THE ACTION PATTERN OF AMYLOMALTASE

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Received 5 June 1968

1. Introduction

Amylomaltase [E.C.2.4.1.3], first described by Monod and Torriani [1], is an enzyme which is induced in *Escherichia coli* ML by maltose. The initial step in the sequence of transglucosylation reactions reported to be catalysed by this enzyme may be represented by:

$$\text{maltose} + \text{maltose} \rightleftharpoons \text{maltotriose} + \text{glucose} \quad \text{(i)}$$

If the reaction is driven to the right with glucose oxidase, successive transglucosylations lead to the formation of 1 → 4-linked α-glucans that stain blue with iodine. The induction of amylomaltase is accompanied by the formation of a maltose permease [2] and a maltodextrin phosphorylase [3]. The further purification and physicochemical properties of amylomaltase, together with its general properties and mechanism of action have also been documented [4,5]. Our own interest in this enzyme originated primarily in its possibilities as a tool for studying the fine structures of glycogen and amylopectin. In the isolation and assay procedures, however, several unexpected findings prompted further investigation. Experimental evidence is produced which casts doubt on the previously postulated mechanism of reaction, implying the rate limitation of reaction (i) under conditions where chromatographically pure maltose is used as substrate for the enzyme.

2. Experimental

E. coli ML 30, kindly supplied by Professor G.V. Schulz [5], was grown, and the enzyme purified, by a procedure to be described later, but purification was essentially as by Weismeyer and Cohn [4], with an additional fractionation on DEAE-Sephadex to remove maltodextrin phosphorylase. The final enzyme preparation had a specific activity of 2020 units/ml protein when assayed with maltose purchased from Kerfoot Ltd, Vale of Bardsley, Lancs, England ("Commercial maltose"). Glucose release was measured with glucose oxidase [6] and 1 unit of activity is defined as 1 μmole of glucose released/hr at 28°, pH 6.8 [5]. The oligosaccharides used were prepared by published methods, i.e. maltodextrins [7], panose [8], isopanose and 6-α-maltotriosylglucose [9].

3. Results and discussion

Amylomaltase was incubated with "commercial maltose", maltose purified by chromatography, maltotriose, maltotetraose and maltopentaose and the rates of reaction were measured by following glucose release. The results in fig. 1 indicate that the action of amylomaltase on the two preparations of maltose is different and that a lag phase is observed when purified maltose is employed. This lag is followed by a phase of rapid glucose release. When, however, maltotriose and higher maltodextrins are

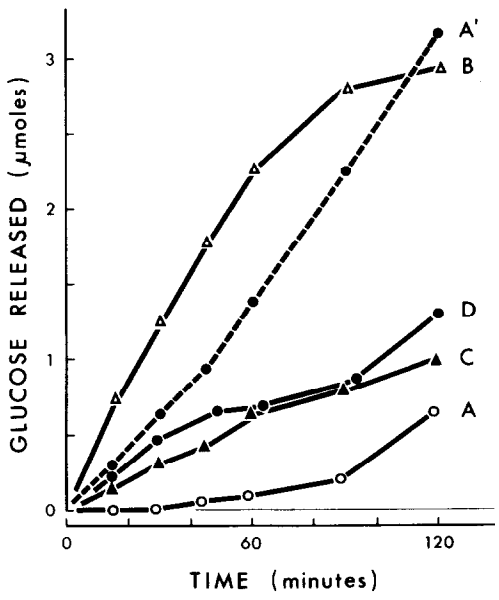

Fig. 1. Action of amylomaltase on A, maltose, purified by chromatography; A', "commercial maltose"; B, maltotriose; C, maltotetraose; D, maltopentaose. Reaction mixtures (1 ml) contained 15 μmoles maltodextrin in 200mM-Tris-maleate, pH 6.8, and 7.5 units amylomaltase, at 28°. Glucose released is per ml of digest.

used in place of maltose there is an immediate rapid linear release of glucose with no lag period. This latter observation, coupled with the fact that the lag phase noted with chromatographically pure maltose is not evident with "commercial maltose", which contains trace quantities of maltotriose [10], led to the supposition that the lag phase represented a rate-limiting step [reaction (i)] during which maltotriose or higher maltodextrins were being synthesized.

To test this possibility reaction systems containing varying proportions of maltose and maltotriose were tested for rate of glucose release. Fig. 2 shows that the lag phase with maltose is shortened by the addition of small amounts of maltotriose and the rate of reaction increases as the proportion of maltotriose increases.

