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IMMUNOCHEMICAL CHARACTERIZATION OF THE SPECIFICITIES OF TWO HUMAN MONOCLONAL IgM's REACTING WITH CHONDROITIN SULFATES*,†

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ABSTRACT

We have studied the specificities of two human monoclonal, IgM containing sera, s/IgM^{MAC} and s/IgM^{FIS}, from patients with polyneuropathy. s/IgM^{MAC} precipitates only with chondroitin sulfate C and not with A and B whereas s/IgM^{FIS} is precipitated by chondroitins A, B (dermatan sulfate), and C. Inhibition assays using 2-acetamido-2-deoxy-3-*O*-(4-deoxy-β-L-*threo*-hex-4-enopyranosyluronic acid)-D-galactose and its 6- and 4-sulfate derivatives showed that the disaccharide 6-sulfate was the best inhibitor of precipitation of s/IgM^{MAC} by chondroitin sulfate C, and the disaccharide 4-sulfate the best inhibitor of precipitation of s/IgM^{FIS} by either chondroitin sulfates C or B. The nonsulfated disaccharide was a good inhibitor in each instance. D-Glucose 6-sulfate, Na_2SO_4, several sugar phosphates, and phosphate buffer also inhibited but to different extents with the s/IgM^{MAC} and s/IgM^{FIS}. All studies were carried out in 0.15M NaCl. The data indicate that both monoclonal proteins are antibodies comparable to the phosphorylcholine-binding myeloma proteins, and that the reactions show specificities above and beyond charge effects. The relation of various cross-reacting macromolecules to the monoclonal antibody was studied by diffusion in gels.

INTRODUCTION

An earlier study from these laboratories showed that the monoclonal IgM's from two patients with axonal polyneuropathy were reactive with chondroitin sulfates[1]. Serum from one, s/IgM^{MAC}, precipitated with chondroitin sulfate C but not with chondroitin sulfates A and B (dermatan sulfate), whereas serum from the

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other, s/IgM^{FIS}, precipitated with chondroitin sulfates A, B, and C. The monoclonal peak in both sera was removed by chondroitin sulfate C. s/IgM^{FIS} reacted with keratan sulfate, only ~50% of the maximum was precipitated. The monoclonal peak of s/IgM^{FIS} was removed by addition of a preparation from human axons[1]. The present investigation was an attempt to study further the specificities of the combining sites of these two monoclonal macroglobulins. They were found to differ in their specificity for sulfated 2-acetamido-2-deoxy-3-*O*-(4-deoxy-L-*threo*-hex-4-enopyranosyluronic acid)-D-galactoses, s/IgM^{MAC} reacting best with the 6-sulfate in accord with its reactivity with chondroitin sulfate C, whereas precipitation of s/IgM^{FIS} by chondroitin sulfates C and B was inhibited best by the disaccharide 4-sulfate. However, s/IgM^{FIS} was also inhibited by SO_4^{2+} and phosphate ions, and by sugar phosphates at micromolar concentrations comparable to the disaccharide 4-sulfate, whereas, with s/IgM^{MAC}, these compounds were considerably less effective than the disaccharide 6- or 4-sulfate. With both monoclonals, the nonsulfated disaccharide inhibited, but less effectively than the disaccharide sulfates. Unlike many studies with charged polysaccharides that were carried out at low salt concentrations, the precipitin and inhibition data were all obtained at a 0.15M concentrations of sodium chloride.

EXPERIMENTAL

Monoclonal proteins. — Serum containing monoclonal proteins s/IgM^{FIS} and s/IgM^{MAC} were available in the laboratory[1]. s/IgM^{FIS} contained 6.2 mg of the monoclonal IgM/mL and s/IgM^{MAC} 2.0 mg/mL.

