Note

Effect of dimethyl sulfoxide on amylase activity*

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Quantitative analyses for starch are generally accomplished by polarimetric or hydrolytic (enzymic or acid) techniques. The starch must be dissolved or dispersed prior to reactions or measurements. The solubilization of starches with anhydrous and aqueous dimethyl sulfoxide at room temperature has been investigated[1] in the quest for a chemical solvent that will slowly dissolve granular starches without appreciably swelling them or causing their molecular degradation. Recently, Libby reported[2] a quantitative method for analyzing corn, wheat, and potato starch, and wheat flour, by extracting the starch with Me_2SO. The starch extract is then hydrolyzed in an Me_2SO–citrate buffer with glucoamylase [(1→4)-α-D-glucan glucohydrolase, E.C. 3.2.1.3], and the amount of D-glucose produced is measured colorimetrically. Initial extraction of starch with Me_2SO has also been reported[3] in a rapid determination of amylose in maize.

We determined the effect of Me_2SO on the activities of three common amylases, to ascertain whether this solvent would affect the enzymes used to analyze and characterize starch. The amylases, each representative of a major type, were glucoamylase I and II from *Aspergillus niger*, sweet potato β-amylase [(1→4)-α-D-glucan maltohydrolase, E.C. 3.2.1.2.], and porcine pancreatic α-amylase [(1→4)-α-D-glucan glucanohydrolase, E.C. 3.2.1.1.].

RESULTS AND DISCUSSION

The effect of Me_2SO, at various concentrations, on each amylase is shown in Table I. The values are averages of four determinations, each done in triplicate, except those for 2.5M Me_2SO, which are averages of duplicate determinations, each done in duplicate. The values for glucoamylase I and II in 2.5M Me_2SO were obtained with enzymes of slightly lower specific activity than those used for the other determinations, and these values have been increased proportionally to their control values for purposes of comparison.

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TABLE I

EFFECT OF METHYL SULFOXIDE ON AMYLASE ACTIVITY

Me_2SO concentration (M)	*Enzyme activity*			
	Glucoamylase I[a]	*Glucoamylase II*[a]	*α-Amylase*[b]	*β-Amylase*[b]
0	2.8	3.0	138	269
0.00045	2.8	2.9	115	255
0.0045	2.8	2.9	117	257
0.045	2.9	3.0	116	255
0.095	2.8	2.8	120	263
1.0	2.2	2.7	146	255
2.5	2.7	2.9	137	278
5.0	1.5	1.6	79	99
10.0	0.5	0.6	26	0

[a]Activity given in mg of D-glucose formed per mg of protein per min. [b]Activity given in μg of maltose formed per ml of enzyme per min.

The activity of glucoamylase I was not affected by Me_2SO concentrations below 1M. At 1M Me_2SO, the activity decreased by about 20%, but at 2.5M Me_2SO the enzyme activity was again comparable with the control value. The slight decrease in activity at the 1.0M concentration may be real (nearly three standard deviations from the mean) but is close to the range of experimental error. The standard deviations for the controls were 0.21 for glucoamylase I; and 0.19 for glucoamylase II. Results were similar with glucoamylase II except that the activity was unaffected at 1.0M Me_2SO. The decrease of glucoamylase II activity with 5.0 and 10.0M Me_2SO was essentially the same as with glucoamylase I. Libby reported[2] no change in activity for a *Rhizopus* glucoamylase in a solution containing 18% of Me_2SO (2.5M) during 4 months. The specific activity of the glucoamylase in 18% Me_2SO was not determined, but the hydrolysis of starch was reported to be rapid (complete in 10 min) under the conditions used. Results from this study indicate that the activity of glucoamylase I and II from *A. niger* is unaffected by 2.5M Me_2SO.

The activity of α-amylase and β-amylase showed the same general trend as glucoamylase II; unchanged at Me_2SO concentrations of 2.5M or less but diminished at higher concentrations. The decrease in enzyme activity at 5.0 and 10.0M Me_2SO was approximately the same as for the glucoamylases, except that 10.0M Me_2SO completely suppressed the activity of β-amylase. Low concentrations of Me_2SO appeared to decrease slightly the activity of α-amylase, but within the range of experimental error (two standard deviations), and, to a lesser extent, that of β-amylase also.

