

IMMUNOCHEMICAL STUDIES ON MOUSE MYELOMA PROTEINS
REACTIVE WITH DEXTRANS OR WITH FRUCTOSANS AND
ON HUMAN ANTILEVANS*

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Heterogeneity of antibody-combining sites to homopolysaccharide antigens can readily be demonstrated. With human antidextran, specificities involving $\alpha(1 \rightarrow 6)$ - and $\alpha(1 \rightarrow 2)$ -, $\alpha(1 \rightarrow 3)$ -, $\alpha(1 \rightarrow 4)$ -linked dglucopyranosyl units have been described (1-9). In addition it has been shown that antidextran of $\alpha(1 \rightarrow 6)$ specificity produced in several individuals differed in their relative affinities for oligosaccharides of the isomaltose series up to isomaltohexaose (IM6) or -heptaose (IM7) (2, 10, 11 cf. 6, 12). This is thought to reflect differences in the extents of the complementary areas of the antibody-combining sites with the upper limit of complementarity being a chain of six or seven $\alpha(1 \rightarrow 6)$ -linked glucose units (2, 11 cf. 12). Indeed the antidextran of $\alpha(1 \rightarrow 6)$ specificity from a given individual has been fractionated into two populations that differ in the ease in which they are inhibited by small relative to large oligosaccharides of the isomaltose series (13-16 cf. 12). Similar data have been obtained with various mannans (17-19).

The immunochemistry of levan has not been as extensively studied (20 cf. 6). Levans are homopolymers of Dfructose with predominantly $\beta(2 \rightarrow 6)$ glycosidic linkages and $\beta(2 \rightarrow 1)$ branches. Another major class of fructosans is $\beta(2 \rightarrow 1)$ linked as in inulin. Their relatively simple structures thus also lend themselves to immunochemical investigations concerning the size and heterogeneity of antibody-combining sites.

In BALB/c mice immunoglobulin-secreting plasmacytomas can be induced by intraperitoneal injection of mineral oil or implantation of solid plastic material (21, cf. 22, 23). A relatively large number of homogeneous BALB/c myeloma proteins has antibody specificity for naturally occurring substances in the environment of inbred mouse strains. This has led to the hypothesis (23-25) that plasmacytomas in mice are chiefly derived from clones of cells that are stimulated by natural antigens. Since dextrans and levans occur widely in nature and microorganisms producing dextran and levan are present in the gastrointestinal tract (26), it is not surprising that myeloma proteins have been found that react specifically with these polysaccharides (27-29).

Myeloma proteins with antibody activity have shown ligand binding characteristic

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of homogeneous combining sites (30-34) while the antibody response to immunization with polysaccharides is generally heterogeneous, frequently yielding a spectrum of antibodies with combining sites varying in their complementary areas (cf. 12). However, if one were to study a number of myeloma proteins reactive with a given antigen, it could be anticipated that the individual myeloma-producing clones might differ in the specificity and site size of their secreted immunoglobulin since each clone is presumably selected from a heterogeneous cell population by a random malignant transformation.

The present report is a study of eight mouse myeloma proteins, four with antidextran and four with antifructosan specificity, and of human antilevan produced by immunization of two individuals with levan. The data clearly show that combining sites of individual mouse myeloma proteins reactive with dextran can differ from one another in specificity, size, and extent of their complementary areas. They also indicate that the antifructosan-specific myelomas fall into two classes with $\beta(2 \rightarrow 6)$ or $\beta(2 \rightarrow 1)$ specificity and that, in the latter class, individual proteins differ in the sizes and extents of the complementary areas of their combining sites. This is consistent with the hypothesis that each myeloma protein represents a population of molecules with homogeneous combining sites produced by a clone selected at random from the diverse population of cells that usually give rise to heterogeneous populations of antibody molecules after antigenic stimulation.

Materials and Methods

Myeloma Proteins.—Myeloma proteins were produced in BALB/c mice, screened for antibody activity, and typed at the National Institutes of Health or the Salk Institute (Table I). Protein W3129 was purified on a Sephadex G-75 column (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) and supplied by Dr. Martin Weigert of the Salk Institute, San Diego, Calif.

Human Antilevans.—Two human antilevan sera were studied. Antilevan from subject 1 (1 D₃₃L₂₄ + D₃₄L₂₅) (20) had been previously absorbed with insoluble blood group substance (35) and with Sephadex (13) to remove anti-A antibodies and antidextrans, respectively. Antilevan C.S. was prepared by immunization with levan P6 (20) and was kindly supplied by Doctors Valee Harisdangkul and Charles L. Christian, Cornell University Medical College, New York.

TABLE I
Myeloma Proteins Reactive with Dextran or Fructosans

Myeloma protein	Reacts with	Type	Present in	Origin
W3129	Dextran	IgA κ	Serum	Salk
W3434	Dextran	IgA κ	Serum	Salk
QUPC 52	Dextran	IgA	Ascitic fluid	NIH
UPC 102	Dextran	IgA λ	Ascitic fluid	NIH
W3082	Fructosan	IgA κ	Serum	Salk
UPC 61	Fructosan	IgA	Ascitic fluid	NIH
Y5476	Fructosan	IgA κ	Serum	Salk
UPC 10	Fructosan	IgG2a	Ascitic fluid	NIH

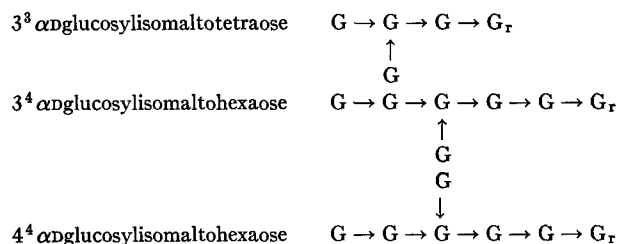
Antigens.—The dextrans and levans used in this study have been described previously (20, 36 cf. 6). The proportions of $\alpha(1 \rightarrow 6)$, $\alpha(1 \rightarrow 3)$ -like, and $\alpha(1 \rightarrow 4)$ -like linkages in the dextrans used are given in Fig. 2. Inulin was from Nutritional Biochemicals Corp., Cleveland, Ohio.

Mono- and Oligosaccharides.—Glucose, fructose, sucrose, isomaltose (IM2), isomaltotriose (IM3), isomaltotetraose (IM4), isomaltopentaose (IM5), isomaltohexaose (IM6), isomaltoheptaose (IM7), maltose, kojibiose, methyl α Dglucopyranoside, methyl β Dglucopyranoside, methyl α Dmannopyranoside, methyl 3-O-methyl α Dglucopyranoside, turanose (3-O- α DglucosylDfructose), and the branched oligosaccharides 3³ α Dglucosylisomaltotetraose, 3⁴ α Dglucosylisomaltohexaose, and 4⁴ α Dglucosylisomaltohexaose¹ were those previously described (4, 8, 13, 36–38). α DGlc(1 \rightarrow 6) β DGlcNAc (6-O- α Dglucosyl-N-acetylDglucosamine) was from Dr. A. L. Tarentino, Division of Laboratories and Research, New York State Dept. of Health, Albany, N. Y. (39). The $\alpha(1 \rightarrow 3)$ -linked series of glucose oligosaccharides (nigerodextrins) were provided by Dr. I. R. Johnston, University College, London, England (40). The di-, tri-, and tetrasaccharides, β Dfructofuranosyl(2 \rightarrow 6) β Dglucopyranose (1F1G), β Dfructofuranosyl(2 \rightarrow 1) β Dfructofuranosyl(2 \rightarrow 6) β Dglucopyranose (2F1G), and β Dfructofuranosyl(2 \rightarrow 1) β Dfructofuranosyl(2 \rightarrow 1) β Dfructofuranosyl(2 \rightarrow 6) β Dglucopyranose (3F1G), were obtained from Dr. F. Arcamone, Istituto Ricerche Farmitalia, Milano, Italy (41). The preparation of their corresponding alditols has been described (29). Levanbiose (6-O- β DfructosylDfructose) and a branched levantriose, β Dfructofuranosyl(2 \rightarrow 1)[β Dfructofuranosyl(2 \rightarrow 6)]Dfructose, in which the fructose units were joined by one $\beta(2 \rightarrow 1)$ and one $\beta(2 \rightarrow 6)$ linkage, were kindly supplied by Dr. S. T. Bauer, The Hebrew University, Jerusalem, Israel.

