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IMMUNOCHEMICAL STUDIES ON DEXTRAN-SPECIFIC AND LEVAN-SPECIFIC MYELOMA PROTEINS FROM NZB MICE*†

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ABSTRACT

Two dextran-specific (PC 3858 and PC 3936) and one levan-specific (PC 3660) NZB myeloma proteins were studied by quantitative precipitin and precipitin-inhibition assays. Both myeloma antidextrans were α-D-(1→6) specific and precipitated strongly with a synthetic, linear dextran, molecular weight 36,500, and with other dextrans. The two myeloma antidextrans differed with respect to their relative reactivities with dextrans containing various proportions of α-D-(1→6), α-D-(1→4)-like, and α-D-(1→3)-like linkages. In inhibition assays, the two antidextran myeloma proteins behaved differently from each other, from α-D-(1→6)-specific BALB/c myeloma antidextrans, and from the human antidextrans previously studied. Isomalto-oligosaccharides IM3, IM4, and IM5 were all equal in inhibitory power but were only about 60% as potent as IM6 and IM7, which also inhibited equally on a molar basis. Although precipitation with linear dextran suggests that both may have groove-type sites, as previously inferred for QUPC 52, the size of their combining sites is uncertain. It is not clear whether the sites are only as big as three glucose residues with the increased inhibition by six and seven glucose residues being attributable to partial bivalence and to their ability to combine in several ways along the chain, or whether the site is as big as six glucose residues with the increment in binding by the fourth and fifth glucose residues being minimal and the sixth contributing considerable additional binding-energy. The fructan-specific myeloma protein did not react with inulin, but reacted with many levans and with perennial rye-grass levan containing only β-D-(2→6) links. The levan–antilevan reaction was not inhibited by β-D-(2→1)-linked oligosaccharides. The findings suggest that PC 3660 has a specificity for (2→6)-linked chains.

*Dedicated to Dr. Allene Jeanes on the occasion of her retirement.

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INTRODUCTION

Myeloma proteins and Waldenström macroglobulins constitute selected monoclonal products derived from the progeny of a single, neoplastic, immunoglobulin-secreting plasma cell. In BALB/c mice, immunoglobulin-secreting plasmacytomas can be induced by intraperitoneal injection of mineral oil or implantation of solid plastic material[1–5], and this has recently been accomplished in NZB mice[6]. BALB/c and NZB mouse myeloma proteins having different antibody specificities have been found[3,6,7]. Amino acid sequences of the first 23 residues of the two NZB myeloma antidextrans differ[8] from those of BALB/c myeloma antidextrans W3129 and W3434. The immunochemical properties of NZB myeloma proteins have not been described, nor have any data been published on the combining sites of NZB myeloma proteins with a given antibody specificity.

This investigation compares, by quantitative precipitin and precipitin-inhibition assays, two antidextrans and one antilevan from NZB mice with those previously described in BALB/c mice and with human antibodies to dextran and levan.

EXPERIMENTAL

Materials and methods. — Myeloma proteins. All three NZB myeloma proteins PC 3858, PC 3936, and PC 3660 were IgAκ. Antidextrans PC 3858, PC 3936, and BALB/c myeloma antidextran W3129 did not show idiotypic cross-reactivity, nor did antilevan myeloma PC 3660 show idiotypic cross-reactivity with J606 or with eleven other myeloma antilevans. BALB/c myeloma proteins W3129 and QUPC 52, obtained from the National Institutes of Health and the Salk Institute, respectively, have been described previously[9].

Antigens. The dextrans and levans* were those used earlier[9–12]. Synthetic linear dextran D3 was provided by Dr. Conrad Schuerch, University of Syracuse, and dextran B-1254 S[Ł]† was provided by Dr. M. E. Slodki, Fermentation Laboratory, United States Dept. of Agriculture, Peoria, Illinois[13,14]. Inulin was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. The proportions of α-D-(1→6), α-D-(1→3)-like, and (1→4)/(1→2)-like linkages in the dextrans are given‡ in Fig. 1.

*B1299 Fr. S, B742 Fr. S, and B1355 Fr. S are the same dextran fractions designated as B1299-S-3, B742-L-R, B742-C-3R, and B1355-S-4 in earlier publications (see Fig. 1). Levan B512 Fr. B was previously designated[10] as levan B512 PP2 FR. B.

