10. Participation of a Sulfhydryl Group in the Activity of Taka-amylase A

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It has been known that most α-amylases contained at least one atom of essential calcium for their activities.1) The essential calcium was shown to be reversibly removed from the enzymes with the exception of Taka-amylase A. Taka-amylase was irreversibly inactivated when the essential calcium was removed either on dialysis of the native enzyme against aqueous solution of EDTA for a long time,2) on electrodialysis,3) or on denaturation with EDTA and 8 M urea. It has therefore been assumed that the calcium firmly bound with unidentified amino acid residues in the amylase molecule and performed as a key role to maintain the conformation of the molecule essential for its function.2,4)

We have attempted to remove the essential calcium reversibly from Taka-amylase A by elevating temperature of the incubation mixture of the enzyme with EDTA under alkaline conditions. When the amylase (2.5 × 10^{-7} M) was incubated with EDTA (10^{-3} M) at 50°C and pH 8.4 for 2 hours, the activity was lost as shown in Fig. 1. The activity was assayed at pH 5.3 and 30°C using amylose

![Fig. 1. Inactivation of Taka-amylase by EDTA and its reactivation by the addition of calcium.](image)

The amylase (2.5 × 10^{-7} M) was incubated with EDTA (10^{-3} M) at 50°C and pH 8.4. Dotted line shows the control experiment without EDTA. At the point indicated by the arrow, calcium acetate (10^{-3} M) was added at pH 8.4 and 50°C. Closed triangles show the activities when calcium acetate was added after the lowering the pH to 7.0 and the temperature.
as a substrate. The lost activity was recovered on addition of calcium, of which amount was slightly in excess of the remaining EDTA, prior to lowering the temperature and the pH of the reaction mixture. The recovered activities at periods of 2 hours and 16 hours after the addition of calcium were 50% and 70% of the intact amylase respectively. No activity was recovered when calcium was added after lowering the temperature and the pH of the incubate. These results suggested that the partial unfolding of the enzyme molecule was required for removal or for re-binding of the essential calcium.

It has also been known that Taka-amylase A contained one residue of cysteine, which existed as a masked state.$^6$,$^7$ The chelating agent, EDTA, was shown to be the most effective to unmask the sulfhydryl group. We have therefore attempted to elucidate the correlation of the unmasking of the sulfhydryl group with the above reversible inactivation of the amylase with EDTA. On incubation of the amylase with iodoacetate or iodoacetamide at pH 8.0 and 50°C, no inactivation resulted. In the presence of EDTA, however, quick inactivation occurred with iodoacetamide and the activity was not recovered after addition of calcium as shown in Fig. 2. The extent of inactivation of the enzyme with iodoacetamide was in parallel to the amount of introduced S-carboxyamidomethyl group, which was estimated as S-carboxymethyl-cysteine after acid hydrolysis of the protein, as shown in Fig. 3.
The lost activity of the amylase by incubating with EDTA and iodoacetate, however, was recovered on the addition of calcium as shown in Fig. 2. The recovered activity was about 15% of that of the native amylase. The velocities of the inactivation with EDTA (10^{-3} M) at pH 7.0 and 50°C of the native enzyme (4.3 \times 10^{-7} M) and the reactivated S-carboxymethyl amylase (SCM-amylase) (4.3 \times 10^{-7} M) were identical, suggesting that no damage occurred at the calcium binding sites. This result appears to suggest that one of the binding sites of calcium is not the sulfhydryl group. The binding sites of calcium in the amylase still remain unknown. The values of Km and Vmax, in the Michaelis-Menten equation measured at pH 5.3 and 30°C with amylose as a substrate, of the native amylase and the reactivated SCM-amylase were 0.15% and 1.34, and 0.05% and 0.39, respectively. These results suggest that carboxymethylation of the sole sulfhydryl group in the amylase has affected both of the states of the catalytic site and the substrate binding site. At any rate, the present results demonstrate the participation of the sulfhydryl group in the enzymatic function of Taka-amylase A. The amino acid sequence of the vicinity of the essential cysteine residue has previously been reported.7)

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References