# A New Amylolytic Enzyme

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To date, the only starch-hydrolysing plant enzymes characterized are α- and β-amylases, limit dextrinase, R-enzyme and phosphorylase.[1] A new amylolytic enzyme has been found in barley and malt, when these were tested with immunoelectrophoresis and using barley and malt protein antiserum made in the horse.[2] It was observed that this new enzyme bears similarities to some enzymes detected earlier in germinating barley.[3] This paper reports on the steps of purification involved in the preparation of the new amylolytic enzyme, its immunological behaviour, and its action on starch.

*Experimental. Purification.* The enzyme was extracted from ground, acetone treated Balder-barley, with 0.01 M phosphate buffer pH 7.0 containing 2.5 % sodium chloride. The extract was precipitated with 40 % ammonium sulphate saturation, the precipitate was dialysed free from salts and freeze-dried. The enzyme was then purified from the more basic β-amylase by the application of ion-exchange chromatography on CM-cellulose with 0.05 M acetate buffer pH 4.95 by the method of LaBerge and Meredith.[4] The active fractions were collected, dialysed, freeze-dried, and further purified on Sephadex G-75. The fractions containing enzyme were dialysed and freeze-dried, and run immunoelectrophoretically; their hydrolytic action against potato starch was tested by means of paper chromatography.

*Immunoelectrophoresis* was carried out by the method described in an earlier report.[5] The antiserum was the EBC reference serum 2 (made in the horse against barley and malt soluble proteins).[6]

*Determination of the activity.* The enzymatic activity of the fractions in ion-exchange chromatography and exclusion chromatography was determined at the pH of the eluting buffer as the reducing groups were liberated from β-limit dextrin, using 3,5-dinitrosalicylic acid as reagent.[7] The activities are reported as absorbance at 540 mμ. β-Limit dextrin was made by the digestion of potato starch with commercial β-amylase (Wallerstein),[8] filtration of the hydrolysate on Sephadex G-25, and freeze-drying.

*Paper chromatography* was carried out with propanol:ethyl acetate:water (14:2:7) as solvent and silver nitrate:sodium hydroxide as detecting reagent.[9] The enzyme digests were made by incubation of the purified enzyme and potato starch at pH 7.0 for 3 days, inactivation of the enzyme by boiling for 15 min, and separation of the digests from buffer salts with mixed bed ion-exchange resin MB 3 (BDH) and freeze-drying of the hydrolysate. The known samples used were wort, maltohexaose (kindly supplied by Dr. Enevoldsen) and maltose (Merck). For comparative digests, barley β- and malt α-amylases were purified from corresponding extracts precipitated with ammonium sulphate saturation, adsorbed on DEAE-cellulose from 0.01 M phosphate buffer pH 7.5, eluted at 0.1 M acetate pH 5.2 buffer, dialysed and freeze-dried. In the preparation of α-amylase, β-amylase was inhibited by the addition of $10^{-5}$ g/ml mercuric chloride to the extract and heating it at 70°C for 15 min.[1]

*Results and discussion.* The elution curve of the purified enzyme is illustrated in Fig. 1. According to the elution volumes of blue dextran, ovalbumin, and cyt. c (indicated as arrows) the relative molecular weight of the new enzyme is 50 000. It does

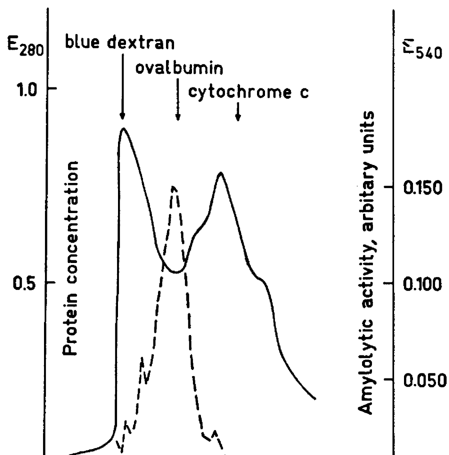


*Fig. 1.* Elution curve of the enzyme on Sephadex G-75. Protein ———; activity – – –. The arrows indicate the elution volumes of the markers.

not differ markedly from that of the smallest $\beta$-amylase component 55 500, and that of malt $\alpha$-amylase 45 000, reported earlier.[1,7]

Comparative immunoelectrophoresis of the new enzyme and $\alpha$- and $\beta$-amylase is indicated in Fig. 2. It can be stated that

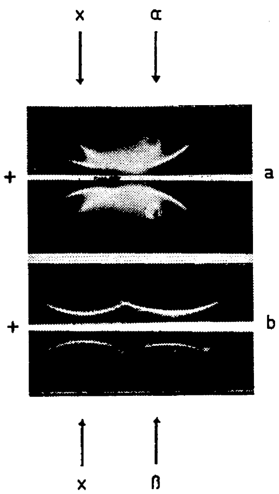


*Fig. 2.* Amylase activity detection after comparative immunoelectrophoresis of the new enzyme (x), $\alpha$- and $\beta$-amylase. a. Concentrated malt extract after inactivation of $\beta$-amylase with mercuric chloride. b. Concentrated barley extract. Samples applicated into the round holes.

the new enzyme is more acidic than both $\alpha$- and $\beta$-amylase. It also differs from them in respect of its antigenic determinants; the immunoarcs of the new enzyme and $\alpha$-amylase and the new enzyme and $\beta$-amylase cross each other.

Fig. 3 reproduces the chromatograph of the starch digests with the new enzyme, $\alpha$- and $\beta$-amylase. It appears as though the new enzyme attacks the starch in a way different from $\alpha$- and $\beta$-amylase; products obtained with the new enzyme always contain maltotriose, followed by two unknown sugars, one travelling a little faster, and the other a little slower than maltotriose. The digests obtained with $\alpha$- and $\beta$-amylase do not contain these three sugars together.

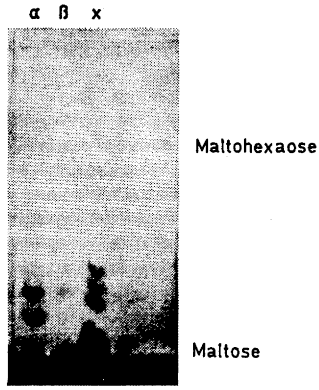


*Fig. 3.* Paper chromatography of products yielded from potato starch after hydrolysis with the new enzyme (x), $\alpha$- and $\beta$-amylase.

These results lead to the conclusion that barley contains an amylolytic enzyme other than $\beta$-amylase. This enzyme also exists in malt, and differs from $\alpha$- and $\beta$-amylase in regard to its electrophoretic mobility, antigenic structure and hydrolytic action on starch.

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