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A NEW, MOUSE-MYELOMA IMMUNOGLOBULIN A HAVING SPECIFICITY FOR β-D-(1→6)-LINKED D-GALACTOPYRANOSYL RESIDUES*

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ABSTRACT

The discovery of T601, a new mouse-myeloma immunoglobulin A having specificity for β-D-(1→6)-linked D-galactopyranosyl residues, brings the total number of known antigalactan immunoglobulins to seven. The interaction of T601 with a number of ligands has been investigated. For those ligands showing interaction with the immunoglobulin, the affinity constants have been quantitatively measured by tryptophanyl fluorescence. The values show that protein T601 behaves very similarly to protein X24.

INTRODUCTION

A large number of myeloma immunoglobulins having antibody activity have been described. Several of these proteins have similar ligand-binding specificity. Examples of such proteins are the antidextrans[1–3], antiphosphorylcholines[4–6], antifructans[3], and antigalactans[7,8]. There has been a great interest in the study of these proteins, as comparative studies of the individual members of any one group of proteins can be expected to provide valuable information in understanding the problem of antibody diversity. We have been involved for some time in the study of Balb/c myeloma immunoglobulins with specificity for β-D-(1→6)-linked D-galactopyranosyl residues[7–11]. Using well defined ligand-probes, we have explored[9] the combining sites of immunoglobulins J539 and X24, and now have seven antigalactans, namely JPC1, SAPC10, X24, X44, J539, T601, and T191. These proteins fall into three groups. On ligand binding, the tryptophanyl fluorescence of proteins X24, J539, and T601 is enhanced, that of protein X44 is quenched, and those of proteins S10, J1, and T191 exhibit no change[8]. In an attempt to understand the combining-site specificity of the various antigalactans, we have studied the ligand-binding affinity of protein T601, and compared the results with those in our earlier reports[9–11].

*Dedicated to Professor Michael Heidelberger in honor of his 87th birthday.
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RESULTS AND DISCUSSION

Table I shows the effect of various saccharide ligands on the tryptophanyl fluorescence of the Fab' fragment[8] of protein T601. A significant increase in fluorescence was observed with D-galactose and with β-D-galactose derivatives, whereas none of the other saccharides tested showed any change in fluorescence. This result is in close agreement with the results obtained[8,9,11] with the antigalactans J539 and X24, suggesting that the saccharide specificity of protein T601 is very similar to that of these other antigalactans.

TABLE I

MAXIMUM CHANGES IN THE TRYPTOPHANYL FLUORESCENCE OF PROTEIN T601 FAB' BROUGHT ABOUT BY LIGANDS

Ligand	*Increase in fluorescence* (%)
Gal_2[a]	27.8
Gal_3[a]	30.6
Gal_4[a]	37.2
6-*O*-β-D-Galactopyranosyl-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose	24.9
Methyl β-D-galactopyranoside	15.3
Methyl 6-*O*-acetyl-β-D-galactopyranoside	18.6
Methyl β-L-arabinopyranoside	none
Methyl α-D-mannopyranoside	none
Methyl β-D-gulopyranoside	none
Isopropyl 1-thio-β-D-galactopyranoside	19.5
D-Galactose	6.7
2-Acetamido-2-deoxy-D-galactose	none
1,4-Anhydro-D-galactitol	none
D-Galactose oxime	none
DL-Inositol	none
iso-Inositol	none
Lactose	none
Cellobiose	none
Melibiose	none
Sophorose (2-*O*-β-D-glucopyranosyl-D-glucose)	none
Epoxypropyl β-D-galactopyranoside	17.3

[a] Gal_2: 6-*O*-β-D-galactopyranosyl-D-galactose. Gal_3 and Gal_4 are the corresponding tri- and tetrasaccharides.

The binding constants of the various ligands that caused an appreciable increase in the fluorescence of T601 Fab' were determined by the fluorescence-titration method[12], and the results are given in Table II. The K_a values for the binding of various ligands to the Fab' fragment of T601, although not identical with those[9] for X24, were nearly so (see Table II). In addition, the similarity to the K_a values for the corresponding ligands and J539 were very close[9] (see Table II). Table III shows the ratio of the free energy (ΔG) of binding (calculated from the expression $\Delta G =$

TABLE II

AFFINITY CONSTANTS OF VARIOUS D-GALACTOSE DERIVATIVES WITH PROTEIN T601 FAB'

Ligand	$K_a \times 10^{-3}$ (*liter. mol*$^{-1}$)
Gal_2[a]	7.3
Gal_3[a]	100
Gal_4[a]	270
6-*O*-*β*-D-Galactopyranosyl-1,2:3,4-di-*O*-isopropylidene-*α*-D-galactopyranose	8.42
Methyl *β*-D-galactopyranoside	0.304
Methyl 6-*O*-acetyl-*β*-D-galactopyranoside	0.614
Isopropyl 1-thio-*β*-D-galactopyranoside	0.37
Epoxypropyl *β*-D-galactopyranoside	1.21

[a]See footnote to Table I.

