Note

Studies on starch-degrading enzymes
Part XIII. The action pattern of porcine, pancreatic alpha-amylase

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In the pH range of 5.0 to 9.0, porcine, pancreatic alpha-amylase hydrolyzes amylose by multiple attack[1], *i.e.*, the enzyme hydrolyzes an α-(1→4)-D linkage randomly, and then a number of linkages in the immediate vicinity of this first point of attack are broken with the liberation of small oligomers, mainly maltose and maltotriose. However, by carrying out the enzymic hydrolysis at high pH values, the action pattern can be altered to multichain, corresponding to a random hydrolysis of the amylose. No satisfactory explanation has been advanced for this pH-dependent action-pattern.

In a previous communication[2], we confirmed that the action pattern of porcine, pancreatic alpha-amylase was indeed a function of pH. Moreover, we found that, at the optimum pH of the enzyme (at which a multiple-attack mechanism is normally favoured), the presence of glycerol led to an essentially random attack. It was tentatively suggested that the two isoenzymes[3] of porcine, pancreatic alpha-amylase might differ slightly in their action patterns, and that one of these enzymes might be suppressed by the use of high pH values, or in the presence of glycerol. However, this explanation is untenable, because the action patterns of the multiple forms of the enzyme are virtually identical[4].

There is no doubt that the change from multiple to multichain attack results in grossly different distributions of product oligomers (compare, for example, samples 3 and 3a of Table IV in Ref. 2). By the use of high pH, or glycerol at pH 5.5, porcine, pancreatic alpha-amylase completely changes its action pattern. Our control experiments showed that this change was not due to a conformational change in the substrate amylose, nor to adventitious interference in any of the assay procedures. The enzyme has been shown to possess only negligible transferase activity[5], and hence it must be concluded that high pH, or the use of glycerol at optimal pH, causes a change in the enzyme which is reflected by a very different hydrolytic action.

We consider that, in its action on amylose, porcine, pancreatic alpha-amylase may be regarded as having two inter-related activities: (1) the random component, which is responsible for the rapid decrease in iodine stain, and (2) the non-random component which produces small oligomers even in the initial stages of hydrolysis, and hence causes an increase in reducing power. At high pH, activity (2) is suppressed,

but this is accompanied by a large decrease in activity (1), relative to measurements carried out at pH 5.5. Similarly, the presence of 40% aqueous glycerol at pH 5.5 completely inhibits activity (2), but also decreases activity (1) to about 30% of the value observed for water. The fact that the changes in the two types of activities are relative, rather than absolute, makes their interpretation difficult.

We suggest that one explanation for the observed behaviour is that porcine, pancreatic alpha-amylase possesses two types of substrate-binding site; one of these binds interior portions of the amylose molecule, whereas the other is adapted for the binding of chain ends. These binding sites could easily involve different amino acids, and hence one would not necessarily expect the effect of pH to be the same on both.

It is, however, more difficult to find an explanation for the mechanism whereby glycerol inhibits activity (2). It is known that alditols are competitive inhibitors for the alpha- and beta-glucosidases[6], because they possess common structural features with the substrates of these enzymes which result in their being bound to the protein. The observation that glycerol caused porcine, pancreatic alpha-amylase to revert from multiple to random attack suggested that alditols might provide a somewhat similar, competitive inhibition by binding to one of the sites in the enzyme. For this reason, we have now investigated the effect of erythritol and methyl α-D-glucopyranoside on the hydrolysis of amylose by porcine, pancreatic alpha-amylase. The results of this study are shown in Fig. 1., in which the iodine stain of the amylose is related to the reducing power of the digest.

