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Immunology

Subunit interactions in mouse myeloma proteins with anti-galactan activity

(light and heavy chains/recombination/fluorescence titration/idiotypes/mice)

B. N. MANJULA, C. P. J. GLAUDEMANS,† E. B. MUSHINSKI, AND M. POTTER

National Institute of Arthritis, Metabolism and Digestive Diseases and the National Cancer Institute, NIH, Bethesda, Maryland 20014

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ABSTRACT **The interactions among the subunits of a unique set of mouse myeloma proteins having specificity for β-D-(1,6) galactans has been studied by making homologous and heterologous recombinants of heavy and light chains. The recombinations were carried out by mixing together the desired heavy and light chains that had been separated on a Sephadex G-100 column in urea-acetic acid and renaturing the chains at near neutral pH. One homologous and six heterologous recombinants have been prepared. All the recombinants prepared possessed a four-chain native-like structure. The ligand binding activity and idiotypic specificity of the homologous recombinant were essentially indistinguishable from those of the original native protein. All the heterologous heavy–light chain combinations also led to the regeneration of functional binding sites. The affinity of the heterologous recombinants towards various galactose ligands was comparable to those of the native molecules. Furthermore, the ligand binding affinity of the recombinants was almost invariably closer to the K_a of the original protein that had a higher affinity. Idiotypic specificity of the heterologous recombinants paralleled that of the original protein that had contributed the heavy chain.**

Workers in our laboratory have been studying a set of eight homogeneous murine Balb/c myeloma immunoglobulins (J 1, S 10, X 24, X 44, T 191, J 539, T 601, and CBPC 4), having specificity for D-galactopyranosyl residues linked β(1,6) (1–3). The availability of this unique set of IgA_κ proteins of specificity for one sugar and one type of linkage prompted us to study the subunit interactions in these proteins by making heavy (H)- and light (L)-chain hybrid recombinants. Recombination studies on various antibody systems have been carried out with a view to understanding the contributions of H- and L-chains to antibody activity (4–12). Although some of the studied systems consisted of homogeneous proteins having the same ligand binding specificity, only homologous recombinants have been reported to show binding activity and idiotypic specificity of the native protein (4, 6–8); heterologous recombinants exhibit little if any binding activity and idiotypic specificity.

The anti-galactan proteins can be broadly classified into three groups, based on changes in tryptophanyl fluorescence observed on addition of ligands: (*a*) proteins X 24, J 539, T 601, and CBPC 4 show an increase in fluorescence on ligand binding; (*b*) protein X 44 shows a small decrease in fluorescence; and (*c*) proteins J 1, S 10, and T 191 have no significant changes in fluorescence on ligand addition.

For three of these proteins, namely, X 24, J 539, and T 601, binding constants (K_a) for different galactose ligands have been determined by fluorescence titration (3, 13, 12). The binding constants for any particular ligand with these proteins were not grossly different.

These anti-galactan proteins have been found to have crossreacting idiotypes (M. Potter and E. B. Mushinski, unpublished results). Proteins J 539 and X 44 shared idiotypes, as did the protein pairs X 24–T 601 (3), and S 10–T 191. Each of these proteins has also been shown by hemagglutination inhibition to possess a unique idiotypic determinant (15).

The light chains of six of these proteins (J 1, S 10, X 24, X 44, T 191, and J 539) have identical sequences to residue 23 and were unique among the known κ subgroups. Their heavy chains were also identical in sequence to residue 30, except for J 1, which had an Ile in place of Leu in position 5 (15). Although the NH_2-terminal sequences of these antigalactans are the same, these proteins show ligand-induced differences in fluorescence, indicating that differences in sequence will be found either in the amino-acid residues that are part of the combining site or in those that indirectly contribute to the binding site. Furthermore, each possesses a unique idiotypic determinant which must be accounted for by sequence differences. It was therefore of interest to study heterologous H- and L-chain interactions among these proteins.