Inhibitors. — The sulfated and nonsulfated disaccharides (*Δ*Di-4-*S*) 2-acetamido-2-deoxy-3-*O*-(4-deoxy-*α*-L-*threo*-hex-4-enopyranosyluronic acid)-D-galactose 4-sulfate; 2-acetamido-2-deoxy-3-*O*-(*α*-L-*threo*-hex-4-enopyranosyluronic acid)-D-galactose 6-sulfate (*Δ*Di-6-*S*); and 2-acetamido-2-deoxy-3-*O*-(*α*-L-*threo*-hex-4-enopyranosyluronic acid)-D-galactose (*Δ*-Di)[2] were from Miles Laboratories Inc. (Elkhart, IN 46515). Other compounds used were *α*-D-glucopyranosyl phosphate (Glc-1-*P*), D-galactose 6-phosphate (Gal-6-*P*), *α*-D-galactopyranosyl phosphate (Gal-1-*P*), D-glucose 6-sulfate (Glc-6-*S*) (Sigma Chemical Co., St. Louis, MO 63178) used as the potassium salts; D-galactose (Sigma), *β*-D-lactose (Eastman Kodak Co., Rochester, NY 14650), D-glucose and sodium pyruvate (ICN K & K Laboratories Inc. Plainview, NY 11803), sodium lactate (neutralized lactic acid from Mallinckrodt Inc., Science Products Div. St. Louis, MO 63134), sodium propionate (Fisher Scientific Co., Pittsburgh, PA 15219), sodium sulfate (Mallinckrodt), and phosphate buffer (pH 7.1).

Polysaccharides. — The following were available: Chondroitin sulfate[3] A, B (dermatan sulfate), and C (Sigma); Dextran sulfate (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, NJ 08854); *λ*-carrageenan (highly fractionated, Marine Colloids, Inc., Rockland, ME 04841); Bacto-agar (Difco Laboratories, Detroit, MI 48232), and successive fractions soluble at 20 and 50°

prepared from it[4]; and commercial agarose (Fisher). D-Galactans from the snail *Helix pomatia* and its first stage of partial periodate oxidation and Smith degradation[5] were obtained, as was pig pneumogalactan[6,7], from Dr. C. Glaudemans. Polysaccharides from K7[8], K21[9], K32[10], *Rhizobium trifolii* TA_1[11] and (through Dr. M. Heidelberger) types IV, XIV, and XXVII pneumococcal polysaccharides[12], and pneumococcal group C polysaccharide from the late Professor S. M. Beiser, gum ghatti, heparin, keratan sulfate (Sigma); preparations from chicken tumor hyaluronic acid[13]; and Hog A +H (ref. 14), and human A (MSS 10% 2X) blood-group substances[15] were available. Cross reactions in gels also included poly G and poly I (Miles).

Immunochemical assays[16,17]. — Quantitative precipitin assays were carried out by a microtechnic using a volume of serum containing ~5–8 μg N of the monoclonal protein for each determination. Increasing known quantities of the various polysaccharides were added, the volume was adjusted to 200 μL with saline (0.15M NaCl), and the contents of each tube mixed and placed for 1 h at 37°, and then for one week at 4° with daily mixing. The tubes were centrifuged at 4° at 2000 r.p.m., the supernatants decanted, and the precipitates washed twice with 0.5 mL of chilled saline. Total N in the washed precipitates was determined by the ninhydrin method[18]. Quantitative-inhibition assays were performed with quantities of antigen and serum giving maximum precipitation. Varying quantities of the sulfated disaccharides and other inhibitors were added to the serum diluted with saline; after 1 h at 37°, the polysaccharide was added, the contents of each tube mixed and kept for 1 h at 37°, and for one week in the refrigerator, washed, and the precipitate analyzed for N as just described. Percent inhibition was calculated from the quantity of N precipitated in the presence and absence of inhibitor. Gel diffusion was by the Ouchterlony technique[19] using 1% agarose in 0.15M NaCl with 0.02% sodium azide; in some instances 0.15M NaCl plus 10mM phosphate buffer at pH 7.2 was used. s/IgM^{MAC} was used undiluted and had to be concentrated 3-fold for the cross reactions to be more distinct; s/IgM^{FIS} was used as undiluted serum and diluted 1:5.

