The Mode of Action of Dandelion Root β -Fructofuranosidases on Inulin

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Soluble protein extracts prepared from roots of the common dandelion (*Taraxacum officinale* Weber) contain hydrolases (Rutherford & Deacon, 1972) that act on the inulin series of carbohydrates present in the roots (Bacon & Edelman, 1951). Hydrolysis of a polysaccharide may proceed either by endo action, resulting in an essentially random hydrolysis of glycosidic linkages, or by exo action, in which the molecule is degraded in a stepwise manner from the non-reducing end of the polymeric chain. This communication describes a simple and sensitive method for distinguishing between exo and endo actions of hydrolases on inulin.

Each member of the inulin series has the general formula Glc-Fru-Fru_n , where Glc-Fru represents a sucrose group and n the number of additional $(2 \rightarrow 1')$ -linked β -D-fructofuranosyl residues (Percival, 1962); n may be any value from 0 (sucrose) to about 35 (inulin). Hence each member is non-reducing. Hydrolysis of a single glycosidic linkage between fructose residues of the inulin molecule results in the production of two fragments: Glc-Fru-Fru_{n-m} , belonging to the inulin series, and the other, Fru_m , differing in that it contains a terminal reducing group. It is well known that reducing sugars undergo a complex series of reactions in hot alkali, whereas non-reducing sugars are relatively stable. With polysaccharides attack commences at the reducing end and proceeds throughout the chain (Whistler & BeMiller, 1958). Therefore alkali treatment should destroy only the Fru_m fragment. If attack by an enzyme is exo, then $m = 1$, and so the amount of fructose destroyed should be equal to that of the reducing sugar released by the enzyme; if attack is endo, then m is between 1 and 35 and the amount of fructose destroyed will be much greater. By comparing the alkali-labile fraction of the total reducing sugar present with that released by the action of the enzyme after a limited amount of hydrolysis has occurred, it should be possible to distinguish between exo and endo attack.

Inulin obtained from Hopkin and Williams Ltd., Chadwell Heath, Essex, U.K., was found to contain significant amounts of reducing sugar that could not be removed by dialysis, and so it was subjected to the alkali treatment described below, then dialysed against distilled water before use. The product was free of any significant reducing sugar and was stable to further alkali treatment. From measurements of

both reducing sugar and glucose after complete hydrolysis the average degree of polymerization was estimated to be 34. Reducing sugar was measured by the cuprimetric method of Nelson (1944) as modified by Asatoor & King (1954); glucose was determined by the glucose oxidase method, with a Biochemica Test Combination supplied by Boehringer Corp. (London) Ltd., London W.5, U.K. Soluble protein extracts were prepared from dandelion roots as described previously (Rutherford & Deacon, 1972). Inulin (5 mg) was incubated at 25°C with root extract (containing approx. 10 mg of protein) dissolved in 1 ml of buffer (0.1 M-citric acid adjusted to pH 4.5 with 0.2 M- Na_2HPO_4) until the determination of reducing sugar in samples revealed that approx. 10% of the total reducing sugar had been released. A 0.5 ml sample was removed, added to 4 M-NaOH (0.5 ml) and heated in a boiling-water bath for 10 min. Hydrolysis was then achieved by the addition of 1 M-HCl (3.0 ml) and heating in a boiling-water bath for 10 min. After cooling, portions were removed for the determination of reducing sugar. A correction was found to be necessary for some reducing compounds present in the protein preparation, both initially and after the alkali treatment and acid hydrolysis.

The reducing sugar released (0.63 mg) was in good agreement with that destroyed by the alkali treatment (0.64 mg), suggesting that attack is exo. A control was set up containing inulin (5 mg) and 0.01 M-oxalic acid in a total volume of 1 ml at 65°C to act as a model of endo attack. After a similar amount of hydrolysis had occurred the reducing sugar released (0.62 mg) was much less than that which was alkali-labile (2.53 mg). Further, after only 0.03 mg had been released 0.46 mg was alkali-labile. Therefore, if even 1 in 20 of the glycosidic linkages attacked were internal, then the alkali-labile reducing sugar (approx. 1.07 mg) would be significantly different from that released by the hydrolytic enzymes (0.63 mg). It may be concluded that soluble protein extracts prepared from dandelion roots contain only hydrolases whose mode of action is exo. Further, since exo attack at the glucose end of the molecule would leave the remainder labile to alkali, attack must occur only at the fructose end and proceed sequentially along the inulin molecule with the concurrent release of free fructose residues until a molecule of sucrose remains; presumably this sucrose is then hydrolysed by the invertase

known to be present (Rutherford & Deacon, 1972). This mode of hydrolysis is consistent with the occurrence of the inulin series of carbohydrates in dandelion roots (Bacon & Edelman, 1951) and with Edelman's model of fructosan metabolism in inulin-storing roots and tubers (Edelman & Jefford, 1968).

This technique does not require the chemical modification of the substrate, nor the isolation of intermediates. The sensitivity depends on the extent of the hydrolysis when the measurements are made, which must be carefully selected according to the degree of polymerization of the substrate. In principle the method is applicable to any carbohydrase acting on a non-reducing polysaccharide.

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