The heterologous recombination experiments were designed with the following objectives: (*i*) Will a heterologous recombinant bind haptenic ligands with affinity comparable to the native protein? (*ii*) Does a heterologous recombinant show an affinity constant related to either original H- or L-chain donor? (*iii*) Is the idiotype of the recombinant derived predominantly from the H- or the L-chain donor? (*iv*) Can a heterologous recombinant be made with fluorescence properties and idiotype indistinguishable from an already known monomer? (*v*) In the combination of donor H- or L-chains from a ligand-induced fluorescing protein with an L- or H-chain from one lacking such property, which chain will exert a predominant influence on the fluorescence behavior of the hybrid?

MATERIALS AND METHODS

Isolation of Protein. The galactan-binding proteins were isolated from the corresponding ascites fluid by immunoadsorption on a Sepharose-bovine serum albumin galactoside column (2, 16).

Fluorescence Titration. The binding constants of proteins towards galactose-ligands were determined by the fluorescence titration method (17). Protein solutions ($A_{280} < 0.05$) in 0.05 M Tris buffer, pH 7.4 were excited at 295 nm and the tryptophanyl fluorescence, in the presence of increasing amounts of ligand, was monitored at 340 nm. Increments in fluorescence (ΔF) were measured as a function of ligand ad-

Abbreviations: Gal_2, 6-*O*-β-D-galactopyranosyl-D-galactose (Gal_3 and Gal_4 are the corresponding tri- and tetrasaccharides); EPG, epoxypropyl β-D-galactopyranoside; DIP-Gal_2, 6-*O*-β-D-galactopyranosyl-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranoside; H- and L-chain, heavy- and light-chain, respectively; phosphate-buffered saline, 0.01 M phosphate buffer containing 0.9% NaCl; $NaDodSO_4$, sodium dodecyl sulfate.

† To whom inquiries should be addressed.

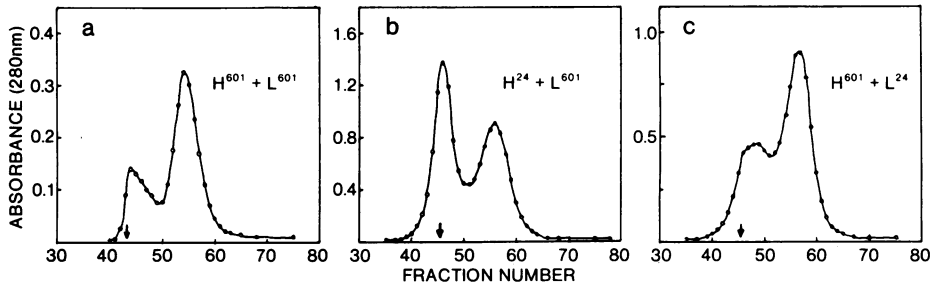

FIG. 1. Fractionation of recombinants on Sephadex G-200 (a) in NaOAc buffer, pH 5.5; (b) and (c) in phosphate-buffered saline, pH 7.4. The void volume of the column, as determined by Blue Dextran, is indicated by the arrow.

dition, until this value reached a maximum (ΔF_{max}). At this point all sites are saturated with ligand. At any concentration of free ligand (C), $\bar{\nu} = \Delta F/\Delta F_{max}$. Since $\bar{\nu}$ can be related to K_a by the equation $\bar{\nu}/C = K_a - \bar{\nu}K_a$, the slope of the Scatchard plot ($\bar{\nu}/C$ versus $\bar{\nu}$) of the titration data gives the value of $-K_a$.

Separation of H- and L-Chains. Purified myeloma proteins were partially reduced with dithiothreitol (0.01 M) and alkylated with iodoacetamide (0.022 M). The solutions were then dialyzed against 6 M urea–1 M acetic acid, and the H- and L-chains were separated on a Sephadex G-100 column (2.5 × 125 cm) in 6 M urea–1 M acetic acid. The purity of the H- and L-chains was checked either by rechromatography on analytical column of Sephadex G-100 in 6 M urea–1 M acetic acid or by polyacrylamide gel electrophoresis in 0.1% sodium dodecyl sulfate ($NaDodSO_4$) (18).