†The dextran designated B1254 fraction L in ref. 13 has been redesignated by Seymour *et al.* (in press) as B-1254 fraction S [Ł].

‡Recent methylation studies[13] give the following percentage values:

	(1→6)	(1→3)	(1→2)/(1→4)
B 512	95	5	0
B 1299 Fr. S	65	0	35
B 1355 Fr. S	65	35	0
B 1254 S [Ł]	77	0	23

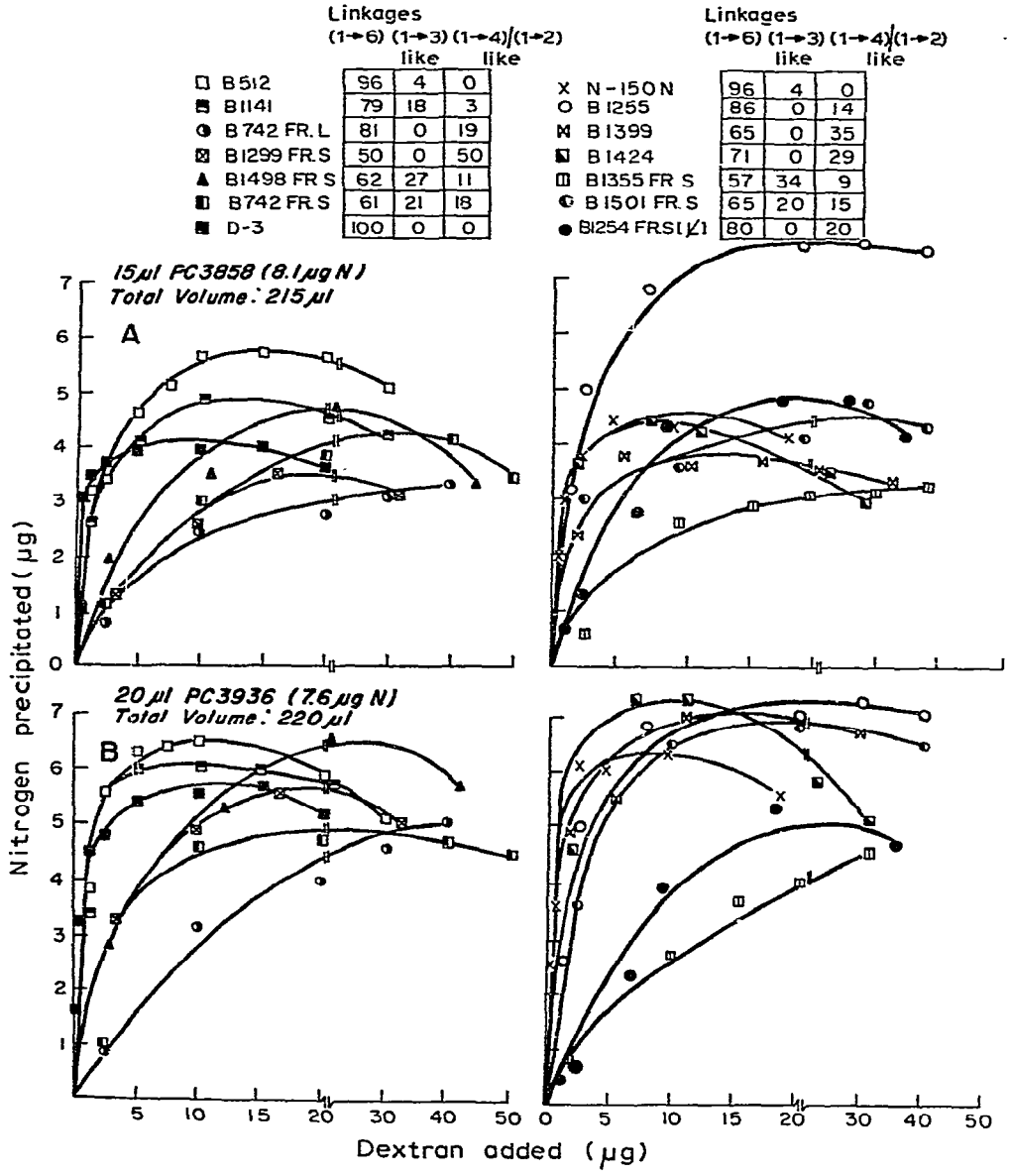


Fig. 1. Quantitative precipitin-curves of NZB mouse-myeloma proteins PC 3858 and PC 3936 with various dextrans.

