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# Effect of interchain disulfide bond on hapten binding properties of light chain dimer of protein 315

(conformational transition/cooperativity/allostery/longitudinal interaction)

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**ABSTRACT** **The hapten binding characteristics of the covalent light chain dimer, derived from the murine IgA secreted by plasmacytoma MOPC-315, to two nitroaromatic compounds, ϵ-*N*-(2,4-dinitrophenyl)-L-lysine and 4-(α-*N*-alanine)-7-nitrobenz-2-oxa-1,3-diazole, were investigated by differential spectroscopic titrations. The binding curves for both haptens were found to display sigmoidity similar to that reported earlier for the reduced and alkylated dimer held together by noncovalent bonds only. However, the presence of the interchain disulfide bond in the covalent dimer was found to cause marked changes in its binding properties. The data, like those obtained for the noncovalent dimer, fit the allosteric model of Monod, Wyman, and Changeux in which binding of the first hapten to the dimer causes a conversion of both sites of the protein molecule from a lower to a higher affinity conformation. However, the binding parameters show that both the affinity and the positive cooperativity in the interaction between haptens and the covalent dimer are significantly enhanced. The differences in the parameters of the binding and of the allosteric transition caused by the presence of the interchain disulfide bond demonstrate the existence of longitudinal interactions in immunoglobulin derivatives. These properties of the light chain dimer make it a potential model for the receptors present on thymus-derived lymphocytes.**

A close structural homology between the Fab fragment and the light chain dimer (L2) of immunoglobulins has been shown by x-ray crystallography (1–4). This structural homology together with the hapten binding capacity of the L2s (1) led to the suggestion that they are a model of a primitive antibody, which could have existed prior to the divergence of independent light and heavy chains (1, 2, 5). The L2 derived from IgA secreted by murine plasmacytoma MOPC 315 has been shown to bind the same nitroaromatic haptens as the parent protein 315 (HL) sites (6, 7). We have reported (8) that a salient feature of the hapten binding properties of L2 315 is a marked positive cooperativity in its interaction with the two hapten molecules that bind to each dimer. Thus, the analysis of the binding data according to the Adair formalism reveals a 100-fold difference in the two intrinsic association constants for 4-(α-*N*-L-alanine)-7-nitrobenz-2-oxa-1,3-diazole (NBDA), which gives a Hill coefficient of 1.83 (8). This cooperativity was explained in terms of a conformational transition induced in the protein upon binding of the hapten. The results were found to fit the Monod, Wyman, and Changeux (MWC) (9) model in which the binding of the first hapten to L2 315 leads to the conversion of both sites from a low-affinity conformer T to a high-affinity conformer R. It has been pointed out earlier (8) that it is difficult to explain this transition in terms of local changes limited to only a few contact residues in the binding site, but it is possible in terms of a hapten-induced change in the overall configuration of the dimer. The cooperative hapten binding was observed in studies carried out on L2 315 prepared from the reduced and alkylated protein 315. Because this protein is an IgA with a λ2 light chain, there is a disulfide bridge between the light chains of the native protein but there is no disulfide bridge between heavy and light chains (10). The disulfide bond is cleaved as a result of the reduction and alkylation procedure. Hence, the chains of this dimer associate by noncovalent bonds only. Recently it was shown (11, 12) that the interchain disulfide bond arrests the interconversion between conformational isomers of the L2 of human IgG1 Mcg (1, 5), whereas the reduction of this bond allows the reversal of the hapten-induced conformational change. This observation together with the above proposed change in the configuration of the L2 of MOPC-315 upon hapten binding prompted us to investigate the significance of the disulfide bond for the behavior of L2 315.

Here we report that the covalent L2 of protein 315 (L2cov) binds ϵ-*N*-(2,4-dinitrophenyl)-L-lysine (DNPL) and NBDA with a markedly higher affinity and higher positive cooperativity than does the noncovalent dimer (L2ncov). This effect of the covalent bond on the behavior of L2 315 shows that a change in the configuration of the protein extends over more than 60 Å from the disulfide bond at the COOH terminus to the hapten binding site in the variable region.

