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BINDING CONSTANTS OF LEVANS AND D-FRUCTO-OLIGOSACCHARIDES TO BALB/c AND NZB D-FRUCTAN-SPECIFIC, MYELOMA PROTEINS, DETERMINED BY AFFINITY ELECTROPHORESIS*

SHUNJI SUGII AND ELVIN A. KABAT

Departments of Microbiology, Human Genetics and Development, Neurology, and the Cancer Center/Institute for Cancer Research, Columbia University College of Physicians and Surgeons, New York, NY 10032 (U.S.A.)

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ABSTRACT

The association constants for the interaction of BALB/c (UPC 10, Y5476, W3082, and UPC 61) and NZB (PC 3660) myeloma anti-D-fructans in pure form, or in ascitic fluids, with high-molecular-weight levans (K^a) and with such low-molecular-weight compounds as rye-grass levan, inulin, sucrose, and D-fructo-oligosaccharides (K_i^a) were determined by affinity electrophoresis, measuring the extent of retardation of the D-fructan-specific band by levan and its restoration by the low-molecular-weight compounds and oligosaccharide haptens. With different levans, K^a values ranged from 1.14×10^5 to 1.52×10^6 mL/g for PC 3660, 1.35×10^5 to 6.45×10^5 mL/g for UPC 10, 1.0×10^4 to 7.9×10^4 mL/g for Y5476, 7.63×10^3 to 6.38×10^4 mL/g for W3082, and 3.35×10^3 to 1.54×10^4 mL/g for UPC 61. The retarded, D-fructan-specific bands of W3082 and UPC 61 were restored by inulin, having β-D-(2→1)-linkages, and rye-grass levan, having β-D-(2→6)-linkages, and those of PC 3660, UPC 10, and Y5476 by rye-grass levan. The K_i^a values of inulin with W3082 and UPC 61 were 10 times those of rye-grass levan. The K_i^a values of inulin (3.65×10^5 M^{-1} for W3082, and 4.44×10^5 M^{-1} for UPC 61) were very similar to those of [β-D-Fru*f*-(2→1)]$_2$-β-D-Fru*f*-(2→6)-D-Glc (**3**) (3.95×10^5 M^{-1} for W3082, and 4.5×10^5 M^{-1} for UPC 61). With sucrose and the D-fructo-oligosaccharides, the order of K_i^a values of W3082 and UPC 61 was **3** > β-D-Fru*f*-(2→1)-β-D-Fru*f*-(2→6)-D-Glc > sucrose > β-D-Fru*f*-(2→6)-D-Glc. With rye-grass levan, K_i^a values were 4.25×10^5 M^{-1} for PC 3660, 2.7×10^5 M^{-1} for UPC 10, and 8.77×10^4 M^{-1} for Y5476. These results confirm earlier findings that W3082 and UPC 61 have dual specificity for β-(2→1) and β-(2→6) D-fructofuranosyl linkages, that PC 3660, Y5476, and UPC 10 have specificity for β-(2→6) D-fructofuranosyl linkages, and that the combining sites of W3082 and UPC 61 are most complementary to the tetrasaccharide **3**. That W3082 and Y5476 share the same, cross-reacting

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idiotype, although their combining sites differ in specificity, provides further evidence that these two properties do not run parallel.

INTRODUCTION

The BALB/c and NZB lines of inbred mice produce myeloma proteins having different specificities after intraperitoneal injection of mineral oil or solid plastic material[1-7]. The immunological properties of BALB/c and NZB D-fructan-specific, myeloma proteins have been extensively studied by quantitative precipitin and precipitin-inhibition assays[8-10]. These D-fructan-specific, myeloma proteins are classified into two groups on the basis of their specificities. The members of the first group, BALB/c UPC 10 and Y5476 and NZB PC 3660, precipitate with rye-grass levan, having β-D-(2→6)-linkages, but not with inulin, having β-D-(2→1)-linkages, whereas those in the second, BALB/c W3082 and UPC 61, react with inulin but not with rye-grass levan[9,10,12,13]. W3082 and UPC 61 have the same idiotypic specificity[14,15], and their combining sites are most complementary[9,11] to the tetrasaccharide [β-D-Fru*f*-(2→1)]$_2$-β-D-Fru*f*-(2→6)-D-Glc (3). These specificities have been confirmed by determination of the binding properties in fluorescence studies of purified anti-D-fructans[11] and their Fab fragments[12,13]. Affinity electrophoresis using poly(acrylamide) gel has been developed for measurement of the binding constants of hapten–antibody with antibody in ascitic fluid, or with purified antibody[16,17], and of ligand–lectin or lectin in crude extracts[18-20]. Association constants for dextran–anti-dextran interactions determined by this method[16] using ascitic fluid were very similar to those of dextran-purified anti-dextran determined by equilibrium dialysis[11] and of the synthetic linear dextran D3-Fab fragment by fluorescence studies[21], establishing affinity electrophoresis as a useful method for determining binding-constants of antibodies in ascitic fluid and for studying their specificities. In the present investigation, attempts were made to determine the association constants of BALB/c and NZB anti-D-fructans in pure form, or in ascitic fluid, by affinity electrophoresis. The findings are in good agreement with previous reports[11-13] on the binding properties of myeloma anti-D-fructans.

