Faust, C. H., Diggelmann, H., and Mach, B. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 2491.
Gally, J. A., and Edelman, G. M. (1972), *Annu. Rev. Genet. 6,* 1.
Harrison, P. R., Birnie, G. D., Hell, A., Humphries, S., Young, B. D., and Paul, J. (1974), *J. Mol. Biol. 84,* 539.
Honjo, T., Packman, S., Swan, D., Nau, M., and Leder, P. (1974), *Proc. Natl Acad. Sci. U.S.A. 71,* 3659.
Honjo, T., Swan, D., Nau, M., Norman, B., Packman, S., and Leder, P. (1976), *Biochemistry* (preceding paper in this issue).
Hood, L., Barstad, P., Loh, E., and Nottenburg, C. (1974), The Immune System; Genes, Receptors, Signals, Sercarz, E. E., Williamson, A. R., and Fox, C. F., Ed., New York, N.Y., Academic Press, pp. 119–139.
Leder, P., Honjo, T., Packman, S., Swan, D., Nau, M., and Norman, B. (1974a), The Immune System; Genes, Receptors, Signals, Sercarz, E. E., Williamson, A. R., and Fox, C. F., New York, N.Y., Academic Press, pp. 299–317.
Leder, P., Honjo, T., Packman, S., Swan, D., Nau, M., and Norman, B. (1974b), *Proc. Natl. Acad. Sci. U.S.A. 71,* 5103.
Leder, P., Honjo, T., Swan, D., Packman, S., Nau, M., and Norman, B. (1975), Molecular Approaches to Immunology, Miami Winter Symposia, Vol. 9, Smith, E. E., and Ribbons, D. W., Ed., New York, N.Y., Academic Press, pp. 173–188.
Milstein, C., Brownlee, G. G., Cartwright, E. M., Jarvis, J. M., and Proudfoot, N. J. (1974), *Nature (London) 252,* 354.
Packman, S., Aviv, H., Ross, J., and Leder, P. (1972), *Biochem. Biophys. Res. Commun. 49,* 813.
Premkumar, E., Shoyab, M., and Williamson, A. R. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 99.
Rabbitts, T. H., Bishop, J. P., Milstein, C., and Brownlee, G. G. (1974), *FEBS Lett. 40,* 157.
Rabbitts, T. H., Jarvis, J. M., and Milstein, C. (1975), *Cell 6,* 5.
Rabbitts, T. H., and Milstein, C. (1975), *Eur. J. Biochem. 52,* 125.
Ross, J., Gielen, J., Packman, S., Ikawa, Y., and Leder, P. (1974), *J. Mol. Biol. 87,* 697.
Schulenberg, E., Simms, E., Lynch, R., Bradshaw, R., and Eisen, H. (1971), *Proc. Natl. Acad. Sci. U.S.A. 68,* 2623.
Stavnezer, J., Huang, R. C. C., Stavnezer, E., and Bishop, J. M. (1974), *J. Mol. Biol. 88,* 43.
Storb, U. (1974), *Biochem. Biophys. Res. Commun. 57,* 31.
Tonegawa, S., Bernardini, A., Weimann, B. J., and Steinberg, C. (1974), *FEBS Lett. 40,* 92.
Weigert, M., Cesari, I., Yonkovich, S., and Cohn, M. (1970), *Nature (London) 228,* 1045.
Wigzell, H. (1973), *Scand. J. Immunol. 2,* 199.

Binding of 2,4-Dinitrophenyl Derivatives by the Light Chain Dimer Obtained from Immunoglobulin A Produced by MOPC-315 Mouse Myeloma†

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ABSTRACT: The light chains, but not the heavy chains, obtained from immunoglobulin A produced by the MOPC-315 mouse myeloma bind the 2,4-dinitrophenyl (DNP) group. Specific interaction with the DNP group was determined by using several immunoadsorbents, including DNP-L-lysine-Sepharose, and elution of the adsorbed light chain by DNP-glycine. Equilibrium dialysis experiments showed that the M-315 light chain in the form of dimer (45 260 daltons) has two identical and homogeneous binding sites that bind DNP-L-lysine with an intrinsic association constant of 6.3×10^3 M^{-1}. This is the first report, to our knowledge, in which the light chain binding data permit reliable determination of the binding constant and valency of the isolated light chain, and which suggests a predominant role for the light chain in construction of the binding site in the intact immunoglobulin molecule.