RESULTS

Quantitative precipitin curves with various polysaccharides, and sera MAC and FIS are presented in Fig. 1A and 1B. s/IgM^{MAC} was most highly specific: it reacted strongly with chondroitin sulfate C and not with chondroitin sulfates A and B. It also reacted, but to a much lesser extent, with dextran sulfate and with λ-carrageenan. It did not react with a substantial number of other polysaccharides including keratan sulfate, heparin, hyaluronic acid, blood-group substances, the 20°-aqueous-extract of agar, dextrans of different structures, and various *Klebsiella* polysaccharides (Fig. 1A).

By contrast, s/IgM^{FIS} (Fig. 1B) showed less specificity, reacting strongly with chondroitin sulfates A, B, and C, with dextran sulfate, and somewhat less effec-

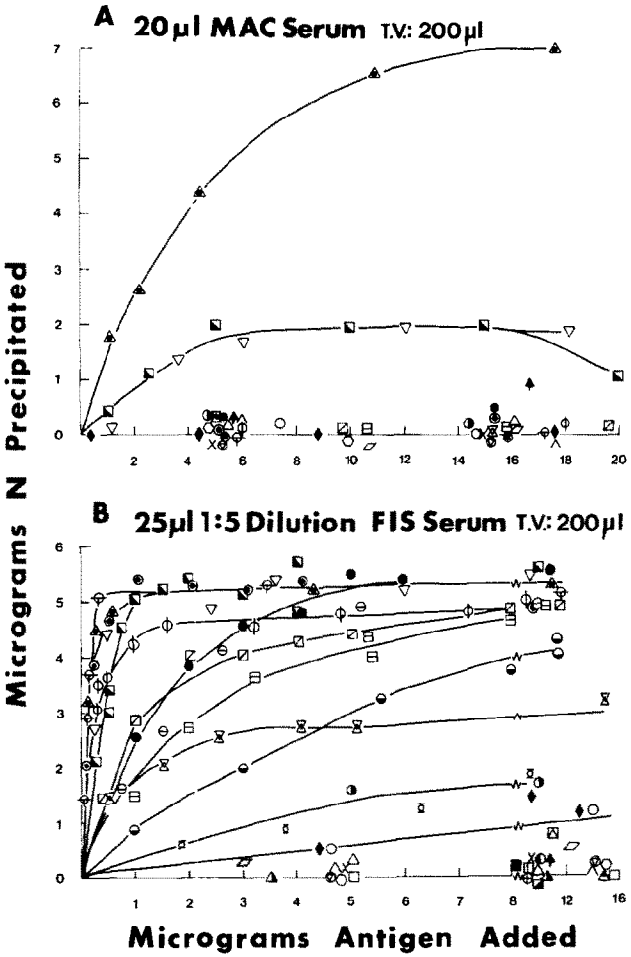

Fig. 1. Quantitative precipitin curves of serum MAC and serum FIS with various polysaccharides.

tively, per unit weight of antigen, with Bacto-agar and the 20°- and 50°-aqueous-extracts of agar, but not with agarose, and only slightly with the residue from the 50° aqueous extract of agar. FIS serum also gave good precipitin curves on addition of moderate amounts of Hog A + H substance and type XIV pneumococcal polysaccharide, which removed part of the monoclonal peak (data not shown). If sufficient polysaccharide was added, most of the curves reached the same maximum for total N precipitated, the only exceptions being keratan sulfate, *Klebsiella* polysaccharide K21, and hyaluronic acid. A variety of other polysaccharides did not react (Fig. 1B).