## MATERIALS AND METHODS

Protein 315 was purified without reduction from the ascitic fluid of BALB/c mice bearing the MOPC-315 tumor according to a slightly modified procedure of Goetzl and Metzger (13). The previously reported low yields of the unreduced protein were attributed to the difficulty in eluting the IgA oligomers which was caused by their polyvalency. Therefore, we used 1 M propionic acid as the eluant and obtained a yield (3 mg of protein per 1 ml of ascitic fluid) comparable to that obtained when the protein was reduced and alkylated prior to loading on the affinity column. The protein was concentrated to 15 mg/ml by ultrafiltration (UM10 membrane, Amicon, Lexington, MA) immediately after elution and applied to a Sephadex G-150 column equilibrated with 1 M propionic acid. The elution pattern and the sodium dodecyl sulfate/polyacrylamide gels (14) of each peak are shown in Fig. 1. The L2cov fractions were pooled, dialyzed against 0.02 M sodium phosphate, pH 7.4/0.15 M NaCl, and concentrated by ultrafiltration to about 3 mg/ml. The solution was kept at 4°C in the presence of 0.02% sodium azide.

DNPL was purchased from Sigma. NBDA was prepared as described (15).

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Abbreviations: NBDA, 4-(α-*N*-L-alanine)-7-nitrobenz-2-oxa-1,3-diazole; DNPL, ϵ-*N*-(2,4-dinitrophenyl)-L-lysine; MWC, the Monod, Wyman, and Changeux allosteric model; L2, light chain dimer; L2cov, covalent L2 of protein 315; L2ncov, noncovalent L2 of protein 315; HL, intact protein 315.

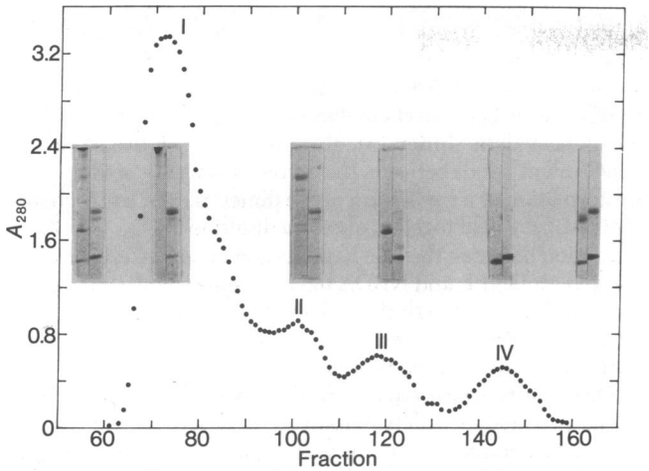


FIG. 1. Purification of L2cov. HL (10 ml at 15 mg/ml) was applied to a Sephadex G-150 column (4 × 90 cm) equilibrated with 1 M propionic acid and 4.3-ml fractions were collected. Sodium dodecyl sulfate/5–20% polyacrylamide gels were prepared for each peak. In each pair of gels the left one was run without and the right one with a reducing reagent (mercaptoethanol). The gels on the far left show unreduced HL after purification on an affinity absorbent. The gels on the far right show HL reduced and alkylated before purification. Notice the slight retardation of the bands in the presence of mercaptoethanol. Peak III represents L2cov.

Spectrophotometric measurements were carried out on a Cary 118 spectrophotometer at 4°C with a matched pair of tandem cells as described (7).

The data were analyzed according to the Adair formalism (8, 16). The Adair approach is a general one and does not involve any assumptions other than the existence of two identical binding sites on L2cov in the absence of ligand and it provides a means to calculate their consecutive association constants ($K_1$ and $K_2$) (17). A nonlinear least square fitting procedure* was used to obtain the best fit parameters of the data ($\Delta A$ vs. concentration of hapten added) to the Adair formalism: $K_1$, $K_2$, and $\Delta\epsilon$, the difference in extinction coefficient between the bound and the free hapten. Two modes of data presentation were used in the fitting procedure: a binding saturation curve and a double reciprocal plot. Although the saturation curve [$(\Delta A/\Delta A_{max})$ vs. free hapten] is useful for determining the $\Delta\epsilon$ and the half-saturation point ($S_{0.5} = 1/\sqrt{K_1 \times K_2}$), it did not allow a satisfactory resolution between curves with the same half-saturation point but different $K_1$ and $K_2$ values. On the other hand, a double reciprocal plot in the region of high cooperativity is very sensitive to variations in the ratio of $K_1$ to $K_2$ and therefore to variations in the Hill coefficient [$n_H = 2/(1 + \sqrt{K_1/K_2})$]. However, because a double reciprocal plot gives too much weight to the initial, least-precise points in a titration (18), it was used only for establishing $n_H$ after $S_{0.5}$ and $\Delta\epsilon$ were determined by using the saturation binding curve. These parameters ($S_{0.5}$ and $n_H$) define both equilibrium constants, $K_1$ and $K_2$.

