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Short Communication

A Simple Method to Differentiate between α- and β-Amylase

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Physical and chemical properties often have been used to differentiate α-amylase from β-amylase (2–4, 7). α-Amylase is insensitive to high temperatures and heavy metal ions, requires Ca^{2+} for activity, and is inactivated at a low pH. β-Amylase is sensitive to high temperatures and heavy metal ions, does not require Ca^{2+}, and is stable at a low pH.

Enzymatic differences between the two forms of amylase have been used to a lesser extent. One enzymatic characteristic that has been used is the dissimilarity of the end products of amylolytic hydrolysis (3, 7).

β-Amylase hydrolyzes soluble starch or amylose, yielding only maltose as an end product. Hydrolysis of soluble starch or amylose by α-amylase occurs randomly, releasing a large amount of oligosaccharides or dextrin. The dextrin is then hydrolyzed, yielding a large quantity of maltose and little glucose and maltotriose (1). At this point, hydrolysis by α- or β-amylase would yield essentially similar end products. If the further hydrolysis of maltose by α-amylase is rate-limiting and is dependent upon the concentration of the enzyme and pH (7), little or no glucose would be expected. As a result, the chromatographic display of the end products would be similar to that obtained from β-amylase hydrolysis.

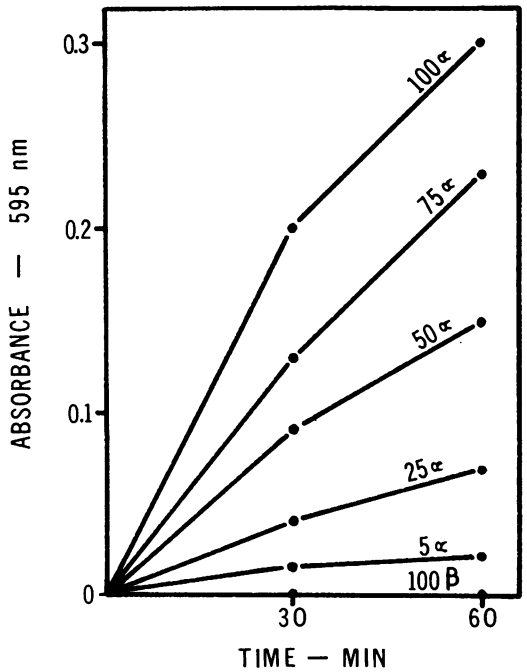

FIG. 1. The hydrolysis of β-limit dextrin azure by α- or β-amylase. The amylase concentration, in micrograms, is shown in the figure.

Another seldom used enzymatic characteristic relies upon the fact that β-amylase can only partially hydrolyze amylopectin. Since β-amylase cannot bypass the branch points of amylopectin, the polysaccaride, β-limit dextrin, remains, which is resistant to any further hydrolysis by the enzyme. On the other hand, α-amylase can bypass the branch points and hydrolyze the β-limit dextrin. Few workers have used β-limit dextrin as a substrate to distinguish α- from β-amylase (4, 6).

In 1967, Rinderknecht, Wilding, and Haverback (5) described a new method for the determination of α-amylase. A blue dye was covalently bonded to the glucose subunits of starch. The concentration of α-amylase was found to be proportional to the amount of soluble sugar-dye complexes released. The following method is a modification of this assay utilizing a β-limit dextrin azure to distinguish between α- and β-amylases.

A substrate specific for α-amylase, β-limit dextrin azure, was prepared by adding 500 mg of amylopectin azure (Calbiochem Co.) and 100 mg of β-amylase (α-amylase free, Calbiochem Co.) to 25 ml of sterile 0.05 M sodium acetate buffer, pH 4.8. Chloramphenicol (20 μg/ml) was added to retard microbial growth. The amylopectin azure was kept in suspension by continuous shaking at 25 C. After 24 hr, the suspension was centrifuged at 30,000g for 15 min, and the precipitate was washed with the sodium acetate buffer. The procedure was repeated by resuspending the precipitate in 25 ml of the sodium acetate buffer containing 100 mg of β-amylase and shaking for another 24 hr. After centrifugation the β-limit dextrin azure was washed with methanol and brought to dryness in a desiccator using a vacuum pump.

The amylase assay consisted of adding an aliquot of the enzyme preparation (0.1 ml) to 0.5 ml of 2% (w/v) freshly prepared β-limit dextrin azure suspended in 0.05 M sodium acetate buffer, pH 4.8, and incubating at 30 C for 30 min or 1 hr. The substrate was kept in suspension by shaking every 5 min. The reaction was terminated by diluting the reaction mixture with 2.5 ml of water and passing it through a Millipore filter into a spectrophotometer tube. The reaction tube was washed with 3 ml of water and filtered. The absorbance of the combined filtrate was read at 595 nm with a Bausch and Lomb spectrophotometer.

The substrate, β-limit dextrin azure, is highly specific for α-amylase (Fig. 1). When 100 μg of β-amylase (α-amylase free, Calbiochem Co.) are incubated with the substrate for 1 hr, no soluble sugar-dye complexes are released, and, as a result, no change in the absorbance is observed. However, the incubation of 100 μg of *Bacillus subtilis* α-amylase (Mann Research Laboratories) with the substrate results in a large increase in the absorbance. The assay is sensitive to small amounts of α-amylase. The rate of hydrolysis is linear for 30 min. Although this initial rate of hydrolysis is not maintained after 1 hr, the

Table I. *Effect of β-Amylase upon Absorbance*

The enzyme preparations contained 25 or 50 μg of α-amylase and various concentrations of β-amylase. The enzyme preparations were incubated for 30 min with β-limit dextrin azure, and the absorbance was read at 595 nm.

Concn. of β-Amylase	Absorbance in the Presence of α-Amylase	
	25 μg	50 μg
μg		
0	0.04	0.09
25	0.05	0.09
50	0.05	0.12
75	0.07	0.16
100	0.07	0.15
150	0.05	0.12
200	0.04	0.09

absorbance remains proportional to the concentration of α-amylase in the reaction mixture.

The presence of a small amount of β-amylase (25 μg) in the α-amylase enzyme preparation has little effect upon the release of sugar-dye complexes (Table I). When α-amylase is kept constant at 25 or 50 μg and the concentration of β-amylase progressively increased, the absorbance first increases, reaching a maximum of 1.8 times the absorbance resulting from α-amylase alone, and then declines. An absorbance comparable to that resulting from α-amylase alone is observed when large amounts of β-amylase (200 μg) are present with α-amylase.

The characteristics of the assay can be utilized to estimate the concentration of amylases in a crude extract. As an example, a crude extract yielding an absorbance of 0.12 could correspond to 65 μg of α-amylase, a mixture of 50 μg of α-amylase and 50 μg of β-amylase, or a mixture of 50 μg of α-amylase and 150 μg of β-amylase. If the addition of a small amount of β-amylase to the crude extract had no effect upon the absorbance, then the predominant amylase would be α-amylase. If there was an increase in the absorbance, then the amylase extract contains about 50 μg of β-amylase. A decrease in the absorbance would indicate that the crude extract contains about 150 μg of β-amylase.

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