MATERIALS AND METHODS

Materials. — Myeloma D-fructan-specific protein BALB/c W3082 (IgAκ), UPC 61 (IgA), Y5476 (IgAκ) and UPC 10 (IgG$_{2a}$κ), and NZB PC 3660 (IgAκ)[9,10] and levans used in this study[9,22] were described previously. Myeloma proteins W3082 and Y5476 were those purified earlier[9] by gel filtration on Sephadex G-75. Myeloma proteins in ascitic fluids were studied after the fluids had been clarified by centrifugation. To determine the association constants (K^a and K_i^a), the minimum amount of detectable, D-fructan-specific, myeloma protein, or D-fructan-specific protein in ascitic fluid, was used, namely, 0.5 μg of N in ascitic fluid per tube for UPC 10, UPC 61, and PC 3660, and 0.4 μg of N per tube for purified W3082 and Y5476.

Sucrose was dialyzed, and recrystallized from alcohol before use. Inulin was purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio. The di-, tri-, and tetra-saccharides, namely, *O*-β-D-fructofuranosyl-(2→6)-D-glucose (**1**), *O*-β-D-fructofuranosyl-(2→1)-*O*-β-D-fructofuranosyl-(2→6)-D-glucose (**2**), and *O*-β-D-fructofuranosyl-(2→1)-*O*-β-D-fructofuranosyl-(2→1)-*O*-β-D-fructofuranosyl-(2→6)-D-glucose (**3**), were kindly provided by Dr. F. Arcamone, Instituto Richerche Farmitalia, Milano[23], and Dr. G. Cassinelli, Farmitalia Carlo Erba, Richerca e Sviluppo Chimico, Milano, Italy.

Electrophoresis and calculation of association constants. — As reported previously[16,17], association constants by affinity electrophoresis are calculated from the extent of retardation or restoration of the mobility of the ligand-specific band as a function of ligand or inhibitor concentration in the separating gel. Affinity electrophoresis was conducted in 5% poly(acrylamide) gel by the modified procedure of Ornstein[24]. The samples of myeloma proteins were applied in 1:9 glycerol–water containing the solution for the stacking gel. To determine the association constant (K^a), levan at an appropriate concentration was added to the separating gel. To determine the association constants (K_i^a) of oligosaccharides and low-molecular-weight compounds, various concentrations of such inhibitors as rye-grass levan, inulin, sucrose, and D-fructo-oligosaccharides were added to the separating gel, together with an amount of levan P6 giving ~55% retardation of the D-fructan-specific band, and restoration of mobility by the inhibitor was measured. The final concentrations of levan P6 in the separating gel were, in μg/mL, 2.5 for PC 3660, 3.1 for UPC 10, 9.8 for Y5476, 40.4 for W3082, and 68.7 for UPC 61.

The dissociation constant (K^d) of the anti-D-fructan–levan complex was calculated by Eq. *1*, as described previously[16,17,25–28].

$$K^d = cRm_i/(Rm_0 - Rm_i), \qquad (1)$$

where Rm_0 and Rm_i are the relative mobilities of the D-fructan-specific band in the absence and the presence, in the separating gel, of levan of concentration (c).

When an inhibitor was added to the separating gel containing levan P6, the dissociation constant was calculated by Eq. *2*.

$$K_i^d = iK^d/\{[cRm_i^d/(Rm_0 - Rm_i^d)] - K^d\}, \qquad (2)$$

where i and Rm_i^d are, respectively, the concentrations of inhibitor in the separating gel containing a constant concentration (c) of levan P6, and the relative mobility of the D-fructan-specific band in the presence of both levan P6 and inhibitor.

Eqs. *1* and *2* can be transformed into Eqs. *3* and *4*, respectively.

$$1/Rm_i = (1 + c/K^d)/Rm_0 \qquad (3)$$

$$Rm_i^d/(Rm_0 - Rm_i^d) = K^d(1 + i/K_i^d)/c \qquad (4)$$

Therefore, if $1/Rm_i$ and $Rm_i^d/(Rm_0 - Rm_i^d)$ are plotted against c and i, respectively, straight lines will be obtained. The intercepts on the c-axis and i-axis

give the negative K^d and K_i^d values, respectively. Binding constants were expressed as association constants (K^a and K_i^a), the reciprocals of the dissociation constants.

RESULTS

Association constants (K^a) of various levans with myeloma anti-D-fructans. — The staining patterns of BALB/c and NZB myeloma anti-D-fructans were similar to those previously illustrated[16,17]. Mobility of the D-fructan-specific bands was retarded proportionally to levan concentration when levan was added to the separating gel. With an excess of levan, the D-fructan-specific band was completely retarded, and observed at the top of the separating gel. Two bands of W3082 and three or four bands of UPC 61 at 0.4 to 0.5 μg of N were retarded with levans, whereas only one band of PC 3660, UPC 10, or Y5476 at 0.4 to 0.5 μg of N was retarded. PC 3660, UPC 10, and Y5476 showed the wavy bands[17] with levans more frequently than did W3082 and UPC 61. In this study, only the main band corresponding to the monomer fraction was used for determination of the association constants (K^a). When the reciprocal values of the relative mobility (Rm) of the D-fructan-specific band were plotted against the concentration of levan in the separating gel, straight lines were obtained, as shown in Fig. 1. Correlation coefficients of the plots were not lower