## RESULTS

The difference absorption titration of L2cov with DNPL is shown in Fig. 2 *upper*. The observed spectrum of the bound hapten is different from that obtained with L2ncov (7, 8) but markedly resembles that of the DNPL complex with HL (8, 19). The positive band centered at 470 nm, characteristic of the complexes between DNPL and anti-DNP antibodies (19–21)

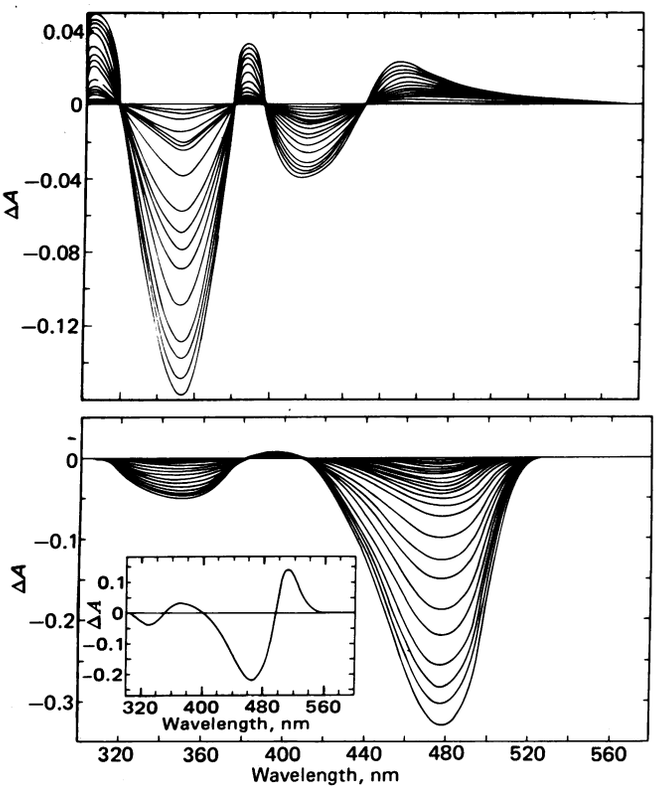


FIG. 2. (*Upper*) Difference spectrophotometric titration of L2cov with DNPL at 4°C. $\Delta A$ is the change in hapten absorption on binding to L2cov [$\Delta A = A$ (bound) $- A$ (free)]. Initial L2cov concentration was 0.115 mM sites, assuming two sites per L2. The concentration of DNPL varied from 5.40 $\mu$M to 0.217 mM. (*Lower*) Difference spectrophotometric titration of HL with NBDA. Initial HL concentration was 0.129 mM sites. The concentration of NBDA varied from 0.8 $\mu$M to 0.11 mM. (*Inset*) Difference spectrum of NBDA bound to L2cov vs. free NBDA at the end of a titration. The concentration of L2cov was 0.115 mM sites and the concentration of NBDA was 0.21 mM.

and usually attributed to a charge-transfer complex between an indole side chain and the nitroaromatic hapten (7, 15, 22), is missing in the spectrum of the L2ncov complex yet is present in that of L2cov. The positive peak at 385 nm is also well resolved. The maximal extinction coefficient difference of the L2cov–DNPL complex is larger than that of the L2ncov–DNPL complex (8) but is smaller than that observed for HL–DNPL (7). It is noteworthy that the shape of the difference spectrum remains constant throughout the titration.

Fig. 2 *lower* shows the absorption difference titration of reduced and alkylated HL plus NBDA and the difference spectrum of L2cov plus NBDA (*inset*). The difference spectrum of L2cov plus NBDA closely resembles the spectrum of the hapten bound to L2ncov (8), but differs from the spectrum of the HL–NBDA complex. In the latter case the positive band at 515 nm, which also could result from a charge transfer interaction (15), is not seen.

