The Metabolic Role of Debranching Enzymes

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A debranching enzyme (branched 1,4-β-glucan 6-glucanohydrolase) specifically hydrolyses the 1,6-α-glucosidic linkages in branched 1,4-α-glucans, primarily glycogen and amylopectin (for review see Lee & Whelan, 1971). The IUB/IUPAC Commission of Enzyme Nomenclature (1972) recognizes four distinct categories of debranching enzymes, namely amylo-1,6-glucosidase (EC 3.2.1.33), pullulanase (EC 3.2.1.41), iso-amyrase (EC 3.2.1.68) and amylopectin 6-glucanohydrolase (EC 3.2.1.69). The classification must be regarded as somewhat tentative but, nevertheless, provides a basis for the present discussion. The primary aim of this joint contribution is to discuss the pathways of branched 1,4-α-glucan catabolism in vivo and, specifically, to pinpoint the roles of debranching enzymes in vivo.

Branched 1,4-α-glucans serve a dual metabolic function in bacteria. Many bacteria can utilize branched 1,4-α-glucans as sole exogenous carbon source. On the other hand, many bacteria accumulate glycogen as a reserve polysaccharide (Preiss, 1969). The glycogen is not metabolically inert but is catabolized in carbon starvation (Dawes & Ribbons, 1962). It is evident that any scheme for the catabolism of branched 1,4-α-glucans in bacteria must, by necessity, incorporate two seemingly separate catabolic pathways; one for the utilization of exogenous branched 1,4-α-glucans and one for the endogenous catabolism of glycogen. Investigations to date (Palmer, 1973; Palmer et al., 1973) support the integrated scheme shown in Fig 1.

Exogenous pathways of branched 1,4-α-glucan catabolism

In Escherichia coli ML, Klebsiella pneumoniae, Streptococcus mutans (Palmer et al., 1973), Pseudomonas stutzeri (Wöber, 1973) and Pseudomonas saccharophila (Norrman & Wöber, 1974) a pullulanase activity is implicated in the hydrolysis of 1,6-α-linkages in exogenous branched 1,4-α-glucan catabolism. The pullulanase is cell-bound and, in Kl. pneumoniae at least, exists in a lipopolysaccharide–phospholipoprotein particulate complex in association with the outer membrane system (Bender, 1970; Palmer et al., 1973).

Our report of an inducible pullulanase activity in E. coli strain ML (Palmer et al., 1973) has recently been questioned (Dessein & Schwartz, 1974). Dessein & Schwartz (1974) found no significant pullulanase activity in a systematic investigation of 51 E. coli strains. This observation, however, hardly justifies the conclusion that the species E. coli lacks pullulanase. Although unable to re-isolate the E. coli strain ML used in the previous investigation (Palmer et al., 1973) preliminary studies confirm the presence of pullulanase in at least one E. coli strain, E. coli Mainz (serotype O13 K! H7) (T. N. Palmer, unpublished work).
The reported specificity of pullulanase is, at first glance, difficult to reconcile with the assigned metabolic role of the enzyme. *Kl. pneumoniae* pullulanase hydrolyses the 1,6-\(\alpha\)-linkages in amylpectin but has little, if any, action on glycogen (Lee & Whelan, 1971). The specificity of maltodextrin permease (Fig. 1) imposes yet another restriction on the function of pullulanase in vivo. Maltodextrins of an average chain length of 20 glycosyl units (analogous to debranched amylpectin) are poor substrates for the permease (Palmer et al., 1973; Norrman & Wöber, 1974). An exogenous catabolic pathway comprising pullulanase and maltodextrin permease (*E. coli* ML and *Strep. mutans*), accordingly, cannot catabolize glycogen and can support only slow growth on amylpectin. Specificity studies with purified pullulanase (Lee & Whelan, 1971) show complete hydrolysis of amylpectin or glycogen by the enzyme in the presence of either \(\alpha\)- or \(\beta\)-amyrase. This fact provides the key to an understanding of the role of pullulanase in vivo. In *Kl. pneumoniae* (Palmer et al., 1973; Katanuma & Suzuki, 1971), *Ps. stutzeri* (Wöber, 1973) and *Ps. saccharophila* (Norrman & Wöber, 1974), an amylase and a pullulanase are co-induced. A catabolic pathway comprising the enzymes amylase, pullulanase and maltodextrin permease permits efficient utilization of branched 1,4-\(\alpha\)-glucans. The lack of an amylase in *E. coli* ML and *Strep. mutans* is problematical. The strains are isolated from human intestinal tract and oral cavity respectively. It has been argued that the presence of host \(\alpha\)-amylase in both environments obviates the need for the organism to elaborate an amylase (Palmer et al., 1973).

It is strictly a working hypothesis that the catabolic pathway comprising the enzymes amylase, pullulanase and maltodextrin permease is normal among bacterial species capable of growth on branched 1,4-\(\alpha\)-glucans. Exceptions exist; no detectable amylase or pullulanase activity is present in *Pseudomonas amylophilosa* (Norrman & Wöber, 1974) or *Cytotrophaga* sp. N.C.I.B. 9417 (T. N. Palmer, unpublished work). Instead, a cell-bound isoamylase is present. Isoamylase and pullulanase are distinct enzymic entities with markedly different specificities (Lee & Whelan, 1971). An isoamylase hydrolyses the 1,6-\(\alpha\)-linkages in amylpectin and glycogen. The enzyme has no action on pullulan. Accordant with the specificity of isoamylase, *Ps. amylophilosa* and *Cytotrophaga* sp. can utilize glycogen and amylpectin as sole exogenous carbon source but show no growth on pullulan.

