IMMUNOCHEMICAL STUDIES ON A MOUSE MYELOMA PROTEIN HAVING SPECIFIC BINDING AFFINITY FOR 2-ACETAMIDO-2-DEOXY-D-MANNOSE*

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ABSTRACT

A transplantable BALB/c mouse plasmacytoma, MOPC 406, produces an immunoglobulin of the IgA class that has been studied immunochemically. Inhibition studies indicate that, among a series of haptens tested, the one which seems to satisfy best the antibody-combining site is methyl 2-acetamido-2-deoxy-β-D-mannopyranoside. The implications of this finding to the elucidation of the antibody-combining site are discussed.

INTRODUCTION

Myeloma proteins having antibody-like activity against stereochemically defined determinants have proved valuable in studies of the nature of antibody-combining sites[1–3].

In the course of screening 86 myeloma proteins for their ability to precipitate Salmonella lipopolysaccharides, it was found that the serum and the ascitic-fluid protein from animals having transplantable tumor MOPC 406 reacted by the Ouchterlony technique with the lipopolysaccharides of *Salmonella weslaco*, *S. kampala*, and *Escherichia coli* 031 (ref. 4). These lipopolysaccharides have been found to contain about 5 to 10 percent of 2-acetamido-2-deoxy-D-mannose[4,5]. The present paper presents experimental data on the immunochemical behavior of this myeloma protein.

MATERIALS

Myeloma protein. — Ascitic fluid from BALB/c mice bearing MOPC 406 was used for the studies to be described. The MOPC 406 protein is IgA and has a kappa-type, light chain[6] (see Fig. 1).

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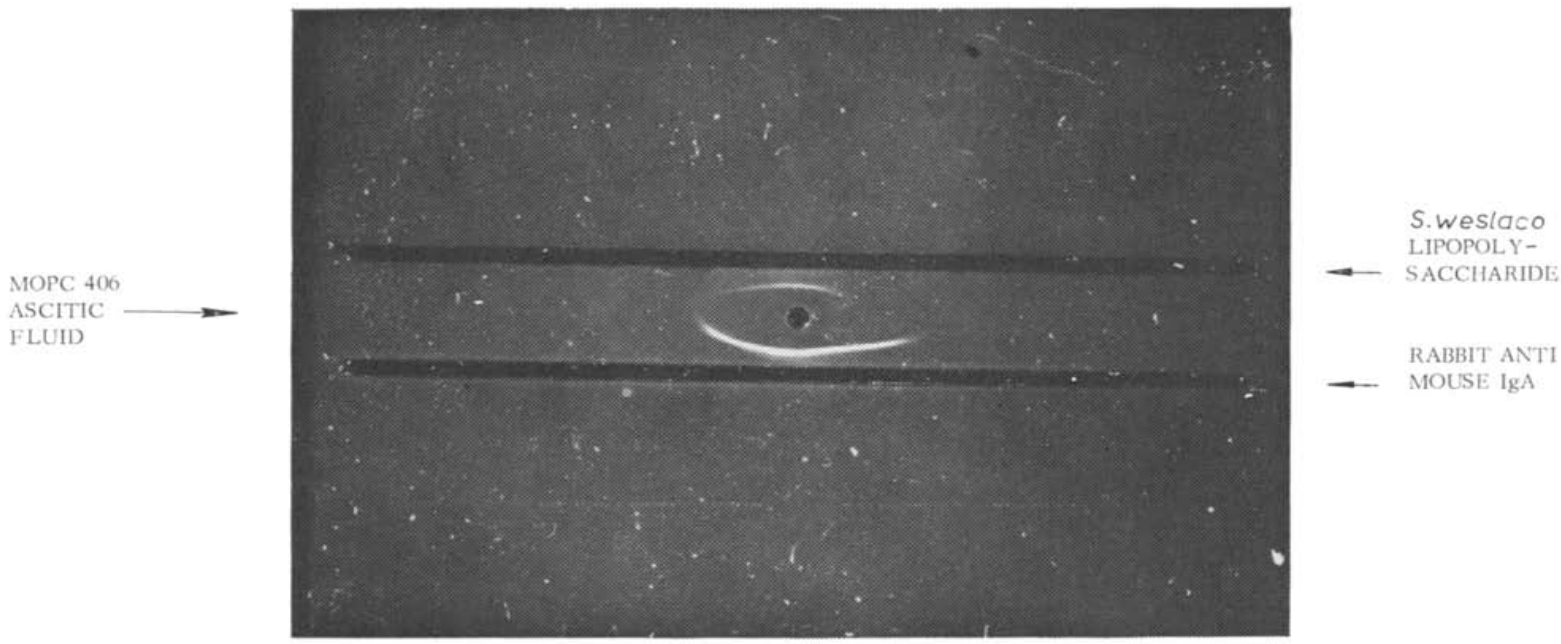