Antibody molecules are made up of two heavy (H^1) and two light (L) polypeptide chains held together by interchain disulfide bonds and noncovalent forces. Numerous experiments have shown that, in the intact 7S molecule, both H and L chains are involved in determining antibody specificity. These included affinity labeling, radioiodination of antibodies in the presence and absence of the specific hapten, and a variety of studies on the recovery of binding activity in recombinants made from the isolated chains (reviewed by Fleischman, 1966; Porter and Weir, 1966; Dorrington and Tanford, 1970). Attempts were also made to assign antibody activity to isolated chains obtained from native antibody molecules that were mildly reduced and alkylated. Essentially in all systems investigated, the isolated H chain bound the homologous hapten or antigen, although to a lesser extent than the intact Ig. These results indicated that the H chain contributes contact residues in the binding site of the intact antibody (see Utsumi and Karush, 1964; Painter et al., 1972). Antibody activity in isolated L chains was not readily demonstrable (Fleischman, 1966; Porter and Weir, 1966). In many systems there was no detectable activity in the L chains (e.g., Fleischman et al.,

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[1] Abbreviations used are: DNP, 2,4-dinitrophenyl; Ig, immunoglobulin; MOPC-315 and MOPC-321 are abbreviated to M-315 and M-321, respectively; pH 7.4 buffer, 0.15 M NaCl–0.01 M sodium phosphate (pH 7.4); PBS, phosphate buffered saline; H and L chains, heavy and light chains, respectively.

1963; Utsumi and Karush, 1964). In other systems the L chains bound specifically the hapten or antigen, but with very low affinity. Binding by the isolated L chains was estimated to be several orders of magnitude less than binding by H chains isolated from the same antibody (Goodman and Donch, 1965; Yoo et al., 1967; Painter et al., 1972; Nakamura et al., 1973). In the present study we measured the interaction of DNP-Lys with the H and L chains of the M-315 IgA which binds DNP-Lys (Eisen et al., 1968). The results showed significant binding of the ligand by the L chain, and none by the H chain. The data permit reliable determination of the binding constant and the number of binding sites per L chain.

Experimental Section

Materials. The M-315 tumor which secretes IgA (λ_2-type L chain) and the M-321 tumor which secretes κ-type L chain were generously provided by Dr. M. Potter (National Cancer Institute, Bethesda, Md.). The plasmacytomas were maintained as solid tumors in female BALB/c mice (Potter, 1967). [^{3}H]DNP-Lys (1.1 Ci/mmol) was purchased from New England Nuclear, Boston, Mass. DNP-Lys and DNP-Gly were from Nut. Biochem. Corp., Cleveland, Ohio.

Immunoadsorbents. DNP-Lys and normal goat Ig were coupled to Sepharose 4B (Pharmacia, Uppsala) via cyanogen bromide according to published procedures (Cuatrecasas, 1970). One milliliter of the DNP-Lys-Sepharose conjugate could adsorb at least 1.5 mg of M-315 IgA.

Plasmacytoma Proteins. The M-321 L chain was isolated from the urine of mice bearing the M-321 tumor as described (Potter, 1967). The M-315 IgA was purified from the serum of mice bearing the M-315 tumor by specific immunoadsorption and elution according to Inbar et al. (1971), with some modifications. Briefly, the Ig from 100 ml of serum was precipitated at 45% saturation of $(NH_4)_2SO_4$. The centrifuged precipitate was washed with 45% $(NH_4)_2SO_4$; it was then dissolved in 60 ml of water, dialyzed against the pH 7.4 buffer (three times 6 l.), and finally against 0.2 M Tris-HCl (pH 8.2). After dialysis the proteins were reduced with 0.01 M dithiothreitol for 1 h at room temperature and alkylated with 0.04 M ethylenimine for 30 min. The alkylated protein solution was applied onto a column containing DNP-Lys-Sepharose conjugate (200 ml slurry), equilibrated, and run with 0.05 M NaCl–0.003 M sodium phosphate (pH 7.4). The column was washed with 0.05 M NaCl–0.003 M sodium phosphate (pH 7.4) until the absorbance at 280 nm was 0.03. The adsorbed M-315 IgA was specifically eluted by 50 ml of 0.05 M DNP-Gly (titrated to pH 7.4) and fractions of 15 ml were collected. Each of the yellow fractions was passed through a column (2 × 4 cm) of Dowex 1-X8 (200–400 mesh) equilibrated with 0.05 M NaCl–0.003 M sodium phosphate (pH 7.4), to remove DNP-Gly quantitatively, and the protein-containing fractions were pooled. For optimal separation of the chains, the M-315 IgA was again subjected to mild reduction and alkylation, as follows. To the protein solution was added 0.15 volume of 1 M Tris-HCl (pH 8.2), and it was reduced and aminoethylated as described above. The protein was then concentrated by precipitation at 65% saturation $(NH_4)_2SO_4$. The centrifuged precipitate was dissolved in 8 ml of water and dialyzed against the pH 7.4 buffer (three times 1 l.) to yield a solution of mildly reduced and aminoethylated M-315 IgA.

