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Note

Comparison of the trehalase of *Trichoderma reesei* with those from other sources

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Recently we have purified a trehalase (EC 3.2.1.28) from *Trichoderma reesei*[1] and shown it to have a high substrate specificity. It is the purpose of this report to compare this enzyme with those obtained from other sources, and to show relationships between trehalases, α-D-glucosidases (EC 3.2.1.20), and α-D-(1→4)-glucan glucohydrolases (EC 3.2.1.3). The trehalases reported in the literature are chiefly from three sources: insects, mammalian tissue, and microorganisms[2]. Some characteristics of these enzymic reactions are as follows.

(a) Specificity. — All of the trehalases are highly specific for α,α-trehalose. That of *T. reesei* is also specific[1], showing no action on other α-D-glucosides or on the 6,6′-bis(phosphate) of α,α-trehalose. Recently, α-D-glucopyranosyl fluoride has been found[3] to be a substrate for a mammalian trehalase and for a yeast trehalase. α-D-Glucosidases, on the other hand, act on a wide variety of α-D-glucopyranosides, including α-D-glucopyranosyl fluoride, but excluding α,α-trehalose.

(b) Transfer action. — Trehalases show little transfer when acting on the natural substrate, α,α-trehalose. Hehre *et al.*[3] using the "unnatural" system with β-D-glucopyranosyl fluoride as substrate and α-D-glucose as acceptor did obtain small proportions (0.25%) of transfer product. With the *T. reesei* trehalase, we could detect no transfer product on chromatograms of digests of 4% trehalose, or of 2% trehalose containing 4% maltose as an acceptor. This is in marked contrast to D-glucosidases where the transfer product may exceed 30% of the starting substrate[4].

(c) Configuration of hydrolysis product. — Since α-D-glucosidases act with retention of configuration[5], it was interesting to find that trehalases, with one exception, that from pig liver[6], act by inversion[3,7,8] to liberate β-D-glucose (plus of course an equal amount of α-D-glucose). Our analysis of the per-*O*-trimethylsilyl derivatives of the products resulting from the action of *T. reesei* trehalase (Table I) indicates that this trehalase also acts by inversion. The high ratio of β- to α-D anomer obtained at early stages of this enzymic hydrolysis clearly indicated that the mechanism resulted in the liberation of both α- and β-D-glucose. The mutarotation of α-D-glucose in buffer at the same temperature is not fast enough to give these

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TABLE I

RATIOS OF β-D-GLUCOSE TO α-D-GLUCOSE UNDER VARIOUS CONDITIONS[a]

System	*Time (min)*		
	2	5	∞
α,α-Trehalose–enzyme–buffer (30°)	1.39	1.38	
α-D-Glucose–buffer (30°)	0.57	0.67	1.35
α-D-Glucose–enzyme (30°)		0.71	

[a]After incubation, in 0.01M citrate buffer, samples (2 mL) were removed, frozen in dry ice, and lyophilized; giving 10–15 mg of solids. Tri-sil "Z" (1 mL) was added to each dry sample, and the mixture warmed briefly to ensure complete silylation. Samples (1–2 μL) were analyzed by g.l.c. in a capillary column (30 m × 0.26 mm) of fused silica DB-1 (SE-30), programmed from 200° to 310° at 12°/min. Helium was used as carrier gas, flow rate ~1 mL/min, and detection was by flame ionization. The ratio of β- to α-D-glucose was determined from the relative peak areas.

TABLE II

INHIBITORS OF TREHALASES

Inhibitor	*Source of enzyme*	K_i/K_m[a]	*Reference*
α-D-Glucopyranosyl 1-thio-α-D-glucopyranoside	Cockchafer	0.08	12
α-D-Glucopyranosyl 1-thio-α-D-mannopyranoside	Cockchafer	0.09	12
α-D-Glucopyranosyl α-D-mannopyranoside	Cockchafer	0.01	12
α,α-Trehalose 6-phosphate	Yeast	0.20	13
Nojirimycin	*T. reesei*	0.04	"
D-Glucono-1,5-lactone	*T. reesei*	0.21	*b*
D-Galactono-1,4-lactone	*T. reesei*	1.3	"

[a]Values estimated from reported data. [b]This report.

high values; and the ratio (0.71:1) of anomers after 5 min for a mixture of α-D-glucose and enzyme indicated that the enzyme preparation did not contain a mutarotase. The action of trehalase by inversion therefore resembles that of α-D-(1→4)-glucan glucohydrolase[5].

(d) Inhibition by nojirimycin and other compounds. — Products and modified substrates are often good competitive inhibitors. Nojirimycin (5-amino-5-deoxy-D-glucose) has previously been shown to inhibit fungal trehalase[9], and our tests with the *T. reesei* enzyme gave similar results (Table II). Where nojirimycin is effective[9], D-glucono-1,5-lactone has a similar, but less potent effect. These compounds have not been widely tested with trehalases from other sources. D-Glucono-1,5-lactone had no effect[10] on the enzyme from the cockchafer (June bug, *Melolontha vulgaris*), but this may be due to the rapid rate at which this compound is hydrolyzed at pH 7.0 (half-life <1 min), as compared to that at pH 4.0–5.0 (half-life ~1 h). 1-Deoxynojirimycin inhibited the trehalase of *Chaetomium aureum* and of rabbit[11].

The cockchafer trehalase is strongly inhibited[12] by some modified "sub-

strates" (Table II), their effectiveness being comparable with that of the modified "product", nojirimycin. Trehalose 6-phosphate was found[13] to be a fairly good inhibitor of yeast trehalase (Table II), but was without effect on cockchafer trehalase[12]. Trehalose 6,6′-bis(phosphate) was without effect on the *T. reesei* enzyme. Sucrose has been reported to be a competitive inhibitor (K_i/K_m = 0.4–2.5) of the trehalases of silk moth[14], ants[15], and honeybees[16], but it was without effect on the *T. reesei* enzyme. Mannitol is a competitive inhibitor (K_i/K_m = 1.0) of the trehalase of *Aspergillus oryzae*[17] but had no effect on the *T. reesei* trehalase.

α-D-Glucosidases are more strongly inhibited by nojirimycin than are trehalases[9]; *i.e.*, the K_i/K_m values are lower (0.004–0.013 for α-D-glucosidases *vs.* 0.48–1.6 for trehalases). The value for *T. reesei* trehalase (0.04) falls between these reported values.

(e) Other properties of trehalases. — The range of K_m values for trehalases[2] is 0.4–20mM; the K_m value for *T. reesei* trehalase is 3.1mM. The range of pH values for optimum activity[2] is 4.0–6.9 for trehalases, and the value for *T. reesei* trehalase is 4.4. The range of values of specific activity[1] is 0.4–80 for trehalases, and the value for *T. reesei* trehalase is 50 μmol/mg/min.

Conclusion. — The aforementioned data indicate several differences between trehalases and α-D-glucosidases. The properties of the trehalases more closely resemble[18] those of the α-D-(1→4)-glucan glucohydrolases (EC 3.2.1.3) than they do those of the α-D-glucosidases. Trehalases and glucohydrolases are alike in their high specificity, low transfer-ability, action by inversion, and in the degree of inhibition shown by nojirimycin.

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