Diffusion in gels was used with both s/IgM^{MAC} and s/IgM^{FIS} to establish the relationship of the cross-reacting macromolecules to chondroitin sulfate C. The bands with chondroitin sulfate C were quite diffuse, whereas those with the 20° ex-

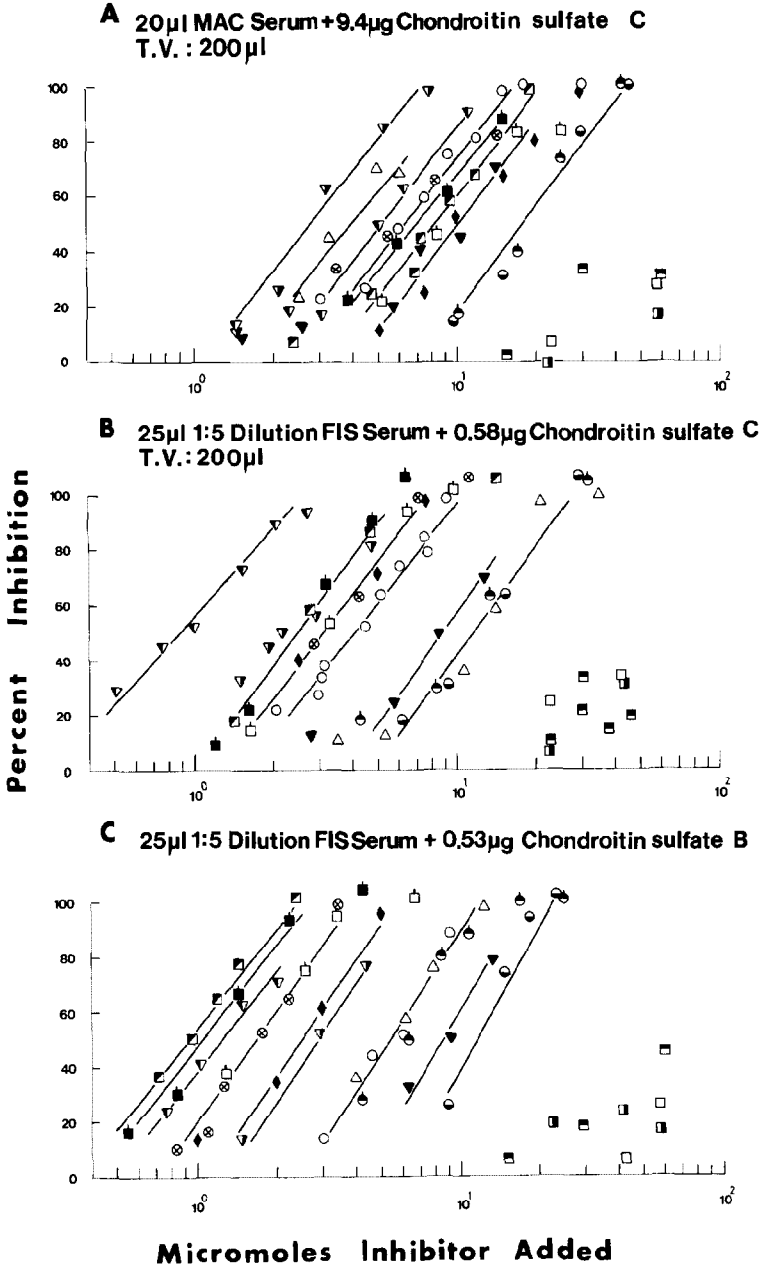

Fig. 2. Inhibition by sulfated and nonsulfated disaccharides of chondroitin sulfate and by other substances of precipitation of s/IgM^{MAC} and s/IgM^{FIS} by chondroitin sulfate C and of s/IgM^{FIS} by chondroitin sulfate B: ▽, Δ*Di*-6*S*; ▽, Δ*Di*-4*S*; ▼, Δ*Di*; ■, Glc-1-*P*; □, Gal-6-*P*; ○, Gal-1-*P*; △, Glc-6-*S*; ○, sodium pyruvate; ○, sodium lactate; ○, sodium propionate; □, sodium sulfate; ◆, phosphate buffer; □, Gal; □, lactose; and □, Glc.

