Note

Effects of Monoclonal Antibody on Guinea Pig Liver Transglutaminase Activity

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Transglutaminase (EC 2.3.2.13) catalyzes three reactions; incorporation of primary amines into glutamine residues of proteins, polymerization of proteins through the formation of intermolecular ϵ -(γ -glutamyl) lysine crosslinks between lysine and glutamine residues, and deamidation of glutamine residues in proteins. Transglutaminases are widely distributed in various tissues and organs,^{1,2)} and a number of biological functions have been proposed for them (reviewed in ref. 3). Characteristics of the catalytic actions of transglutaminase have also been shown to be suitable for applied enzymology, such as improvement of the functional properties and nutritive values of food proteins,^{4~7)} and the formation of soluble-insoluble interconvertible NAD⁺ and enzymes.^{8,9)}

We have recently succeeded in obtaining hybridoma clones that produce monoclonal antibodies against guinea pig liver transglutaminase¹⁰⁾ and have used them for a specific immobilization¹¹⁾ and purification of this enzyme. In this paper we report that the monoclonal antibody produced by one of the hybridoma clones inhibits only one of the three reactions manifested by transglutaminase, polymerization of protein, but does not influence the other two. An attempt to figure out the mechanism of the observed inhibitory effect is also described.

Transglutaminase was purified from guinea pig liver by the method of Connellan *et al.*¹²⁾ α_{s1} -Casein was prepared from cow's milk by the method of Zittle and Custer.¹³⁾ Acetylation of α_{s1} -casein was done by the method of Fraenkel-Conrat.¹⁴⁾ Monoclonal antibodies were produced in mice using a hybridoma clone, 8D, and were purified as described previously.¹¹⁾ Transglutaminase-monovalent antibody complex was prepared by incubating the enzyme (0.1 mg/ml) with a saturating amount of monoclonal antibody (1 mg/ml) in 10 mM Tris-HCl buffer (pH 7.5) containing 0.15 M NaCl, 1 mM EDTA, and 0.1 mM dithiothreitol at 25°C for 90 min before use; virtually

complete (>95%) complexation of the enzyme with the antibody was verified by the immunoprecipitation assay.¹⁰⁾ The prepared complex was stable in every transglutaminase assay system used in this study. The standard transglutaminase assay mixture contained 50 mM Tris-HCl buffer (pH 7.5), 5 mM CaCl₂, 10 mM dithiothreitol, 170 μ M native or acetylated α_{s1} -casein, and the enzyme sample. The amine incorporation into native and acetylated α_{s1} -casein was assayed by the filter paper method¹¹⁾ using [³H]histamine as a primary amine substrate. The deamidation of glutamine residues in acetylated α_{s1} -casein was assayed by measuring ammonia released during the reaction.⁴⁾ The polymerization of native α_{s1} -casein was followed by analyzing the products with SDS*-polyacrylamide gel electrophoresis.¹⁵⁾

In the incorporation of amines into protein using histamine and acetylated α_{s1} -casein as substrates, the binding of the monoclonal antibody to transglutaminase did not affect the *K_m* of the amine substrate (histamine; 0.35 mM) and the protein-bound glutamine substrate (acetylated α_{s1} -casein; 0.76 μ M), and *V_{max}* value. The rate of deamidation of glutamine residues in acetylated α_{s1} -casein was also not affected by the binding of the monoclonal antibody to the enzyme (data not shown).

FIG. 1. SDS-Polyacrylamide Gel Electrophoresis Patterns of α_{s1} -Casein Incubated with Free and Monoclonal Antibody-bound Transglutaminase.

The reactions were done with 10 μ g/ml transglutaminase protein. (A) Free enzyme. (B) Monoclonal antibody-enzyme complex. Other conditions for the reaction were described in the text. Electrophoresis was done in a 3% stacking gel and 10% separating gel at constant voltage (40 V for 14 hr). Migration is from top to bottom. Samples on panels (A) and (B) are, from left, 0, 10, 20, 30, 60, and 120-min products. The arrow indicates bands of monomeric α_{s1} -casein.