Immunochemical Methods.—Quantitative precipitin and inhibition assays were done by a microprecipitin technique (6) using 3–8 μ g of protein N per determination. Total N in the washed precipitates was measured by the ninhydrin method (42). The solubility of precipitates was determined as previously described (6, 29).

Protein W3129 (2.9 mg of N in 0.6 ml) was separated into monomer- and polymer-rich fractions by filtration at 4°C through an 80 \times 1.5 cm Bio-Gel P-300 (100–200 mesh) column (Bio-Rad Laboratories, Richmond, Calif.) equilibrated with 0.01 M phosphate-buffered saline (PBS), pH 7.4 (Fig. 1). The contents of tubes 27–31 and 35–40 were pooled to give polymer and monomer fractions, respectively. A Spinco model E analytical ultracentrifuge equipped with ultraviolet optics and automatic temperature control was used for sedimentation velocity measurements. Analysis of sedimentation patterns at the 25, 50, and 75% levels of ultraviolet absorbance gave $s_{20,w}$ values of 7.7S, 6.9S, and 6.1S with the monomer fraction (70 μ g N/ml) and 26S, 17.7S, and 13.1S with the polymer fraction (108 μ g N/ml). The material in the polymer fraction sedimenting at 26S or faster (25% of the protein) apparently resulted from aggregation since only 12 and 25% of unfractionated W3129 sedimented at or faster than 18.1S and 14.6S, respectively. With the polymer fraction, 9% of the protein was 9.8S or smaller

¹ The structures of the branched oligosaccharides may be represented as follows: G, Dglucopyranosyl; G_r, reducing Dglucose; \rightarrow , $\alpha(1 \rightarrow 6)$ link; \uparrow , $\alpha(1 \rightarrow 3)$ link; \downarrow , $\alpha(1 \rightarrow 4)$ link.



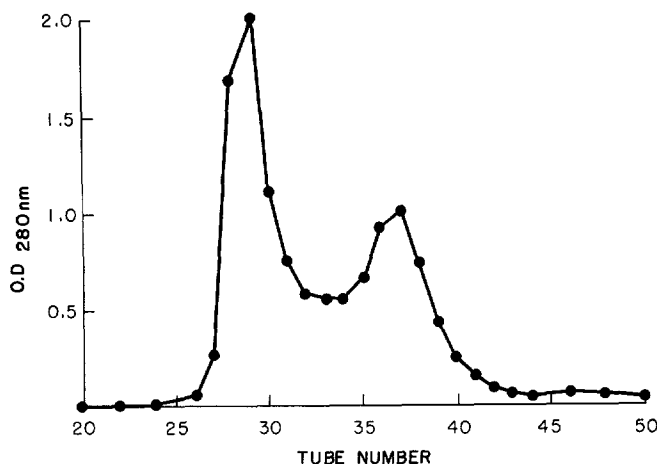


FIG. 1. Separation of myeloma protein W3129 into polymer-rich (tubes 27-31) and monomer-rich (tubes 35-40) fractions by chromatography on Bio-Gel P-300.

while 8% of the monomer fraction was 9.2S or larger. Immunoabsorption experiments using 3 mg Sephadex G-75 were done with approximately 10 μ g of myeloma N in a total volume of 250 μ l. The percentage of myeloma protein N adsorbed to the Sephadex or precipitated by dextrans was determined by analyses of supernatants.

RESULTS

Quantitative Precipitin Studies with Mouse Myeloma Proteins Reactive with Dextran.—Initial screening of myeloma proteins W3434, W3129, QUPC 52, and UPC 102 with several antigens revealed specificity for dextrans. To establish the pattern of reactions for each myeloma protein, quantitative precipitin assays were performed with dextrans differing in their proportions of $\alpha(1 \rightarrow 6)$, $\alpha(1 \rightarrow 4)$ -like, and $\alpha(1 \rightarrow 3)$ -like glycosidic linkages.

Myeloma proteins W3434 and W3129 reacted similarly with the dextrans tested (Fig. 2 A and B, respectively). Both were precipitated most efficiently by native dextrans high in $\alpha(1 \rightarrow 6)$ glycosidic linkages (B512 or N236 and B1141) or by those high in $\alpha(1 \rightarrow 6)$ and $\alpha(1 \rightarrow 4)$ -like linkages that are largely $\alpha(1 \rightarrow 2)$ (B1399, B1299-S-3, and B1424) and 50% precipitation required approximately 4 μ g of these dextrans; B1399 precipitated about 2 μ g more N from W3434 than did any of the other dextrans. Larger quantities of dextrans high in $\alpha(1 \rightarrow 3)$ -like linkages (B1498S, B1355-S-4, and B1501S) were required for 50% precipitation, 11 μ g with W3434, and 8 μ g with W3129. Dextran B742L-R with 81% $\alpha(1 \rightarrow 6)$ linkages did not react like the other dextrans with high $\alpha(1 \rightarrow 6)$ contents and approximately 8 μ g were required for 50% precipitation of protein W3129; similar results were obtained with a pool of W3434 serum other than that used for Fig. 2 A: the atypical behavior of this dextran has been repeatedly shown (3 cf. 6).

	LINKAGES				LINKAGES				LINKAGES		
	1→6	1→3 -like	1→4 -like		1→6	1→3 -like	1→4 -like		1→6	1→3 -like	1→4 -like
□ B512 or N236	96	4	0	M B 1399	65	0	35	▲ B 1498 S	62	27	11
X N-150N	96	4	0	⊠ B1299-S-3	50	0	50	⊠ B1355-S-4	57	34	9
■ B 1141	79	18	3	■ B 1424	71	0	29	⊠ B742C-3R	61	21	18
○ B 1255	86	0	14					● B 1501S	65	20	15
● B 742L-R	81	0	19								

