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The Chemical Modification of Biopolymers. IV. The Introduction of Succinyl Groups into Bacterial α-Amylase

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The introduction of functional groups into enzymes has been studied for its effect on enzymic properties. As has been shown previously, the mercaptosuccinylation of Taka-amylase A[1] (EC. 3.2.1.1) caused an increase in the enzymic activity, but the enzymic activity was not elevated, but was lowered, by succinylation.

However, the introduction of succinyl groups into bacterial α-amylase (*Bacillus subtilis*, EC. 3.2.1.1) raised the activity.

The preparation of succinyl α-amylase and their properties has been studied.

Experimental

Activity Measurements. The hydrolytic activity of the native and modified α-amylase was measured by studying the decrease in absorbance at 700 mμ due to iodine-amylose complex formation (the blue-value method),[2] and by studying the increase in the reducing groups (the Somogyi-Nelson method).[3,4] The digestion was conducted at 37°C for 30 min in a 0.07 M acetate buffer.

The protein concentration was estimated by spectrophotometry with the extinction coefficient, $E^{1\%}_{1cm}$ of 25.3 at 280mμ,[5] or by the method of Lowry.[6]

Preparation of Succinyl α-Amylase. Succinyl α-amylase was prepared as follows: One milliliter of a succinic anhydride solution in dioxane (2.2×10^{-6} mol to 2.2×10^{-4} mol) was added to 20 m*l* of the buffered protein solution (4.4×10^{-7} mol in a 0.2 M phosphate buffer, pH 8.0) at 2—4°C. After 30 min, 6 m*l* of a 0.4M hydroxylamine solution in a 0.2M phosphate buffer (pH 7.5) were added in order to stop the reaction. The reaction mixture was then dialyzed against distilled water for 24 hr at 5°C. The extent of succinylation was estimated by studying the decrease in the amino groups by the method of Matsushima *et al.*[7]

Results and Discussion

Determining the Extent of Succinylation of α-Amylase. Figure 1 shows the effect of the concentration of succinic anhydride on succinylation at 2°C. The extent of the succinylation of lysyl residues increased in accordance with the increase in the concentration of succinic anhydride. Fifteen succinyl groups per mole of enzyme was obtained when a 500-fold mole excess was used.

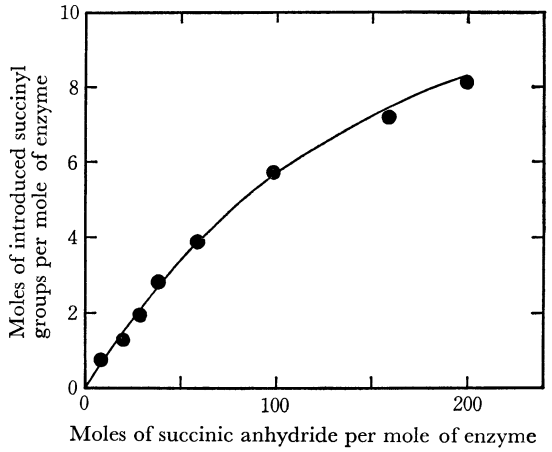

Fig. 1. The effect of concentration of succinic anhydride on degrees of succinylation of α-amylase.

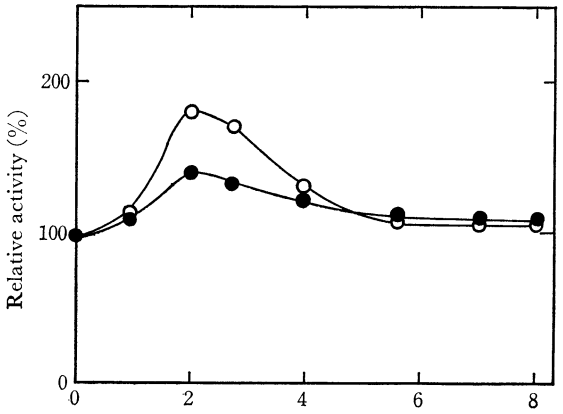

Fig. 2. The activities of the native and modified α-amylase on amylose measured at these pH optima in a 0.07 M acetate buffer and a 4.1×10^{-3}M amylose solution by the blue-value method (Curve A, open circle) and by the Somogyi-Nelson method (Curve B, closed circle).