TABLE I

INHIBITION BY SUGARS AND OLIGOSACCHARIDES OF PRECIPITATION OF s/IgM^{MAC} AND s/IgM^{FIS} BY CHONDROITIN SULFATE C, AND OF s/IgM^{FIS} BY CHONDROITIN SULFATE B

Inhibitor	*MAC serum (20 μL) + chondroitin sulfate C (9.4 μg)*	*FIS serum (25 μL) + chondroitin sulfate C (0.58 μg)*	*FIS serum (25 μL) + chondroitin sulfate B (0.53 μg)*
Δ Di-6*S*	2.8	2.5	2.9
Δ Di-4*S*	5.1	0.89	1.25
Δ Di	10.1	9.0	8.6
Glc-6-*S*	4.0	11.5	5.5
Na_2SO_4	8.2	2.5	0.95
Gal-1-*P*	6.4	3.1	1.7
Gal-1-*P*	7.0	2.5	1.0
Gal-6-*P*	9.0	3.1	1.7
Phosphate buffer	10.0	3.1	2.5
Sodium pyruvate	6.4	4.1	5.5
Sodium lactate	18.0	11.5	6.0
Sodium propionate	18.0	11.5	12.0

[a]In a total volume of 200 μL.

tract of agar, dextran sulfate and λ-carrageenan were quite sharp. With s/IgM^{MAC} λ-carrageenan, dextran sulfate, and poly G fused with the band formed with chondroitin sulfate C. With s/IgM^{FIS}, dextran sulfate, λ-carrageenan, and the 20° extract of agar formed sharp bands that spurred strongly over chondroitin sulfate C; however, owing to the diffuseness of the band with chondroitin sulfate C, reciprocal spurring of chondroitin C over these compounds could not be observed. The bands with Hog A + H, pneumococcal S XIV, heparin, and poly I fused with that of chondroitin sulfate C with no spurring. With hyaluronic acid and keratan sulfate, no bands were seen.

The inhibition data are shown in Fig. 2 and Table I. The three chondroitin disaccharides (Δ Di-6*S*, Δ Di-4*S*, and Δ Di) inhibited in each instance as would be expected from the precipitin data given in Table I. Thus, with s/IgM^{MAC}, which reacts only with chondroitin sulfate C having a 6-sulfate, the disaccharide having a 6-sulfate (Δ Di-6*S*) gave a stronger reaction than did the disaccharide having a 4-sulfate (Δ Di-4*S*). s/IgM^{FIS}, which reacts with chondroitin sulfate A, B, and C, showed a specificity for the 4-sulfate group, Δ Di-4*S* being two to three times more active than Δ Di-6*S*, whether chondroitin sulfate C or B was used in the inhibition assays. Both s/IgM^{MAC} and s/IgM^{FIS} were inhibited by the nonsulfated disaccharide (Δ Di), but it was only ~28, 10, and 14% as effective as the most potent disaccharide in each assay (columns 1, 2, and 3, respectively, of Table I). The remaining inhibition data established additional differences in specificity between s/IgM^{MAC} and s/IgM^{FIS}. With s/IgM^{MAC}, D-glucose 6-sulfate was slightly less active than Δ Di-6*S*, but more active than Δ Di-4*S*, with s/IgM^{FIS} D-glucose 6-sulfate was less effective than Δ Di when chondroitin sulfate C was used as precipitant, but was somewhat

better with chondroitin sulfate B; with chondroitin sulfates B and C, ΔDi was clearly less potent than ΔDi-4*S* and ΔDi-6*S*. s/IgM^{MAC} and s/IgM^{FIS} also behaved differently toward Na_2SO_4 as an inhibitor; with s/IgM^{MAC}, the activity of this compound was between those of ΔDi-4*S* and the ΔDi, whereas, with s/IgM^{FIS}, it was as active as ΔDi-6*S* in inhibiting precipitation by chondroitin sulfate C, and it was the most active inhibitor of precipitation with chondroitin sulfate B. The three sugar phosphates, phosphate buffer, and sodium pyruvate were comparable as inhibitors to sodium sulfate with both s/IgM^{MAC} and s/IgM^{FIS}. Sodium lactate and sodium propionate were somewhat less potent, but sodium lactate was as active as sodium pyruvate when chondroitin sulfate B was used. Little or no inhibition was obtained with D-galactose, lactose, and D-glucose.