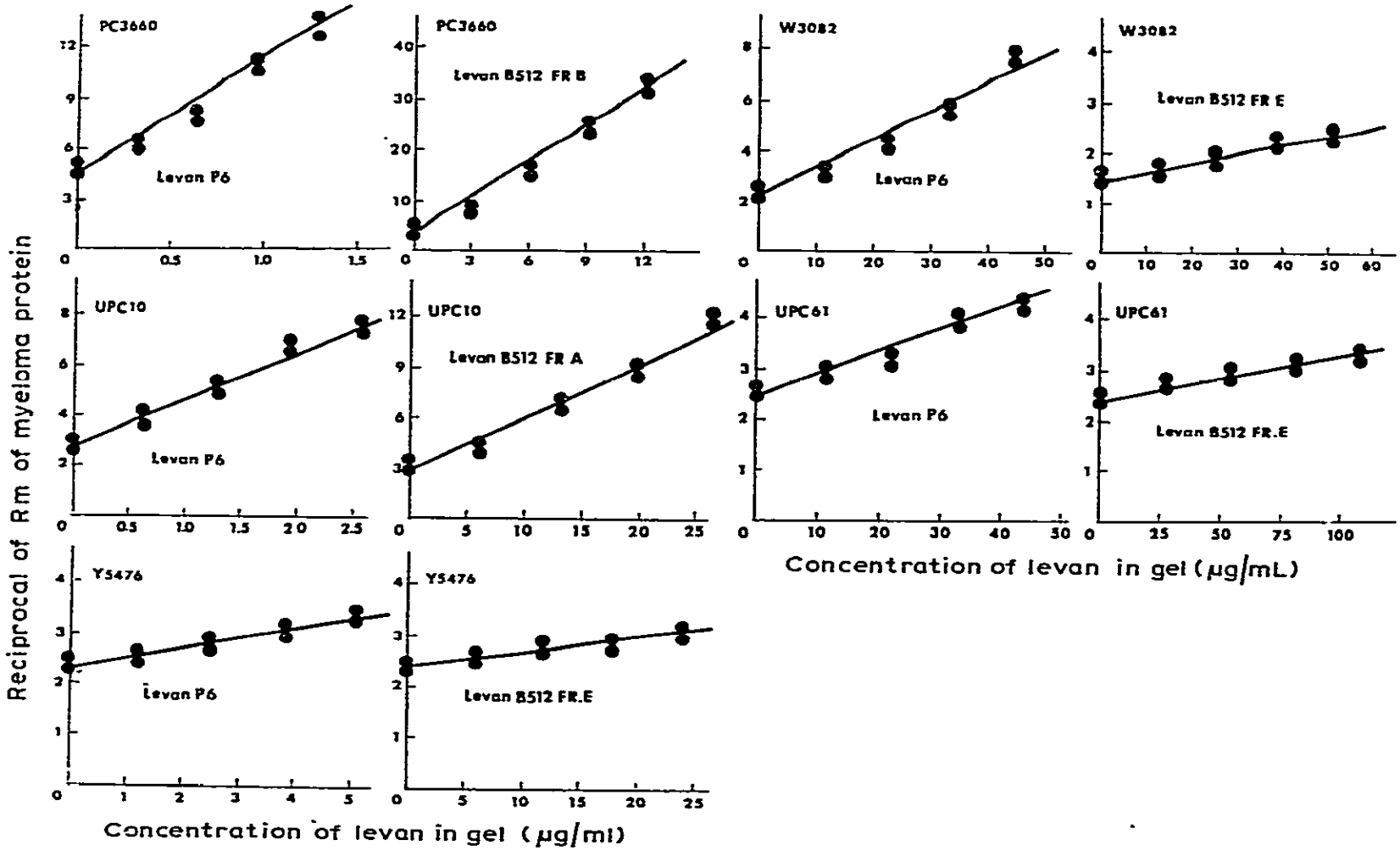

Fig. 1. Reciprocal values of relative migration distance (Rm) of myeloma anti-D-fructans against concentration of levans.

TABLE I

ASSOCIATION CONSTANTS (K^a) FOR THE BINDING OF VARIOUS LEVANS TO MYELOMA PROTEINS, DETERMINED BY AFFINITY ELECTROPHORESIS

Levan	*Association constants (mL/g)*				
	PC 3660	*UPC 10*	*Y5476*	*W3082*	*UPC 61*
P-6	1.52×10^6	6.49×10^5	7.9×10^4	6.38×10^4	1.54×10^4
B512 Fr. A	1.56×10^5	1.35×10^5	3.65×10^4	2.2×10^4	4.66×10^3
B512 Fr. B	1.14×10^5	1.44×10^5	2.45×10^4	1.26×10^4	7.51×10^3
B512 Fr. E	5.49×10^5	1.41×10^5	1.0×10^4	7.66×10^3	3.35×10^3
Hestrin levan (native)	8.6×10^5	4.67×10^5	4.8×10^4	3.51×10^4	1.47×10^4
Hestrin levan (Fr. A)	1.16×10^6	4.35×10^5	1.05×10^5	5.73×10^4	9.35×10^3
Hestrin levan (Fr. B)	6.02×10^5	4.62×10^5	5.8×10^4	2.94×10^4	5.46×10^3

than 0.93. Table I summarizes the K^a values obtained from the graphs shown in Fig. 1. The K^a values of PC 3660 and UPC 10 were always higher than those of Y5476, W3082, and UPC 61. The highest K^a values were obtained with levan P6 (1.52×10^6 mL/g for PC 3660, 6.49×10^5 mL/g for UPC 10, 7.9×10^4 mL/g for Y5476, 6.38×10^4 mL/g for W3082, and 1.54×10^4 mL/g for UPC 61), and the lowest K^a values with levan B512 fractions (1.14×10^5 to 5.49×10^5 mL/g for PC 3660, 1.35×10^5 to 1.44×10^5 mL/g for UPC 10, 1.0×10^4 to 3.65×10^4 mL/g for Y5476, 7.7×10^3 to 2.2×10^4 mL/g for W3082, and 3.35×10^3 to 7.51×10^3