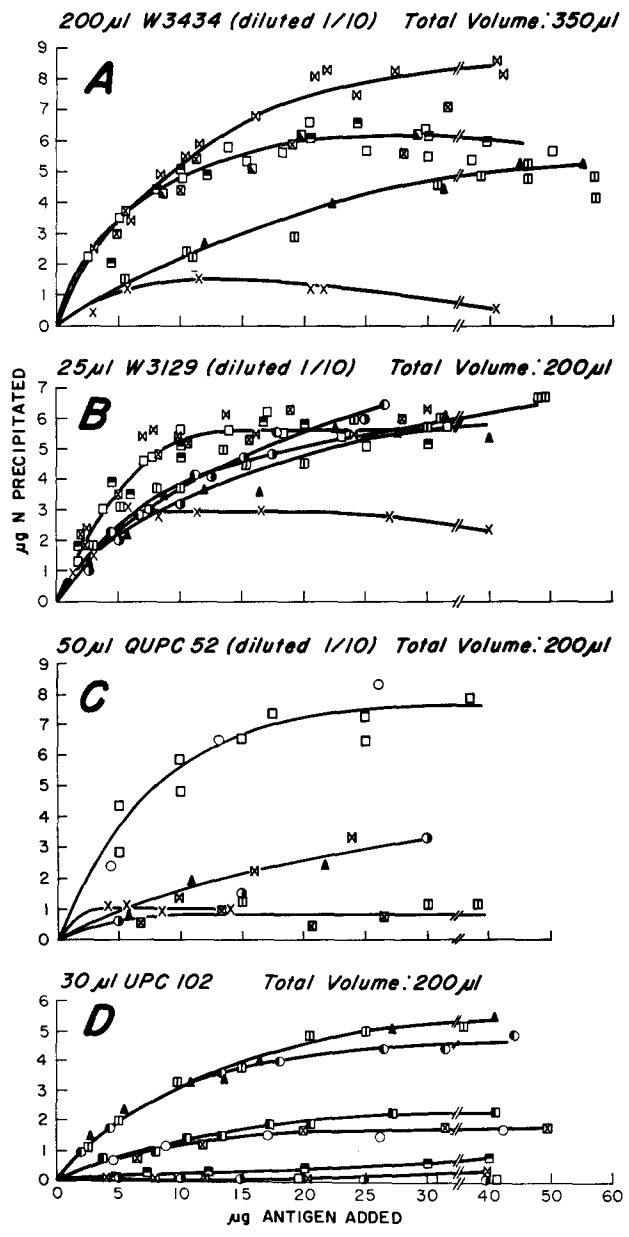


FIG. 2. Quantitative precipitin curves of mouse myeloma proteins with various dextrans.

Limited data with myeloma protein QUPC 52 indicated a pattern of precipitin reactions that differed from that of W3434 or W3129 (Fig. 2 C). Dextran B512 and B1255 with 96 and 86% $\alpha(1 \rightarrow 6)$ linkages, respectively, reacted well and approximately 5 μg gave 50% precipitation. Dextran B1399 and B1498S with 65 and 62% $\alpha(1 \rightarrow 6)$ linkages reacted intermediately, while B1299-S-3 and B1355-S-4 with 50 and 57% $\alpha(1 \rightarrow 6)$ linkages reacted poorly. As noted for W3434 and W3129, dextran B742L-R reacted less well with QUPC 52 than did the other $\alpha(1 \rightarrow 6)$ -linked dextrans.

Mouse myeloma protein UPC 102 differed from the three previous antidextran myelomas and precipitated best with those dextrans containing high proportions of $\alpha(1 \rightarrow 3)$ -like glycosidic linkages (B1498S, B1355-S-4, and B1501S) (Fig. 2 D). Dextran B742C-3R and B1141 were exceptional and although they contained 21 and 18%, respectively, of $\alpha(1 \rightarrow 3)$ -like linkages, they precipitated only a portion of the total UPC 102 N. Those dextrans with low $\alpha(1 \rightarrow 3)$ -like and high $\alpha(1 \rightarrow 6)$ and/or $\alpha(1 \rightarrow 4)$ -like linkages reacted poorly.

With $\alpha(1 \rightarrow 6)$ -specific human antidextrans, clinical dextran N-150N (mol wt 60,000) (43) precipitated 15% less N than did native dextran B512 (mol wt 10^7 - 10^8) (cf. 6); however, N-150N precipitated 50, 75, and 85% less N than did B512 with myeloma proteins W3129, W3434, and QUPC 52, respectively, (Fig. 2). Solubilities of precipitates of protein W3129 with N-150N and B512 were similar (0.8 and 1.0 μg N/ml, respectively) and indicated that the failure of dextran N-150N to precipitate all of the myeloma protein did not reflect a high solubility of the specific precipitates.

Since IgA myeloma proteins consist of monomeric and polymeric molecules and isolated monomer fractions, although capable of antigen binding have been found not to precipitate (30, 44), the influence of monomeric IgA on precipitin curves obtained with dextrans B512 and N-150N was investigated. Purified W3129 and the previously described monomer- and polymer-rich fractions of this myeloma protein were equally reactive with dextran since 85-90% of each could be adsorbed on to Sephadex G-75. Quantitative precipitin curves with dextrans B512 and N-150N are shown in Fig. 3. Unfractionated W3129 was 64% precipitable with dextran B512 and 24% precipitable with dextran N-150N. The polymer fraction of W3129 was 88% precipitable with B512 and 54% precipitable with N-150N. The monomer fraction was 45 and 5% precipitable with B512 and N-150N, respectively. Thus the percentage of N-150N to B512-precipitable N was 40% with unfractionated W3129, 60% with the polymer-rich fraction, and 10% with the monomer-rich fraction of W3129.

Quantitative Inhibition Assays of Myeloma Proteins Reactive with Dextrans.—The abilities of various oligosaccharides to inhibit precipitation between proteins W3434 or W3129 and dextran B512 were assayed. Both proteins displayed specificity for $\alpha(1 \rightarrow 6)$ -linked glycosyl residues since kojibiose, nigerose, and maltose were poorer inhibitors on a molar basis than methyl α glucoside, which in turn was less effective than IM2 (Fig. 4 A and B). With W3434 the

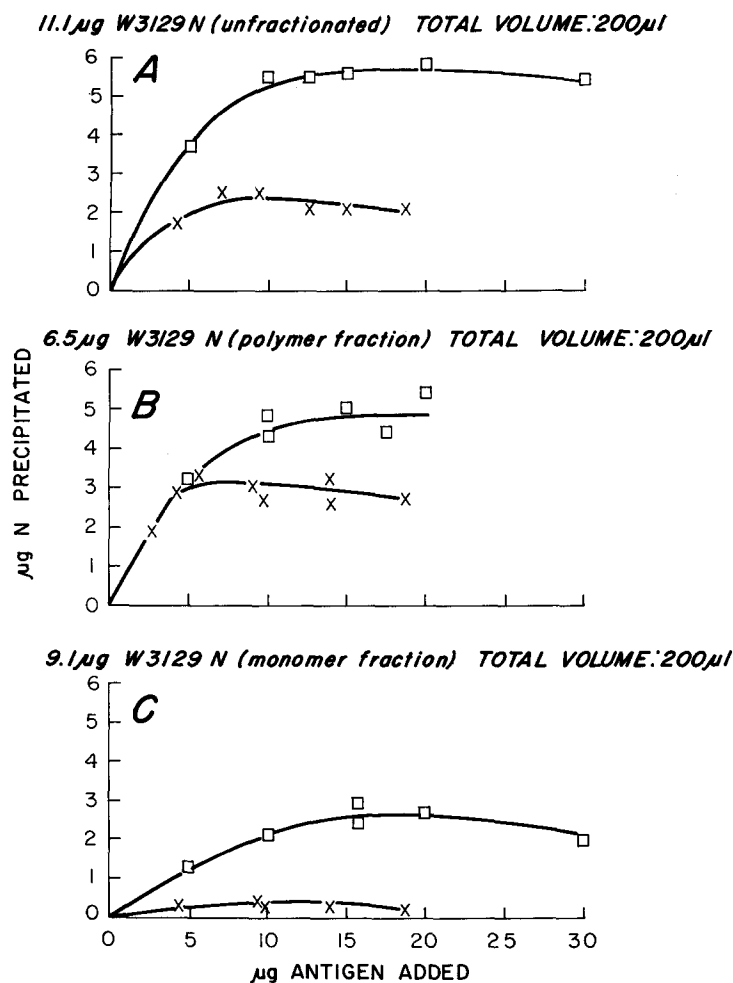


FIG. 3. Quantitative precipitin curves of protein W3129 (A) and its polymer- (B) and monomer-rich (C) fractions (cf. Fig. 1) with: □, native dextran B512; ×, clinical dextran, N-150N.

isomaltose oligosaccharides inhibited in the following order: IM7 = IM6 = IM5 > IM4 > IM3 ≫ IM2 (Fig. 4 A). The order of inhibition with protein W3129 was: IM7 = IM6 = IM5 > IM4 = IM3 ≫ IM2 (Fig. 4 B). Thus both proteins showed complementarity to a terminal nonreducing chain of five glycosyl residues in $\alpha(1 \rightarrow 6)$ linkage. However, W3129 and W3434 differed in their relative affinities for IM4, W3129 showing no additional inhibition by IM4 over IM3 on a molar basis.