Mono- and oligo-saccharides. D-Fructose, sucrose, isomaltose (IM2), isomaltotriose (IM3), isomaltotetraose (IM4), isomaltopentaose (IM5), isomaltohexaose (IM6), isomaltoheptaose (IM7), maltose, kojibiose (α-D-glucosyl-(1→2)-D-glucose), methyl α-D-glucopyranoside, and the branched oligosaccharides**, 3^4-α-D-glucosyl-isomaltohexaose and 4^4-α-D-glucosylisomaltohexaose were previously described[9,15–18]. Nigerose (α-D-glucosyl-(1→3)-D-glucose) was obtained from Dr. I. R. Johnston, University of Cambridge, England[19]. The di-, tri-, and tetra-saccharides, β-D-fructofuranosyl-(2→6)-D-glucopyranose (1F1G), β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-(2→6)-D-glucopyranose (2F1G), β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-(2→1)-β-D-fructofuranosyl-(2→6)-D-glucopyranose (3F1G) were kindly supplied by Dr. F. Arcamone, Istituto Richerche Farmitalia, Milano, Italy[20]. Levanbiose (β-D-fructofuranosyl-(2→6)-D-fructose) was a gift from Dr. S. T. Bauer, The Hebrew University, Jerusalem, Israel.

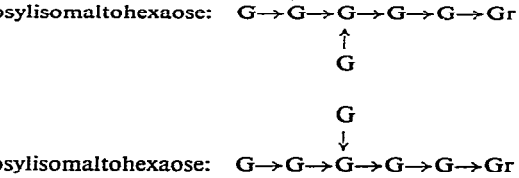
Immunochemical assays. Quantitative precipitin and inhibition assays were performed by a microprecipitin technique[21] using 7–8 μg of protein nitrogen for each determination; total nitrogen in the washed precipitates was estimated by the ninhydrin method[22]. Dextran B512 was chosen for inhibition assays; 8.1 μg was used with PC 3858, and 7.5 μg with PC 3936; levan P6 was chosen for the levan-specific inhibition assays and 8.9 μg was used.

RESULTS

Quantitative precipitin-studies on dextran-specific, NZB myeloma proteins. Both myeloma proteins precipitated well with all dextrans. The various dextrans differ in the maximum amounts of myeloma protein nitrogen that each could precipitate; this is generally ascribable[9] to the myeloma proteins being mixtures of monomeric and polymeric IgA. As shown in Fig. 1, PC 3858 and PC 3936 gave similar, quantitative precipitin-curves with various dextrans differing in α-D-(1→6), α-D-(1→3)-like, and α-D-(1→4)/(1→2)-like linkages. Synthetic, linear D3 (refs. 12 and 14) and primarily α-D-(1→6)-linked clinical dextran, N-150 N (mol. wt. 60,000, Commercial Solvents Corp.) were most effective per unit weight in precipitation, 50% precipitation requiring less than 0.7 μg. Native dextrans B512 and B1141 also were very potent precipitants, less than 1.2 μg giving 50% precipitation. From the amounts required

**The structures of the branched oligosaccharides[15,17] are depicted as follows:
G, D-glucopyranosyl; Gr, reducing D-glucose.
→, α-D-(1→6) link; ↑, α-D-(1→3) link; ↓, α-D-(1→4) link.

3^4-α-D-glucosylisomaltohexaose:



4^4-α-D-glucosylisomaltohexaose:

Superscripts denote the substituted glucose residue, counting from the reducing end.

for 50% precipitation of the maximum nitrogen that each dextran could precipitate, the dextrans fell into several groups in order of decreasing potency. 1: linear D3, N-150 N, B512 (PC 3936), and B1424 (PC 3858)>2: B512 (PC 3858), B1141, B1399, and B1424 (PC 3936)>3: B1255, B1501Fr.S, B1254Fr.S[Ł] (PC 3858), and B1299Fr.S (PC 3936)>4: B1498Fr.S and B742Fr.S for both proteins, B1299Fr.S, B742Fr.L and B1355Fr.S for PC 3858, and B1254Fr.S[Ł] for PC 3936>5: B742Fr.L and B1355Fr.S (PC 3936).

