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A HIGHLY STABLE WATER-INSOLUBLE DERIVATIVE OF RIBONUCLEASE T_1

Hisashi ITO, Masaaki HAGIWARA, Iwao ICHIKIZAKI, and Kenji TAKAHASHI*
Department of Chemistry, College of Science and Engineering, Aoyama Gakuin University, Chitosedai, Setagaya-ku, Tokyo 157
*Department of Biochemistry, Primate Research Institute, Kyoto University, Inuyama, Aichi 484

A water-insoluble derivative of ribonuclease T_1 (RNase T_1) was prepared by chemically combining RNase T_1 with a copolymer of acrylamide and divinylbenzene (Enzacryl AH), and its enzymatic properties were examined. The enzyme derivative retained much of its original activity and was considerably more stable to heat and extremes of pH than the native enzyme.

Immobilized derivatives of RNase T_1 have been prepared by Kuriyama and Egami[1] and Lee[2] using polysaccharide derivatives as carriers, but their value in practical use appears to be somewhat limited because of their low activity toward RNA. We have succeeded in immobilizing RNase T_1 by binding the enzyme chemically to a cross-linked copolymer of acrylamide and divinylbenzene (Enzacryl AH, Koch-Light Co., Ltd.), and examined several characteristics of the preparation.

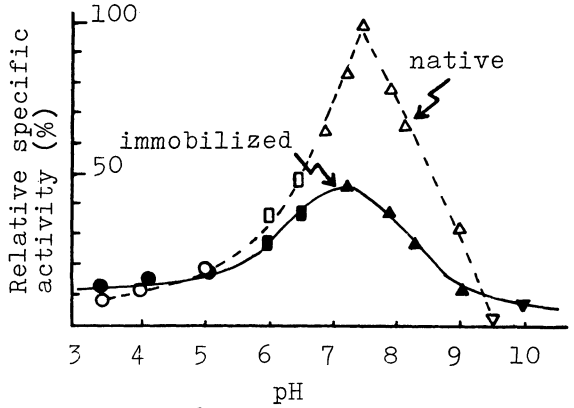
Preparation of Immobilized RNase T_1. Binding of RNase T_1 to Enzacryl AH was performed essentially by the method of Baker and Somers.[3] RNase T_1 (2.0 mg) in borate buffer (50 mM, pH 8.5, 9 ml) was allowed to couple with the acid azide derivative of Enzacryl AH (200 mg) at 0-5 °C for 48 hrs. The protein content of the preparation was estimated to be 3.2 mg per g (dry weight) by amino acid analysis of a portion of the preparation after acid hydrolysis (6 N HCl, 110 °C, 24 hrs.).

Determination of RNase Activity. a) With yeast RNA. The method of Takahashi[4] measuring the absorption of acid soluble hydrolysate at 260 nm was adopted. b) With 2',3'-cyclic guanosine monophosphate (2',3'-cyclic GMP). The method used was a modification of that of Takahashi et al.[5] The reaction was performed in 0.05 M Tris-sulfate buffer, pH 7.3, containing 0.1 % 2',3'-cyclic GMP. After 30 min of incubation, 0.025 M $ZnCl_2$ solution was added to the reaction mixture, then 3'-GMP formed were determined by high pressure liquid chromatography.

Properties of Immobilized RNase T_1. The activity of the immobilized RNase T_1 toward RNA at pH 7.5 was 45 % of that of the native enzyme (Fig. 1). On the other hand, the activity of the immobilized enzyme using CM-cellulose as a carrier was reported to be only 2 %.[2] The activity of our preparation toward 2',3'-cyclic GMP was 77 % of that of the native enzyme. pH dependences of the enzymatic activity of the native and bound enzymes toward RNA are shown in Fig. 1. The optimal

pH of the immobilized enzyme was shifted slightly to acidic pH. Immobilized RNase T_1 showed an apparent Km value (about 1 mg/ml) toward RNA roughly similar to that of the native enzyme. It exhibited a very large increase in stability in solution over a wide pH range (i. e. pH 1-10). Figure 2 demonstrates the stability of immobilized RNase T_1 at 100 °C. Further, the activity toward RNA of the immobilized enzyme assayed at 80 °C was 67 % of that at 37 °C, whereas the native enzyme was inactive when assayed at 80 °C. The emzyme derivative was able to be used repeatedly for digestion of RNA without significant loss of activity.

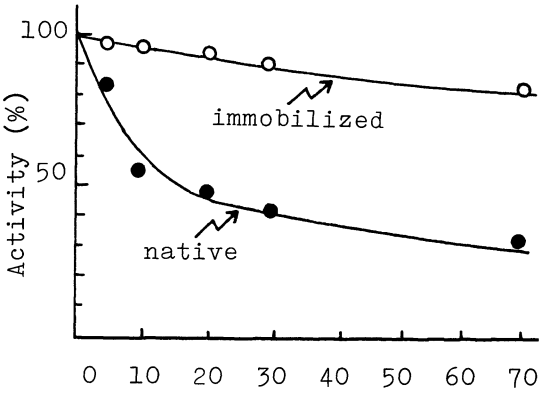
Fig. 1 Enzymatic activity of native and immobilized RNase T_1 as a function of pH



○,●: Sodium acetate buffer
□,■: Sodium acetate-Tris-HCl buffer
△,▲: Tris-HCl buffer
▽,▼: Glycine-NaOH buffer

The maximum value for native RNase T_1 is taken as 100 %.

Fig. 2 Heat stability of immobilized RNase T_1



Each sample was heated at 100°C in 0.05 M Tris-HCl buffer, pH 7.5, at a protein concentration of about 0.5 μg/ml, then assayed under the standard conditions at 37°C.

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