Additional data with W3129 indicated that $\alpha(1 \rightarrow 6)$ -linked oligosaccharides containing a single glucose branch on carbon 3 or 4 of different glycosyl residues

- | | | |
|--------------------|---|--|
| ◇ GLUCOSE | ◇ ME α D GLUCOSIDE | ♣ NIGEROSE |
| △ ISOMALTOSE | ○ ME β D GLUCOSIDE | ▲ NIGEROTRIOSE |
| △ ISOMALTOTRIOSE | ◆ ME α D MANNOSIDE | ■ NIGEROTETRAOSE |
| □ ISOMALTOTETRAOSE | ⊞ ME 3-O-ME α D GLUCOSIDE | ▲ NIGEROPENTAOSE |
| △ ISOMALTOPENTAOSE | X α D Glc(1 \rightarrow 6) D Glc NAc | ◆ NIGEROHEXA-HEPTAOSE |
| ○ ISOMALTOHEXAOSE | ▼ MALTOSE | ○ 3 ³ α D GLUCOSYLISOMALTOTETRAOSE |
| ● ISOMALTOHEPTAOSE | ⊙ KOJIBIOSE | ○ 3 ⁴ α D GLUCOSYLISOMALTOHEXAOSE |
| | | ○ 4 ⁴ α D GLUCOSYLISOMALTOHEXAOSE |

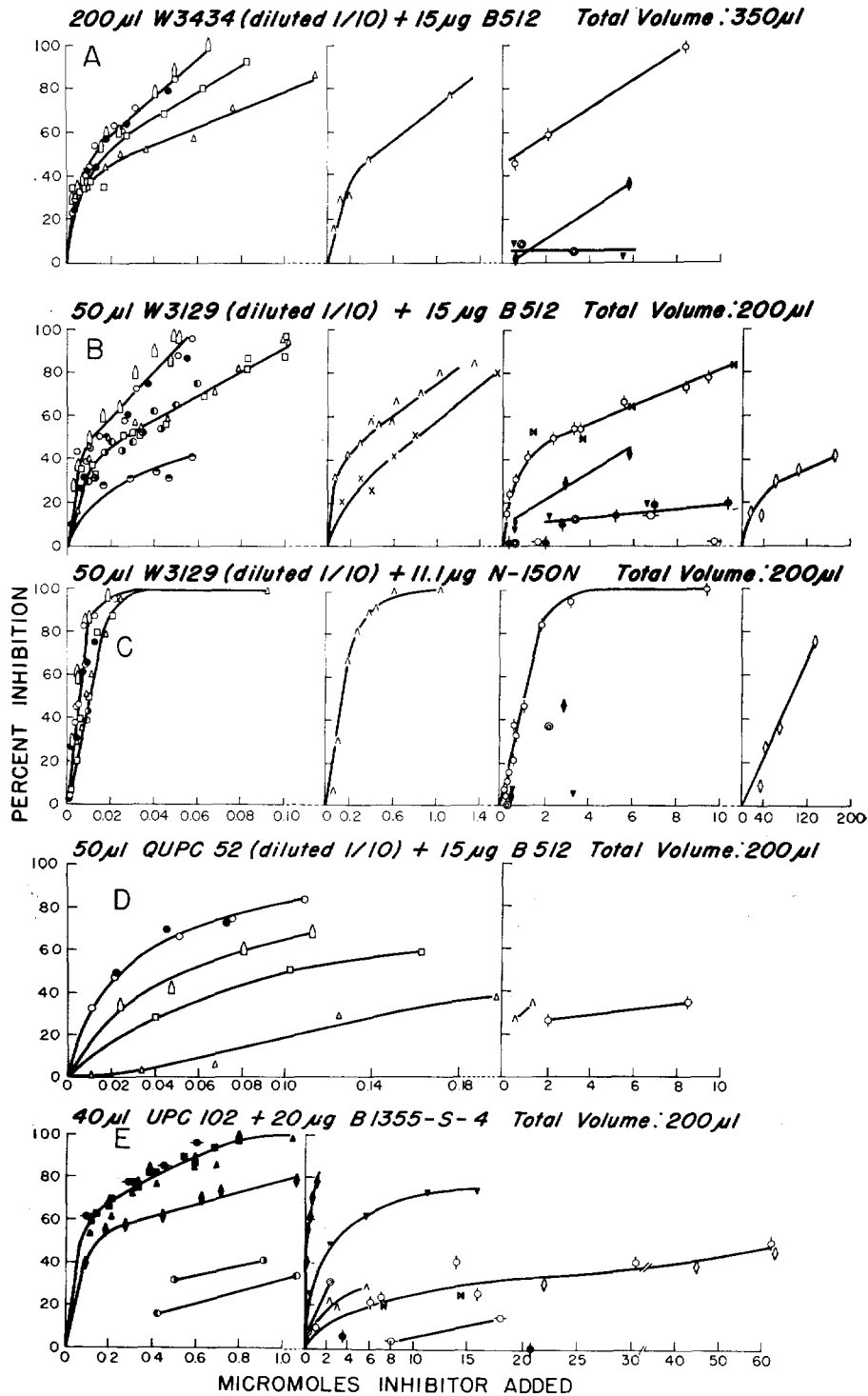


FIG. 4. Inhibition by various oligosaccharides of precipitation of mouse myeloma proteins by dextran.

were poorer inhibitors than the corresponding $\alpha(1 \rightarrow 6)$ -linked oligosaccharides. Thus $3^{\alpha}\text{Dglucosylisomaltohexaose}$ and $4^{\alpha}\text{Dglucosylisomaltohexaose}$ were slightly less inhibitory than IM6 and were similar to IM3 or IM4, while $3^{\alpha}\text{Dglucosylisomaltotetraose}$ was a poorer inhibitor on a molar basis than IM4 (Fig. 4 B). The importance of the second glucose residue from the nonreducing end was also shown by finding that $\alpha\text{DGlc}(1 \rightarrow 6)\text{DGlcNAc}$ was less potent than IM2. Methyl $\alpha\text{Dglucoside}$ was a much better inhibitor than methyl $\beta\text{Dglucoside}$ and methyl $\alpha\text{Dmannoside}$ indicating, respectively, that the α -glycosidic linkage and the equatorial position of the hydroxyl group on the second carbon of the terminal nonreducing glucose residue were critical for complementarity. Methyl 3-*O*-methyl $\alpha\text{Dglucoside}$ and methyl $\alpha\text{Dglucoside}$ were equally potent.

Precipitation of myeloma protein QUPC 52 with dextran B512 was effectively inhibited by the isomaltose series of oligosaccharides and the order of inhibition was: IM7 = IM6 > IM5 > IM4 \gg IM3 \gg IM2 (Fig. 4 D). Since IM7 and IM6 were both twice as inhibitory as IM5, the combining region of QUPC 52 clearly showed complementarity to a terminal chain of six glucosyl residues in $\alpha(1 \rightarrow 6)$ linkage and was thus larger than those of proteins W3434 and W3129.

