CONJUGATION OF MONOCLONAL ANTIBODIES 5B4D6 IMMOBILIZED ON IMMUNOSORBENT WITH CHELATING POLYMER

N. M. Mukhamedova and M. A. Slinkin

UDC 615.339:547.962.4].015.4.07

KEY WORDS: monoclonal antibodies; chelating polymer; chemical modification.

Selectivity of action of a preparation on damaged cells is the exception rather than the rule, as a result of the existence of similarity between membrane structures, functions, and metabolic pathways of normal and abnormal cells requiring treatment. In the search for a solution to the problem of targeted transport of drugs a number of alternative possibilities have been examined: regional administration, release due to changes in normal physiological parameters (pH, temperature) or of exposure to external factors (magnetic field), and binding with vector molecules, for which the affected zone serves as a natural target. The use of antibodies of polyclonal and monoclonal origin as the vector part of the transported system seems to be the most promising solution. Active agent/vector complexes have found clinical application not only in the treatment of various diseases, but also in diagnosis. The introduction of the gamma-camera has led to widespread use of preparations labeled with gamma-isotopes (^{123}I, ^{131}I, ^{111}In, ^{99m}Tc). The overwhelming majority of methods of labeling proteins with metals are based on preliminary modification of the protein by a chelating agent and subsequent binding of the chelating agent with the metal. The main criteria when choosing a chelating agent are: stability of the metal-ligand bond in vivo and maintenance of specificity of the antibodies.

This paper describes the study of changes in specificity of antibodies as a result of their modification by a chelating agent, namely the cyclic anhydride of diethylenetriaminepenta-acetic acid (ca-DTPA) and a chelating polymer (CP), namely a complex of succinylated polylysine with ca-DTPA (PL-DTPA).

EXPERIMENTAL METHOD

Typical conjugation of monoclonal antibodies (McAb) 5B4D6 to light λ-chains of human immunoglobins and polyclonal rabbit antibodies to acid glial fibrillar protein (AGFP) with the above-mentioned modifiers was carried out in accordance with the plan described in [5]. To create more sparing conditions for the reaction between McAb and PL-DTPA to proceed, a method of conjugation of these compounds was developed, including preliminary immobilization of the antibodies on the immunosorbent BrCN-sepharose 4B ("Pharmacia") with immobilized human IgG. The ligand, in this case human IgG, was fitted in accordance with recommendations of the manufacturer. The ready-made sorbent was kept in the form of a suspension in borate buffer. The final capacity of the carrier was 5 mg/ml. To 0.5 ml of sorbent was added 0.5 mg McAb in 0.05 M borate buffer, pH 8.6 (concentration 2 mg/ml), and the mixture was incubated for 2 h with constant stirring at room temperature. The sorbent was then washed 3 times with borate buffer, the optical density of the supernatant being measured each time (λ = 280 nm). Of the total quantity of antibodies added 80% bound with the matrix ligand. The chelating polymer was activated by the standard method [5] and mixed with the immunosorbent The molar ratio polymer/protein = 1/2 was observed, allowing for

Institute of Experimental Cardiology, Cardiologic Scientific Center, Russian Academy of Medical Sciences, Moscow (Presented by Academician of the Russian Academy of Medical Sciences V. N. Smirnov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 12, pp. 590-592, December, 1992. Original article submitted May 27, 1992.

 0007-4888/92/0012-1776$12.50

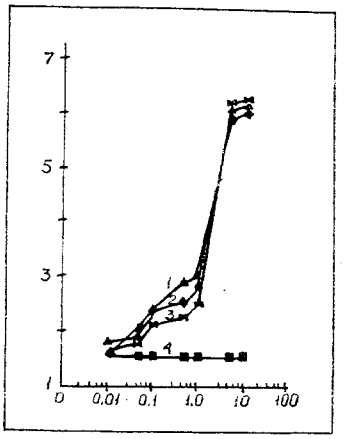

Fig. 1. Titration curves of native and modified antibodies to AGFP in RIA. Here and in Fig. 2 and 3, abscissa: quantity of antibodies introduced into well (μg); ordinate, radioactivity (conventional units). 1) Native anti-AGFP; 2) anti-AGFP-DTPA; 3) anti-AGFP-PL-DTPA; 4) BSA.