DISCUSSION

As the concept developed that monoclonal immunoglobulins in multiple myeloma and related plasma-cell dyscrasias were antibodies[20,21], the proteins that were most difficult to be accepted as *bona fide* antibodies were those reacting with polysaccharides, such as heparin, agar, keratan sulfate, that contain charged groups, especially sulfate[22–28]. This was particularly a problem because such polysaccharides generally precipitate immunoglobulins as well as other proteins nonspecifically in media of low ionic-strength. A murine BALB/c IgG2a myeloma protein[29], SAPC-15, was precipitated by dextran sulfate, heparin, chondroitin sulfates A, B, and C, hyaluronic acid, *H. influenzae* type B, polysaccharide, calf thymus DNA, *Klebsiella* polysaccharide K63, and poly-L-glutamic acid in 50mM Tris at pH 7.4. However, in 0.15M sodium chloride containing 10mM phosphate buffer, only dextran sulfate precipitated. In several instances[22], precipitation with Fc was also seen.

Other studies[23–26,28,30] involved human monoclonal IgGκ and IgMκ which bound to heparin and, in several instances, neutralized the action of heparin *in vivo* and *in vitro*. Levy *et al.*[30] found only six of 100 monoclonal human IgG to precipitate in agar gels but not in agarose; this was ascribed to agaropectin. The 100 sera were examined further, and eight including five of the six that reacted in agar were found to precipitate with heparin in gels with agarose containing 3% poly(1,2-ethanediol) 6000 and 50mM phosphate buffer, pH 7.4; in quantitative precipitin assays, inhibition of precipitation by heparin increased with increasing ionic-strength.

Nevertheless, the finding that the reactions showed reasonable specificity, in that only a few of the monoclonal proteins reacted with these polysaccharides in 50mM sodium chloride and that the precipitation was generally associated with the Fab'_2 fragment and not with Fab, Fab', or Fc fragments, led to their cautious acceptance as equivalent to the other myeloma antibodies, although the anomalous solubilities caused concern[30].

The recent preparation of monoclonal mouse antibodies reacting specifically with keratan sulfate indicates that many, if not all, of the human monoclonal pro-

teins reacting with sulfated polysaccharides may be specific antibodies[31]. The present study tends to support this interpretation. All precipitin and inhibition assays were carried out in 0.15M sodium chloride. The two macroglobulins, s/IgM^{MAC} and s/IgM^{FIS}, reacted differently, *i.e.*, s/IgM^{MAC} reacted only with chondroitin sulfate C (~80% of 6-sulfate and 20% of 4-sulfate)[32], and not with chondroitin sulfate A (~80% of 4-sulfate and 20% of 6-sulfate)[32] or B, whereas s/IgM^{FIS} reacted well with all three suggesting that the two IgM's differ in their reactive sites, s/IgM^{MAC} being more specific. These specificities were further supported by the numerous polysaccharides (Fig. 1) that did not react over a wide range with s/IgM^{MAC}. The partial reactivity of keratan sulfate and other polysaccharides with s/IgM^{FIS}, and of λ-carrageenan and of dextran sulfate with s/IgM^{MAC} could be due to differences in solubility of the polysaccharide–immunoglobulin complexes[17].