* SDS, sodium dodecyl sulfate.

When native α_{s1} -casein is incubated with transglutaminase, it is easily polymerized through the formation of intermolecular ϵ -(γ -glutamyl) lysine crosslinks (Fig. 1A). However, the binding of the monoclonal antibody to the enzyme significantly inhibited this polymerization (Fig. 1B). The monoclonal antibody to human erythropoietin,¹⁶⁾ which is the same immunoglobulin subclass (IgG₁) with the monoclonal antibody used here and was used as a control for this experiment, showed no repressive effect (data not shown).

Transglutaminase has two substrate sites; the amide site for the protein-bound glutamine substrate, and the amine site for the amine substrates, including the protein-bound lysine.¹⁷⁾ The catalytic reaction of this enzyme follows a modified double displacement mechanism of three steps¹⁷⁾: the first step is the formation of an acyl-enzyme intermediate between the enzyme and a protein-bound glutamine substrate (acyl donor); the second is the subsequent formation of a ternary complex of the acyl-enzyme intermediate and an amine substrate (acyl acceptor); and the final step is the acyl-transfer reaction. The results described above indicated that the binding of the monoclonal antibody to the enzyme causes no change in either the affinity of the amide site for the protein-bound glutamine substrate or that of the amine site for a small amine substrate such as histamine. Taking these findings into account, we guessed two mechanisms for the monoclonal antibody-induced inhibition of the protein polymerization; (I) the binding of the monoclonal antibody to the enzyme results in a decrease in the affinity of the amine site for the protein-bound lysine substrate, and (II) the monoclonal antibody bound to the enzyme hampers the final step of the crosslinking between glutamine and lysine residues, the acyl-transfer step.

Since it is difficult to measure the rate of protein polymerization quantitatively, we analyzed kinetically the histamine incorporation into native α_{s1} -casein catalyzed by free and monoclonal antibody-bound transglutaminases to examine the effects of the monoclonal antibody on the affinity of the amine site for the protein-bound lysine substrate. In these reactions, the lysine residues of native α_{s1} -casein competes with histamine for the binding to the amine site of the enzyme, and so the apparent K_m value of histamine is expressed by $K_m(1 + [I_o]/K_i)$, where $[I_o]$ is the initial concentration of protein-bound lysine substrate and K_i is the dissociation constant of the lysine substrate at the amine site. With both free and monoclonal antibody-bound enzymes, K_m values of histamine are identical as described above. Therefore, the apparent K_m value of histamine found at a constant $[I_o]$ depends on the value of K_i , which is a parameter reflecting the affinity of the amine site for the protein-bound lysine substrate. Figure 2 shows that the apparent K_m values of histamine measured under the same conditions with both free and the antibody-bound enzymes are exactly the same, indicating that the binding of the monoclonal antibody to transglutaminase does not affect the interaction between

FIG. 2. Lineweaver-Burk Plots for the Measurement of the Apparent K_m Value of Histamine in the Reaction of Histamine Incorporation into Native α_{s1} -Casein.

The concentration of enzyme protein was 10 μ g/ml. Assay conditions were described in the text. Initial velocity (v) is expressed as the increase in radioactivity (cpm) incorporated into native α_{s1} -casein. (●), free transglutaminase; (○) monoclonal antibody-transglutaminase complex. With both enzyme systems, the apparent K_m value of histamine was 0.48 mM.

the amine site of enzyme and the protein-bound lysine substrate. Thus mechanism (I) is unlikely. At present, although we do not have any direct evidence for mechanism (II), it seems reasonable to conclude that the binding of monoclonal antibody to transglutaminase inhibits the intermolecular crosslinking of proteins by hampering the acyl-transfer reaction, the final step of this catalytic process. The monoclonal antibody tested here probably, through its binding to the epitope of transglutaminase, causes steric hindrance of the acyl-transfer reaction in which a bulky acyl-acceptor like a protein-bound lysine is involved.

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