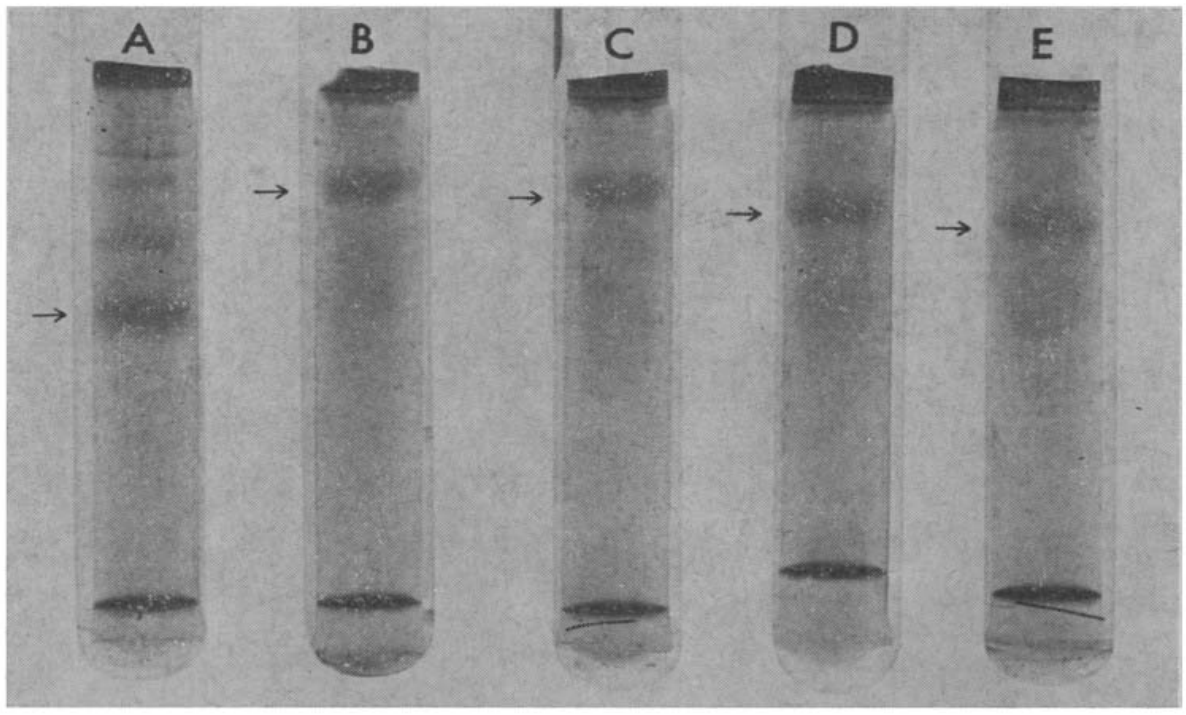

Fig. 2. Disc-electrophoresis patterns of myeloma anti-D-fructan UPC 61 in the presence of levan P6 and 1 in the gels. (A: no levan or 1; gels B to E contained 68.7 μg of levan P6 per mL. In addition, gels from C to E contained 1 at 0.18, 0.36, and 0.54mM, respectively.)