Separation of M-315 Heavy and Light Chains. The mildly reduced and aminoethylated M-315 IgA was chromatographed on Sephadex G-100 column (5 × 95 cm) equilibrated with 6 M urea–1 M acetic acid (Bridges and Little, 1971). The resolved chains were dialyzed against the pH 7.4 buffer (five times 6 l.) and then concentrated to about 0.5% protein by negative pressure ultrafiltration.

Immunoadsorption Experiments. The sample (1–1.5 mg of protein in 0.8 ml of pH 7.4 buffer) was loaded onto the immunoadsorbent (2 ml packed volume) that was equilibrated with the pH 7.4 buffer. After the sample penetrated into the immunoadsorbent, the flow through the column was stopped for 5 min, and then it was developed with the pH 7.4 buffer (16–20 ml) and 0.25 N NH_4OH. The eluate was collected in 1-ml fractions. In one case (Figure 1B), after washing with the pH 7.4 buffer, the column was developed with 1.5 ml of the buffer containing 0.05 M DNP-Gly, it was washed with 15 ml of the pH 7.4 buffer, and then with 0.25 N NH_4OH. Protein absorbance at 280 nm in the yellow eluate was determined after removal of the DNP-Gly with Dowex 1-X8 (Inbar et al., 1971). All manipulations were carried out at room temperature.

Equilibrium Dialysis. Equilibrium dialysis was carried out in small leucite chambers. Protein in 0.075 ml of the pH 7.4 buffer and [^{3}H]DNP-Lys in 0.075 ml of the same buffer were added to opposite sides of the membrane. After equilibration was attained (30 h at 4 °C) 10–25-μl samples withdrawn from each compartment were mixed with 0.5 ml of water and counted in 10 ml of Bray's solution (Bray, 1960) in a Packard liquid scintillation counter.

The [^{3}H]DNP-Lys (1.1 Ci/mmol) was used as such for equilibration with the M-315 IgA. When the other proteins were studied, higher concentrations of the ligand were required. In these cases unlabeled DNP-Lys was added to the labeled ligand. In all experiments the radioactivity actually measured ranged between 2 000 and 300 000 cpm. The following values were used in computation of the data (all measurements performed in the pH 7.4 buffer). DNP-Lys: ϵ_{360nm}^{M} 17 500 (Underdown et al., 1971). M-315 IgA: molecular weight (7S monomer), 153 000 (Underdown et al., 1971); $\epsilon_{278nm}^{0.1\%}$ 1.35 (Underdown et al., 1971). M-315 L chain (monomer): molecular weight, 22 630 (from amino acid sequence, Dugan et al., 1973); $\epsilon_{280nm}^{0.1\%}$ 1.4. M-315 H chain (monomer): molecular weight, 54 500 (Underdown et al., 1971); $\epsilon_{280nm}^{0.1\%}$ 1.4. M-321 L chain (monomer): molecular weight, 24 020 (from amino acid sequence, McKean et al., 1973); $\epsilon_{280nm}^{0.1\%}$ 1.4.

