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Introduction of sulfhydryl groups into Taka-amylase A and changes in enzymic activity

The introduction of biologically active groups into enzymes by treatment with various chemical reagents has been studied in order to change the enzymic characteristics or to increase the specific activities of enzymes. Recently it was observed that the thiolation[1] of Taka-amylase A (EC 3.2.1.1) by *S*-acetylmercaptosuccinic anhydride resulted in increasing enzymic activity on amylose.

Taka-amylase A was purified and crystallized from the Taka-diastase of Sankyo Ltd. (Tokyo), according to the method of AKABORI, IKENAKA AND HAGIHARA[2]. Amylose was obtained from Nagase Sangyo Co., and *S*-acetylmercaptosuccinic anhydride was synthesized from maleic anhydride and thioacetic acid by the method of HOLMBERG AND SCHJÅNBERG[3].

The following procedure was employed in the experiments to prepare mercaptosuccinyl Taka-amylase A. A protein solution was prepared by adding $3.6 \cdot 10^{-7}$ moles of Taka-amylase A to 6 ml of 0.2 M phosphate buffer (pH 8.0). 1 ml of *S*-acetylmercaptosuccinic anhydride solution in dioxane ($3.6 \cdot 10^{-6}$–$1.8 \cdot 10^{-4}$ moles) was then added to the protein solution at 2°. After 30 min, hydroxylamine was added to the reaction mixture to remove the acetyl groups which blocked the sulfhydryl groups. This reaction mixture was then dialyzed against distilled water for 24 h at 20°. The protein concentration was determined spectrophotometrically by the method of LOWRY *et al.*[4]. The degree of mercaptosuccinylation was estimated by determining sulfhydryl groups of mercaptosuccinyl residues, and the determination of sulfhydryl groups was carried out spectrophotometrically by measuring the increase in absorbance at 255 mμ due to the mercaptide formation of sulfhydryl groups with *p*-chloromercuribenzoate, according to the method of BOYER[5]. The enzymic activity was measured by two different ways, using the blue-value method[6] and the SOMOGYI–NELSON method[7,8].

The enzymic activities of native and modified Taka-amylase A are shown in Fig. 1.

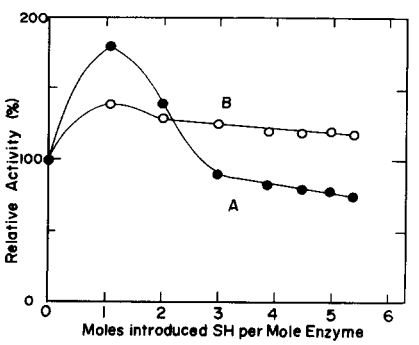

Fig. 1. The enzymic activities of native and mercaptosuccinylated Taka-amylases. The enzymic activity of native Taka-amylase at its pH optimum is taken as 100%. Curve A, activity measured by the iodine staining method of FUWA[6]; Curve B, activity measured by the SOMOGYI–NELSON method[7,8] for reducing power.

The enzymic activity of the modified Taka-amylase A on amylose measured by the blue-value method (Curve A in Fig. 1) increased to 180% of the original activity when 1 mole of sulfhydryl group was introduced into 1 mole of Taka-amylase A. Similar results were obtained for the activity by the SOMOGYI–NELSON method; when one or two mercaptosuccinyl groups were attached to the enzyme, the activity was 130–140% compared with that of native enzyme, and 110% of the original activity was retained even after attachment of 6 sulfhydryl groups per mole Taka-amylase A. From this result, it may be concluded that the rate of hydrolysis for long-linear-chain substrates has been increased more than the corresponding one for short-chain substrates by the mercaptosuccinylation procedure.

However, the succinylation of Taka-amylase A by treatment with succinic anhydride did not result in increases in either of the enzymic activities, in contrast to mercaptosuccinylation.

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