

## Isoelectric Properties of Barley $\beta$ -Amylase

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It has been shown that purified barley  $\beta$ -amylase monomer will polymerize spontaneously to form a series of polymers in solutions in which no reducing agents are present.<sup>1</sup> Crude extract contains a series of at least four  $\beta$ -amylase components of different molecular size but immunologically identical.<sup>2,3</sup> The nature of these natural polymers has not yet been clarified.

Isoelectric focusing electrophoresis resolves the purified  $\beta$ -amylase monomer into four distinct peaks whilst other methods show it to be homogeneous.<sup>4</sup>

Isoelectric focusing electrophoresis was used to study the  $\beta$ -amylase in crude extract by comparing the isoelectric properties of purified  $\beta$ -amylase monomer, of the purified polymer and of the  $\beta$ -amylase components in crude extract.

*Experimental.* Preparation of samples: Pirikka, a Finnish six-row barley, was extracted with phosphate buffer<sup>2</sup> and desalted with Sephadex G-25 in water. The monomer was purified<sup>4</sup> and the purified polymer was prepared by dialysing the monomer for 3 days against distilled water. The product of dialysis was a series of polymers of which the highest aggregate contained approximately eleven monomer units.

*Isoelectric focusing electrophoresis:* This was done according to the method described by Vesterberg and Svensson.<sup>5</sup> The volume of the electrophoresis apparatus was 110 ml. Into the column were transferred glycerol (50% w/v) to give a density gradient, synthetic ampholytes (ampholine pH 5–7, from LKB as 1% solution) and the sample, by means of an LKB gradient mixer. The focusing time was 70 h, voltage 500 V. The elution rate was 0.3 ml/min and the volumes of the fractions collected were 1.5 ml. The  $\beta$ -amylase activities of the fractions were determined<sup>2</sup> and the pH measured at room temperature.

*Results and discussion.* The figures show the isoelectric pattern of the purified

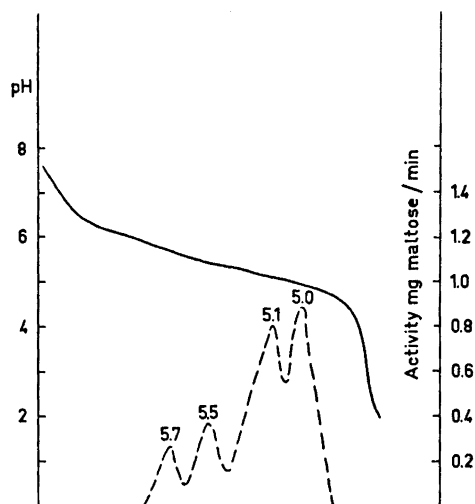


Fig. 1. The isoelectric focusing electrophoresis of purified barley  $\beta$ -amylase monomer. Amylase activity — —, pH-gradient ———.

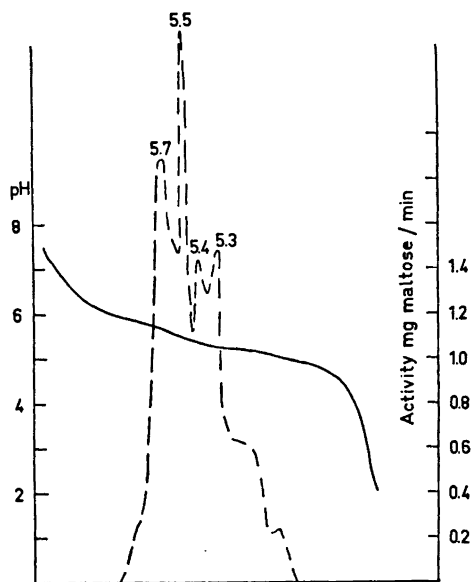


Fig. 2. The isoelectric focusing electrophoresis of polymerized purified barley  $\beta$ -amylase. Amylase activity — —, pH-gradient ———.

monomer (Fig. 1), the purified polymer (Fig. 2), and crude extract (Fig. 3).

The purified  $\beta$ -amylase monomer focused as four peaks<sup>4</sup> having pI-values 5.0, 5.1, 5.5, and 5.7 (Fig. 1). The polymer focused at 5.3, 5.4, 5.5, and 5.7 (Fig. 2) and  $\beta$ -amylase in the crude extract at 5.1, 5.2, 5.4, 5.5, and 6.0 (Fig. 3).

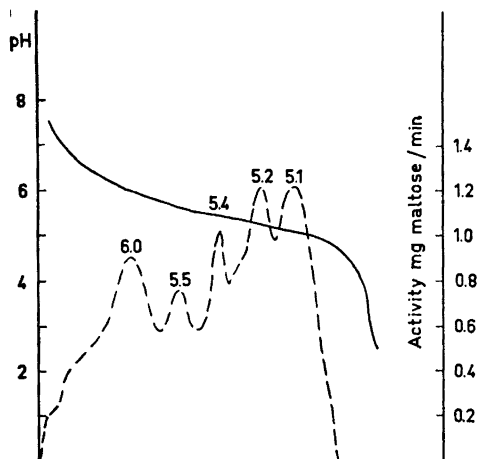


Fig. 3. The isoelectric focusing electrophoresis of barley  $\beta$ -amylase in crude extract. Amylase activity — —, pH-gradient ———.

The figures indicate that during polymerization of the purified monomer the two most acid peaks at 5.0 and 5.1 move towards more alkaline pH, to 5.3 and 5.4. It has been assumed that SH-groups participate in the polymerization of  $\beta$ -amylase.<sup>4</sup> This focusing pattern supports the idea that some acid groups are blocked during polymerization. Some  $\beta$ -amylase molecules evidently do not participate in the blocking reaction because the peaks at pH 5.5 and 5.7 are present in both monomer and purified polymer. Thus the changes in the protein structure of  $\beta$ -amylase during polymerization are so small that they do not affect the electrophoretic mobility and antigenic properties of dif-

ferent polymerization products, as has been stated earlier.<sup>3</sup>

The focusing pattern of  $\beta$ -amylase in crude extract differs from those of both the purified monomer and the purified polymer. All the peaks of the crude extract have shifted towards the alkaline pH when compared with the monomer. If compared with the purified polymer it can be seen that in the crude extract there are peaks at more acid (5.1, 5.2) and also at more alkaline pH (6.0) than in the purified polymer. This shows that the polymers in the extract have not been formed by the same mechanism as in the spontaneous polymerization of the monomer. This is not surprising because in the extract there are numerous small molecules which can react with the  $\beta$ -amylase to block polymerization, or act as reducing agents which inhibit the polymerization. Indeed it has earlier been shown that in the crude extract the four different molecular forms of  $\beta$ -amylase are split to the monomer during storage.<sup>2</sup> On the basis of the earlier observations and of the results presented here, it seems that in the crude extract  $\beta$ -amylase is not only polymerized but also has reacted with small molecules with consequent changes in its isoelectric properties. Neither these reactions nor the polymerization in general change the electrophoretic mobility or the antigenic properties and protein structure of  $\beta$ -amylase.<sup>3</sup>

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