Fig. 3 shows the saturation curves and the corresponding double reciprocal plots of L2cov with DNPL and NBDA and of HL with NBDA. Both haptens display pronounced sigmoidal binding curves with L2cov but not with the parent HL protein. The best fit parameters for these curves are given in Table 1. The binding constants of DNPL and NBDA to L2cov are the same and are significantly higher than those to L2ncov. The most pronounced difference in binding of DNPL between L2cov and L2ncov is expressed in the sharp increase of positive cooperativity exhibited by the L2cov, resulting in a Hill coefficient of 1.97 compared to that of 1.33 for L2ncov.

* Powell, M. J. D. (1971) in *Harwell Subroutine Library, Atomic Energy Research Establishment, Harwell, U.K.* (subroutine VA04A).

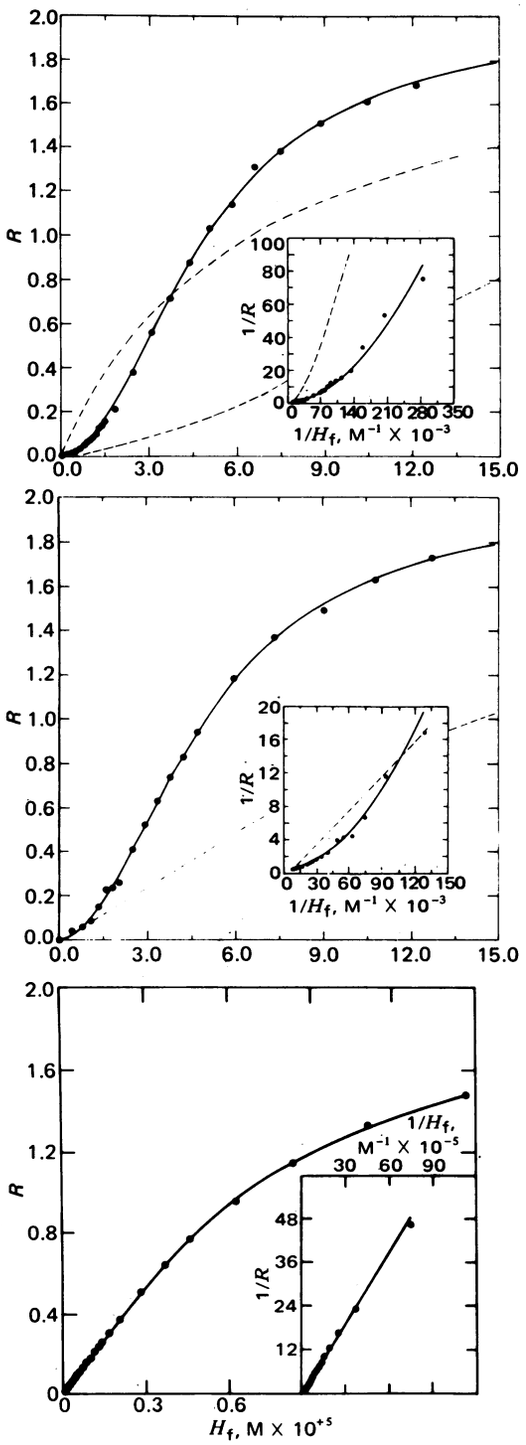


FIG. 3. Hapten saturation curves. $R$, Average number of moles of hapten bound per mol of protein dimer ($R \leq 2$). $R$ is plotted vs. free hapten concentration ($H_f$). (*Top*) L2cov plus NBDA; $\Delta A$ was measured at 464 nm. (*Middle*) L2cov plus DNPL; $\Delta A$ was measured at 354 nm. (*Bottom*) HL plus NBDA; $\Delta A$ was measured at 478 nm. —, Drawn according to binding parameter values in Table 1; - - -, drawn according to binding parameters of L2ncov; -·-·-, drawn according to parameters of binding of HL to NBDA. (*Insets*) Same data replotted on a double reciprocal plot.

## DISCUSSION

The strong positive cooperativity of hapten binding to L2cov can be explained only by an allosteric conformational transition of the protein. The two alternatives considered previously (8)—that hapten binding is coupled to a chain-dissociation equilibrium of the form L2 ⇌ 2L, or, that the first bound hapten exerts a direct attractive interaction on the second—can now be excluded on the following grounds: (*i*) The chain dissociation is prevented in the L2cov by the disulfide bond. Furthermore, because chain dissociation has been excluded for the noncovalent dimer (8), the possibility of breaking the noncovalent bonds between the domains, which is required for the formation of a *trans* form of the dimer, is even less probable for the L2cov held together also by a disulfide bridge. (*ii*) Direct attraction between the two haptens is even less likely here because both DNPL and NBDA have the same binding parameters to L2cov, although their chemical nature is different (8). Therefore, the observed cooperativity must arise from a structure change in the protein.