TABLE III

RATIO OF THE FREE ENERGY OF BINDING FOR SEVERAL LIGANDS TO THE FREE ENERGY OF BINDING OF 6-*O*-*β*-D-GALACTOPYRANOSYL-D-GALACTOSE WITH IMMUNOGLOBULINS T601, X24, AND J539

Ligand	$\Delta G_{ligand}/\Delta G_{Gal_2}$[a]		
	T601	*X24*	*J539*
Gal_2[b]	1.00	1.00	1.00
Gal_3[b]	1.29	1.40	1.28
Gal_4[b]	1.41	1.45	1.40
6-*O*-*β*-D-Galactopyranosyl-1,2:3,4-di-*O*-isopropylidene-*α*-D-galactopyranose	1.02	—	1.09
Methyl *β*-D-galactopyranoside	0.64	0.69	0.74
Methyl 6-*O*-acetyl-*β*-D-galactopyranoside	0.72	—	0.79
Isopropyl 1-thio-*β*-D-galactopyranoside	0.67	0.71	0.75
Epoxypropyl *β*-D-galactopyranoside	0.80	0.89	0.92

[a]From data presented in this paper and in refs. 9 and 11. [b]See footnote to Table I.

$-RT\ln K_a$) of a ligand to that of the disaccharide 6-*O*-*β*-D-galactopyranosyl-D-galactose. As the differences in the free energy of interaction between ligand and protein reflect differences in binding (and, hence, structure), several proteins can be compared, using the same ligand-probe, to gain an insight into structural differences between proteins. Thus, it appears that the contact amino acids in the combining site of protein T601 are very similar to those in the combining site of proteins X-24 and J539.

To compare the structural similarity among the antigalactans exhibiting change in fluorescence on ligand binding, completely reduced and alkylated proteins were analyzed by isoelectric focusing on poly(acrylamide) gel in 6M urea (see Fig. 1). The general pattern was comparable to that obtained with mouse antiphosphoryl-

choline myeloma protein[13]. The light chains gave 3–4 bands, and their pattern was very similar for all of the proteins studied. On the other hand, the heavy chains gave a complex pattern, but distinct differences for individual proteins could be seen. The heavy chains of proteins T601 and X24 gave very similar patterns, but these were distinctly different from those of proteins X44 and J539. The patterns obtained for X44 and J539 were very similar to each other. These results suggest that protein T601 is structurally more similar to protein X24 than to proteins J539 and X44.

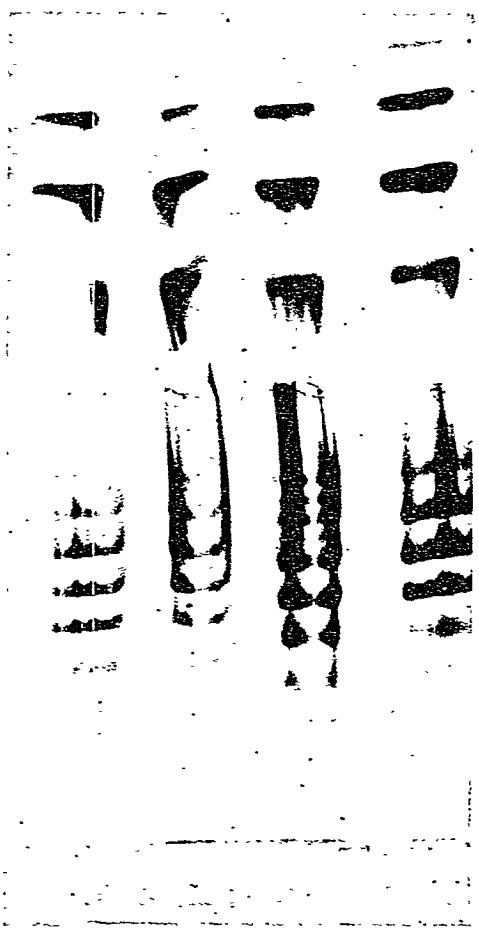

Fig. 1. Isoelectric focusing of anti-galactan immunoglobulins. (From left to right: X-44, J539, X24, and T601. The top three, major bands are from the light chains. The bottom half of the picture shows the pattern of the heavy chains.)