Reconstitution of Immunoglobulins. H- and L-chains were recombined by mixing together the chains while still in the urea-acetic acid solvent in the H:L chain-absorbance ratio at 280 nm of 2:1. The final A_{280} of the solutions was about 1.0 or less. No albumin was present in the recombination mixture. The chains were allowed to recombine at 4° by dialysis against either 0.1 M sodium acetate buffer, pH 5.5, (5) or against distilled water followed by phosphate-buffered saline, pH 7.4 (4, 7). The buffer was changed at least four times during a period of 48–60 hr‡. The solutions were then concentrated to a suitable volume by ultrafiltration in an Amicon cell using a UM 10 membrane, and the recombinants were chromatographed on a Sephadex G-200 column (2.5 × 125 cm) in phosphate-buffered saline, pH 7.4 (0.1% NaN_3) to separate the recombinant monomers from aggregates and free chains.

Molar H/L Chain Ratio. The molar H- to L-chain ratio in the recombinants was determined either by rechromatography of the recombinant on an analytical column of Sephadex G-100 in 6 M urea–1 M acetic acid or by weighing out the area of each peak from the densitometric tracing of an $NaDodSO_4$-polyacrylamide gel electrophoresis pattern.

Idiotypic Assay. Idiotypic antisera to the individual myeloma proteins were prepared in AL/N mice as described earlier (3, 15). For the hemagglutination inhibition assay, sheep erythrocytes were coupled with the immunoadsorbant-purified myeloma protein by the glutaraldehyde method (19). The anti-idiotypic antisera were absorbed with sheep erythrocytes and T-15 (an IgA with anti-phosphorylcholine specificity) before the hemagglutination titer for the individual systems was determined. A concentration of antiserum four times the end point in the hemagglutination test was used in the hemagglutination inhibition studies. In the hemagglutination inhibition test, purified myeloma proteins or H-L recombinants were seriallly diluted (2-fold) and a constant amount of antiserum was added to each dilution. After 15 min of incubation the coupled sheep erythrocytes were added. The end point reported is the reciprocal of the highest $\log_2$ dilution of protein that gave complete inhibition.

‡ When 0.1 M sodium acetate buffer, pH 5.5, was used for the recombination of homologous chains of T 601, the protein remained in solution during the recombination process. However, when the same buffer was used for the heterologous chain recombinations, there was a considerable amount of precipitation of protein during recombination. On the other hand, when phosphate-buffered saline, pH 7.4, was used for the recombination, there was no significant precipitation during dialysis. Hence, in these studies the use of phosphate-buffered saline was preferred for recombination of H- and L-chains.

RESULTS

Homologous Recombination of T 601. The capacity of the separated chains of antigalactans to recombine and form a native-like four-chain structure was first tested by making a homologous recombinant from the chains of protein T 601. The separated chains in the urea-acetic acid solvent were recombined by dialysis against sodium acetate buffer, pH 5.5. The elution profile of the recombined material on a Sephadex G-200 column showed two peaks (Fig. 1a). The first peak is close to the void volume of the column and therefore might represent aggregates. The elution position of the second peak is the same as the elution position of the reduced-alkylated T 601 monomer. The protein in this peak was found to have a H/L chain molar ratio near unity (Table 1) and therefore represents the recombinant having a four-chain structure (H_2L_2) similar to the native reduced-alkylated protein. This protein will be referred to as the recombinant monomer.

Heterologous Recombination. Heterologous recombinants were prepared from three protein pairs (*i*) X 24–T 601; (*ii*) X 24–J 539; and (*iii*) X 24–S 10. Two reciprocal recombinants were obtained from each original pair, yielding a total of six hybrid immunoglobulins: $H^{24}L^{601}$, $H^{601}L^{24}$, $H^{24}L^{539}$, $H^{539}L^{24}$, $H^{24}L^{10}$, and $H^{10}L^{24}$. Although the antigalactans are a set of closely related proteins, each of the three protein pairs used to make the heterologous recombinants has different characteristics. The pairs X 24–S 10 and X 24–J 539 consist of proteins with non-crossreacting idiotypes, while the X 24–T 601 pair is an example of proteins with crossreacting idiotypes. Determination of the idiotypic specificity of heterologous recombinants from a pair of proteins with non-crossreacting idiotypes can be expected to provide information about which chain makes the major contribution to the idiotype determinants. With respect to ligand inducible fluorescence, the pairs X 24–J 539 and X 24–T 601 are derived from proteins, both of which exhibit increased fluorescence on binding ligand; in the pair S 10–X 24, only X 24 shows ligand-induced increase in fluorescence. Thus, heterologous S 10–X 24 recombinants should provide infor-