With myeloma protein UPC 102 nigerose was a better inhibitor than maltose, kojibiose, or isomaltose (Fig. 4 E). Methyl $\alpha\text{Dglucoside}$, methyl 3-*O*-methyl $\alpha\text{Dglucoside}$, and glucose were equally inhibitory and were more effective on a molar basis than methyl $\beta\text{Dglucoside}$ and methyl $\alpha\text{Dmannoside}$. Assays with the nigerose series of oligosaccharides showed nigerotriose, -tetraose, -pentaose, and -hexa-heptaose to be equal on a molar basis and more effective than nigerose or the branched oligosaccharides $3^{\alpha}\text{Dglucosylisomaltohexaose}$ and $4^{\alpha}\text{Dglucosylisomaltohexaose}$. Thus the combining site of UPC 102 was most complementary to nigerotriose.

With protein W3129 the relative inhibitory powers of the isomaltose oligosaccharides were approximately the same as judged by their abilities to compete with determinants on native dextran B512 or clinical dextran N-150N. However, the shapes of inhibition curves obtained with B512 (Fig. 4 B) were strikingly different from those with N-150 N (Fig. 4 C). With B512, a biphasic curve was obtained in that the initial 40% of inhibition required relatively low oligosaccharide concentrations compared with those needed to inhibit the remaining 60%. This biphasic character was not observed in the N-150N system in which inhibition curves resembled those usually found with antibodies. Inhibition curves with purified W3129 and B512 were also biphasic and indicated that 20–30% of the precipitable myeloma N was easily inhibited by IM6 or IM3 (Fig. 5 A). Studies with W3129 polymer- and monomer-rich fractions (Fig. 5 B and C, respectively) resolved the biphasic inhibition curve into typical quantitative precipitin curves and indicated that the biphasic curves were attributable to the preferential inhibition of W3129 monomers over polymers from B512 precipitates.

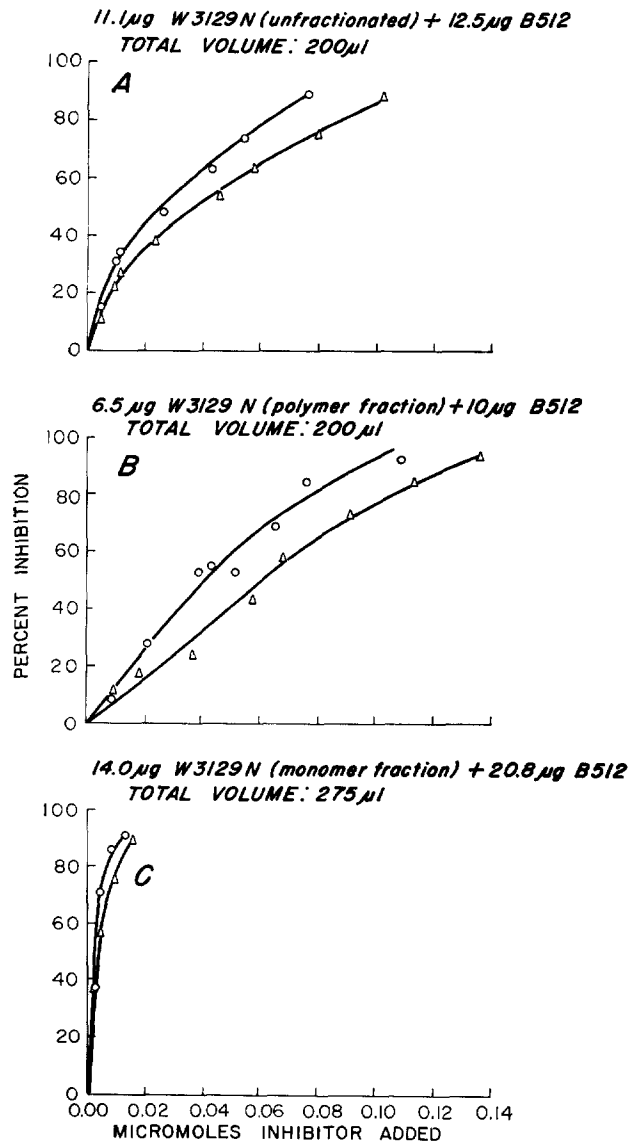


FIG. 5. Inhibition by oligosaccharides of precipitation of protein W3129 (A) and its polymer (B) and monomer-rich (C) fractions (cf. Fig. 1) by dextran. Δ , isomaltotriose; \circ , isomaltohexaose.

Quantitative Precipitin Studies with Mouse Myeloma Proteins Reactive with Fructosans.—Proteins UPC 61 and W3082 reacted similarly with various levans and with inulin (Fig. 6 A and B). Approximately 1.5 μ g of Hestrin levan A or Hestrin levan B and 3–4 μ g of Hestrin native levan, levan P6, and levan

- | | | |
|------------------------|-------------------------|-----------------------|
| □ HESTRIN LEVAN A | ○ LEVAN P6 | △ LEVAN B 523 FR. M |
| ● HESTRIN LEVAN B | ● LEVAN B 512 PP2 FR. B | ○ PERENNIAL RYE GRASS |
| ■ HESTRIN NATIVE LEVAN | ■ LEVAN B 512 FR. E | X LEVAN INULIN |

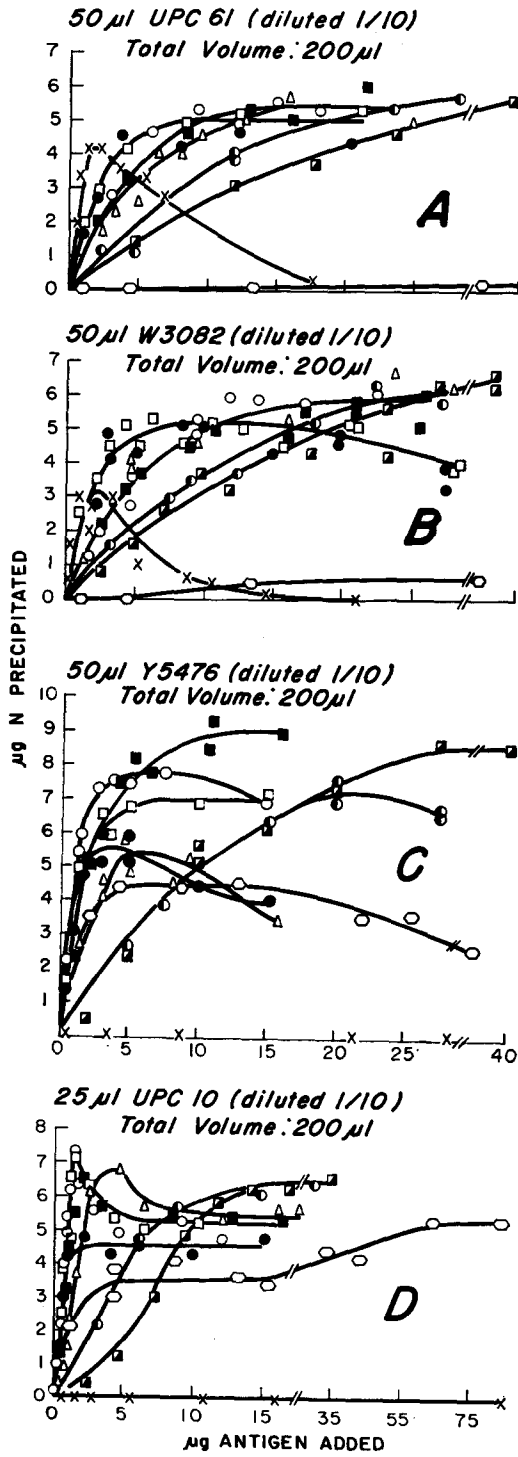


FIG. 6. Quantitative precipitin curves of mouse myeloma proteins with various levans and with inulin.