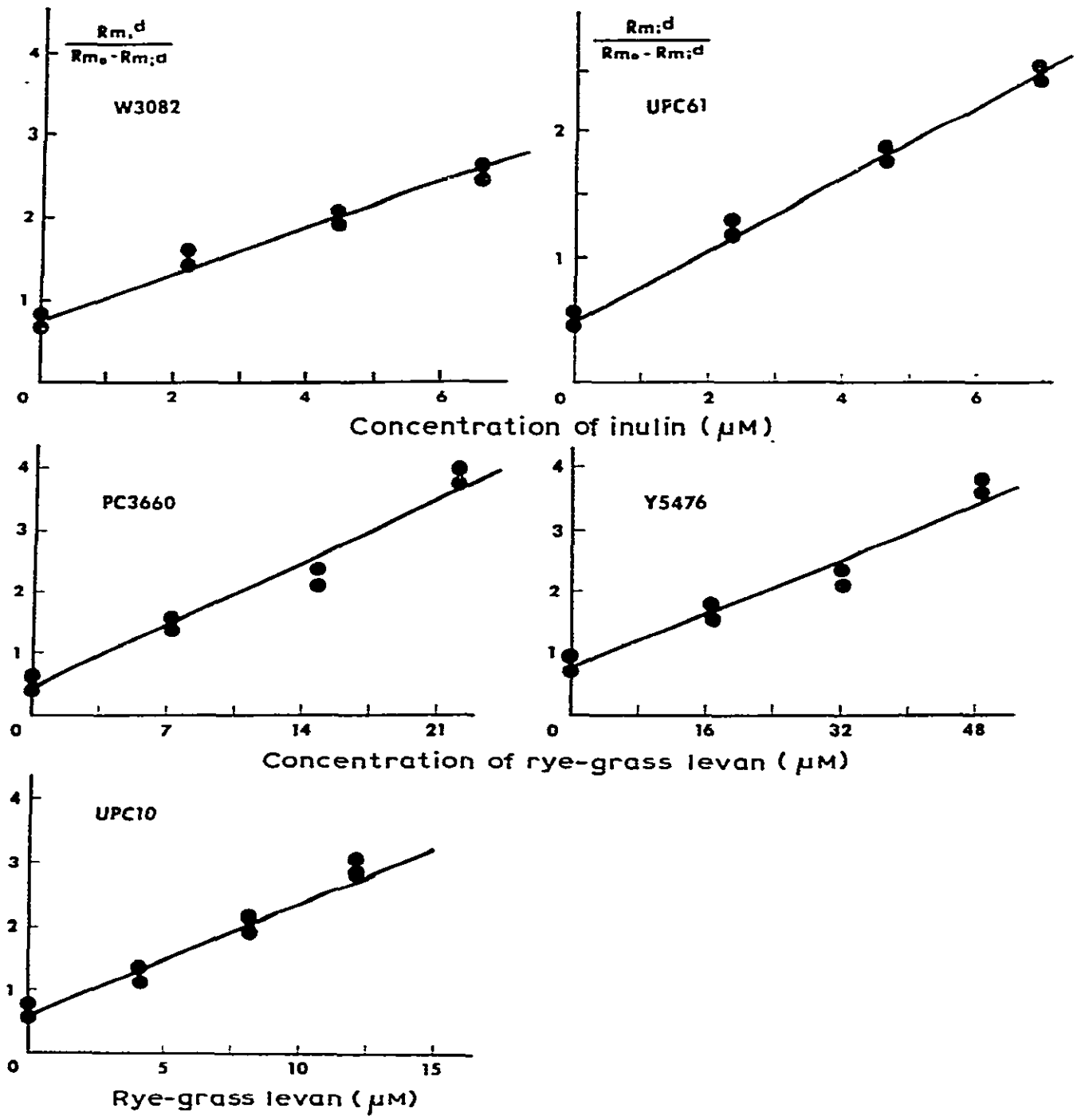

Fig. 3. Plots of myeloma anti-D-fructan–inulin or –rye-grass levan interaction.

mL/g for UPC 61). Intermediate K^a values were obtained with Hestrin levans (6×10^5 to 1.16×10^6 mL/g for PC 3660, 4.35×10^5 to 4.67×10^5 mL/g for UPC 10, 4.8×10^4 to 1.07×10^5 mL/g for Y5476, 2.94×10^4 to 5.73×10^4 mL/g for W3082, and 5.5×10^3 to 1.47×10^4 mL/g for UPC 61). When used with inulin or rye-grass levan at 500 μg/mL, values 7 to 200 times those of the levans giving 55% retardation, no retardation of the D-fructan-specific bands of five myeloma anti-D-fructans was observed.

Association constants (K_i^a) *of rye-grass levan, inulin, sucrose, and* D-*fructo-oligosaccharides and myeloma anti-*D-*fructans.* — The mobility of the D-fructan-specific band was restored proportionally to hapten concentration when hapten was added together with a constant concentration of levan P6 in the separating gel (see Fig. 2). With addition of an excess of hapten to the separating gel, the mobility of the D-fructan-specific band was completely restored, and corresponded to that of

TABLE II

ASSOCIATION CONSTANTS (K_i^a) FOR THE BINDING OF RYE-GRASS LEVAN, INULIN, SUCROSE, AND D-FRUCTO-OLIGOSACCHARIDES TO MYELOMA PROTEINS, DETERMINED BY AFFINITY ELECTROPHORESIS, COMPARED WITH THOSE OBTAINED BY PRECIPITATION-INHIBITION TESTS AND FLUORESCENCE STUDIES

Carbohydrate	*Association constants* (M^{-1})											
	W3082			*UPC 61*					*PC 3660*	*UPC 10*		*Y5476*
	AE[a]	*PI*[b]	*FQ*[b]	*AE*	*PI*[b]	*FQ*[b]	*FQ*[c]	*FQ*[d]	*AE*	*AE*	*FQ*[e]	*AE*
Rye-grass levan	4.76×10^4			4.36×10^4					4.24×10^5	2.7×10^5	1.97×10^5	8.77×10^4
Inulin	3.65×10^5			4.4×10^5			3.1×10^5		($<1.4 \times 10^3$)	($<1.4 \times 10^3$)		($<1.4 \times 10^3$)
Sucrose	8.47×10^2	6.3×10^2	2.6×10^2	1.03×10^3	6.3×10^2	8×10^2	5.3×10^2	6.8×10^2				
1	6.4×10^2	6.3×10^2	2.6×10^2	8.2×10^2	6.3×10^2	8×10^2	5.3×10^2	6.8×10^2				
2	2.35×10^5	3.6×10^5	1.9×10^5	2.69×10^5	3.6×10^5	2.5×10^5		1.32×10^4				
3	3.95×10^5	3.6×10^5	3.6×10^5	4.5×10^5	3.6×10^5	3.6×10^5	1.5×10^5	1.34×10^5				

[a]AE, affinity electrophoresis; PI, precipitin-inhibition tests; and FQ, fluorescence quenching. [b]See ref. 11. [c]K_i^a value of Fab fragment; ref. 12. [d]K_i^a values of monomer; these measurements, and those in footnote *c*, were made on the corresponding D-fructofuranosyl (2→1)-oligosaccharides, but linked β-(2→1) to D-Glc. [e]K_i^a value of Fab fragment; ref. 13.