For comparison, quantitative precipitin-curves of BALB/c mouse myeloma proteins W3129 and QUPC 52 with dextrans B1255, B1254Fr.S[Ł], and B742Fr.L,

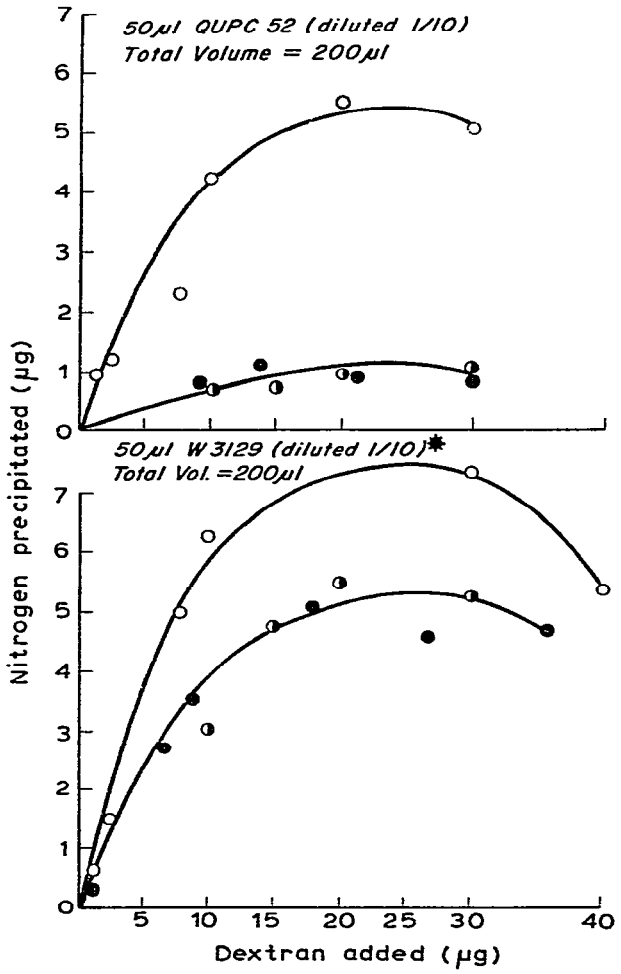


Fig. 2. Quantitative precipitin-curves of BALB/c mouse-myeloma proteins with dextrans B742 L and B1254S[Ł]. Symbols as for Fig. 1. *The graph of the earlier study[9] (Fig. 2B) stated that 25 µl W 3129 diluted 1:10 had been used. This was an error; 50 µl of 1:10 was actually used.

which were not studied previously[9] but which have a high proportion of α-D-(1→6) linkages, were also tested (Fig. 2). Dextran B1255 precipitated well with the dextran-specific NZB myeloma proteins, PC 3858 and PC 3936, and with the BALB/c myeloma proteins W3129 and QUPC 52. More B1254Fr.S[Ł] is required for 50% precipitation as compared with other dextrans having a similar proportion of α-D-(1→6) linkages, and less than the maximum amount of myeloma protein is precipitated. With QUPC 52, only about 18% of the maximum is precipitated by B1254Fr.S[Ł]. The NZB myeloma proteins and W3129 differed significantly from QUPC 52 in their activity with B1254Fr.S.[Ł] and B742Fr.L (Fig. 1 and 2). With QUPC 52, dextran B1255 gave a precipitin curve indistinguishable from that[9] of the most reactive dextran B512, whereas dextrans B1254Fr.S[Ł] and B742Fr.L showed unusually poor precipitating power (Fig. 2).

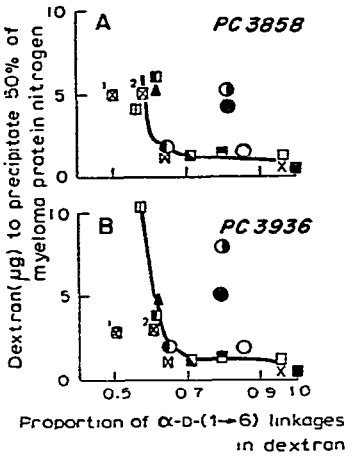


Fig. 3. Relation between precipitating power of dextran for antidextran and the proportion of α-D-(1→6) linkages. Symbols as for Fig. 1. (1) The proportion of α-D-(1→6) linkages was reported[9–12] as 50%. 2. The proportion of α-D-(1→6) linkages was reported[13] as 65%.