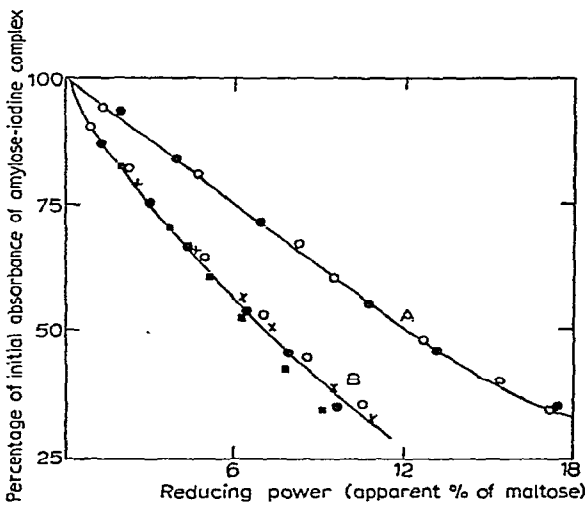

Fig. 1. Change in absorbance of the amylose–iodine complex as a function of the apparent reducing power for the action of porcine, pancreatic alpha-amylase on amylose under different experimental conditions. Line A: ○, pH 5.5; ●, pH 7.0. Line B: ○, pH 10.6; ●, 40% aqueous glycerol at pH 5.5; ×, 0.3M erythritol at pH 5.5 (typical of results for the pH-range 5.5–9.0); ■, 0.3M methyl α-D-glucopyranoside at pH 5.5 (typical of results for the pH-range 5.5–9.0).

All the experimental results were found to lie on one or other of the two curves shown in Fig. 1. Line *A* represents the iodine stain–reducing power relation for the

action of porcine, pancreatic alpha-amylase on amylose at pH 5.5 and 7.0; line *B* shows the corresponding relation when the digestion is carried out (*a*) at pH 10.6, (*b*) in the presence of 40% aqueous glycerol at pH 5.5, (*c*) in the presence of 0.3M erythritol in the pH range 5–9, and (*d*) in the presence of 0.3M methyl α-D-glucopyranoside, again in the pH range 5–9. The fact that the results obtained when erythritol and methyl α-D-glucopyranoside are present fall on line *B* indicates that these materials do alter the action pattern of this alpha-amylase, changing it from multiple to random attack.

Additionally, in the presence of 0.3M erythritol or 0.3M methyl α-D-glucopyranoside, it was found that the random component (**1**) of the enzyme action was the same as in water, even although the non-random component (**2**) was completely suppressed.

The above results are not inconsistent with our contention that porcine, pancreatic alpha-amylase possesses more than one type of substrate-binding site.

EXPERIMENTAL

Materials and methods. — Crystalline, porcine, pancreatic alpha-amylase was purchased from the Sigma Chemical Company, London. Linear amylose was obtained by the aqueous leaching of potato starch[7]. The unit of enzyme activity used in this work is that of Briggs[8]. Reducing power was measured by means of the alkaline ferricyanide–ceric sulphate method[9]. Iodine stain was determined on samples (0.05 ml) that were added to iodine (1.0 ml, 0.2%, in 2% potassium iodide) and hydrochloric acid (0.2 ml, 5M) in a total volume of 50 ml.

Digest conditions. — Digests (total volume 30 ml) were set up as follows. (*a*) Amylose (90 mg), acetate buffer (3.0 ml, 0.2M, pH 5.5), alpha-amylase (0.8 unit). (*b*) Amylose (90 mg), acetate buffer (3.0 ml, 0.2M, pH 5.5), glycerol (12 ml), alpha-amylase (2.5 units). (*c*) Amylose (90 mg), acetate buffer (3.0 ml, 0.2M, pH 5.5), erythritol (9 ml, M), alpha-amylase (0.8 unit). (*d*) Amylose (90 mg), acetate buffer (3.0 ml, 0.2M, pH 5.5), methyl α-D-glucopyranoside (9 ml, M), alpha-amylase (0.8 unit). (*e*) Amylose (90 mg), glycine–sodium hydroxide buffer (3.0 ml, 0.2M, pH 10.6), alpha-amylase (5 units). The digests were incubated at 40°, and samples were withdrawn as a function of time for the estimation of reducing power (3.0 ml) and iodine stain (0.05 ml).

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