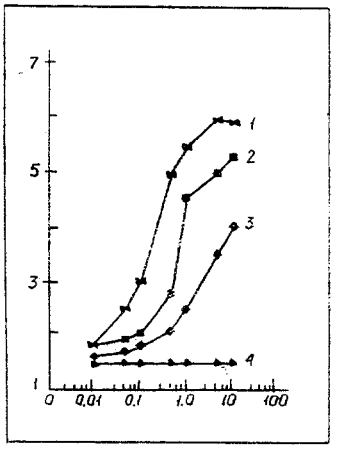

Fig. 2. Titration curves of native and modified monoclonal antibodies 5B4D6 in mixture in RIA. 1) Native 5B4D6; 2) 5B4D6-DTPA; 3) 5B4D6-PL-DTPA; 4) BSA.

the total quantity of protein, i.e., both monoclonal antibodies 5B4D6 and human IgG. After incubation for 2 h at room temperature, 1 ml of a solution of glycine (0.2 M NH_2CH_2COOH, 0.15 M NaCl, pH 2.8) was added to the reaction mixture to remove the conjugate from the matrix. Neutralization was carried out with 0.2 ml of 1 M Tris solution. After centrifugation (5 min, 3000*g*) the supernatant was dialyzed against phosphate buffer (0.5 M Na_2HPO_4, 0.15 M NaCl, pH 7.5, three changes in the course of 15 h). Unbound reagents and the resulting conjugates were

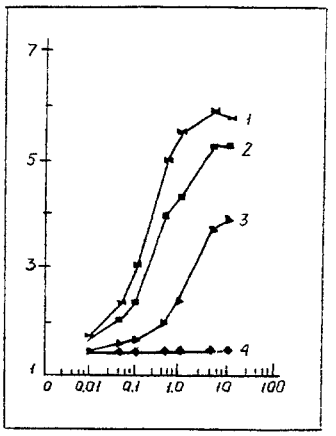

Fig. 3. Titration curves of monoclonal antibodies 5B4D6, modified under different conditions, in RIA. 1) Native 5B4D6; 2) 5B4D6 modified on BrCN-sepharose; 3) 5B4D6 modified in mixture; 4) BSA.

separated by successive gel-filtration and ion-exchange chromatography, the working procedure of which is described in detail in [1]. The degree of inactivation of the modified antibodies was determined by radioimmunoassay (RIA). As the antigen, solutions of AGFB or of IgG light chains were used. To allow for nonspecific binding of antibodies with the polystyrene of the planchet, instead of antigen a solution of bovine serum albumin (BSA) was introduced into the well as the control.

EXPERIMENTAL RESULTS

As Figs. 1 and 2 show the ability of the antibodies to bind with the antigen was reduced after modification with both ca-DTPA and PL-DTPA. However, the degree of inactivation depends, first, on the type of antibodies and, second, on the type of modification carried out. For instance, polyclonal antibodies to AGFB preserved their antigen-binding activity virtually completely after the two above-mentioned modifications (Fig. 1), whereas the monoclonal antibodies 5B4D6 underwent a marked decrease in their ability to bind with the antigen, a fourfold decrease in the case of binding with ca-DTPA, and a more than 20-fold decrease after conjugation with PL-DTPA (Fig. 2). To avoid this kind of inactivation we suggested a method of modification of antibodies immobilized on an immunosorbent by means of a chelating polymer. The results are given in Fig. 3. Clearly, with this method of chemical modification the antigen-binding capacity of 5B4D6 was preserved better than with antibodies modified in mixture (by 10 times), which can evidently be explained by the protection of the active center of $F(ab')_2$-region at the stage of ligation of the activated chelating polymer. Alternative versions of this protection are possible. Knight and co-workers, for instance, used ca-DTPA to treat a fibrinogen complex consisting of two molecules of the D-domain and one molecule of the E-domain. The active center located on the E-region and bound with the D-domain by a noncovalent bond, thus did not undergo chemical modification. Later the complex was dissociated in 3 M urea, and the E-subunits, carrying ca-DTPA on the inactive segment, formed protofibrlls [3]. It is better to use reagents reversibly bound with NH_2-groups of antibodies, such as, for example, dimethylmaleic anhydride [2]. Partial loss of the immunologic activity of 5B4D6 during this sparing modification can be explained by a change in conformation of the antibody molecule as a result of addition of the negatively charged polymer. A similar mechanism of inactivation in

fact takes place in the case of addition of polymers of the PL-DTPA type to antibodies, and this can be avoided by "ligation" of neutral CP [4].

REFERENCES

1. N. M. Mukhamedova, S. K. Obukhov, M. A. Slinkin, et al., Byull. Éksp. Biol. Med., No. 12, 705 (1989).
2. N. Endo, N. Umomito, Y. Kato, et al., J. Immunol. Meth., **104**, No. 12, 253 (1987).
3. L. C. Knight, M. Kollman, A. H. Maurer, et al., Biochim. Biophys. Acta, **924**, No. 1, 45 (1987).
4. "Preparation of At-211-IgG conjugate which is stable in vivo," Int. J. Appl. Radiat. Isot., **25**, No. 11, 1005 (1984).
5. V. P. Torchilin, A. L. Klibanov, and M. A. Slinkin, Appl. Biochem. Biotechnol., **29** (1989).