ANTI-GLYCOSYL ANTIBODIES: PREPARATION AND CHARACTERIZATION OF RABBIT ANTI-GALACTOSE AND ANTI-LACTOSE ANTIBODIES

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SUMMARY: Anti-galactose and anti-lactose antibodies have been isolated from the antisera of rabbits immunized with non-viable cells of Streptococcus faecalis, strain N containing an antigenic diheteroglycan of glucose and galactose in the cell wall. The anti-galactose antibodies are specific for the galactosyl moiety while the anti-lactose antibodies are specific for the lactosyl moiety of the diheteroglycan. Hapten inhibitions with galactose and lactose, the sedimentation constant, the immunoglobulin type, the carbohydrate content, the electrophoretic mobility and the amino acid composition have been determined for the two new types of anti-glycosyl antibodies.

Anti-glycosyl antibodies with specificity for carbohydrate antigens from the Pneumococci (1), the Streptococci (Group A and C)(2), and the Leuconostoc (3) have been available for some time and a few of these antibodies have been shown to possess homogeneity in molecular size and structure (4-6). The latter preparations are of special value for studies pertaining to antibody diversity and the genetic regulation of antibody synthesis (7,8). Two new types of anti-glycosyl antibodies have now been isolated from antiserum of rabbits immunized with a vaccine of a Group D organism, Streptococcus faecalis strain N, which contains an antigenic glycan of glucose and galactose in the cell wall (9). One type, anti-galactose (anti-gal), possesses specificity for the galactose moiety while the other, anti-lactose (anti-lac), possesses specificity for the lactosyl moiety of the glycan. The isolation procedure involved chromatography of the antiserum on a lactosyl-sepharose column, removal of the serum protein with phosphate buffer and elution of the antibodies with galactose and lactose solutions. The anti-gal and anti-lac antibodies yield positive

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precipitin reactions with the diheteroglycan when tested by the capillary precipitin and the double diffusion in agar tests. Hapten inhibition studies with galactose and lactose showed that the lactose was an extremely potent inhibitor of both antibody preparations but the galactose was inhibitory only for the anti-gal preparation. Both the anti-gal and the anti-lac antibodies exhibit a high degree of molecular uniformity on sedimentation in sucrose density-gradient columns. The sedimentation constant calculated from these data was 6.9S for both antibodies. The proteins in the rabbit antibody preparations are of the IgG type as shown by reactivity with anti-IgG serum. The latter is in contrast with equine antibodies against the same organism which were shown to be of the IgM type (10). The carbohydrate constituents of the antibodies comprise less than 1% of the total molecule and consist of galactosamine and neuraminic acid most likely in the acetylated form. The carbohydrates, fucose, mannose, galactose and glucosamine, normally present in immunoglobulins (11,12) were not detectable in the new antibodies. Gel electrophoresis revealed the presence of six distinct proteins in the anti-gal preparation and nine proteins in the anti-lac preparation. Since the proteins in each preparation possess antibody activity and react with the same structural unit of the antigen, the term isoantibodies is appropriate for members of each group. The amino acid composition of the two antibody preparations is remarkably similar raising some questions about the mechanism and the regulation of antibody synthesis.

METHODS

Isolation of Anti-gal and Anti-lac Antibodies - The preparation of the vaccine of Streptococcus faecalis, strain N and the immunization regime has been described in previous publications (9,13). Rabbit antiserum samples from the twelvth to fourteenth week of immunization were used for the isolation experiments. A sample of 2 ml of the antiserum was passed through a lactosyl-sepharose column (14) and the unabsorbed serum proteins were washed through the column with saline - 0.1M phosphate buffer of pH 7.2. The antibodies which were absorbed on the column were then eluted with 0.5M galactose solution and 0.5M lactose solution. Finally, the column was washed with 0.1M acetic acid solution. The eluates from the column were monitored for UV absorbing components by an ISCO UV analyzer. A portion of a typical elution pattern is shown in Fig. 1. The fractions corresponding to the UV absorbing material eluted by the galactose and the lactose were combined separately and

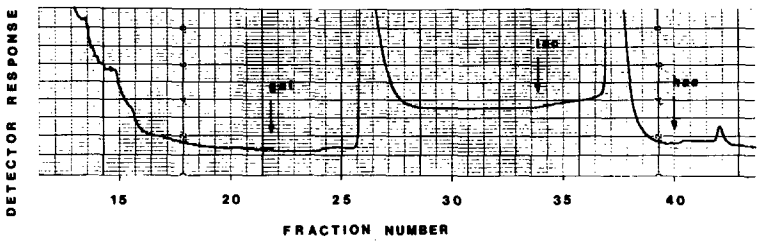

Fig. 1 Elution pattern for rabbit antiserum from a lactosyl-sepharose column: gal (0.5M galactose solution), lac (0.5M lactose solution) and hac (0.1M acetic acid solution).

stirred with an equal volume of saturated ammonium sulfate solution. The precipitates which formed on refrigeration overnight were collected and dissolved in a small volume of saline - 0.1M phosphate buffer of pH 7.2. The anti-gal and anti-lac antibodies from five runs were combined and the resulting solutions were dialyzed exhaustively against the saline-phosphate buffer to remove traces of galactose and lactose. The dialyzed solutions contained about 50 mg of protein at a concentration of 0.5%. These solutions constitute the anti-gal and the anti-lac antibody preparations.