B523 fraction (Fr.) M gave 50% precipitation of either protein. Larger amounts of levans B512, PP2 Fr. B, and B512 Fr. E were required for 50% precipitation. The $\beta(2 \rightarrow 1)$ -linked fructosan, inulin, reacted very well with both proteins, and 2 μg precipitated approximately 70% of the UPC 61 N and 50% of the W3082 N. By contrast, perennial rye grass levan, which is predominantly $\beta(2 \rightarrow 6)$ -linked (45, 46), reacted hardly at all.

Precipitin reactions with myeloma proteins Y5476 and UPC 10 (Fig. 6 C and D) differed in two basic respects from those described for UPC 61 and W3082. When the antifructosan myeloma proteins were adjusted to the same amount of precipitable N, the quantities of each levan required for 50% precipitation of Y5476 and UPC 10 were roughly one-half to one-quarter those with UPC 61 and W3082. Secondly, Y5476 and UPC 10 did not react with inulin but were precipitated by perennial rye grass levan. The precipitin reactions of Y5476, an IgA protein, and UPC 10, an IgG, were not identical; however, both proteins reacted well with levan P6 and the Hestrin levans while greater amounts of B512 PP2 Fr. B and B512 Fr. E were required for 50% precipitation; B523 Fr. M was intermediate.

Oligosaccharide Inhibition Studies with Antifructosan Mouse Myeloma Proteins.—Inhibition studies with UPC 61 and W3082 (Fig. 7 A and B) indicated that their specificities were very similar. Both were inhibited by $\beta(2 \rightarrow 1)$ -linked fructose oligosaccharides and 3F1G was the best inhibitor with about 1.8 nM giving 50% inhibition. When 3F1G was reduced to its corresponding alditol, its inhibitory power decreased to that of 2F1G; both 3F1G reduced and 2F1G were approximately 60% as effective as 3F1G. Reduction of 2F1G to its alditol resulted in a fivefold decrease of inhibitory power for UPC 61 and a ninefold decrease with W3082. With both proteins the branched levantriose, containing a $\beta(2 \rightarrow 1)$ - and a $\beta(2 \rightarrow 6)$ -linked terminal fructofuranosyl residue, was somewhat more effective than 2F1G reduced. Levanbiose, the $\beta(2 \rightarrow 6)$ -linked disaccharide, was approximately $\frac{1}{15}$ as inhibitory as 2F1G reduced but four to five times more effective than sucrose and 1F1G, which were similar. Turanose, fructose, and glucose were very poor inhibitors of precipitation.

Mouse myeloma proteins UPC 10 and Y5476 were not inhibited by any of the oligosaccharides assayed (Fig. 7 A and B symbols with asterisks). The highest amounts tested were greater than those required for 50% inhibition of W3082 or UPC 61 by factors from 100 to 1,000 with 3F1G, 10 to 60 with branched levantriose, 4 to 10 with levanbiose, 1 to 7 with 1F1G, 40 to 100 with sucrose, and 0.5 to 3 with turanose. Fructose in amounts greater than 10^6 nM appeared to inhibit both myeloma proteins; however, under these conditions the sugar concentrations were between 10 and 20% and increased viscosity may have affected the quantitative inhibition assays.

Quantitative Precipitin and Oligosaccharide Inhibition Studies with Human Antilevans.—Levan P6 precipitated 2.5 μg of N from 0.5 ml of human antilevan C.S. and 50% precipitation required 1.5 μg of levan. Perennial rye grass levan

- | | | |
|----------------|------------------------|------------|
| ■ 3F1G | V 1F1G | ● TURANOSE |
| ○ 3F1G reduced | □ LEVANBIOSE | ◆ FRUCTOSE |
| ▽ 2F1G | ▼ BRANCHED LEVANTRIOSE | X GLUCOSE |
| ● 2F1G reduced | □ SUCROSE | |

50 μ l UPC61 (diluted 1/10) + 11.1 μ g LEVAN P6 Total Volume: 200 μ l
 25 μ l UPC10 (diluted 1/10) + 1.1 μ g LEVAN P6 Total Volume: 200 μ l *
 1.5 ml 1D₃₃L₂₄ + 1 D₃₄L₂₅ (dextran and A absorbed) + 5.1 μ g LEVAN P6 Tot. Vol.: 1.6 ml †

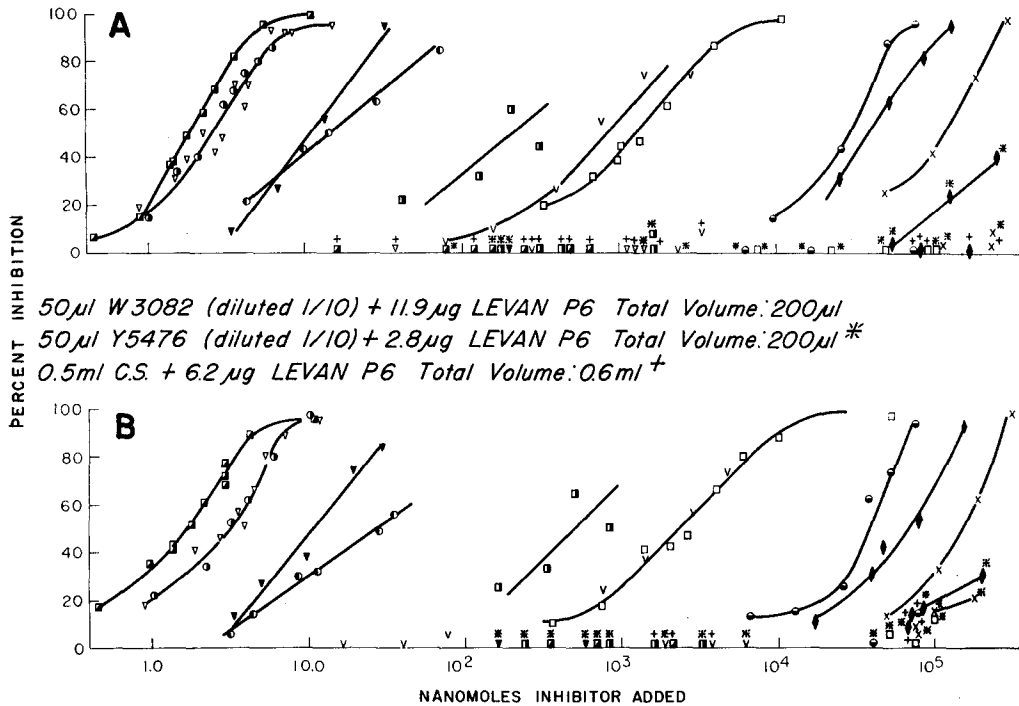


FIG. 7. Inhibition by various oligosaccharides of precipitation of mouse myeloma proteins and human antilevans by levan.

and inulin in amounts up to 9 μ g did not precipitate with antilevan C.S. Quantitative precipitin reactions with various fructosans and the antilevan from subject 1 D₁₁L₂ have been reported previously (20); with 1.5 ml of 1 D₃₃L₂₄ + 1 D₃₄L₂₅ serum, taken 9 yr later, levan P6 precipitated 6 μ g of antilevan N with 1 μ g of levan P6, giving 50% precipitation. Small amounts (1–5 μ g) of perennial rye grass levan precipitated 0.5 μ g of N while inulin failed to precipitate. With both antilevans, precipitation by levan P6 was effectively inhibited with excess perennial rye grass levan (mol wt approximately 5,000) (45) and 160 μ g gave 40–50% inhibition. Increased inhibition could not be observed as greater amounts of perennial rye grass levan precipitated a serum protein that apparently was distinct from antilevan, as had previously been

noted with inulin for the earlier sample (20). With both antilevans, inulin (mol wt approximately 5,000) was completely inactive as an inhibitor in amounts up to 360 μ g. Thus like myeloma proteins UPC 10 and Y5476, the human antilevans were reactive with determinants on perennial rye grass levan but not on inulin. Nor were the antilevans inhibited significantly by the oligosaccharides that were active with myeloma proteins W3082 and UPC 61 (Fig. 7 symbols with crosses).