the myeloma anti-D-fructan alone. The retarded, D-fructan-specific bands of W3082 and UPC 61 were restored by inulin and rye-grass levan, and those of PC 3660, UPC 61, and Y5476 were restored only by rye-grass levan. The plots calculated from Eq. *4* gave straight lines, as shown in Fig. 3. Correlation coefficients were not lower than 0.93. Table II shows the association constants (K_i^a) of rye-grass levan, inulin, sucrose, and D-fructo-oligosaccharides for the myeloma anti-D-fructans determined by affinity electrophoresis as compared with those determined by fluorescence studies on purified anti-D-fructans[11] and Fab fragments[12,13]. With inulin, sucrose, and D-fructo-oligosaccharides, the K_i^a values of W3082 were similar to those of UPC 61. The highest K_i^a value was obtained with **3** (3.95×10^5 M^{-1} for UPC 61), whereas the lowest was with **1** (6.4×10^2 M^{-1} for W3082 and 8.2×10^2 M^{-1} for UPC 61). The K_i^a values of inulin, 3.67×10^5 M^{-1} for W3082, and 4.44×10^5 M^{-1} for UPC 61, were quite similar to those of **3**. The K_i^a values of **2** were ~200 to 400 times those of sucrose or **1**, and 1/1.7 times those of **3**. Although the retarded, D-fructan-specific bands of W3082 and UPC 61 were also restored with rye-grass levan, the K_i^a values of rye-grass levan (4.76×10^4 M^{-1} for W3082, and 4.36×10^4 M^{-1} for UPC 61)

TABLE III

ASSOCIATION CONSTANT OF UPC 10 WITH NATIVE, HESTRIN LEVAN

	Association constant (mL/g)
1	3.12×10^5
2	2.46×10^5
3	3.79×10^5
4	4.42×10^5
5	3.21×10^5
6	2.76×10^5
7	2.81×10^5
8	3.00×10^5
9	4.46×10^5
Mean	3.36×10^5
S.d.	$3.36 \pm 0.72 \times 10^5$

TABLE IV

ASSOCIATION CONSTANT OF W3082 WITH **3**

	Association constant (M^{-1})
1	3.95×10^5
2	5.98×10^5
3	6.03×10^5
4	5.18×10^5
5	6.85×10^5
6	4.06×10^5
7	2.33×10^5
Mean	4.91×10^5
S.d.	$4.91 \pm 1.43 \times 10^5$

were 1/10th those of inulin. With rye-grass levan, the K_i^a values of PC 3660, UPC 10, and Y5476 were 4.24×10^5 M^{-1}, 2.7×10^5 M^{-1}, and 8.9×10^4 M^{-1}, respectively. Inulin at 3.15 mg/mL (corresponding to 1.4×10^3 M^{-1}) did not restore the retarded, D-fructan-specific bands of PC 3660, UPC 10, and Y5476.

The reproducibility of association constants determined by affinity electrophoresis. — As reported previously[17], the K_i^a value of isomalto-oligosaccharides to NZB myeloma antidextran PC 3936 varied over a 4-fold range, as PC 3936 usually showed a wavy band. To determine the reproducibility of the K^a and K_i^a values obtained in this study, the K^a and K_i^a values were calculated by making nine independent determinations with UPC 10 and native Hestrin levan, and seven independent determinations with W3082 and **3**. As shown in Tables III and IV, the K^a and K_i^a values ranged from 2.46×10^5 to 4.46×10^5 mL/g (mean value 3.36×10^5 mL/g) and from 2.33×10^5 to 6.85×10^5 M^{-1} (mean value, 4.91×10^5 M^{-1}).

DISCUSSION

The specificity and the combining-site size of a myeloma protein or plant lectin is usually determined by quantitative precipitin and precipitin-inhibition assays[6]. The binding properties of antigen–antibody or hapten–antibody and of ligand–lectin interactions are expressed as association constants determined by equilibrium dialysis, fluorescence quenching, and affinity electrophoresis. The specificity and the combining-site size of BALB/c myeloma anti-D-fructans determined by quantitative, precipitin-inhibition assays[8,9] have been established by measurement of their binding properties by fluorescence quenching[11–13]. BALB/c W3082 and UPC 61 have specificity for inulin, having β-D-fructofuranosyl (2→1)-linkages[9], whereas BALB/c UPC 10 and Y5476 and NZB PC 3660 are specific for rye-grass levan, having β-D-fructofuranosyl (2→6)-linkages[9,10,12,13]. By the results of measurement of the binding constants in affinity electrophoresis, the present study confirmed the earlier findings.

The D-fructan-specific bands of BALB/c and NZB myeloma anti-D-fructans were retarded in affinity electrophoresis by all levans tested, which were homopolymers of D-fructose with mainly β-D-(2→6)-linkages and β-D-(2→1) branches[29], similar to findings with BALB/c and NZB myeloma anti-dextrans and dextrans[16,18]. The binding properties (K^a values) of myeloma anti-D-fructans to levans are in good agreement with their precipitating properties as described previously[9,10]. When used with either inulin or rye-grass levan, the D-fructan-specific bands of these myeloma anti-D-fructans were not retarded in affinity electrophoresis, although BALB/c and NZB myeloma anti-D-fructans are precipitated with either inulin or rye-grass levan[9,10]; this is probably due to the low molecular weights of inulin[30,31] and rye-grass levan[13,32]. Similar findings were obtained in affinity electrophoresis with the completely linear dextran D3 and NZB myeloma anti-dextrans[17] PC 3858 and PC 3936. Dextran D3 (mol. wt. 36,500) is the most potent precipitinogen[10] for PC 3858 and PC 3936, but the K^a values of D3 to PC 3858 and PC 3936 determined by affinity electrophoresis were lower than those of the least-branched dextrans, such as

