Note

Dissociation and Reassociation of *Xanthomonas* α -Amino Acid Ester Hydrolase

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In the previous work on α -amino acid ester hydrolase,¹⁾ we purified the enzyme from *Xanthomonas citri* IFO 3835 and investigated its enzymological properties. The molecular weight of the purified enzyme was estimated to be 270,000~280,000. In the presence of denaturing agents such as sodium dodecyl sulfate and guanidine HCl, the enzyme dissociated into 4 subunits each having a molecular weight of 72,000. In the present paper we describe the mild conditions used to induce tetramer-monomer interconversion of the enzyme molecule and the change in catalytic activity in the course of the interconversion.

Incubation of the enzyme with a high concentration of NaCl caused a drastic decrease in the enzyme activity (Fig. 1). The degree of inactivation depended on the con-

FIG. 1. Effect of Incubation with NaCl on the Activity of *Xanthomonas* α -Amino Acid Ester Hydrolase.

The purified enzyme solution (1.5 mg/ml in 5 mM potassium phosphate buffer, pH 6.0) was kept at -20°C or 5°C for 16 hr in the presence of various concentrations of NaCl. The residual enzyme activity was determined as described previously.¹⁾

○—○, 5°C ; ●—●, -20°C .

centration of NaCl, and the lower temperature accelerated the inactivation. On polyacrylamide gel electrophoresis with 5% acrylamide columns,²⁾ the inactivated preparation showed a single protein band (Fig. 2B) that migrated faster than that of the native enzyme (Fig. 2A). After this inactivated preparation was dialyzed against water, the electrophoretic mobility changed again and coincided with that of the native enzyme (Fig. 2C). These results suggested that the enzyme dissociated into subunits in the presence of NaCl, and that on removal of NaCl by dialysis they reassociated to form the native molecule. This was confirmed by gel filtration on a Sephadex G-200 column. As shown in Fig. 3, the inactivated preparation showed a protein peak (peak II) with a small shoulder at the higher molecular weight region, whereas the preparation obtained after dialysis of this inactivated preparation against water was eluted faster (peak I). The molecular weights of the peak I and peak II proteins were estimated to be 280,000 and 72,000, respectively (data not shown). The specific activities of the preparations corresponding to the peak I and peak II proteins as determined by the hydrolysis of D- α -phenylglycine methyl ester¹⁾ were 85% and 1.5% of that of the native enzyme, respectively.

The results of the present experiments show that the

FIG. 2. Polyacrylamide Gel Electrophoretograms of *Xanthomonas* α -Amino Acid Ester Hydrolase and Its Derivative Proteins.

(A), native enzyme; (B), inactivated preparation obtained by keeping the enzyme (1.5 mg/ml) at -20°C for 16 hr in 1 M NaCl-5 mM potassium phosphate buffer (pH 6.0); (C), preparation obtained after dialysis of preparation (B) against water at 5°C for 48 hr. Proteins were stained with Coomassie Brilliant Blue G.

FIG. 3. Gel Filtration on a Sephadex G-200 Column of the Enzyme-derived Proteins.

Protein samples (1 ml) were applied to a Sephadex G-200 column (2.3 × 25 cm) previously equilibrated with 0.01 M potassium phosphate buffer (pH 6.0) containing 1 M NaCl, and eluted with the equilibration buffer at a flow rate of 15 ml/hr.

○—○, inactivated preparation; ●—●, preparation obtained after dialysis of the inactivated preparation against water at 5°C for 48 hr.

Xanthomonas α -amino acid ester hydrolase dissociates into 4 inactive subunits each having a molecular weight of 72,000 in the presence of high concentrations of NaCl, and that removal of NaCl leads to reassociation of the subunits into a native tetrameric molecule having almost full catalytic activity. In our previous work on the enzymatic synthesis of amoxicillin with whole cells of *X. citri* IFO 3835, NaCl inhibited the reaction.³⁾ This may be explained

by inactivation of the enzyme through its dissociation into subunits described here.

Inactivation of the enzyme was also observed to a lesser extent in the presence of other salts such as NH_4Cl and KCl, which was accompanied by partial dissociation of the enzyme into subunits. These salts may diminish the ionic interaction on the surface of the enzyme molecule causing its dissociation. Acceleration at the lower temperature of dissociation of the enzyme in the presence of NaCl may be due to reduction of the hydrophobic bonding in the enzyme molecule. Horse hemoglobin was also shown to dissociate reversibly in NaCl solutions above 0.5 M.⁴⁾ The mechanism of dissociation and reassociation of the α -amino acid ester hydrolase depending on the salt concentration remains to be elucidated.

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