Our results can be equally well analyzed in terms of either of the two models widely employed for description of cooperative interactions of proteins with ligands—the more general model of Koshland *et al.* (23) and that of Monod *et al.* (9). However, because generally one cannot distinguish between the above two models on the basis of static binding data alone (24) and because we have already analyzed our earlier results for L2ncov according to the MWC model, we have used it also for the analysis of the present results obtained for the L2cov.

In the MWC model it is postulated that the protein exists in two conformations, which are designated R (relaxed) and T (taut). These conformers differ in their ability to bind a hapten; the association constant of the R conformer, $K_R$ is larger than the association constant of the T conformer, $K_T$. In the absence of ligands, the two conformers exist in an equilibrium, $T_0 \rightleftharpoons R_0$, which is characterized by the allosteric transition equilibrium constant $L = T_0/R_0$.

Another postulate of the MWC model is that all the binding sites of a given molecule are in the same conformation—i.e., the conformational transition occurs in all of them simultaneously. MWC parameters of the binding ($K_T$, $K_R$) and of the allosteric transition ($L$) were calculated from the Adair constants according to ref. 17. In this procedure three MWC parameters ($K_T$, $K_R$, $L$) are derived from two parameters of the Adair formalism ($K_1$, $K_2$). The resulting uncertainty makes exclusive ($K_T = 0$) and nonexclusive ($K_T > 0$) binding mathematically indistinguishable. However, in the case of nonexclusive binding, in which both conformers bind the hapten, two different complexes should be present and the shape of the absorption spectrum of the system could be expected to change during the titration. In our case, the shapes of the difference spectra of complexes of L2cov with NBDA and DNPL were constant from less than 1% to 85% saturation of the protein, which is best illustrated by the well-resolved isosbestic points in both titrations (Fig. 2). Thus, we conclude that the L2cov exhibits exclusive binding and we can obtain unique values for $L$ and $K_R$ (Table 1).

The thermodynamic (Table 1) and spectral (Fig. 2) data allow a comparison between the modes of ligand binding to L2cov and to the two closely related proteins, the noncovalent light chain dimer of 315 (7, 8) and the parent protein 315 (6, 7, 19). One fact is immediately apparent: the presence of the inter-light chain disulfide bond at its COOH terminus causes a very profound change in the properties of the binding site at the variable domain. Thus, L2cov shows extremely high positive cooperativity in binding either DNPL or NBDA (Table 1). This is expressed in the large value of the $K_2$ to $K_1$ ratio and the consequently large Hill coefficient [$n_H = 2/(1 + \sqrt{K_1/K_2}) = 1.97$)], which virtually assumes the maximum theoretical value of 2. The large $K_2/K_1$ also results in a large equilibrium constant for the conformational transition [$L = (K_2/K_1 - 1) = 4300$]. The free energy of the conformational transition ($\Delta G_c = 4.6$

Table 1. The best fit parameters for curves in Fig. 3

| Protein | Hapten | Intrinsic Adair constants, $M^{-1}$ | | $n_H$ | $L$ | Intrinsic MWC constants, $M^{-1}$ | | $\Delta G_c$,* kcal/mol | $\Delta G_b$,† kcal/mol | $\lambda$, nm | $\Delta\epsilon_\lambda$, $cm^{-1}\,M^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $K_1$ | $K_2$ | | | $K_T$ | $K_R$ | | | | |
| L2cov | NBDA | $3.0 \times 10^2$ | $1.3 \times 10^6$ | 1.97 | $4.3 \times 10^3$ | 0 | $1.3 \times 10^6$ | 4.6 | −7.7 | 464 | −14,400 |
| L2cov | DNPL | $3.0 \times 10^2$ | $1.3 \times 10^6$ | 1.97 | $4.3 \times 10^3$ | 0 | $1.3 \times 10^6$ | 4.6 | −7.7 | 354 | −5,400 |
| L2ncov‡ | NBDA | $5.0 \times 10^2$ | $5.6 \times 10^4$ | 1.83 | 110 | 0 | $5.6 \times 10^4$ | 2.8 | −6.0 | 464 | −12,400 |
| L2ncov‡ | DNPL | $3.5 \times 10^3$ | $1.4 \times 10^4$ | 1.33 | 110 | $2.9 \times 10^3$ | $6.7 \times 10^4$ | 2.8 | −6.1 | 354 | −4,500 |
| HL | NBDA | $1.5 \times 10^5$ | $1.5 \times 10^5$ | 1 | 0 | 0 | $1.5 \times 10^5$ | 0 | −6.5 | 478 | −18,200 |
| HL§ | DNPL | $5.4 \times 10^6$ | $5.4 \times 10^6$ | 1 | 0 | 0 | $5.4 \times 10^6$ | 0 | −8.5 | 345 | −6,600 |

