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IMMUNOCHEMICAL PROPERTIES OF IMMUNOGLOBULIN G, CONJUGATED WITH DEXTRAN

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Theoretical developments in physicochemical biology have demonstrated the possibility of deliberate modification of the pharmacologic properties of various drugs. As a result of progress in engineering enzymology (a rapidly developing field of physicochemical biology), success has been achieved in the creation of unique therapeutic substances by conjugating medically important enzymes with various polymers [6, 8, 10].

Such a deliberate change in the physiological and pharmacological activity of immunoglobulins [5, 14], proteins with an important role in the maintenance of homeostasis, could be of great practical value. The good prospects for the creation of long-acting immune preparations (of the immobilized enzyme type) are largely determined by preservation of the therapeutic and prophylactic activity of antibodies after incorporation into the polymer matrix.

The aim of this investigation was to study specific antigen-binding activity of antibodies and their effector functions after conjugation of immunoglobulin molecules with dextran.

EXPERIMENTAL METHOD

Immunoglobulin G (IgG) from the blood serum of hyperimmunized rabbits, specific for sheep's red blood cells (SRBC), and IgG from horse serum, isolated by ion-exchange chromatography in DEAE-cellulose [3], served as the model proteins in our experiments. The IgG were conjugated as described previously [9] (with certain modifications), with dextran (mol. wt. 35-50 kD), with protein and dextran in the reacting mixture in a ratio (w/w) of 1:6.

The physicochemical properties of the conjugates were studied by gel-chromatography on Toyopearl gel, ultracentrifugation in a sucrose density gradient [12], and polyacrylamide gel

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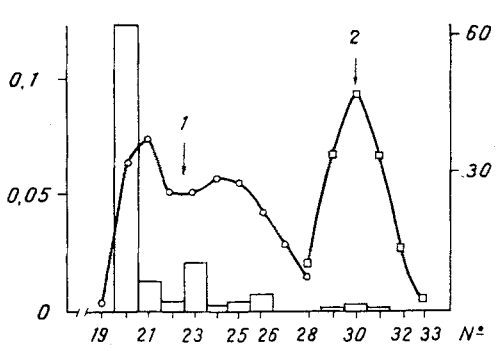

Fig. 1

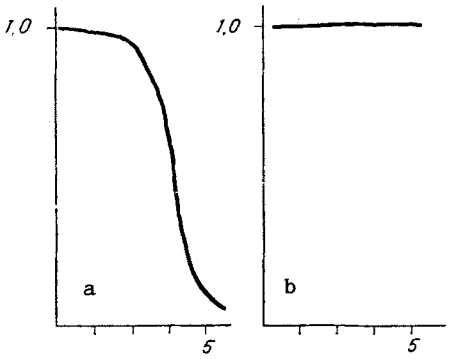

Fig. 2

Fig. 1. Distribution of hemagglutinating activity (columns) by gel-chromatography profiles, of native rabbit IgG against SRBC (2), and of IgG conjugated with dextran (1). Abscissa, nos. of fractions; ordinate: on left — optical absorbance at wavelength of 280 nm (relative units); on right — hemagglutinating activity of IgG — reciprocal of minimal quantity of protein inducing a visible hemagglutination phenomenon (mg^{-1}).

Fig. 2. Typical experimental curves of immune hemolysis of erythrocytes sensitized with native specific IgG (a) and IgG conjugated with dextran (b). Abscissa, time after beginning of recording (in min); ordinate: optical density at wavelength of 500 nm (relative units).

gradient electrophoresis with sodium dodecylsulfate [13]. The specific activity of antibodies against SRBC was tested by the double dilutions method with a microtitrator in the hemagglutination and immune hemolysis test, using traditional (panels of the microtitrator) and kinetic methods in the latter case [2]. The anticomplementary activity of the conjugates was tested by kinetic titration and estimation of its level by means of the inhibition index of complement [4].

The ability of IgG to interact with Fc-receptors of mouse peritoneal cells was studied in the rosette formation test [11].

EXPERIMENTAL RESULTS

As a result of modification of rabbit IgG by dextran conjugates with different molecular weights were obtained (Fig. 1). Sedimentation analysis of the individual fractions obtained by chromatographic separation demonstrated changes in the sedimentation coefficients from fraction to fraction: fraction 20 contained a conjugate with sedimentation coefficient of 15.2S, fraction 21 — 13.8S, 22 — 10.1 and 11.1S, 23 — 8.5 and 10.7S, 24 — 8.6 and 10.7S, and 25 — 8.4 and 10.7S; the content of the component with a coefficient of 8.5S, moreover, was increased considerably on passing from fraction 23 to fraction 25. The initial IgG protein had an average sedimentation coefficient of 7.6S, and contained traces of aggregates with a sedimentation coefficient of 9.6S.