Farkas and Gobel[4] have reported that addition of Me_2SO (2–30%) increased the activity of barley D-glucose 6-phosphate dehydrogenase, as measured by reduction of NADP, by several hundred percent.

With that enzyme the V_{max} was increased after Me_2SO was added, whereas the K_M for both NADP and D-glucose 6-phosphate was decreased. Under similar

conditions, low concentrations of Me_2SO did not stimulate yeast D-glucose 6-phosphate dehydrogenase, but high levels of Me_2SO inhibited activity.

Our results are similar to those cited for yeast D-glucose 6-phosphate dehydrogenase. In no case was enzyme activity affected by low concentrations of Me_2SO, but in each case, activity was inhibited by concentrations of Me_2SO above 2.5M. Thus, when Me_2SO is used to extract starch from natural sources, the starch may be determined by digestion with glucoamylase, or amylolysis limits may be measured in the normal manner without affecting enzymic activity, if the concentration of Me_2SO is 2.5M or less. If the concentration of Me_2SO is above 2.5M allowances must be made for decreased enzyme activity, or the solution may be diluted to bring the Me_2SO below 2.5M.

EXPERIMENTAL

Enzymes. — Porcine pancreatic α-amylase (twice crystallized, DFP-treated) and sweetpotato β-amylase (crystalline) were obtained from Worthington Biochemical Corporation, Freehold, N. J. The α-amylase was then dialyzed for 15 h at 4° against a 0.02M sodium glycerophosphate hydrochloride buffer, pH 6.9, containing the desired concentration of Me_2SO, 10mM chloride ion, and 3mM calcium ion. β-Amylase was diluted with 0.5 mM reduced glutathione and 0.05% serum albumin to the desired enzyme concentration and then dialyzed for 15 h at 4° against 0.02M acetate buffer, pH 4.8, containing the desired concentration of Me_2SO. Glucoamylase I and II from *A. niger* were isolated and purified by the gradient-elution procedure of Lineback *et al*[6]. Specific activities of the two purified enzymes agreed with values for a highly purified preparation[6]. The purified glucoamylase solutions were dialyzed for 15 h at 4° against 0.05M acetate buffer, pH 4.8, containing the desired concentration of Me_2SO. During all dialyses, the solution was replaced at 5-h intervals with fresh buffer. Upon completion of dialysis, the initial pH value was maintained at the desired value by addition of M potassium hydroxide or citric acid, as necessary.

Enzyme assays. — Glucoamylase activity was measured by incubating a 0.5-ml aliquot of the enzyme solution for one h at 30° with 1.5 ml of 4% starch (Lintner soluble) in 0.05M acetate buffer, pH 4.8. The D-glucose thus formed was assayed in the manner previously described for the coupled D-glucose oxidase–chromophore system[6].

α-Amylase activity was measured[7] by incubating a 1.0-ml aliquot of the enzyme solution for 10 min at 25° with 1.0 ml of 1% starch (Lintner soluble) in 0.02M sodium glycerophosphate buffer, pH 6.9. The reducing value of the digest was determined by the Nelson colorimetric copper method, as described by Robyt and Whelan[7].

β-Amylase activity was measured[3] by incubating a 2.0-ml aliquot of the enzyme for 30 min at 35° with 28 ml of starch solution (25 ml of 0.6% starch plus 3.0 ml of 0.2M acetate buffer, pH 4.8). The maltose formed in the digest was determined by the Nelson colorimetric copper method used for the α-amylase assay.

In all of the enzyme assays, the starch substrate also contained the desired concentration of Me_2SO at the pH value indicated.

Methyl sulfoxide, over the range of concentrations used in this investigation,

was found to be without significant effect on the Nelson colorimetric copper method or the coupled D-glucose oxidase–chromophore system. At 10M Me_2SO, precipitates were observed in some of the Nelson reducing-value determinations, but apparently these did not affect the results significantly.

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