L, allosteric transition equilibrium constant, 1 cal = 4.184 J.

* Free energy of the conformational transition, calculated as $\Delta G_c = -RT\ln(1/L)$.

† Free energy of the binding, calculated as $\Delta G_b = -RT\ln K_R$.

‡ From Lancet *et al.* (8).

§ From Schechter *et al.* (6) and Licht *et al.* (7).

kcal/mol) of L2cov is significantly higher than that for the noncovalent dimer ($\Delta G_c$ = 2.8 kcal/mol) (8). This suggests that the allosteric transition of L2cov involves a more substantial rearrangement of the protein structure.

The structural transition of L2cov leads to an unprecedented result: the R state of L2cov has an affinity for DNPL that is comparable to that of HL [$K_a = 5.4 \times 10^6\ M^{-1}$ (6)] and an affinity for NBDA that is even higher than that of the parent protein (Table 1). The free energy of the binding (−7.7 kcal/mol), which is much larger in absolute value than that of the free energy of the conformational transition, allows the equilibrium between the T and R forms to shift from the 99.98% of the protein in the T state in the absence of a ligand, $T_0 = [1 - 1/(1 + L)]$, to 100% of the protein in the R form at full saturation. The large energy of the structural change required to bring both sites from T to R state and the influence of the interchain disulfide bond on the magnitude of the allosteric effect suggest that the binding of a ligand indeed causes an overall change in the structure of the L2. This is in agreement with the crystallographic and spectroscopic data obtained for another L2, Mcg (12). Ligand binding to the variable domains of this protein was shown to lead to a conformational transition assigned to distal regions close to the disulfide bridge at the constant domains.

The resemblance between the structure of Fab and L2 has been amply illustrated by crystallographic studies (1–4). On the basis of these reports we have previously suggested that the two sites in L2 315 are related to each other by a 2-fold rotation symmetry, although the molecule as a whole is not strictly symmetrical. We have also proposed (8) that each hapten bound to L2 315 interacts with residues of both light chains of the dimer in a fashion analogous to the interaction of complementarity residues of sites present in HL (1, 5, 7, 25–27). This, together with the similarity between the interchain interaction in the Fab and L2 mentioned above (1, 3, 4, 26), raises the possibility that similar conformational transitions are induced in the native Fab upon hapten binding. Such changes may not be resolved by static binding measurements because the Fab usually binds only one hapten per two chains (8, 28). However, kinetic measurements have resolved hapten-induced conformational transitions in an increasing number of different homogeneous immunoglobulins (28–30).

The remarkable fact is that the effect of the covalent bond between the light chains at their COOH termini on the hapten binding properties of the binding site at the variable region is communicated over a distance of more than 60 Å. This clearly reflects the presence of longitudinal interactions along the domains of this protein. Indirect evidence for such interactions is also available from studies of the role of the interchain disulfide bond in cryoprecipitation of Bence-Jones proteins (31) and measurements of the binding of variable and constant domains of light chains to Fd (32) as well as from studies of the Mcg L2 mentioned above. This type of interaction among distal domains of immunoglobulins may constitute a further extension of the functional homology between the L2 and the parent HL molecules. Furthermore, the long-range interaction found in the L2 could be of significance for the mechanism of triggering of the effector functions of antibodies by antigen binding (33).

The positive cooperativity observed in the ligand binding to L2 315 allows one to speculate that it constitutes a potential basis for the interactions between the T-cell receptor and the two antigenic determinants to which it is known to bind (34, 35). It has been reported that no significant binding of any one of the two determinants (either the self determinant, H-2, or the foreign antigen determinant) can be detected unless both of them are present (36, 37). Thus, it is conceivable that the T-cell receptor could also exhibit positive cooperativity in binding the above two determinants.

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