Fig. 1. Agar-gel immunoelectrophoresis of ascitic fluid from mice bearing MOPC 406.

Sugars, glycosides, and oligosaccharides. — Methyl 2-acetamido-2-deoxy-α- and β-D-mannopyranoside and methyl 2-acetamido-2-deoxy-α- and β-D-mannofuranoside were the products previously isolated in this laboratory[7] by treatment of 2-acetamido-2-deoxy-D-mannose with methanol in the presence of Amberlite IR-120 (H^+) ion-exchange resin, and separation, followed by preparative paper-chromatography. 2-Acetamido-2-deoxy-D-mannose was a commercial sample obtained from Pfanstiehl Co., Waukegan, Ill. The other monosaccharides and acetylated amino sugars used have been described earlier[8]. Methyl α-D-mannopyranoside was obtained from Mann Chemical Co., New York, N. Y.

Antigens. — The *S. weslaco* lipopolysaccharide (aqueous phase of the 55-percent phenol extract) was also available[4]. In addition, we tested MOPC 406 ascitic fluid for its ability to precipitate with the following antigens known to contain 2-acetamido-2-deoxy-D-mannose; group A meningococcus polysaccharide RV XV normal *O*-acetylated and FI, II low *O*-acetylated[9], pneumococcal polysaccharides type IV (ref. 10), type XIX (ref. 11), and type IX (ref. 12).

METHODS

Quantitative precipitin assays. — Quantitative precipitin tests and inhibition assays were performed on a microscale by using 25 μl of diluted, 1:3 ascitic fluid[13]. The total nitrogen in the washed, specific precipitates was determined by the ninhydrin method[14].

Immunoelectrophoresis. — Ascitic fluid from mice bearing the MOPC 406 plasmacytoma was electrophoresed in agar gel at pH 8.2 in 0.05M tris acetate buffer. A rabbit antiserum specific for mouse IgA (R93) was added to one trough, and the lipopolysaccharide of *S. weslaco* (4 mg/ml) was added to the other. The precipitin component for *S. weslaco* corresponds to the cathodic part of the IgA arc (see Fig. 1). A similar result was obtained[1] with the MOPC 315 protein by Eisen, Simms, and Potter.