DISCUSSION

The findings presented on myeloma proteins reactive with dextrans and those reacting with the fructosans levan and inulin provide a crucial link in the chain of evidence relating specificity of myeloma proteins with antibody specificity. The mouse myeloma proteins QUPC 52, W3434, and W3129 reacted with the isomaltose oligosaccharides in a manner remarkably similar to that of the $\alpha(1 \rightarrow 6)$ -specific human antidextrans. Earlier studies with human antidextrans established the upper limit for an antidextran-combining site as complementary to a terminal chain of six or seven $\alpha(1 \rightarrow 6)$ -linked glucose units (10, 11 cf. 6, 12). Inhibition assays with isomaltose oligosaccharides showed the antidextran produced by a given individual to be heterogeneous in that molecules varied in the sizes of their combining sites (2 cf. 12). Further studies showed that the antidextrans from a given individual could be fractionated into two populations with combining sites of different sizes (13-16). The present studies on myeloma proteins reactive with dextrans have clearly shown that these proteins also have combining sites of different sizes, one having a site complementary to six glucoses, two others to five glucoses, and one to three glucoses. The following paper by Weigert et al. (47) clearly establishes that isomaltopentaose, the oligosaccharide which best satisfies the combining site of a myeloma protein specific for dextran, is also the best inhibitor of its idiotypic specificity. Thus, idiotypic specificity is clearly seen to be progressively affected by a conformational change, involving each residue of the determinant that interacts with its specific combining site. Moreover myeloma proteins reactive with dextran differ in their idiotypic specificities if they differ in the sizes of their combining sites (QUPC 52 vs. W3434 or W3129) and even if the sizes of their combining sites are similar but the relative contribution of each residue to the binding energy varies (W3434 and W3129). Also UPC 102, an IgA protein, and MOPC 104E, an IgM protein (27) whose precipitation was inhibited best by an $\alpha(1 \rightarrow 3)$ -linked trisaccharide, nigerotriose, both inhibited the J558 idio type reaction equally but were much less effective than J558 itself, which was shown (29) to be most complementary to an $\alpha(1 \rightarrow 3)$ -linked pentasaccharide. With both UPC 102 and J558, maltose was a better inhibitor than isomaltose; however, with MOPC 104E, the reverse was noted.

With myeloma proteins reactive with fructosans, two W3082 and, UPC 61, both of which react with inulin and are inhibited best by the tetrasaccharide

3F1G, are identical in idiotype specificity and differ in idio type from J606, which also reacted with inulin but was most complementary to trisaccharide 2F1G (29). The other two fructosan-specific proteins UPC 10 and Y5476, which like human antilevans appear to have $\beta(2 \rightarrow 6)$ specificity, differ in idio type from W3082.

The detailed data given above for myeloma proteins reactive with dextran or with fructosans favor a high degree of similarity for these sites and antibody-combining sites. Thus the concentrations of isomaltose oligosaccharides required for inhibition of precipitation of the myeloma proteins by dextran were in the same range as those previously reported for human antidextran of $\alpha(1 \rightarrow 6)$ specificity (2, 8, 9, 13, 15, 16, 48 cf. 6). Also, the dextrans used for quantitative precipitin curves behaved in a quite comparable manner with the mouse myeloma proteins as with human antidextrans. It is highly significant that the two dextrans found to react differently with human $\alpha(1 \rightarrow 6)$ antidextrans, with Type II horse and rabbit antipneumococcal antibody, and with Type XX antipneumococcal antibody (3, 36 cf. 6) showed the identical anomalous behavior with two of the $\alpha(1 \rightarrow 6)$ -specific myeloma proteins (W3129 and W3434) studied. Thus, more dextran B742L-R was required for precipitation than expected from its content of $\alpha(1 \rightarrow 6)$ linkages, while B1299-S-3 was much more effective in precipitating than expected from its content of $\alpha(1 \rightarrow 6)$ linkages. Protein QUPC 52 with the largest size site reacted poorly with both B1299-S-3 and B742L-R, indicating its high specificity for long linear chains. These quantitative findings, in addition to the lower potencies of $\alpha(1 \rightarrow 4)$ -, $\alpha(1 \rightarrow 3)$ -, and $\alpha(1 \rightarrow 2)$ -linked disaccharides, provide important evidence that the isomaltose oligosaccharides are the actual determinants for which the sites on the myeloma proteins are specific.

The specificity of W3129 was examined in greater detail than those of the other two myeloma proteins. The only structural deviation from the isomaltose series that was tolerated was the substitution of a methoxyl group on C-3 of methyl α Dglucoside, which did not reduce complementarity. That methyl α Dmannoside and methyl β Dglucoside were less inhibitory than methyl α Dglucoside and that α DGlc(1 \rightarrow 6)DGlNAc, although a better inhibitor than methyl α Dglucoside, was less potent than IM2 indicated that the W3129 site required a terminal isomaltose determinant. The findings with the isomaltose oligosaccharides containing a single glucosyl branch at the second or third residue from their nonreducing ends are strikingly similar to those with $\alpha(1 \rightarrow 6)$ -specific human antidextrans (8) in that branching inhibited sterically to some extent but did not prevent access to the W3129-combining region.

It is well known that the immunodominant group, which in polysaccharides is often the terminal nonreducing end, contributes most to the binding energy with each successive residue of the determinant contributing a smaller increment (cf. 6, 12). Thus with human antidextran, assuming a ΔF° for the hexasaccharide of $-7,500$ cal., the first five sugars from the nonreducing end

contributed 97 and 98%, the first four 91 and 95%, and the first three 75 and 90% (2); with myeloma protein QUPC 52, most complementary to six glucoses, the values for the first five, four, and three were 93, 88, and 74% while with the two complementary to five glucoses (W3129 and W3434) the corresponding values for four, three, and two glucoses were 95 and 98%, 95 and 94%, and 75 and 74%, respectively. Since the antibodies were mixtures of molecules with different-sized combining sites, the values are in good agreement. Thus the relative binding energies of the homogeneous myeloma proteins are quite comparable to those for the heterogeneous antibodies. Using a value of $-6,400$ cal. for ΔF° (16), changes from the above percentages were not significant.

Myeloma proteins specific for fructosans were of two types as distinguished by their reactivity with inulin. Those precipitated by inulin (W3082 and UPC 61) appeared most specific for $\beta(2 \rightarrow 1)$ -linked fructose oligosaccharides since the disaccharide levanbiose, the only member of the $\beta(2 \rightarrow 6)$ -linked series available, was a much poorer inhibitor than 2F1G reduced and only slightly better than sucrose and 1F1G. In addition, with both proteins, maximum complementarity was for three $\beta(2 \rightarrow 1)$ -linked fructose units plus an additional β -linkage (3F1G). W3082 and UPC 61 appear to have virtually identical specificities but differ from myeloma protein J606 (29) in the size of their combining regions as measured with $\beta(2 \rightarrow 1)$ -linked oligosaccharides and in their approximately 20-fold greater affinity for sucrose and for 1F1G than for turanose (3-*O*- α DglucosylDfructose), while with J606, turanose was about twice as potent as sucrose and 1F1G.

UPC 10 and Y5476 as well as the human antilevans did not react with inulin nor were they inhibited by any oligosaccharides available; thus their specificities could not be studied. It is likely that these myeloma proteins and the human antilevans have $\beta(2 \rightarrow 6)$ specificities as they reacted well with the $\beta(2 \rightarrow 6)$ -linked perennial rye grass levan (45, 46). Previous findings with the antilevan from subject 1 also suggested a $\beta(2 \rightarrow 6)$ specificity (20).