Results

Immunoadsorption of Ig and Ig Chains. The anti-DNP activity of the various proteins was first studied by measuring their capacity to interact with immobilized DNP. As expected, the M-315 IgA was adsorbed on DNP-Lys-Sepharose and could be eluted either by 0.25 N NH_4OH or by 0.05 M DNP-Gly in the pH 7.4 buffer, while normal rabbit Ig was not retained on this immunoadsorbent (data not shown). The data given in Figure 1 show that the M-315 L chain behaves similarly to the intact M-315 IgA. The M-315 L chain is adsorbed on the DNP-Lys-Sepharose, and can be eluted either by 0.25 N NH_4OH (Figure 1A) or by 0.05 M DNP-Gly in the pH 7.4 buffer (Figure 1B). The specificity of this interaction was further ascertained from: (1) the lack of adsorption of M-321 L chain on DNP-Lys-Sepharose (Figure 1A); (2) the lack of adsorption of M-315 L chain on Sepharose alone or on Sepharose conjugated to normal goat Ig (data not shown).

The results obtained with the M-315 H chain are summarized in Figure 2. On first passage on DNP-Lys-Sepharose, 26% of the H chain was retained on the immunoadsorbent (range, 20–27% in four experiments using different batches of M-315 H chain). The material that was not retained on the first passage (peak 1, Figure 2A) was not retained when it was

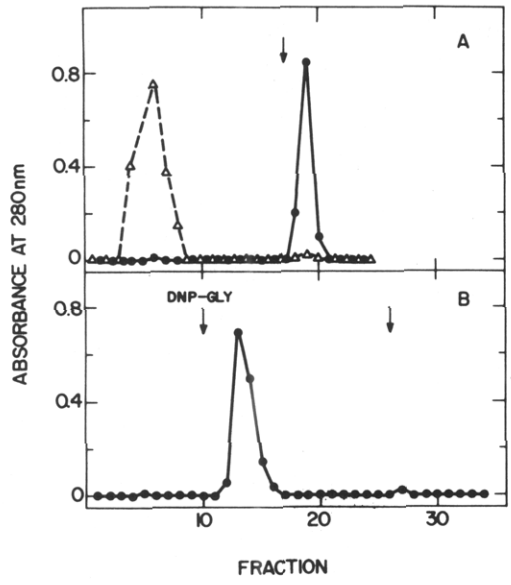

FIGURE 1: Elution pattern of M-315 L chain (●) and M-321 L chain (△) from DNP-Lys-Sepharose. Samples were loaded onto the immunoadsorbent in the pH 7.4 buffer, the column was developed with this buffer, and then the solvent was changed. In A, 0.25 N NH_4OH was applied (indicated by the arrow). In B, 0.05 M DNP-Gly in the pH 7.4 buffer was applied, and then 0.25 N NH_4OH (indicated by an arrow).

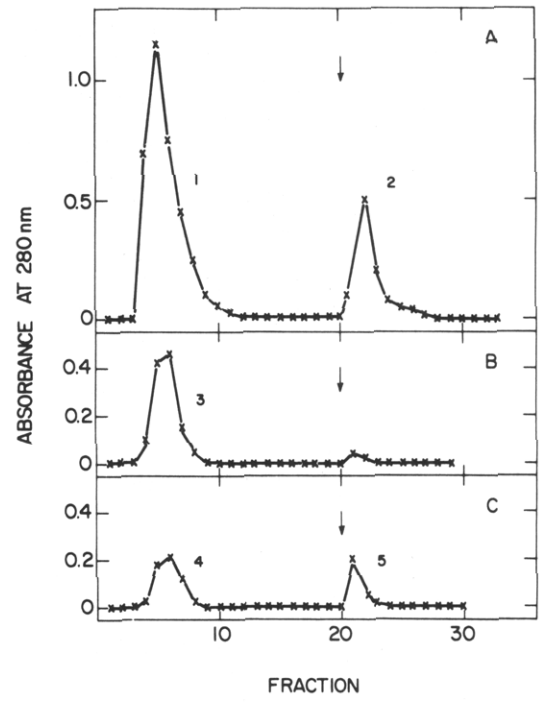

FIGURE 2: Elution pattern of M-315 H chain from DNP-Lys-Sepharose. The column was developed first with the pH 7.4 buffer and then with 0.25 N NH_4OH (indicated by the arrows). Samples are: (A) original M-315 H chain; (B) H chain that was not retained on first passage (peak 1 of A); (C) H chain that was retained on first passage (peak 2 of A).