Consistent with this were the inhibition studies with the chondroitin disaccharides; the best inhibitor with s/IgM^{MAC} was Δ*Di-6S* (6-sulfate), whereas Δ*Di-4S* (4-sulfate) was the best inhibitor with s/IgM^{FIS}. Most significant was the finding that the nonsulfated disaccharide (ΔDi) inhibits well in both systems, which is consistent with a binding site having a substantial residual specificity for the sugar component, as might be expected for an antibody-combining site. The ability of the low-molecular-weight sugars to inhibit, despite the specificity of the precipitin reactions, may be ascribed to being free from the conformational constraints imposed on the macromolecular polysaccharides.

Since the quantitative precipitin reactions were carried out with whole serum, it is possible that certain of the precipitin reactions might have been due to antibodies other than the monoclonal IgM. This was studied by gel diffusion. With s/IgM^{MAC}, the bands with dextran sulfate and λ-carrageenan fused with that of chondroitin sulfate C. With s/IgM^{FIS}, however, dextran sulfate, the 20° extract of agar, and λ-carrageenan formed sharp bands that spurred over that of chondroitin sulfate C, whereas the bands with Hog A + H, pneumococcal SXIV, and heparin fused with the band formed by chondroitin sulfate C. Further evidence for the difference in specificities of the binding sites of s/IgM^{MAC} and s/IgM^{FIS} was seen in that s/IgM^{MAC} gave a band with poly G, whereas s/IgM^{FIS} gave a band with poly I; in each instance the bands fused with the chondroitin C band.

Caterson and assoc.[33,34] have just reported monoclonal, mouse-hybridoma antibodies to rat chondrosarcoma or bovine, nasal-cartilage proteoglycan monomers that had been extensively treated with chondroitinase ABC. These proteoglycans retain stubs of disaccharides. Three monoclonal hybridoma cell-lines were obtained; one was specific for Δ*Di-6S*, one for Δ*Di-4S*, and the third for ΔDi, results in complete accord with our findings. It would be of interest to compare the specificities of the mouse and human monoclonal antibodies in detail.

The inhibition by sulfate and phosphate is not as surprising as it might seem. In the well characterized, monoclonal IgAκ myeloma-protein McPC603, which specifically binds phosphorylcholine and which has been studied by X-ray crystallography in 42% saturated sodium sulfate, it was found[35] that a sulfate ion oc-

cupied the combining site in the crystal and had displaced the phosphate residue of phosphorylcholine. Thus, the same situation probably obtains with s/IgM^{MAC} and s/IgM^{FIS} with respect to the inhibition by sulfate, phosphate, sugar sulfates and phosphates, and perhaps to some extent to pyruvate, lactate, and propionate ions as well. It would be important to extend these studies by working with the purified IgM antibodies.