From Fig. 3, it may be seen that the two NZB myeloma antidextrans were α-D-(1→6)-specific, since reactivity increased as the proportion of α-D-(1→6) linkages increased. Three dextrans, B742Fr.L, B1254Fr.S[Ł], and B1299Fr.S behaved atypically. All three deviated from the curve in exactly the same way as they had[9] with BALB/c myeloma protein W3129; with four α-D-(1→6)-specific human antidextrans[23], B742Fr.L and B1299Fr.S had also deviated from the curves in the same way as they did in Fig. 3. B1254Fr.S[Ł] had not been studied. Dextrans B742Fr.L and B1254Fr.S [Ł], containing 19 and 20% of α-D-(1→4) and α-D-(1→2) linkages, are significantly less effective per unit weight in precipitating ability; native dextran B1299Fr.S with 50% α-D-(1→4)/(1→2)-like linkages appeared more effective than expected (Fig. 3).

Oligosaccharide inhibition-assays of dextran-specific myeloma proteins. The ability of various oligosaccharides to inhibit precipitation between PC 3858 or

PC 3936 and dextran B512 is illustrated in Fig. 4. Both proteins were α-D-(1→6)-specific and showed similar patterns with the isomalto-oligosaccharides. Both were also inhibited by the branched oligosaccharides, 3^4-α-D-glucosylisomaltohexaose and 4^4-α-D-glucosylisomaltohexaose. However, the two branched oligosaccharides were less inhibitory on a molar basis than isomaltotriose or any other larger linear α-D-(1→6)-linked oligosaccharide; they differed in that, relative to isomaltotriose, they were twice as potent with PC 3936 as with PC 3858. The α-D-(1→4)-, α-D-(1→3)-, or α-D-(1→2)-linked disaccharides and methyl α-D-glucoside were the weakest inhibitors of all oligosaccharides tested. The order of inhibitory potency was IM7 and IM6 > IM5, IM4, and IM3 > 3^3-α-D-glucosylisomaltohexaose and 4^4-α-D-glucosyl-isomaltohexaose ≫ IM2 ≫ maltose, nigerose, kojibiose, and methyl α-D-glucoside. With