To find out whether protein T601 shares idiotypic specificity with any of the other antigalactans, antiserum to protein T601 (made in AL/N mice) was tested, by the micro-Ouchterlony technique, for its ability to precipitate the various antigalactans (see Fig. 2). Anti T601 (absorbed with normal Balb/c serum) gave a strong precipitin

line with protein 601, and a weak reaction with X24, but did not give a line with any of the other antigalactans tested (see Fig. 2a). The line with protein T601 spurred over the line with protein X24, showing cross reactivity but not identity. Moreover, when the anti-T601 was absorbed with protein X24, the antiserum still gave a precipitin line with protein T601 (see Fig. 2b). These results show that, although

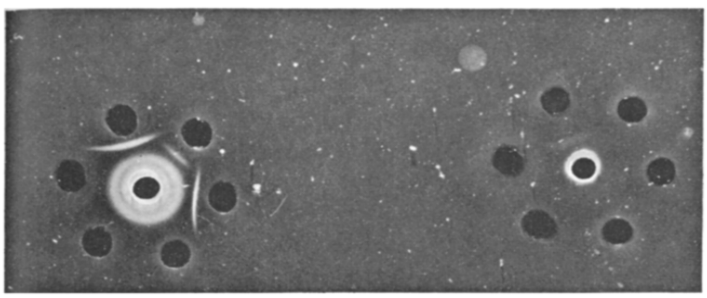

Fig. 2. (a) Double diffusion, in agar, of mouse anti-T601 antibody (center) *versus* (clockwise starting at the top) T601, X24, T601, J539, S10, and J1. (*b*) Mouse anti-T601 absorbed with X24 (center) *versus* (clockwise starting at the top) T601, X24, X44, J539, 510, and J1.

protein T601 shares cross-idiotypic specificities with protein X24, it still contains unique determinants. The results so far obtained here, and those already reported[9], thus indicate that, within the group of BALB/c myeloma immunoglobulins with antigalactan specificity, there are three subgroups. On binding with ligand, the tryptophanyl fluorescence of one subgroup (J539, X24, and T601) is enhanced, that of X44 is quenched, and those of a third group (J1, S10, and T191) show no change. The changed fluorescence is probably caused by a conformational change in the variable regions of the combining area involving a tryptophan residue.

EXPERIMENTAL

Immunoglobulin isolation. — Protein T601 was isolated from TEPC 601 ascites by immunoadsorption on a Sepharose–bovine serum albumin–galactoside column[8,14]. It was found to be homogeneous by immunoelectrophoresis using rabbit antimouse serum. By agar double-diffusion, it gave a reaction with Rabbit anti-IgA. Fab′ fragments of protein T601 were prepared by digesting partially reduced and alkylated protein with pepsin[15]. Fab′ fragment was isolated by chromatography on Sephadex G100 in borate buffer of pH 8.0, and the Fab′ fractions were extensively dialyzed against 0.05M Tris hydrochloride (pH 7.4). The binding constants (see Table I) of the various saccharide ligands were determined by the fluorescence-titration method[12], using the Fab′ fragment of T601. The preparation of the ligands has been reported[9]. Protein solution (A_{280} <0.05) in 0.05M Tris hydrochloride (pH 7.4) was excited at 295 nm, and the tryptophanyl fluorescence in the presence and absence of graded amounts of ligand was monitored at 340 nm.

Isoelectric focusing. — This procedure was performed on 5% poly(acrylamide) gel containing 6M urea and 5% of pH 5–8 ampholytes[16]. The protein samples were completely reduced in 6M guanidine hydrochloride with 0.2M 2-mercaptoethanol for 3 h at room temperature, followed by alkylation with 0.4M 2-iodoacetamide for 30 min in an ice bath. The samples were then dialyzed against 6M urea. After being focused, the protein bands were fixed by means of 10% trichloroacetic acid (TCA), the excess of TCA was removed by washing with 40% ethanol, and the protein bands were stained with 0.1% Coomassie Blue in 6:1:13 alcohol–acetic acid–water.

Idiotypic antisera. — Idiotypic antisera were prepared by immunization of Al/N mice with the myeloma protein that had been partially purified by ammonium sulfate precipitation. The first two injections were given subcutaneously with protein emulsified in complete Freund's adjuvant, the next three were in incomplete Freund's adjuvant, and the rest were in saline.

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