the material as confirmed by the absorption spectrum

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Reversible disaggregation of *Escherichia coli* succinyl-CoA synthetase

Immunodiffusion experiments with homogeneous succinyl-CoA synthetase (succinate CoA ligase (ADP), EC 6 2 1 5, formerly known as succinic thiokinase) from *Escherichia coli* have shown the presence of three molecular species of the enzyme