

Note

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

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(Received February 14th, 1983; accepted for publication, March 14th, 1983)

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 \rightarrow 4)-glucan glucohydrolases (EC 3.2.1.3). The trehalases reported in the literature are chiefly from three sources: insects, mammalian tissue, and microorganisms². 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 specific¹, 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 found³ 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 substrate⁴.

(c) *Configuration of hydrolysis product.* — Since α -D-glucosidases act with retention of configuration⁵, it was interesting to find that trehalases, with one exception, that from pig liver⁶, 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

Actin
Hydrolysis
trehalase

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	

^aAfter 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 μ L) were analyzed by g.l.c. in a capillary column (30 m \times 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 \sim 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	<i>T. reesei</i>	0.04	^b
D-Glucono-1,5-lactone	<i>T. reesei</i>	0.21	^b
D-Galactono-1,4-lactone	<i>T. reesei</i>	1.3	^b

^aValues estimated from reported data. ^bThis 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 \rightarrow 4)-glucan glucohydrolase⁵.

(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 trehalase⁹, and our tests with the *T. reesei* enzyme gave similar results (Table II). Where nojirimycin is effective⁹, 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 effect¹⁰ 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 \sim 1 h). 1-Deoxynojirimycin inhibited the trehalase of *Chaetomium aureum* and of rabbit¹¹.

The cockchafer trehalase is strongly inhibited¹² by some modified "sub-

strates" (Table II), their effectiveness being comparable with that of the modified "product", nojirimycin. Trehalose 6-phosphate was found¹³ to be a fairly good inhibitor of yeast trehalase (Table II), but was without effect on cockchafer trehalase¹². 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 moth¹⁴, ants¹⁵, and honeybees¹⁶, 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 trehalases⁹; *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 trehalases² is 0.4-20mM; the K_m value for *T. reesei* trehalase is 3.1mM. The range of pH values for optimum activity² is 4.0-6.9 for trehalases, and the value for *T. reesei* trehalase is 4.4. The range of values of specific activity¹ is 0.4-80 for trehalases, and the value for *T. reesei* trehalase is 50 μ mol/mg/min.

Conclusion. — The aforementioned data indicate several differences between trehalases and α -D-glucosidases. The properties of the trehalases more closely resemble¹⁸ those of the α -D-(1 \rightarrow 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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