N-279, NRC fractions 8, 6, and 4, and about the same as those with NRC3, all with molecular weights[17] >36,500. Unlike the findings with dextran D3 and antidextran, inulin and rye-grass levan did not retard the mobility of the anti-levan myelomas, probably due to their lower molecular weight. However, as inhibitors, inulin and rye-grass levan restored the retarded D-fructan-specific bands of W3082 and UPC 61, and rye-grass levan, those of PC 3660, UPC 10, and Y5476, showing that BALB/c myeloma anti-D-fructans W3082 and UPC 61 show apparent dual specificity for β-D-fructofuranosyl (2→1)- and (2→6)-linkages, and BALB/c UPC 10 and Y5476 and NZB PC 3660 have specificity for β-D-fructofuranosyl (2→6)-linkages.

The dual specificity of W3082 and UPC 61 is supported by the findings that W3082 and UPC 61 precipitate with bacterial levans having mainly β-D-(2→6)-linkages, and that W3082 shows idiotypic cross-reactivity with Y5476 having a specificity for β-D-(2→6)-linkages[14]. Therefore, the specificities of W3082 and UPC 61 are similar to those of anti-inulin E109 and E4 with dual specificities[13]. That members of the two groups of myeloma anti-D-fructans can show the same idiotypic specificity is a further indication that idiotypic specificity and antibody combining-site specificity do not run parallel, in accord with the findings of Oudin and Cazenave[33], and tends to support the minigene hypothesis for the generation of antibody diversity[34-37]. The location of these cross-reacting, idiotypic determinants in the amino acid sequence, in relation to those amino acids forming the complementarity-determining regions of the antibody combining-site, could provide a test of this hypothesis.

The combining-site sizes of BALB/c W3082 and UPC 61 have been studied by precipitin-inhibition assays with D-fructo-oligosaccharides[9]. The order of inhibitory potency of these oligosaccharides was **3** > **2** > **1** ⩾ sucrose. These findings have been confirmed by the binding properties determined by fluorescence quenching[11-13]. As shown in Table II, the K_i^a values of D-fructo-oligosaccharides obtained by affinity electrophoresis increased as the number of β-D-fructofuranosyl (2→1)-linkages increased. Of the oligosaccharides available, the highest K_i^a values were obtained with **3**, indicating that the combining sites of myeloma W3082 and UPC 61 are most complementary to a chain of at least three β-D-fructofuranosyl units and a β-D-(2→6)-linkage to D-Glc (as in **3**). However, the finding that the K_i^a values of inulin with W3082 and UPC 61 are similar to those of **3** indicated that maximal complementarity was achieved with a tri-D-fructosyl β-D-(2→6)-linkage to D-glucose occupying the combining site[12]. Moreover, the fluorescence studies of Streefkerk and Glaudemans[12] have shown that the binding constants are lower if this (2→1) chain of the D-fructofuranosyl oligosaccharides is β-D-(2→1)-linked to D-Glc. In this connection, BALB/c myeloma W3082 and UPC 61 have the same specificities[9], but differ from BALB/c myeloma J606 in the sizes of their combining sites, as J606 is most complementary[8] to trisaccharide **2**.

The combining-site size of myeloma anti-D-fructans UPC 10, Y5476, and PC 3660, having specificity for β-D-(2→6)-linkages, could not be studied, as they were not inhibited by any D-fructo-oligosaccharides available[9,10]. The K_i^a value of rye-grass levan to UPC 10 (2.7×10^5 M^{-1}), obtained in this study, was similar to that

(1.99×10^5 M^{-1}) of the Fab fragment obtained by fluorescence quenching[13]. The K_i^a values of myeloma anti-D-fructans for rye-grass levan were calculated on the basis of a molecular weight of 5,000 for rye-grass levan[32] in this study, and the K_i^a values of the Fab fragment of rye-grass levan on a molecular weight[13] of 2,000 (from the data on the chemical analysis of rye-grass levan[38]). If the K_i^a values obtained in this study are calculated on the basis of a molecular weight of 2,000, they become 1.69×10^5 M^{-1} for PC 3660, 1.08×10^5 M^{-1} for UPC 10, and 3.49×10^4 M^{-1} for Y5476. In either case, K_i^a of the interaction of rye-grass levan with UPC 10 is similar to that determined by fluorescence quenching[13], as already mentioned.

It is of interest to compare the association constants (K_i^a) determined by affinity electrophoresis with those obtained by fluorescence, described previously[11–13]. The K_i^a values obtained by both methods are in good agreement[11–13], as shown in Table II. Comparable findings were previously reported by affinity electrophoresis using BALB/c myeloma anti-dextrans W3129 and QUPC 52 and isomalto-oligosaccharides[16]. Affinity electrophoresis is a simple, useful, and practical method for determining the interaction of antigen–antibody or hapten–antibody interactions with association constants ranging from 10^2 to 10^6 mL/g or M^{-1}, respectively[16,17]. Although the precision of affinity electrophoresis is somewhat lower than that of other methods, the ability to (*a*) use ascitic or culture fluid instead of purified antibody, and (*b*) determine binding-constants for interaction of macromolecules with antibody, offers substantial advantages.