Table 1. Ligand binding characteristics of recombinant molecules

Protein	V_e/V_0*	Molar H/L ratio	ΔF_{max} Gal_2	K_a¶ Gal_2	Gal_3	DIP-Gal_2	EPG
Native							
S 10	1.2		—	—	—	—	—
X 24§	1.2		32.5	0.71×10^4	1.75×10^5	—	2.28×10^3
J 539§	1.2		28.0	1.25×10^4	1.50×10^5	2.60×10^4	5.18×10^3
T 601§	1.2		20.8	0.71×10^4	0.86×10^5	0.84×10^4	1.21×10^3
Recombinants							
$H^{601}L^{601}$	1.2	1.14†	—	—	0.86×10^5	0.92×10^4	—
$H^{24}L^{601}$	1.2	1.25†	28.6	1.28×10^4	2.12×10^5	—	2.92×10^3
$H^{601}L^{24}$	1.2	—	25.0	0.87×10^4	1.31×10^5	—	2.35×10^3
$H^{24}L^{539}$	1.2	—	54.0	1.41×10^4	—	2.78×10^4	4.50×10^3
$H^{539}L^{24}$	1.2	—	18.3	1.19×10^4	—	1.16×10^4	5.38×10^3
$H^{24}L^{10}$	1.2	0.93‡	20.8	0.73×10^4	1.65×10^5	—	—
$H^{10}L^{24}$	1.2	0.80‡	9.0	0.80×10^4	—	—	—

Gal_2, 6-*O*-β-D-galactopyranosyl-D-galactose; EPG, epoxypropyl β-D-galactopyranoside; DIP-Gal_2, 6-*O*-β-D-galactopyranosyl-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranoside.

* The ratio of the elution position of the recombinant or reduced-alkylated original protein to the void volume of the Sephadex G 200 column in phosphate-buffered saline, pH 7.4.

† H/L ratio determined by rechromatography of the recombinant proteins on Sephadex G 100 column in 6 M urea-1 M acetic acid.

‡ H/L ratio determined by weighing out the area of the peaks from the tracings of the $NaDodSO_4$-gel electrophoresis pattern, standardized with native immunoglobulin.

§ The K_a values given are for the corresponding Fab's.

¶ The range of error in the value for K_a is from ±0.05 to 0.1 (17).

mation as to whether the H- or the L-chain contributes most to the increased fluorescence on addition of ligand. Furthermore, having one protein common to all pairs provides two sets of recombinants, one in which the contribution of H-chain, and the other in which the contribution of the L-chain, is constant.

Recovery of Structure and Activity. The Sephadex G-200 elution profiles of heterologous recombinant preparations obtained from H- and L-chains of proteins X 24 and T 601 are shown in Fig. 1 (curves b and c). The elution profile is very similar to that of the T 601 homologous recombinant preparation (Fig. 1a). Fractionation of the other heterologous recombinant preparations showed very similar elution positions to the ones shown in Fig. 1, but yields of recombinant monomer for the different H- and L-chain combinations ranged from 45 to 60% after separation from the aggregates, no definite preference being apparent for any particular combination. In the present study, only the recombinant monomer peak has been used to study the effect of recombination on the binding activity and idiotypic specificity of anti-galactan proteins.

The gel filtration properties, the molar H/L chain ratio, and the ligand binding characteristics of the various recombinants are summarized in Table 1. The gel filtration property, as characterized by the V_e/V_0 ratio of the various recombinants, was invariably comparable to that of the reduced-alkylated parent proteins. The molar H/L chain ratio of the recombinants ranged from 0.8 to 1.2, and is in good agreement with the values reported for various other recombinants (4, 10). It is, therefore, apparent that the heterologous H- and L-chains can combine almost as efficiently as the homologous chains to give rise to recombinants with the same four-chain subunit structure as the native 7S monomer.