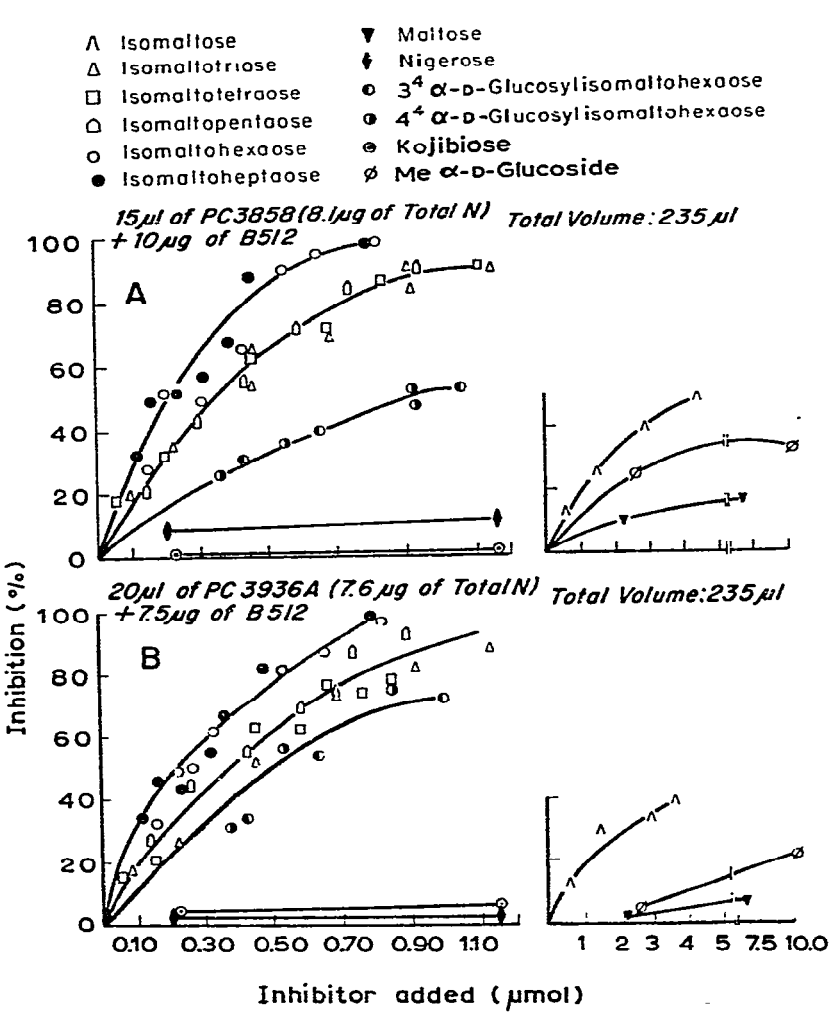


Fig. 4. Inhibition by various oligosaccharides of precipitation of mouse-myeloma proteins by dextran.

QUPC 52, which had not previously been studied with the branched oligosaccharides, 4^4-α-D-glucosylisomaltohexaose was about twice as potent as IM3, whereas 3^4-α-D-glucosylisomaltohexaose was only as effective as IM3.

Quantitative precipitin-studies on a levan-specific myeloma protein. Quantitative precipitin-curves of the NZB mouse myeloma proteins with various levans and with

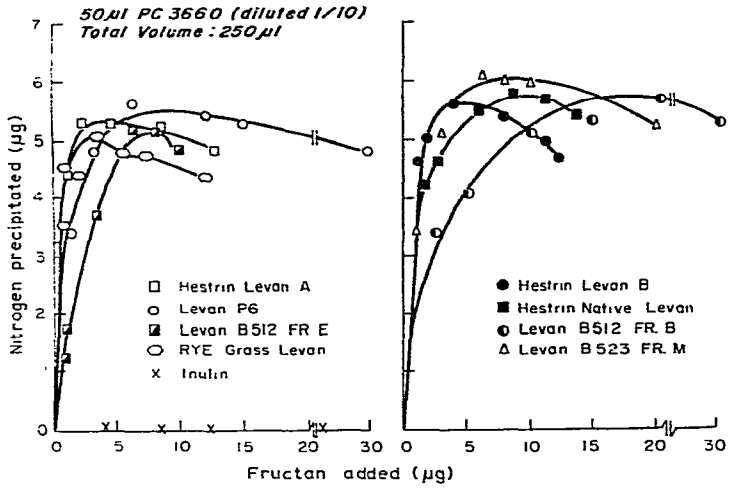


Fig. 5. Quantitative precipitin-curves of NZB mouse-myeloma protein PC 3660 with various levans and with inulin.

TABLE I

NHIBITION BY VARIOUS OLIGOSACCHARIDES OF PRECIPITATION OF MYELOMA PROTEIN PC 3660 BY LEVAN[a]

Inhibitor[b]	*Nmol used*	*Inhibition* (%)
3F1G	2.7×10^2	0
	4.0×10^2	4.8
2F1G	7.6×10	0
	1.1×10^3	3.6
1F1G	7.9×10^3	0
	1.6×10^4	0
Levanbiose	2.5×10^2	0
	4.2×10^2	0
Sucrose	9.8×10^3	0
	5.9×10^4	0
D-Fructose	1.5×10^4	0
	7.6×10^4	4

[a](50 µl Myeloma protein, diluted 1/10) + 8.9 µg of levan P6; total volume = 265 µl. [b]Structures are given in Materials and Methods.

inulin are presented in Fig. 5. All levans, including perennial rye-grass levan, reacted very well, whereas inulin, containing β-D-(2→1) linkages, did not precipitate. The preparation of perennial rye-grass levan used contained mostly β-D-(2→6) linkages[24], although recent studies have yielded a fraction containing only β-D-(2→6) linkages[25]. An amount of 2.5 μg of levan B512Fr.B was needed for 50% precipitation, as compared with less than 1 μg of the others.

Oligosaccharide inhibition-assays with the levan-specific, myeloma protein. The β-D-(2→1)-linked oligosaccharides 3F1G and 2F1G, 1F1G, levanbiose, sucrose, and also D-fructose, were used. As shown in Table I, PC 3660 myeloma protein was not inhibited significantly by any of the mono- and oligo-saccharides tested; the highest concentrations used are given.