REFERENCES

1 M. Potter and C. R. Boyce, *Nature (London)*, 193 (1962) 1086–1087.
2 M. Potter and R. L. Lieberman, *Adv. Immunol.*, 7 (1967) 91–145.
3 M. Potter, *Fed. Proc.*, 29 (1970) 85–91.
4 M. Potter, *Physiol. Rev.*, 52 (1972) 631–708.
5 N. L. Warner, *Immunogenetics*, 2 (1975) 1–20
6 E. A. Kabat, *Structural Concepts in Immunology and Immunochemistry*, 2nd edn., Holt, Rinehart, and Winston, New York, 1976.
7 M. Potter, *Adv. Immunol.*, 25 (1977) 141–211.
8 A. Lundblad, R. Steller, E. A. Kabat, J. W. Hirst, M. G. Weigert, and M. Cohn, *Immunochemistry*, 9 (1972) 535–544.
9 J. Cisar, E. A. Kabat, J. Liao, and M. Potter, *J. Exp. Med.*, 139 (1974) 159–179.
10 A. M. Wu, E. A. Kabat, and M. G. Weigert, *Carbohydr. Res.*, 66 (1978) 113–124.
11 J. Cisar, E. A. Kabat, M. M. Dorner, and J. Liao, *J. Exp. Med.*, 142 (1975) 435–459.
12 D. G. Streefkerk and C. P. J. Glaudemans, *Biochemistry*, 16 (1977) 3760–3765.
13 D. G. Streefkerk, B. N. Manjula, and C. P. J. Glaudemans, *J. Immunol.*, 122 (1979) 537–541.
14 M. Weigert, W. C. Raschke, D. Carson, and M. Cohn, *J. Exp. Med.*, 139 (1974) 437–447.
15 R. L. Lieberman, M. Potter, W. Humphrey, Jr., E. B. Mushinski, and M. Vrana, *J. Exp. Med.*, 142 (1975) 106–119.
16 K. Takeo and E. A. Kabat, *J. Immunol.*, 121 (1978) 2305–2310.
17 S. Sugii, K. Takeo, and E. A. Kabat, *J. Immunol.*, 123 (1979) 1162–1168.
18 H. Hořejší, M. Tichá, and J. Kocourek, *Biochim. Biophys. Acta*, 499 (1977) 290–300.
19 H. Hořejší, M. Tichá, and J. Kocourek, *Biochim. Biophys. Acta*, 499 (1977) 301–308.
20 H. Hořejší, M. Tichá, and J. Kocourek, *Trends Biochem. Sci.*, 4 (1979) N6–N7.
21 L. G. Bennett and C. P. J. Glaudemans, *Carbohydr. Res.*, 72 (1979) 315–319.
22 P. Z. Allen and E. A. Kabat, *J. Exp. Med.*, 105 (1957) 383–394.
23 R. R. Arcamone, W. Barbieri, G. Cassinelli, and C. Pol, *Carbohydr. Res.*, 14 (1970) 65–71.

24 L. ORNSTEIN, *Ann. N. Y. Acad. Sci.*, 121 (1964) 321–349.
25 K. TAKEO AND S. NAKAMURA, *Arch. Biochem. Biophys.*, 153 (1972) 1–7.
26 K. TAKEO, A. KUWAHARA, H. NAKAGAWA, AND S. NAKAMURA, *Protides Biol. Fluids*, 23 (1975) 645–649.
27 K. TAKEO AND S. NAKAMURA, *Proc. Int. Symp. Affinity Chromatogr.*, (1978) 67.
28 K. NAKAMURA, A. KUWAHARA, AND K. TAKEO, *J. Chromatogr.*, 171 (1979) 89–99.
29 B. LINDBERG, J. LÖNNGREN, AND J. L. THOMPSON, *Acta Chem. Scand.*, 27 (1973) 1819–1821.
30 E. L. HIRST, C. I. MCGILVRAY, AND E. G. V. PERCIVAL, *J. Chem. Soc.*, (1950) 1297–1302.
31 L. A. BOGGS AND F. SMITH, *J. Am. Chem. Soc.*, 78 (1956) 1878–1880.
32 R. A. LAIDLAW AND S. G. REID, *J. Chem. Soc.*, (1951) 1830–1834.
33 J. OUDIN AND P. A. CAZENAVE, *Proc. Natl. Acad. Sci. U.S.A.*, 68 (1971) 2616–2620.
34 E. A. KABAT, T. T. WU, AND H. BILOFSKY, *Proc. Natl. Acad. Sci. U.S.A.*, 75 (1978) 2429–2433.
35 T. T. WU, E. A. KABAT, AND H. BILOFSKY, *Proc. Natl. Acad. Sci. U.S.A.*, 76 (1979) 4617–4621.
36 E. A. KABAT, T. T. WU, AND H. BILOFSKY, *J. Exp. Med.*, 149 (1979) 1299–1313.
37 E. A. KABAT, in E. COHEN AND H. KOHLER (Eds.), *Membranes, Receptors, and the Immune Response: Eighty Years After Ehrlich's Side Chain Theory*, Alan R. Liss, New York, 1980, pp. 1–46.
38 J. TOMAŠIĆ, H. J. JENNINGS, AND C. P. J. GLAUDEMANS, *Carbohydr. Res.*, 62 (1978) 127–133.