In conclusion, four groups of organisms may be delineated with respect to the complement of enzymes constituting the exogenous pathway of branched 1,4-\(\alpha\)-glucan catabolism. These four pathways are: (1) pullulanase–amylase–maltodextrin permease; (2) pullulanase–maltodextrin permease; (3) maltodextrin permease (Dessein & Schwartz, 1974); (4) isoamylase–maltodextrin permease.

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**Fig. 1. Schematic representation of the proposed pathways of branched 1,4-\(\alpha\)-glucan metabolism in bacteria**
The pullulanase–amylase–maltodextrin permease pathway is physiologically the most versatile. Pathways (2) and (3) are modifications of pathway (1) and are dependent upon the obligatory presence of amylase or amylase and pullulanase activities in the environment. These modifications prompt the suggestion that a fifth type of pathway exists, comprising the enzymes amylase and maltodextrin permease.

**Endogenous pathway of glycogen catabolism**

Notwithstanding the obvious importance of glycogen as a reserve polysaccharide in bacteria (Preiss, 1969; Dawes & Ribbons, 1962), knowledge of the pathway(s) of glycogen catabolism is scanty. Glycogen catabolism is triggered by transfer to carbon-deficient medium. The mechanism of derepression involves protein biosynthesis de novo (T. N. Palmer, unpublished work). In *E. coli* (a strain formerly listed as N.C.T.C. 5928), the initial step in glycogen catabolism is the specific hydrolysis of component 1,6-α-linkages of the polysaccharide to yield maltodextrins (Palmer et al., 1973). Specificity studies show that the reaction is catalysed by an intracellular isoamylase (Fig. 1). Isoamylase clearly has a central role in glycogenolysis. Glycogen phosphorylase activities are reported in *E. coli* (Chen & Segal, 1968), *Streptococcus salivarius* (Khandelwal et al., 1973) and in *Streptococcus mitis* (Pulkownik & Walker, 1974). The low reported phosphorylase activities belie the fact that the enzyme may have a significant role in vivo.

**Endogenous metabolism of maltodextrins**

The exogenous pathways of branched 1,4-α-glucan utilization and the endogenous pathway of glycogen catabolism both produce maltodextrins. Evidence to date supports the idea that maltodextrins, produced by either catabolic route, are metabolized by a common pathway (Palmer et al., 1973; Norrman & Wöber, 1974). The pathway comprises the enzymes amylomaltase (4-α-glucanotransferase, EC 2.4.1.25) and maltodextrin phosphorylase (EC 2.4.1.1) (Fig. 1). The concerted actions of the two enzymes yield glucose and α-glucose 1-phosphate.

The assigned metabolic roles of the two enzymes in maltodextrin metabolism are consistent with their reported specificities. Growth studies (Palmer et al., 1973) and a reinvestigation of the reaction specificity of amylomaltase (Palmer et al., 1968) both give weight to the conclusion that maltose *per se* is a restricted substrate for the maltodextrin-utilizing system in *E. coli*. The preferred substrates are maltotriose and higher maltodextrins. Hitherto, the concept of the maltose operon (Schwartz, 1967; Dessein & Schwartz, 1974) has traditionally assigned roles to amylomaltase and maltodextrin phosphorylase specific to the metabolism of the disaccharide maltose. This view is no longer compatible with the known specificities *in vivo* and *in vitro* of the maltodextrin-utilizing system in *E. coli*.

**Branched 1,4-α-glucan metabolism in bacteria: a model for plant starch catabolism**

The metabolism of starch in plants is, in many ways, analogous to branched 1,4-α-glucan metabolism in bacteria. In both systems, ADP-glucose pyrophosphorylase is the rate-limiting enzyme in branched 1,4-α-glucan biosynthesis. The enzyme is subject to allosteric control (Preiss, 1969). The analogy extends to the key enzymes of polysaccharide catabolism. The plant enzymes, plant debranching enzyme (R-enzyme), D-enzyme (4-α-glucanotransferase) and plant phosphorylase, are similar in their specificities to pullulanase, amylomaltase and maltodextrin phosphorylase respectively. The α- and β-amylases have a widespread distribution in plants. It has been proposed, as a working hypothesis, that plant debranching enzyme, an α- or β-amylase, D-enzyme and plant phosphorylase constitute the pathway of starch catabolism in plants (Palmer et al., 1973). This hypothesis is shown schematically in Fig. 2.

**Conclusions: the catabolism of the 1,6-α-linkage**

The present discussion has sought to assign tentative roles to pullulanase and isoamylase in bacterial and plant branched 1,4-α-glucan metabolism. The enzymes clearly occupy central positions in metabolism. This self-evident fact, it is hoped, will promote a
Fig. 2. Schematic representation of the proposed pathways of branched 1,4-α-glucan metabolism in plants and bacteria

The Figure has been drawn primarily to emphasize the analogies between plant and bacterial metabolism.

Rethinking of debranching enzymes in the context of metabolic function and regulatory control in vivo. This statement is particularly relevant to the problems that apply to the pathway(s) of starch catabolism in plants and the roles in vivo, not only of debranching enzymes, but also of other enzymes in starch catabolism. Despite the mass of literature on photosynthetic carbon assimilation, starch biosynthesis and related topics, little attention has been devoted to the pathway(s) and enzymes of starch catabolism. In this context, it is necessary to differentiate clearly between starch metabolism in chloroplasts and in amyloplasts, and to recognize the existence of lysosomes in plant tissues and the implied role of lysosomal acid hydrolases in polysaccharide catabolism.

We acknowledge the support of the Deutsche Forschungsgemeinschaft (G. W., grant Wö 197/1), the Science Research Council (T. N. P.) and the University of London Central Research Fund (T. N. P.).

IUB/IUPAC (1972) Enzyme Nomenclature, Elsevier, Amsterdam