To evaluate the reconstitution of the combining site, binding activity of the recombinants towards various galactose ligands was determined by fluorescence titration (17). It has previously been shown that the affinity of ligands to the Fab' fragment of X 24 and J 539 was essentially indistinguishable from the affinity of these ligands to the 7S monomers (2). This has now been confirmed for the binding of Gal_2 to Fab' of T 601 and whole T 601. Therefore, comparisons may validly be made between binding studies on recombinant monomers and Fab' fragments of original H-L donors. The data for the binding of a galactose ligand, Gal_3, to the T 601 homologous recombinant ($H^{601}L^{601}$) are given in Fig. 2a along with the data obtained for the Fab' of T 601. The linearity of the Scatchard plot for the homologous recombinant suggests homogeneity of the reconstituted sites. The association constant (K_a) for the recombinant determined from the curve is 0.86×10^5, and is the same as the K_a of the Fab' of T 601. The maximum increase in fluorescence on Gal_3 binding to $H^{601}L^{601}$ was 22% and is close to the value of 24% obtained with the native T 601 monomer, suggesting nearly quantitative reconstitution of the binding sites in the T 601 homologous recombinant.

The maximum change in fluorescence (ΔF_{max}) obtained on addition of various galactose ligands to the heterologous recombinants was in the same range as the values obtained with the original monomeric proteins. The values obtained with the ligand Gal_2 are given in Table 1. The ΔF_{max} values obtained with the S 10–X 24 recombinants are discussed subsequently. The ΔF_{max} values for ligand binding to the heterologous recombinants therefore indicate the high efficiency in the reconstitution of the combining site from the heterologous H- and L-chains.

The amounts of ligand bound by the recombinant and native immunoglobulins were measured by the method of Farr (20, 21) using radioactive galactotetraose (Gal_4*) in which the reducing residue had been converted to tritium-labeled galactitol by sodium borotritiide. Each immunoglobulin in Table 1 was admixed with the same quantity of Gal_4* in an amount such that, for the four native proteins, the site saturation was about 75%.

The moles of Gal_4* bound per mole of immunoglobulin were derived as follows: the relationship between the theoretical number of moles of ligand bound per mole of immunoglobulin as a function of its K_a was calculated assuming bivalence. Galactitol-terminated Gal_4* was assumed to bind

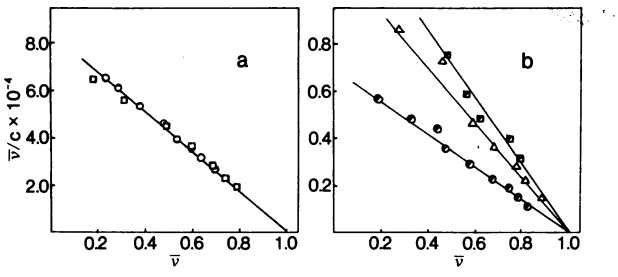

FIG. 2. Scatchard plots of (a) homologous recombinant $H^{601}L^{601}$ (□) compared to ^{601}Fab′ (○) with Gal_3 and (b) a comparison of ^{X24}Fab′ (●) with $H^{539}L^{24}$ △) and $H^{24}L^{539}$ (▲) with Gal_2.

equivalent to the trisaccharide Gal_3. This is essentially correct, as Gal_4 and Gal_3 show very nearly identical binding in all cases studied (13). Thus, for each immunoglobulin for which a K_a for Gal_3 has been measured, a theoretical amount of ligand bound at the concentration of Gal_4* used could be computed. The *actual* amount of Gal_4* bound to the immunoglobulin was measured in quadruplicate by liquid scintillation counting of the precipitate and standardization of the specific activity of the Gal_4* solution. These values for Gal_4* bound were then divided by the amount theoretically required, and are given in the last column of Table 2. Ideal agreement would give a value of 1.0. The technique is not as accurate as one would wish; a value as low as 0.8 was found for one native immunoglobulin, namely, X 24. However, no value was less than 0.7 for either the recombinants or the native immunoglobulins, showing acceptable reconstitution of the two binding sites in all cases observed.