The possibility for two types of fructosan-specific combining sites is compatible with models of $\beta(2 \rightarrow 6)$ - and $\beta(2 \rightarrow 1)$ -linked fructosyl trisaccharides. Thus the terminal nonreducing $\beta(2 \rightarrow 1)$ - and $\beta(2 \rightarrow 6)$ -linked fructofuranosyl residues appear to be very similar while chains of $\beta(2 \rightarrow 6)$ and $\beta(2 \rightarrow 1)$ residues are quite different (Fig. 8). This could account for the $\beta(2 \rightarrow 1)$ specificity of those proteins reactive with inulin and suggests that their reactions with levans occur at the nonreducing ends of $\beta(2 \rightarrow 6)$ chains. Those proteins reactive with levans but not with inulin would probably have sites complementary to chains of $\beta(2 \rightarrow 6)$ -linked fructoses longer than two sugars since inhibition could not be obtained with fairly high amounts of levanbiose or of branched levantriose.

With IgA myeloma protein W3129, the differences from the classical behavior in quantitative precipitin (Fig. 3 A) and inhibition assays (Fig. 5) were accounted for by the presence of a monomer-polymer system. The ability of

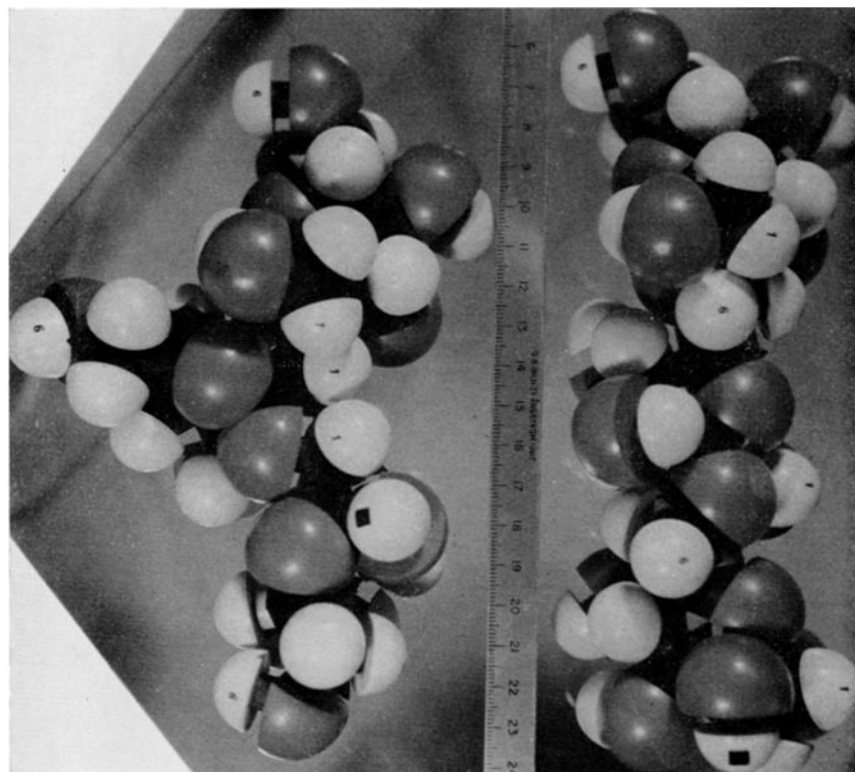


FIG. 8. Models of trisaccharides of fructose. *Left*: β -D-fructofuranosyl(2 \rightarrow 1) β -D-fructofuranosyl(2 \rightarrow 1)D-fructose. *Right*: β -D-fructofuranosyl(2 \rightarrow 6) β -D-fructofuranosyl(2 \rightarrow 6)D-fructose. The reducing end has a square of black tape on the hydrogen of the glycosidic hydroxyl.

W3129 monomer to inhibit competitively precipitation by polymer was much greater with clinical dextran N-150N (Fig. 3 C) than with native dextran B512 (Fig. 3 B). Residual precipitation by the monomer fraction was most likely due to small amounts of polymer present. Thus, inhibition of precipitation of polymer by monomer rather than combining site heterogeneity or solubility was the primary basis for the two dextrans precipitating different amounts of N from W3129. This finding strongly supports the explanation suggested (49) to account for the failure of a soluble antigen to precipitate as much of a myeloma protein as could be removed on an insoluble adsorbent prepared from the same antigen and to account for differences in amounts of myeloma protein precipitated by various antigens. Moreover, since dextrans N-150N and B512 differ only in molecular weight, it appears that IgA monomer acts more effectively as an inhibitor with an antigen having fewer determinants per molecule. Indeed with the IgA myeloma proteins J558 (29) and UPC 102, which were specific for nigerose oligosaccharides, more myeloma protein N was precipi-

tated by dextrans higher in $\alpha(1 \rightarrow 3)$ -like linkages and therefore containing more determinants per molecule.

SUMMARY

Four BALB/c IgA mouse myeloma proteins (W3129, W3434, QUPC 52, and UPC 102) reactive with dextran, four myeloma proteins reactive with fructosans, three IgA (W3082, UPC 61, and Y5476), and one IgG2a (UPC 10), and two human antilevans were studied immunochemically. Quantitative precipitin and inhibition assays showed that W3129, W3434, and QUPC 52 had specificities for isomaltose oligosaccharides similar to those previously found with $\alpha(1 \rightarrow 6)$ -specific human antidextrans. W3129 and W3434 were most complementary to IM5 but W3129 reacted equally with IM4 and IM3 while W3434 had a greater affinity for IM4 than IM3. QUPC 52 had a larger combining region and was most complementary to IM6. Protein UPC 102 (IgA), like MOPC 104E (IgM) (27), was most complementary to the $\alpha(1 \rightarrow 3)$ -linked trisaccharide, nigerotriose, and thus differed from J558 (29), which was inhibited best by nigeropentaose. UPC 102 was similar to J558 but they differed from MOPC 104E in their reactions with non- $\alpha(1 \rightarrow 3)$ -linked disaccharides.

The fructosan-specific myeloma proteins fell into two groups with different specificities. The first group, W3082 (IgA), UPC 61 (IgA), and the previously studied J606 (IgG3) (28, 29), reacted with inulin and W3082 and UPC 61 appeared to have identical specificities for $\beta(2 \rightarrow 1)$ -linked fructofuranosyl residues with maximum complementarity for the tetrasaccharide β Dfructofuranosyl(2 \rightarrow 1) β Dfructofuranosyl(2 \rightarrow 1) β Dfructofuranosyl(2 \rightarrow 6)Dglucose while protein J606 was inhibited best by the trisaccharide β Dfructofuranosyl(2 \rightarrow 1) β Dfructofuranosyl(2 \rightarrow 6)Dglucose. W3082 and UPC 61 also differed from J606 in their behavior toward sucrose and β Dfructofuranosyl(2 \rightarrow 6)Dglucose as compared with α Dglucosyl(1 \rightarrow 3)Dfructose (turanose). The second group containing myeloma proteins UPC 10 (IgG2a) and Y5476 (IgA) behaved similarly to human antilevans in that neither reacted with inulin nor were they inhibited by the $\beta(2 \rightarrow 1)$ -linked fructose oligosaccharides. Unlike the $\beta(2 \rightarrow 1)$ -specific proteins, they reacted with perennial rye grass levan that contained over 90% $\beta(2 \rightarrow 6)$ links. The differences in specificity and site size among homogeneous mouse myeloma proteins reactive with the same antigenic determinant are completely consistent with the concept that they represent products of homogeneous clones selected from the known heterogeneous population of antibody-forming cells.

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