Reaction of the Antibody Preparations with the Diheteroglycan - The antibody preparations were tested with a 0.1% solution of the diheteroglycan by the capillary precipitin method. Copious amounts of precipitate formed rapidly in tests conducted with both types of antibodies. The antibodies and the diheteroglycan solution were also tested for the formation of precipitin bands by the method of double diffusion in agar. Sharp bands of antigen-antibody complexes were obtained with both preparations and under the conditions of the experiments, the diffusion rates of the antibodies in the two preparations were very similar. Quantitative precipitin tests were performed by standard procedures and the optimum concentration of the antigen with both preparations of the purified antibodies was found to be 5 µg. This level of antigen and the solutions of antibodies were used in hapten inhibition studies with varying concentrations of haptens (10^{-3} to 10^{-2}M lactose and 10^{-2} to 10^{-1}M galactose). In the latter experiments, fifty percent inhibition of the anti-gal antibodies was obtained at a concentration of $2.5X10^{-4}$M lactose and of the anti-lac antibodies at a level of $1.5X10^{-3}$M lactose. With galactose 50% inhibition of the anti-gal antibodies was obtained at a level of 10^{-2}M galactose but with the anti-lac antibodies less than 5% inhibition was obtained even at levels of 10^{-1}M galactose.

RESULTS AND DISCUSSION

The sedimentation behavior of the antibodies was determined by centrifugation in sucrose gradient columns (5 to 40%) by procedures previously described (15) and typical sedimentation patterns are shown in Fig. 2. From these data and values for reference proteins (16), the sedimentation constant of 6.9S was calculated for both types of antibodies. Samples of both prep-

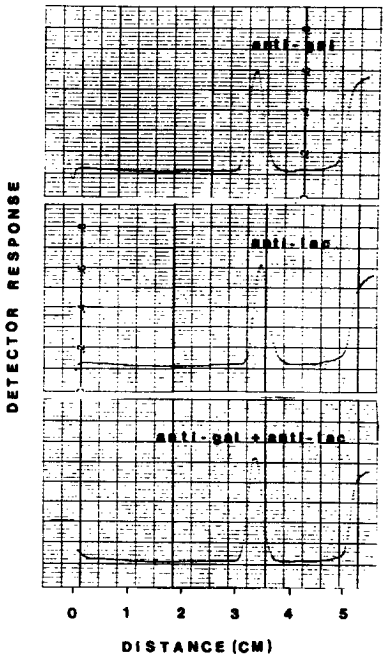

Fig. 2 Density-gradient centrifugation patterns for anti-gal, anti-lac and a mixture of equal parts of anti-gal and anti-lac antibodies in density gradient columns (5 to 40% sucrose): 0, the top of the column and 5, the bottom of the column.

arations were tested by the agar double diffusion method with goat anti-IgA, IgG and IgM sera. The anti-gal and anti-lac preparations reacted only with the IgG antiserum showing that proteins of both antibody preparations are IgG.

On acid hydrolysis of the antibodies, two reducing sugars, galactosamine and neuraminic acid identified by galactose oxidase (9,17) and color reactions (18,19), were liberated from each antibody type. Initial quantitative values show that both preparations possess very low, less than 1%, carbohydrate. Gel electrophoresis revealed that the antibody preparations each consist of isoantibodies. Fig. 3 shows that anti-gal antibodies consist of at least six isoantibodies while the anti-lac antibodies consist of at least nine, with the former isoantibodies reacting with the galactose moiety and the latter with the lactose moiety of the antigen.

The data obtained to date on the amino acid composition of the anti-gal

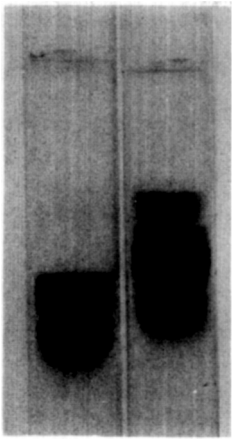

Fig. 3 Gel electrophoresis patterns for preparations of anti-gal and anti-lac antibodies.

and the anti-lac antibodies show that there is a remarkable similarity in the amino acid composition of the two sets of isoantibodies. This similarity exists despite the fact that the anti-gal preparation consists of six proteins and the anti-lac preparation consists of nine different proteins.

We have succeeded in isolating two types of anti-glycosyl antibodies from the antiserum of a rabbit immunized with a vaccine of S. faecalis. One type (anti-gal) is specific for the galactosyl moiety and the other (anti-lac) is specific for the lactosyl moiety of the antigen in the cell wall of the organism. Of special note is the high inhibitory activity of the lactose which has been found to be effective at extremely low concentrations with both preparations. This observation may be of value to X-ray crystallographers for studying conformation changes when an antibody combines with a hapten or an antigen.

The anti-gal antibodies are composed of at least six isoantibodies while the anti-lac antibodies are composed of at least nine isoantibodies. Recently a preliminary report has been published on the isolation of two species of antibodies with specificity for a polypeptide region (residues 53-69) of

staphylococcal nuclease (20). These species of antibodies are an example of another set of isoantibodies, in this case with anti-polypeptide specificity. Studies are being continued in our laboratory on the isolation of the individual isoantibodies by electrofocusing and preparative gel electrophoretic techniques. Preparations of pure isoantibodies will be used for studies on the nature of the light and heavy chains and on the amino acid composition and sequence of these interesting new anti-glycosyl antibodies.

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