The titration data for some heterologous recombinants and Gal_2 are illustrated in Fig. 2b. In all cases, Scatchard plots of the binding data were linear, indicating that just as in the homologous recombinant, the binding sites of the heterologous recombinants are homogeneous. The binding constants of the various heterologous recombinants towards different galactose ligands are quite comparable to those of the native proteins (Table 1). Furthermore, in combinations in which the two proteins differed in their affinity towards a ligand, the K_a of the recombinant appeared closer to the K_a of the protein that had the higher K_a, regardless of whether that protein was the H- or the L-chain donor. For example, the K_a values towards Gal_2 of the recombinants of proteins X 24 and J 539, namely, $H^{24}L^{539}$ and $H^{539}L^{24}$, were closer to the K_a of J 539 ($1.25 \pm 0.1 \times 10^4$ M^{-1}) than to the K_a of X 24 ($0.70 \pm 0.06 \times 10^4$ M^{-1}). It is, therefore, not clear if the H- or the L-chain plays a dominant role in regenerating a more efficient combining site. In the case of $H^{24}L^{601}$, the recombinant possessed a combining site which appeared more efficient than that of either original protein, when judged from affinity constants for Gal_2 and Gal_3 (see Table 1).

Table 2. Reconstitution of the number of binding sites in myeloma antigalactans as determined using radioactive galactotetraose (Gal_4*)

Immunoglobulin	mol Gal_4 bound/mol protein†	$\frac{(\text{mol } Gal_4\text{* bound})_{exp.}}{(\text{mol } Gal_4\text{* bound})_{theor.}}$
S 10	1.32	—
X 24	1.46	0.8
J 539	1.61	0.9
T 601	1.55	1.0
$H^{601}L^{601}$	1.45	0.9
$H^{10}L^{24}$	1.12	—
$H^{24}L^{10}$	1.31	0.8
$H^{24}L^{539}$	1.80	—
$H^{539}L^{24}$	1.64	—
$H^{24}L^{601}$	1.23	0.7
$H^{601}L^{24}$	1.51	0.9

† Average of four determinations.

Reconstitution of Idiotypic Determinants. The idiotypic specificity of the recombinants was analyzed by determining their capacity to inhibit the hemagglutination of myeloma protein-coated sheep erythrocytes by the corresponding idiotypic antisera. The results are presented in Table 3. Except for anti-T 601, all antisera tested are individually specific (13). Anti-T 601 showed significant crossreaction with X 24. The homologous recombinant $H^{601}L^{601}$ was indistinguishable from native T 601 in the T 601–anti T 601 system.

Regeneration of idiotypic determinants in the heterologous recombinants was analyzed by the hemagglutination inhibition assay in the corresponding parent idiotypic systems. For example, the heterologous recombinants $H^{24}L^{539}$ and $H^{539}L^{24}$ were tested in both X 24 and J 539 idiotypic systems. $H^{24}L^{539}$ inhibited the X 24 system significantly, but not the J 539 system. The reciprocal recombinant $H^{539}L^{24}$ inhibited the J 539 system as well as the native protein did; however, it also inhibited the X 24 system to some extent. The behavior of the other recombinants in the corresponding idiotypic systems was rather similar to that observed with the X 24–J 539 recombinants. The heterologous recombinant invariably showed a pattern in the hemagglutination inhibition assay for idiotype which paralleled that of the donor of the H-chain. However, light chains also contribute to the idiotype. For instance, L^{10} contributes substantially to the idiotype of S 10, since $H^{24}L^{10}$ inhibits the agglutination of sheep erythrocytes-S 10/anti-S 10 very well, even though S 10 and X 24 show no idiotypic crossreaction (see Table 2). L^{24} contributes moderately to the idiotype of X 24 (Table 3).

In addition to the homologous idiotypic systems, the heterologous recombinants were also tested for the idiotypic determinants of the other antigalactans to see if any of the heterologous chain combinations studied simulated an already existing H–L pair. None of the recombinants inhibited any idiotypic system other than the one which contributed the chains (Table 3).