Enzymic Activities of Succinyl α-Amylase. The enzymic activities of the modified enzymes are shown as curve A (the blue-value method) and curve B (the Somogyi-Nelson method) in Fig. 2. The enzymic activities were measured in a 4.1×10^{-3}M amylose solution and are expressed in percentages of the native enzymic activity. The enzymic reactions were carried out at the optimal pH values, which were shifted by the succinylation as is shown below. When two moles of succinyl groups were introduced per mole of enzyme, the activity on amylose, as measured by the blue-value method, increased to 180%, while that as measured by the Somogyi-Nelson method increased to 140%, of that of the native enzymic activity.

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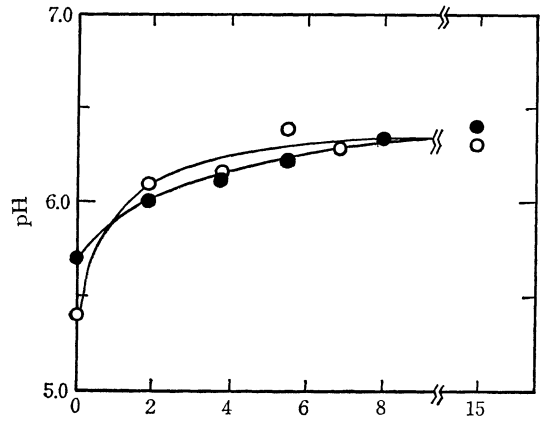

Fig. 3. The effect of the extent of succinylation of α-amylase on pH optima in a 0.07M acetate buffer and a 4.1×10^{-3}M amylose solution by the blue-value method (Curve A, open circle) and by the Somogyi-Nelson method (Curve B, closed circle).

pH Optima of Succinyl α-Amylase. The pH optima of the native and modified α-amylase are plotted against the numbers of the introduced succinyl groups by curve A (the blue-value method) and curve B (the Somogyi-Nelson method) in Fig. 3.

The pH optima were shifted higher with the progress of succinylation. The pH optima of the native enzymes were 5.4 and 5.7, as measured in a 4.1×10^{-3}M amylose solution by the blue-value method and by the Somogyi-Nelson method respectively, while the pH optima of the modified enzyme into which 15 succinyl groups per mole of enzyme has been introduced were 6.3 (the blue-value method) and 6.4 (the Somogyi-Nelson method).

Properties and Characteristics of Succinyl α-Amylase. The kinetic properties of the native and modified amylase with two succinyl groups per mole of enzyme were determined by the Lineweaver-Burk method.

The V_{max} values for the native and modified enzymes were 1.3×10^4 and 1.4×10^4 mol per min per mole of enzyme respectively.

However, succinylation decreased the K_m for amylose from 4.4×10^{-3}M^{-1} to 2.6×10^{-3}M^{-1}.

These results suggest that the increase in enzymic activity is due to the increase in affinity for amylose.

The reaction with succinic anhydride replaces the positively charged ammonium groups, NH_3^+, at a neutral pH with a $NHCOCH_2CH_2COO^-$ function. The changes that had been produced in the viscosity and sedimentation behavior of succinylated proteins would suggest a considerable expansion or unfolding of the molecular structure.

The $S^0_{20,w}$ values for the native and modified enzymes were found to be 4.4 and 5.0 respectively by ultracentrifugal analysis. That this is not primarily due to aggregation of the molecules is likely to be indicated by the fact that the change in the sedimentation constant is small and the ultracentrifugal patterns of the modified amylase showed a single well-defined peak. The native and the succinyl α-amylase show intrinsic viscosities of 0.024 *dl*/g and 0.021 *dl*/g respectively.

From these experiments, we have concluded that the effective volume occupied by the succinyl amylase molecule increased compared to that of untreated α-amylase, and that the increase in the affinity for amylose is likely to be due to the expansion of the molecular structure. As has been reported previously,[1] the succinylation of Taka-amylase A caused a decrease in the enzymic activity. The difference in the effects of succinylation on the two enzymic activities seem to be due to the extent of conformational change taking place by succinylation.