The horse IgG conjugates contained fractions with sedimentation coefficients of 8.7, 11, 13, and 20S; the average sedimentation coefficient of the native protein was 7.6S.

Essential heterogeneity of the conjugates by molecular weight from 90 to 450 kD was discovered by gradient electrophoresis, and just like the data of sedimentation analysis, this indicates the presence of complexes of protein—dextran—protein and protein—dextran type in the total fraction of conjugates. The distribution of antibody activity among fractions in conjugates of IgG and native protein is shown in Fig. 1. The fact will be noted that it was the "heaviest" conjugates, eluted in the zone of void volume of the chromatographic column that possess exceptionally high hemagglutinating activity compared with native IgG. The dextran used for conjugation itself did not possess hemagglutinating activity.

This phenomenon is evidently the result of structural homology of conjugates of protein—dextran—protein type and of multivalent antibodies of the IgA or IgM type, which possess antigen-binding activity 1000 times greater than the bivalent antibody [7].

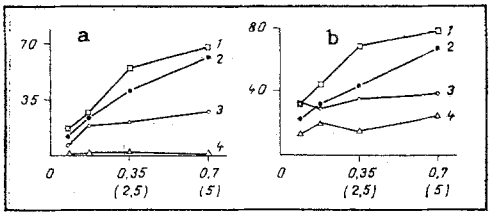

Fig. 3. Anticomplementary activity (ACA) of native IgG (1), of IgG conjugated with dextran (2, 3— different batches), and of dextran (4). Abscissa, concentration of substance, in mg protein or dextran (in parentheses)/ml; ordinate, ACA index (in %). a) Depending on value of induction period, b) on velocity of hemolysis.

In the course of the immune hemolysis reaction on microtitrator panels it was found that IgG specific against SRBC, in the presence of guinea pig complement, possesses marked lytic activity up to an IgG concentration in the well of 40 μg/ml.

Conjugated IgG had virtually none of this type of activity when the initial protein concentration in the composition of the conjugate was 3 mg/ml. A similar result was obtained by the method of kinetic hemolysis (Fig. 2). This experiment showed that hemolysis of erythrocytes sensitized with conjugates does not take place whatever the activity of complement, unlike lysis of erythrocytes sensitized with native protein.

The results are evidence that addition of the glycoside residues of dextran not only screens important functional sites of the IgG molecule mechanically, but also significantly limits the ability of the globular structure of the protein to undergo the usual conformational changes in the course of the antigen–antibody reaction, leading to release of the $C_\gamma 2$ domain, for although the antigen-binding activity is preserved, ability to interact with the complement system (to activate it in the classical way) is virtually absent. These hypotheses were confirmed by investigation of the anticomplementary activity of conjugates of horse serum IgG and native protein (Fig. 3).

The anticomplementary activity of native IgG, associated with the presence of spontaneously aggregated forms (contaminants with sedimentation coefficients of 9.6S) in its composition, was virtually unchanged or reduced in the total fraction of conjugates. Native dextran had virtually no anticomplementary activity. Under the influence of conjugation of IgG with dextran, another effector function of immunoglobulin changed significantly: ability to interact with Fc-receptors of cells. When mouse peritoneal exudate cells and SRBC, sensitized with native IgG, were used in the rosette formation test, the percentage of cells (calculated with the aid of the median [1]) forming rosettes with SRBC was 18 (9.8-20.9; $p \leq 0.05$), whereas when the total fraction of conjugated IgG with the same antibody titer as native IgG was used to sensitize the SRBC, the value of this parameter fell to 8 (7-9.8). The level of spontaneous rosette formation was 1.5%.

To summarize the experimental data it can be concluded that conjugation of IgG with dextran under the chosen conditions leads to the formation of compounds of the protein–dextran–protein and protein–dextran types; under these circumstances specific activity of the antibodies either is unchanged or is intensified, whereas the effector functions are significantly depressed.

The results show that good prospects exist for the use of such compounds as a basis for the creation of immune preparations of a new type, with functional properties controllable in a desired direction.

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