DISCUSSION

In the present investigation, two NZB myeloma proteins specific for dextran (PC 3858 and PC 3936) and one specific for levan were studied by quantitative precipitin and precipitin-inhibition assays and compared with respect to the specificity and sizes of their combining sites with the BALB/c antidextran myelomas and with human antidextrans.

Both NZB myeloma antidextrans reacted with most dextrans in a manner generally similar to the α-D-(1→6) human antidextrans[9,11,12,21,26–29] and the BALB/c myeloma proteins, W3434, W3129, and QUPC 52 (refs. 9 and 12). Both NZB myeloma antidextrans, like QUPC 52 (ref. 12), precipitated strongly with a synthetic, linear dextran (D3) having a molecular weight of 36,500. Most striking are the observations (Fig. 3) that two dextrans, B1254S[Ł] and B742Fr.L, did not behave in the manner expected from their proportion of α-D-(1→6) linkages. B742Fr.L also reacted in the same atypical manner with the BALB/c myelomas[12], human antidextrans[23], and type II and XX horse antipneumococcal sera that cross-react with dextran[11], indicating the same structural relationships of all dextrans in reacting with these different antibody sites.

Despite their general similarity in being α-D-(1→6) specific, the two NZB myelomas differed from each other and also from the four BALB/c myeloma antidextrans previously studied[9]. Quantitative precipitin-curves showed them to precipitate with synthetic, linear, dextran D3 and they could therefore have groove- rather than cavity-type combining-sites[12], with specificity directed toward internal chains of α-D-(1→6)-linked glucose residues. In this respect, they may resemble QUPC 52, and could differ from W3129 (ref. 12). W3434 was not tested. Three antidextran myeloma proteins, the two NZB and BALB/c W3129, react well with B1254Fr.S[Ł] and B742Fr.L whereas QUPC 52 reacts poorly.

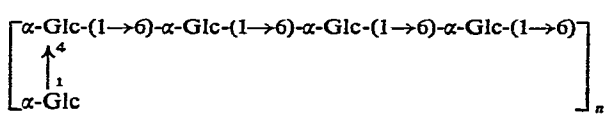
Comparisons of the quantitative precipitin-curves of the different dextrans with the two NZB myelomas is complicated, because these IgA myeloma proteins are usually mixtures of monomers and polymers, with the former tending to inhibit precipitation[9,12]. Assuming that the maximum amount of nitrogen precipitated by the synthetic, linear dextran (D3) relative to that by B512 may be taken as a rough

measure of the relative proportion of monomer to polymer, with PC 3936 this would then be 89%, whereas with PC 3858 it was only 68%. Thus, the latter contained more monomer. This result limits structural inferences from the quantitative precipitin-data as to differences between the two NZB myelomas.

The differences among the myeloma proteins are also supported by the inhibition data. The two NZB myelomas are α-D-(1→6)-specific, both showing a similar inhibition pattern with the isomalto-oligosaccharides. However, their inhibition patterns differ sharply from BALB/c myelomas[9,12] and from human antidextrans[26–29]. With the NZB myelomas, IM3, IM4, and IM5 inhibit equally on a molar basis. This would suggest that the groove-type combining sites are no larger than a trisaccharide. However, IM6 and IM7 are equally active and are about 1.8 times as potent as IM3, IM4, and IM5. This observation creates some ambiguity in defining the size of the combining site. Thus, the site could be complementary to a trisaccharide, with the increased potency of IM6 and IM7 being attributable to the emergence, with increasing chain length, of a second antigenic determinant, creating a statistically enhanced capacity to react with even a single combining site[30]. Alternatively, the site might be considered as big as a hexasaccharide, with the fourth and fifth sugars contributing relatively little to the binding energy, and the sixth making an appreciable contribution. Either of these interpretations shows PC 3936 and PC 3858 to differ from the other myeloma antidextrans, which just reach an upper limit.

The findings with 3^4- and 4^4-α-D-glucosylisomaltohexaoses are also of considerable importance and show the two NZB myeloma proteins to differ among themselves as well as from W3129 and QUPC 52. Thus, with PC 3936, 0.43 μmol of the 3^4- and 4^4-α-D-glucosylisomaltohexaoses were required for 50% inhibition, as compared with 0.37 μmol for IM3, whereas with PC 3858, the corresponding values were 0.95 μmol as compared with 0.35 μmol. With W3129, the branched oligosaccharides gave curves identical to those of IM3 and IM4 as inhibitors[9]; with QUPC 52, the two branched oligosaccharides differed, 4^4-α-D-glucosylisomaltohexaose being only about one-fourth as potent as IM4 but twice as potent as IM3, whereas the 3^4-oligosaccharide was as active as IM3; with human α-D-(1→6) antidextrans they were as active as IM4 (ref. 17). Thus the QUPC 52 myeloma site appears more capable of detecting the steric or conformational changes induced by the glycosyl substitution on O-3 or O-4 of the fourth sugar from the reducing end.