Relative Contribution to Ligand-Induced Change in Fluorescence by the H- and L-Chains. Since proteins X 24, J 539, and T 601 show an increase in fluorescence on ligand binding, it is difficult to assess the relative contribution of the H- and L-chains to such a change in fluorescence in the heterologous recombinants of these proteins. However, in the protein pair S 10–X 24, S 10 does not show any significant changes in fluorescence on ligand binding and X 24 does; thus any ligand-induced increase in fluorescence observed with the recombinants $H^{10}L^{24}$ and $H^{24}L^{10}$ can be viewed as due to contribution from the L- and the H-chains of X 24. The increase in fluorescence found on Gal_2 addition to $H^{24}L^{10}$ was much greater (20.8%) than that observed with $H^{10}L^{24}$ (9%), suggesting that at least in protein X 24 the major contribution to an increase in fluorescence on ligand addition is made by the tryptophanyl residues located on its heavy chain.

DISCUSSION

It is apparent from the results presented above that the heterologous recombinants from a series of galactan-binding proteins in Balb/c mice retain ligand binding activity and idiotypic specificity. All combinations studied regenerated

Table 3. Idiotypic specificity of recombinant molecules

Inhibitor	Hemagglutination inhibition titer ($\log_2$) for the individual systems					
	S 10/ anti-S 10	X 24/ anti-X 24	X 44/ anti-X 44	T 191/ anti-T 191	J 539/ anti-J 539	T 601/ anti-T 601
S 10	14	0	0	0	0	—
X 24	0	14	0	0	0	9
X 44	—	—	—	—	—	—
T 191	—	—	—	10	—	—
J 539	0	0	0	0	12	—
T 601	0	0	0	0	0	14
$H^{601}L^{601}$	—	0	—	0	—	15
$H^{10}L^{24}$	11	3	0	0	0	—
$H^{24}L^{10}$	7	11	0	0	0	4
$H^{24}L^{539}$	0	10	0	0	0	—
$H^{539}L^{24}$	0	6	0	0	12	—
$H^{24}L^{601}$	0	12	0	0	0	6
$H^{601}L^{24}$	0	3	0	0	0	15

functionally active binding sites and idiotypic determinants associated with the native proteins. The heavy chain combined with any light chain of the galactan-binding proteins tested to regenerate idiotypic specificity closely related to that of the original protein. While idiotypic specificity appeared to be dominated by the heavy chain, ligand binding properties did not show any such preference. The K_a values of the recombinants were invariably closer to the K_a of the original protein with the higher ligand binding affinity.

Various heterologous recombinants reported by others exhibited anywhere from 10 to 100-fold reduction in binding affinity for ligand when compared to the homologous immunoglobulin (4, 6, 7, 9). By contrast, the heterologous recombinants of the galactan-binding proteins reported here are, however, nearly as active as the original proteins. A recombination study using mouse myeloma proteins 315 and 460 (IgAs, anti-Dnp activity) showed that the binding specificity of protein 315 and 460 can be restored only when the homologous chains are recombined (4). Absence of activity in these heterologous recombinants, however, may be ascribed in part to differences in light chain type 315 belonging to λ_2 (22) while 460 is κ (23). Huser *et al.* (6) have made recombinants from homogeneous rabbit antibodies to bacterial polysaccharides. The three proteins studied, although all were κ, showed significant variation in their light chain sequences, and heterologous recombinants exhibited little if any of the binding activity and idiotypic specificity of the native molecules. Recombination studies on α(1,3) dextran binding protein J 558 (IgA λ_0) suggested that up to two amino-acid substitutions in the light chain do not alter the J 558 idiotype, while three significantly altered the idiotype (8).

In several of the recombination studies reported by others, in agreement with the results of the present study, it was shown that a four-chain immunoglobulin structure can be readily reconstituted from heterologous H- and L-chains (4–6, 10–12, 24). Mutual affinity of homologous H- and L-chains has been found to exceed that of heterologous H- and L-chains in two cases (11, 12). Incomplete recovery of antibody activity in those studies by heterologous chain recombinations, on the one hand, and recovery of complete activity by homologous chain recombinations, on the other hand, suggest that the requirements to regenerate a unique specificity in an antibody molecule are more stringent than the requirements necessary to regenerate a four-chain immunoglobulin molecule (4–6, 10).

Thus, regeneration of binding activity and idiotypic specificity in heterologous anti-galactan recombinants suggests close similarities in their structures. Since these H-chains make the major contribution to the idiotype, sequences of the light chains in those regions affecting idiotypic specificity may be very similar.

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