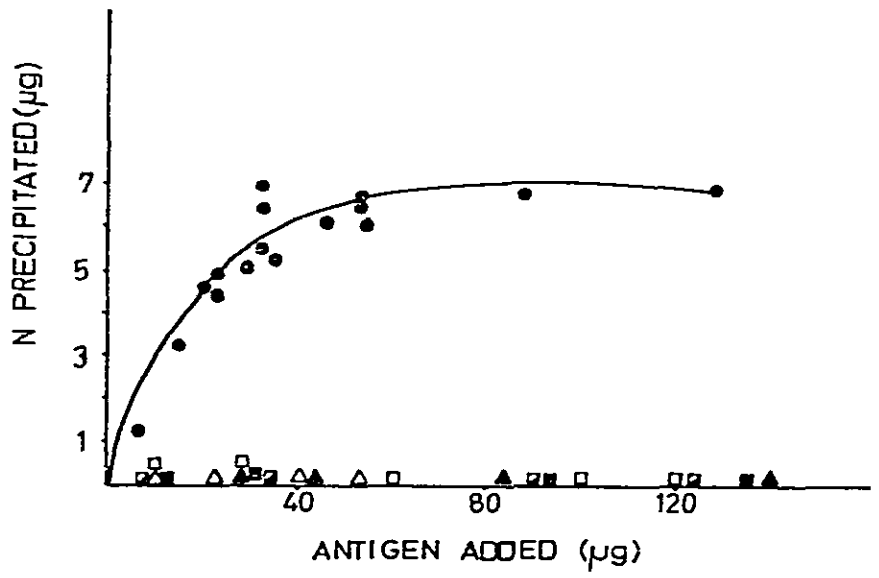

Fig. 2. Quantitative, precipitin curve of MOPC 406 ascitic fluid with *S. weslaco* lipopolysaccharide and various polysaccharides containing 2-acetamido-2-deoxy-D-mannose.
(Key: ● *S. weslaco* lipopolysaccharide; △ Group A RU XV, normal *O*-acetyl; ▲ Group A F I, II, low *O*-acetyl, meningococcus polysaccharides; □ Type IV, ■ Type IX, and ◪ Type XIX pneumococcal polysaccharides.)

RESULTS

The quantitative precipitin curve for the ascitic fluid and *S. weslaco* lipopolysaccharide is shown in Fig. 2. The same sample of ascitic fluid failed to react with the other polysaccharides containing 2-acetamido-2-deoxy-D-mannose.

The relative capacities of various monosaccharides, amino sugars, and methyl glycosides to inhibit the precipitin reaction between ascitic fluid MOPC 406 and *S. weslaco* lipopolysaccharide are shown in Fig. 3.

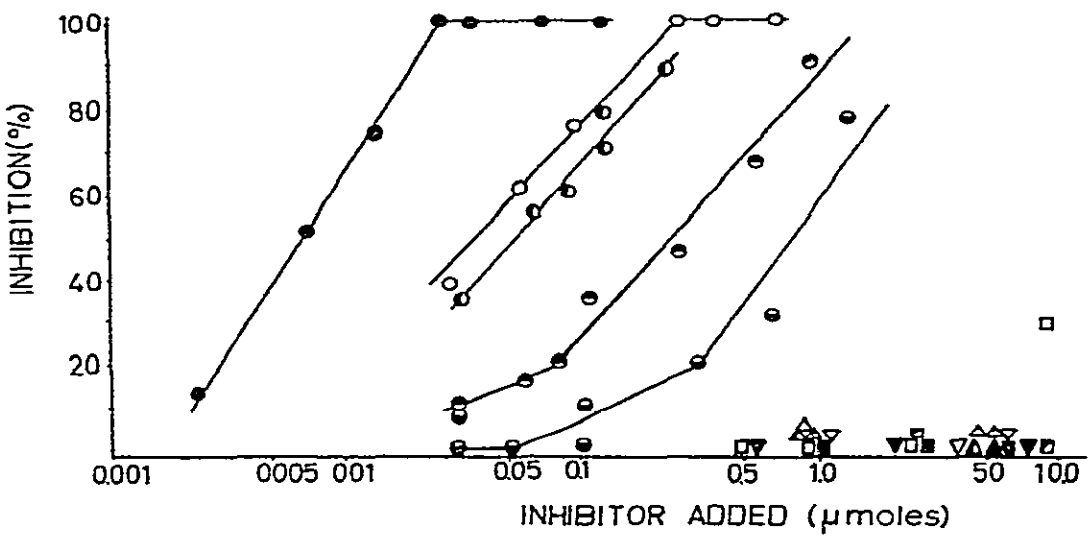

Fig. 3. Inhibition, by monosaccharides and methyl glycosides, of precipitation of 25 μl of MOPC 406 ascitic fluid diluted 1:3 by 36.2 μg of *S. weslaco* lipopolysaccharide; total volume 200 μl.
(Key: ○ 2-Acetamido-2-deoxy-D-mannose; ⊖ methyl 2-acetamido-2-deoxy-α-D-mannofuranoside; ⊖ methyl 2-acetamido-2-deoxy-β-D-mannofuranoside; ◐ methyl 2-acetamido-2-deoxy-α-D-mannopyranoside; ● methyl 2-acetamido-2-deoxy-β-D-mannopyranoside; ▼ methyl α-D-mannopyranoside; ■ D-mannose; □ 2-acetamido-2-deoxy-D-glucose; ◪ 2-acetamido-2-deoxy-D-galactose; ▲ 2-amino-2-deoxy-D-glucose; △ 2-amino-2-deoxy-D-galactose; ◭ D-glucose; ◮ L-fucose; and ▽ D-galactose.)