Recent methylation studies on six atypical dextrans showed substantial structural variation[13]. One dextran (B1254Fr.S[Ł]) had about one terminal nonreducing D-Glc and one α-1,4,6-tri-*O*-substituted branch per three α-(1→6)-linked D-Glc residues plus a small number of α-(1→4)-linked D-Glc residues. Assuming an exclusively α-D-(1→6)-linked backbone chain, an average repeat-unit structure was drawn as shown (Glc is a D-glucopyranosyl group or a D-glucopyranose residue):



Although this structure gives no idea of the number of terminal chains of α-D-(1→6)-linked glucose residues, it explains to some extent the very poor cross-reactivity of QUPC 52 with B1254Fr.S[Ł]. Accepting the site of QUPC 52 as a groove complementary to six α-D-(1→6)-linked glucose residues[12], the repeating unit would provide a chain of but three α-D-(1→6)-linked glucose residues, and the α-D-(1→4) branches on every fourth glucose residue could well introduce substantial steric hindrance.

A much larger proportion of the total antibody is precipitated by B742Fr.L and by B1254Fr.S[Ł] from the two NZB myelomas having groove-type sites, but for which the size is ambiguous as already discussed, than from QUPC 52. However, these two dextrans also precipitate a correspondingly larger proportion of the antibody from W3129 having a cavity-type site. As these IgA myelomas are mixtures of monomers and polymers, and the monomers interfere to some extent with the maximum amounts of antibody precipitable, it is difficult to establish whether or not the two NZB myelomas are reacting better because their proposed groove-type sites are smaller than that of QUPC 52, thus permitting less steric hindrance from the α-D-(1→4) side-chains in the proposed structure. If this were the case, it would permit resolution of the uncertainty already noted in determining site size from the inhibition data. However, as with both NZB myelomas and W3129, B1254Fr.S[Ł] precipitates the same fraction of the total antibody, this inference may not be justified. Although the detailed structure of B742Fr.L is not known, its reactivity resembles that of B1254Fr.S[Ł].

With the myeloma proteins and with human antidextrans[23], and with types II and XX mouse antipneumococcal sera[11], B1299Fr.S showed greater reactivity than expected from its proportion of non-α-D-(1→6) linkages. Recent methylation studies[13] on B1299 showed the presence of 39% terminal, nonreducing D-Glc, 26% α-D-(1→6)-linked and 35% α-D-(1→2,6)-linked D-Glc; the structure proposed is a chain of (1→6)-linked D-glucose residues having alternating, α-D-(1→2), terminal branches. Thus the methylation data give a value of 61% α-D-(1→6) linkages. If this value is used instead of the 50% reported by periodate oxidation, the activity of B1299Fr.S falls exactly on the curve for both NZB myelomas, for the human anti-dextrans[23], and for cross-reactivity with types II and XX antipneumococcal sera[11]. The original and revised positions for the dextran are shown in Fig. 3 with super-scripts 1 and 2 respectively.

The levan-specific NZB mouse-myeloma protein (PC 3660) reacted with the fructans in a manner remarkably similar to that of BALB/c myeloma protein UPC 10 and Y5476; it did not react with inulin, but reacted with many levans and with perennial rye-grass levan, which contains mostly β-D-(2→6) links[24,25]. The levan–antilevan reaction was not inhibited by β-D-(2→1)-specific oligosaccharides. These findings suggest that PC 3660 has a specificity for β-D-(2→6)-linked chains.

No unique immunochemical characteristics were found for NZB as compared with BALB/c myeloma proteins. However, individual proteins showed differences in the sizes and the extents of the complementary area of their combining sites. The

results indicate that each myeloma protein in either BALB/c or NZB mice represents a population of molecules having homogeneous combining-sites produced by a clone selected at random from the diverse population of cells that usually give rise to heterogeneous populations of Ab molecules after antigenic stimulation.

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