REFERENCES

1 W. H. SHERMAN, N. LATOV, A. P. HAYS, M. TAKATSU, R. NEMMNI, G. GALASSI, AND E. F. OSSERMAN, *Neurology*, 33 (1983) 192–201.
2 S. SUZUKI, H. SAITO, T. YAMAGATA, K. ANNO, N. SENO, Y. KAWAI, AND T. FURUHASHI, *J. Biol. Chem.*, 243 (1968) 1543–1550.
3 P. HOFFMAN, A. LINKER, AND K. MEYER, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, 17 (1958) 1078–1082.
4 W. DUCKWORTH AND W. YAPHE, *Carbohydr. Res.*, 16 (1971) 189–197.
5 H. BRETTING, N. F. WHITTAKER, E. A. KABAT, K. KONIGSMANN-LANGE, AND H.-J. THIEM, *Carbohydr. Res.*, 98 (1981) 213–236.
6 M. HEIDELBERGER, Z. DISCHE, W. B. NEELY, AND M. L. WOLFROM, *J. Am. Chem. Soc.*, 77 (1955) 3511–3514.
7 N. ROY AND C. P. J. GLAUDEMANS, *Carbohydr. Res.*, 63 (1978) 318–322.
8 G. G. S. DUTTON, A. M. STEPHEN, AND S. C. CHURMS, *Carbohydr. Res.*, 38 (1974) 225–237.
9 Y.-M. CHOY AND G. G. S. DUTTON, *Can. J. Chem.*, 51 (1973) 198–207.
10 G. H. BEBAULT, G. G. S. DUTTON, N. A. FUNNELL, AND K. L. MCKENZIE, *Carbohydr. Res.*, 63 (1978) 183–192.
11 A. S. CHAUDHARI, C. T. BISHOP, AND W. F. DUDMAN, *Carbohydr. Res.*, 28 (1973) 221–231.
12 R. BROWN, *J. Immunol.*, 37 (1939) 445–455.
13 E. A. KABAT, *J. Biol. Chem.*, 130 (1939) 143–147.
14 E. A. KABAT, *Blood Group Substances. Their Chemistry and Immunochemistry*, Academic Press, New York, 1956, pp. 330.
15 G. SCHIFFMAN, E. A. KABAT, AND S. LESKOWITZ, *J. Am. Chem. Soc.*, 84 (1962) 73–77.
16 E. A. KABAT, *Kabat and Mayer's Experimental Immunochemistry*, C. C. Thomas, Springfield, Illinois, 2nd ed., 1961, pp. 905.
17 E. A. KABAT, *Methods Enzymol.*, 70 (1980) 3–39.
18 G. SCHIFFMAN, E. A. KABAT, AND W. THOMPSON, *Biochemistry*, 3 (1964) 113–120.
19 O. OUCHTERLONY, *Prog. Allergy*, 5 (1958) 1–78; 6 (1962) 30–54.
20 H. G. KUNKEL, *Harvey Lect.*, 59 (1965) 219–242.
21 M. SELIGMAN AND J. C. BROUET, *Semin. Hematol.*, 10 (1973) 163–167.
22 P. BURTIN AND M. C. GENDRON, *Immunochemistry*, 8 (1971) 423–429.
23 D. MILLER, *Blood*, 16 (1960) 1313–1317.
24 H. I. GLUECK, M. R. MACKENZIE, AND C. J. GLUECK, *J. Lab. Clin. Med.*, 79 (1972) 731–744.
25 J.-L. BEAUMONT AND N. LEMORT, *Pathol.-Biol.*, 22 (1974) 67–76.
26 E. POGLIANI, E. COFRANESCO, AND C. PRAGA, *Acta Haemotol.*, 53 (1975) 249–255.
27 H. TOLLESHAUG AND K. HANNESTAD, *Immunochemistry*, 12 (1975) 173–182.
28 M. FREEDMAN, R. MERRETT, AND W. PRUZANSKI, *Immunochemistry*, 13 (1976) 193–202.
29 B. N. MANJULA, M. POTTER, AND C. P. J. GLAUDEMANS, *Mol. Immunol.*, 19 (1982) 913–923.
30 D. E. LEVY, A. A. HORNER, AND A. SOLOMON, *J. Exp. Med.*, 153 (1981) 883–896.
31 B. CATERSON, J. E. CHRISTNER, AND J. R. BAKER, *J. Biol. Chem.*, 258 (1983) 8848–8854.
32 S. R. DELANEY, H. E. CONRAD, AND J. L. GLASER, *Anal. Biochem.*, 108 (1980) 25–34.
33 B. CATERSON, J. R. BAKER, J. E. CHRISTNER, AND J. R. COUCHMAN, *J. Invest. Dermatol.*, 79, Suppl. 1 (1983) 455–505.
34 J. E. CHRISTNER, B. CATERSON, AND J. R. BAKER, *Abstr. Annu. Meet. Soc. Complex Carbohydr.* (1983) 80.
35 E. A. PADLAN, D. M. SEGAL, T. F. SPANDE, D.R. DAVIES, S. RUDIKOFF, AND M. POTTER, *Nature (London), New Biol.*, 145 (1973) 165–167.