Three possible reaction mechanisms can be invoked to explain the rate-limiting nature of reaction (i):

(a) Maltose may be a poor acceptor but an excellent donor in transglucosylation. Thus reaction (i) is unfavourable because of low acceptor affinity of

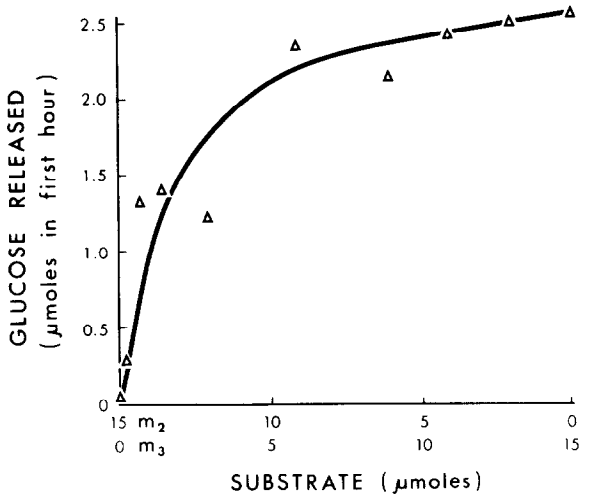

Fig. 2. Action of amylomaltase on varying proportions of maltose (m_2) and maltotriose (m_3). Reaction mixtures (1 ml) contained 15 μmoles maltodextrin in 200 mM-Tris-maleate, pH 6.8, and 7.5 units amylomaltase, at 28°. Glucose released is per ml of digest.

maltose. The maltotriose which is perhaps synthesised during the lag phase then "primes" the reaction by functioning as an acceptor in transglucosylations from maltose.

(b) The enzyme is a transglucosylase which prefers to transfer glucose units in multiples, and has low affinity for the transfer of single glucosyl units. The lag phase again represents maltotriose synthesis, which in this mechanism will act as a donor in transmaltosylation to maltose, forming maltotetraose, and so on.

(c) The maltotriose formed during the lag phase "activates" the enzyme and permits transglucosylation between maltose molecules to occur as in (i).

Incubation of the enzyme with "commercial maltose" and measurement of glucose release showed that at equilibrium the percentage of glucose released was of the order of 25. This level of conversion implies the formation of relatively long polymers. In the presence of glucose oxidase to disturb the equilibrium almost quantitative production of amylaceous (iodine-staining) products can be demonstrated, and similar material is also present in small quantities in the absence of glucose oxidase. These observations imply that glucosyl units are not transferred to all potential acceptors at random, but that there is a preferential chain lengthening of a rela-

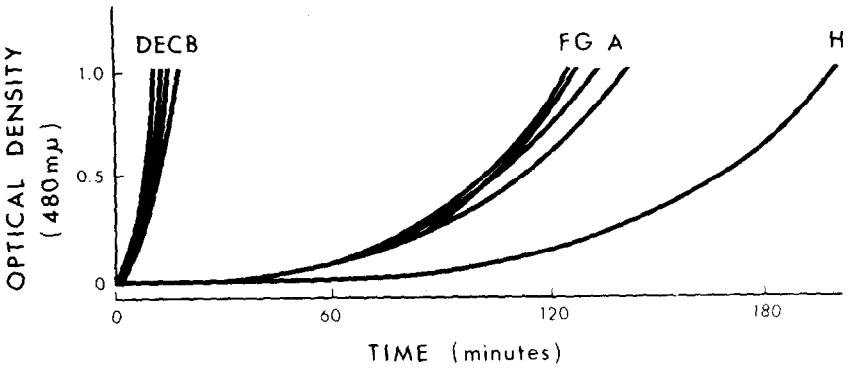

Fig. 3. Effects of various oligosaccharides on the lag phase of glucose release from maltose purified by chromatography. Reaction mixtures (3.1 ml) contained 100 mM-phosphate buffer, pH 6.8, 46.5 μmoles of maltose, oligosaccharide, when present (3 μmoles), glucose oxidase reagent [6] (2 ml) and 50.4 units amylomaltase, at 28°. The intensity of the colour developed by the oxidation of the glucose formed was continuously recorded at 480 mμ on a Beckmann DB2 spectrophotometer. A, Maltose only; B, + maltotriose; C, + maltotetraose; D, + maltopentaose; E, + maltohexaose; F, + panose; G, + 6-α-maltotriosylglucose; H, + isopanose (6-α-maltosylglucose).

tively small proportion of acceptors. This would appear to eliminate (b) but permit (a) or (c), the latter however only under conditions of single-chain attack [11], in which one maltose molecule is lengthened at a time.

In order to examine the "priming" effects of oligosaccharides, the purified maltose was incubated with a selected oligosaccharide, amylomaltase and glucose oxidase reagent, and the O.D. at 480 mμ was continuously recorded against a blank containing maltose and glucose oxidase. The results in fig. 3 show that panose and 6-α-maltotriosylglucose (a potential substrate) have little effect, while isopanose (6-α-maltosylglucose), another potential substrate, increases the duration of the lag phase.

Clearly, therefore, the presence of small amounts of maltotriose or higher homologues has a profound effect on the ability of amylomaltase to utilize maltose. We have not distinguished whether the effects are due to the maltotriose etc. acting as a primer in the reaction, or as a modifier/activator of the enzyme. It is clear, however, that maltose *per se* must now be regarded as a poor substrate of amylomaltase. Indeed, by itself it may not even be a substrate, that is reaction (i) may be impossible. Such reaction as is noted with purified maltose might still be due to contaminating traces of oligosaccharide. Furthermore, maltose should only be used as a substrate for the assay of amylomaltase activity when regulated amounts of maltotriose are present. Better still would be in future to measure and define its activity in terms of maltotriose as substrate.

Acknowledgements

T.N.P. acknowledges the receipt of a Science Research Council Research Studentship. We thank Imperial Chemical Industries Ltd for the loan of apparatus.

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