2-Acetamido-2-deoxy-D-mannose, a mixture of the various possible anomeric structures, gave 100-percent inhibition with 250 nmoles. With the α and β anomers of the furanoside and the pyranoside forms of methyl 2-acetamido-2-deoxy-D-mannose, the order of inhibition is β-pyranoside > 2-acetamido-2-deoxy-D-mannose > α-pyranoside > β-furanoside > α-furanoside. The best inhibitor is the β-pyranoside, which gives 50-percent inhibition with 6 nmoles. On a molar basis, it is 10 times more active than the α-pyranoside, 40 times more active than the β-furanoside, and about 130 times more active than the α-furanoside. Oligosaccharides containing 2-acetamido-2-deoxy-D-mannose were not available. Methyl α-D-mannopyranoside at 10.4 μmoles gave no inhibition

DISCUSSION

In comparing a series of inhibitors, the assumption is made that the one giving the greatest inhibition is the one that best fits into the antibody-combining site. A major limitation to the use of this principle is the difficulty in obtaining all possible variants of the structure under investigation. In the present study, the methyl α- and β-furanosides and α-and β-pyranosides of 2-acetamido-2-deoxy-D-mannose were available; the results given by them provide very strong evidence that the receptor site of the MOPC 406 IgA myeloma protein is specific for a β-D-linked 2-acetamido-2-deoxy-D-mannopyranoside residue, most probably as a nonreducing end-group. This is, of course, the minimum requirement, and the site could well be specific for a structure larger than a single β-D-linked 2-acetamido-2-deoxy-D-mannose residue. However, oligosaccharides of 2-acetamido-2-deoxy-D-mannose were unavailable to us.

It is of interest that a comparison of the best inhibitors in this system with those of myeloma protein J606 having specificity for levan[15], with the human dextran–antidextran system[16], and with *Helix pomatia* A hemagglutinin reacting with periodate-oxidized and Smith-degraded, human blood-group H substance[17], shows that about the same quantities of each were required for 50-percent and for 100-percent inhibition. As, in the first two systems, the sizes of the combining sites were estimated to be complementary to a trisaccharide and a hexasaccharide, respectively, the methyl 2-acetamido-2-deoxy-β-D-mannopyranoside may have as high an affinity for its receptor site as has the trisaccharide for the J606 myeloma protein (which belongs to the IgG3 subclass). Comparison with the J558 IgA myeloma protein[15] and the S117 IgA myeloma protein[3] having specificity for dextran and for nonreducing-terminal, β-D-linked 2-acetamido-2-deoxy-D-glucose residues, respectively, shows that much higher quantities of the respective oligosaccharide inhibitors were needed (300 and 130 nmoles) in these systems as compared with 6 nmoles for the methyl β-D-pyranoside of 2-acetamido-2-deoxy-D-mannose in the MOPC 406 system. Although such comparisons are essentially approximate (because of uncertainties in the number of reactive groups on the antigen and its molecular weight), they nevertheless suggest that the methyl β-D-pyranoside of 2-acetamido-2-deoxy-D-mannose may have a somewhat higher binding-affinity for its myeloma protein than a single

glycosidic unit, properly linked, has in the other myeloma globulin and antibody systems. This could, of course, be a property of the immunoglobulin synthesized by the particular clone represented by MOPC 406.

The finding that one of the four methyl glycosides of 2-acetamido-2-deoxy-D-mannose is much more active than the other three provides additional evidence for the very precise complementarity of the combining site of the MOPC 406 protein. It would be of considerable interest to determine the relative contributions of the hydrophobic portions of the molecule to the binding energy (*cf.* ref. 18).

It is of interest that free 2-acetamido-2-deoxy-D-mannose was only slightly more active than the methyl α-D-pyranoside (see Fig. 3), indicating that the α-D-pyranoside is the preponderant form in solution; this observation is in agreement with the optical rotatory dispersion data[7]. Both the immunochemical data and the o.r.d. and c.d. data clearly indicate that only minimal proportions of the furanosides of 2-acetamido-2-deoxy-D-mannose are present in solution.

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