ENZYMIC PREPARATION OF 3-O-β-CELLOBIOSYL D-GLUCOSE

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Received June 7, 1963

3-O-β-Cellobiosyl D-glucose has been reported as a major component in enzymic hydrolysates of oat and barley β-glucan (2, 3, 4) and of lichenin (1, 6, 7). In these hydrolysates it is always present with other oligosaccharides. β-1,4 glucanases (cellulase) act on these polysaccharides of mixed $1 \rightarrow 3$, and $1 \rightarrow 4$ linkage to give 4-O-β-laminaribiosyl D-glucose, cellobiose, glucose, and other products; β-1,3 glucanases (endo-) to give 3-O-β-cellobiosyl D-glucose, laminaribiose, glucose, etc. (4, 7). These two enzyme types account for most of the hydrolysis of lichenin in nature (8). Recently two enzymes have been discovered which degrade lichenin and barley glucan but are without effect on cellulose or on β-1,3 glucans. Both are components of commercial amylase preparations: (1) Röhm and Haas Diastase No. 57 (7), derived from a fungus, and (2) Bacterial Amylase, Novo Terapeutisk Lab., Copenhagen (2), derived from Bacillus subtilis. Both have been separated from the amylase (2, 7).

A convenient method for the preparation of 3-O-β-cellobiosyl D-glucose utilizes the Bacterial Amylase and a commercially available lichenin. We have found that the Novo preparation without purification hydrolyzes lichenin to 3-O-β-cellobiosyl D-glucose in almost quantitative yield. The absence of β-glucosidase (Tab. 1) probably accounts for the lack of glucose and of disaccharides in the hydrolysate. The lichenin (Fluka AG, Buchs SG, Switzerland) although only about 50% polyglucan, can be used without purification.

In forming 3-O-β-cellobiosyl D-glucase from lichenin, the β-glucanase of B. subtilis (A) resembles closely the β-$(1 \rightarrow 3)$ glucanases (B), but its

specificity cannot be related only to the presence of a 3-substituted β-glucosyl unit in the polymer for it is unable to attack a long chain of such units (Tab. 1). The substrate site in lichenin differs from that in a β-(1 → 3) polyglucan in having 4-substituted β-D-glucosyl units at either side of 3-substituted units (4, 5). Although this alteration of the environment about the center of hydrolysis has little apparent effect on type B glucanase, it affects A markedly. This suggests that the activity of the latter is dependent upon a greater number of sites in lichenin than is type B. The degradation of lichenin by enzyme A also can be viewed as the hydrolysis of bonds joining β-D-glucosyl units through positions -1 and -4. The enzyme, therefore, might be designated as a "β-(1 → 4)glucanase" (C). However, its specificity is not determined by a structure of this type for the enzyme cannot hydrolyze a β-(1 → 4) glucan (Tab. 1).

The current data, together with earlier information on the action of type B and C enzymes on lichenin (6, 7), illustrate the wide range of stereoselectivity that exists among glucanases, that are capable of degrading a common substrate. They emphasize also the difficulty of classifying such enzymes in terms of a specific substrate.

Table 1. Enzymic Composition of Bacterial Amylase*

Enzyme	Substrate	pH of Assay	Enzyme "units" per mg
β-1,3 glucanase	β 1,3 glucan	4.5	< 0.01
		6.3	< 0.01
β-1,4 glucanase	CMC	4.5	< 0.01
		6.7	< 0.01
Lichenase	Lichenin	4.5	0.25
		6.8	8.4
Amylase	Starch	5.6	2.0
β-glucosidase	Salicin	5.0	< 0.01

* TB 50, Novo Terapeutisk Ind. No. 533 acts similarly.

Method

Crude lichenin (4 g.) in 200 ml. M/50 citrate pH 6.5 was added to Bacterial Amylase (Novo TB 50 or No. 533), 50 mg. in 200 ml water. The mixture was incubated at 50° for 5 hrs, or until the reducing value as glucose (Dinitrosalicylic acid method, 9) reached 6 mg/ml. of the reaction mixture. The hydrolysate was passed through a carbon column (Nuchar, granular) and gradient elution was performed with water-ethanol. 3-O-β-Cellobiosyl D-glucose appeared when the alcohol concentration reached 20%. No glucose or disaccharide was detected. The yield of trimer varied from 40-50% of the initial weight of crude lichenin. This represents nearly complete conversion of the "true" lichenin to 3-O-β-cellobiosyl D-glucose.

As an alternate procedure, undigested components present in the hydrolysate can be precipitated with 3 vol. ethanol. The alcoholic supernatant is evaporated to dryness; the residue taken up in water and applied to the carbon column as above.

The isolated trimer (0.5 g) was dissolved in 5 ml water, filtered through charcoal-celite and concentrated to 2 ml. Ethanol was added slowly to the hot solution. Crystallization started on cooling. Yield 360 mg, m.p. 232-237°. The identity was confirmed by comparison of the X-ray powder diagram with that of a known sample of 3-O-β-cellobiosyl D-glucose.

References

1. Cunningham, W. L., and Manners, D. J., Biochem. J. 80:42-43P (1961).
2. Moscatelli, E. A., Ham, E. A., and Rickes, E. L., J. Biol. Chem. 236: 2858-62 (1961).
3. Ono, H., and Dazai, M., Nature 183: 1055 (1959).
4. Parrish, F. W., Perlin, A. S., and Reese, E. T., Can. J. Chem. 38: 2094 (1960).
5. Peat, S., Whelan, W. J., and Roberts, J. G., J. Chem. Soc. 3916-24 (1957)
6. Perlin, A. S., and Suzuki, S., Can. J. Chem. 40: 50-56 (1962).
7. Reese, E. T., and Mandels, Mary. In Enzymic Hydrolysis of Cellulose and Related Materials. Pergamon Press, New York City (1963).

8. Reese, E. T., Andreotti, R., and Parrish, F. W., Arch. Biochem. and Biophys. 96: 187-8 (1962).

9. Sumner, J. B., and Somers, G. F., Laboratory Experiments in Biological Chemistry. Academic Press, Inc., New York City (1944).