NOTE

Purification of Beta-Glucanase for Beta-D-Glucan Assays

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β-Glucanase from *Trichoderma reesei* has been used to selectively hydrolyze β-D-glucan extracted from various cereals (Bamforth 1981, Martin and Bamforth 1981, Prentice and Faber 1981, Prentice et al 1980). The fungus produces a spectrum of enzymes that has the capability to completely convert β-D-glucan to glucose, which can be measured easily. In addition to the β-glucanase, the fungus produces amyloglucosidase, which must be removed from culture filtrates (Prentice et al 1980) or inactivated before use (Bamforth 1981, Martin and Bamforth 1981); otherwise glucose is produced from the starch in cereal extracts as well as from the β-D-glucan.

Removal of the amyloglucosidase by treatment of preparations with diethylaminoethyl cellulose resulted in a high-quality β-glucanase but involved a rather laborious operation (Prentice et al 1980). Preferential inactivation of the amyloglucosidase by suitable exposure to temperature and pH is simple, but the stability of the enzyme appears to vary (Bamforth 1981).

This report describes the effect of pH and temperature on *T. reesei* amyloglucosidase and β-glucanase.

MATERIALS AND METHODS

Enzymes

Commercial cellulases were from Worthington Biochemical Corp., Freehold, NJ, and Yakult Biochemicals Inc., Nishimoniya, Japan.

Crude cellulase was prepared by culturing the following, as described by Prentice and Faber (1981): *T. reesei* QM9414 (U.S. Army Research and Development Command, Natick, MA) for seven and 14 days; and *T. reesei* 3652 (USDA-ARS-NCR Microbial Culture Collection, Northern Regional Research Center, Peoria, IL) for seven days.

Treatment of Enzymes at Various pHs and Temperatures

The enzymes were dissolved (about 300 μg of nondialyzable nitrogen per milliliter) in buffers (0.1*M* tartrate, pH 3.0; 0.1*M* acetate, pH 4–6; and 0.1*M* phosphate, pH 6.2–8.0) and dialyzed against the corresponding buffer. The dialyzed solutions in 10 × 1-cm tubes were heated at 40–60° C for 0.2–24 hr.

Substrates

β-D-Glucan (Biocon Inc., Lexington, KY) was dissolved in 0.1*M* acetate, pH 4.8, such that the concentration was 2.2 mg/ml. Starch substrate was prepared similarly at the same concentration.

Substrate-Enzyme Reactions

Reaction mixtures consisted of 0.15 ml of substrate, 0.3 ml of 0.1*M* acetate buffer (pH 4.8), and 0.12 ml of enzyme solution, which contained 30–40 μg of nondialyzable N. Appropriate enzyme and substrate blanks were included. Reaction mixtures and blanks were incubated for 24 hr at 50° C. Aliquots (50 μl) were assayed for glucose by the glucose oxidase-peroxidase method (Hasegawa and Smolensky 1970). Original substrate concentrations were determined by hydrolyzing the substrates with acid and determining glucose in the hydrolysates (Prentice et al 1980).

RESULTS AND DISCUSSION

Table I summarizes the effect of various pHs on the activity of Onozuka 2S at 40–60° C for 0.2–24 hr. These data show that, although β-glucanase is thermally stable at low pHs, so also is the amyloglucosidase. This contrasts with Bamforth's (1981) results, which show that the amyloglucosidase in his preparation (Worthington) was inactivated at pH 4.0 when heated to 60° C for 10 min. Table I shows that the most effective pH and temperature for preferential inactivation of amyloglucosidase in Onozuka 2S were pH 6.0 and 60° C. Above this pH, unacceptable losses of β-glucanase occurred.

These conditions (pH 6.0 and 60° C) were applied to two preparations each (from seven and 14-day cultures) of crude enzyme from *T. reesei* QM9414, a preparation from *T. reesei* 3652, a Worthington sample, and Onozuka 3S and caused the inactivations shown in Table II. Large variations in the sensitivity

TABLE I
Percent Inactivation of Onozuka 2S Amyloglucosidase and Beta-Glucanase after Exposure to Various pHs and Temperatures

Conditions			Percent Inactivation of	
pH	Exposure Time (hr)	Temperature (°C)	β-Glucanase	Amyloglucosidase
3.0	3	60	0	15
4.0	0.2	60	0	20
4.6	23	60	0	0
5.0	3	60	0	70
5.2	3	50	0	73
5.4	3	50	0	73
5.6	3	50	0	76
5.8	3	50	0	84
6.0	1.5	50	3	72
6.0	3.0	60	3	75
6.0	24	60	0	100
6.2	3	50	27	93
6.3	3	50	29	84
6.4	3	50	22	89
6.6	3	50	54	91
6.8	3	50	88	94
7.0	4.5	50	100	100
8.0	0.5	40	89	33

TABLE II
Percent Inactivation of Beta-Glucanase Preparations at pH6 and 60° C for 24 hr

	Prepared with				
	Trichoderma reesei QM9414				
	7-day culture	14-day culture	*T. reesei* 3652[a]	Worthington	Onozuka 3S
β-Glucanase	75	72	89	3	3
Amyloglucosidase	0	6	48	100	100

[a]Seven-day culture.

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of *T. reesei* amyloglucosidase and the β-glucanase group of enzymes evidently exist in these preparations. The amyloglucosidase of *T. reesei* QM9414 and, to a lesser extent, of 3652 was resistant to these conditions, but the β-glucanase was largely inactivated. On the other hand, the β-glucanases of the Worthington, Onozuka 3S, and Onozuka 2S (Table I) were essentially unaffected, but the amyloglucosidase was completely inactivated. Despite these differences between the enzymes of QM9414 and the Onozuka, previous work has shown that the β-glucanase from both can be purified by removing the amyloglucosidase with the diethylaminoethyl cellulose ion exchanger (Prentice and Faber 1981).

Crude enzyme preparations from *T. reesei* should be considered individually with regard to the purification of the β-glucanase they contain.

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