rerun again on DNP-Lys-Sepharose (Figure 2B). On the other hand, the material retained in the first passage (peak 2, Figure 2A) was resolved into two fractions upon rechromatography on the immunoadsorbent (peaks 4 and 5 in Figure 2C). To clarify these results, the various fractions were reduced and analyzed on sodium dodecyl sulfate–polyacrylamide gels (Figure 3). It was found that the original M-315 H-chain preparation was contaminated by trace amounts of L chain (Figure 3c). Samples from peaks 1, 3, and 4 (see Figure 2), that were not retained on DNP-Lys-Sepharose, were free from L chain (Figure 3d, f, g). On the other hand, samples from peaks 2 and 5 (see Figure 2), that were retained on the immunoadsorbent, showed L-chain contamination (Figure 3e,h). Thus, pure M-315 H chain did not bind to DNP-Lys-Sepharose, while partial adsorption of the M-315 H chain was clearly associated with L-chain contamination. In the retained species, the H:L ratio is considerably higher than the ratio in the native IgA (Figure 3, compare bands in slots e,h with slot i). This indicates that the H chain retained on the immunoadsorbent is not only (if at all) in the form of native IgA (H:L ratio, 1:1), but is mainly present in the form of aggregates containing predominantly H chains and relatively few L chains (H:L ratio > 2:1).

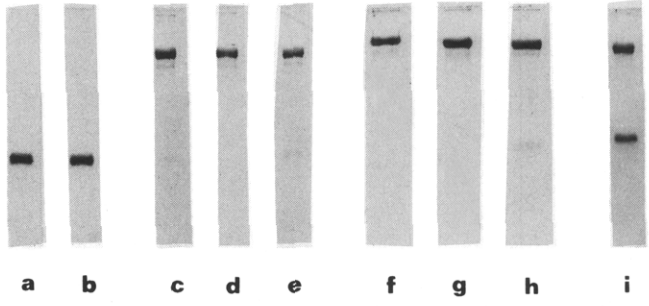

FIGURE 3: Sodium dodecyl sulfate–polyacrylamide gels of M-315 IgA chains. The samples were reduced and analyzed on slab gels (13% acrylamide, 10 V/cm, 2 h) as described (Maizel, 1972). The gels were stained with coomassie blue. (a) Original L chain; (b) L chain eluted from DNP-Lys-Sepharose (Figure 1B); (c) original H chain. Slots d to h contain samples of H-chain fractions eluted from the DNP-Lys-Sepharose columns shown in Figure 2. (d) Peak 1; (e) peak 2; (f) peak 3; (g) peak 4; (h) peak 5. Slot i contains M-315 IgA. Molecular weight standards were as detailed in Figure 4. The H chain migrated similarly to bovine serum albumin; the L chain migrated similarly to chymotrypsinogen A.

Molecular Size of Ig Light and Heavy Chains. In order to determine the valency (n) of the Ig chain, it is necessary to know the molecular weight of the protein at the same reaction conditions in which the equilibrium dialysis experiments are performed. This was done by seizing of the proteins by gel filtration on Sephadex columns equilibrated in the pH 7.4 buffer. The elution patterns of the M-315 and M-321 L chains from Sephadex G-100 were essentially identical, and it corresponded to molecules of 47 000 ± 2000 daltons (see Figure 4A). These results demonstrate that in the pH 7.4 buffer the L chains are in the form of dimers. The M-315 IgA and H chain were analyzed on Sephadex G-150 (Figure 4B). The M-315 IgA was eluted at a position corresponding to molecules of 150 000 ± 3000 daltons, a value comparable with that determined from high-speed sedimentation equilibrium (153 000 ± 2000, Underdown et al., 1971). The M-315 H chain was eluted in the void volume of the column which corresponds to molecules ≥200 000 daltons. In 6 M guanidine hydrochloride, the molecular weight of the H chain was found to be 54 500 ± 1300 (Underdown et al., 1971). Thus, in the pH 7.4 buffer, the M-315 H chain is in the form of aggregates composed of at least four H-chain molecules.

Equilibrium Dialysis Experiments. The crude data for binding DNP-Lys by similar amounts of M-315 L chain, M-315 H chain, and M-321 L chain are given in Figure 5. The M-315 L chain bound the ligand in a pattern characteristic for a saturation process. On the other hand, the M-315 H chain (two preparations) and M-321 L chain exhibited significantly weaker binding. The linear relationship of the weak binding

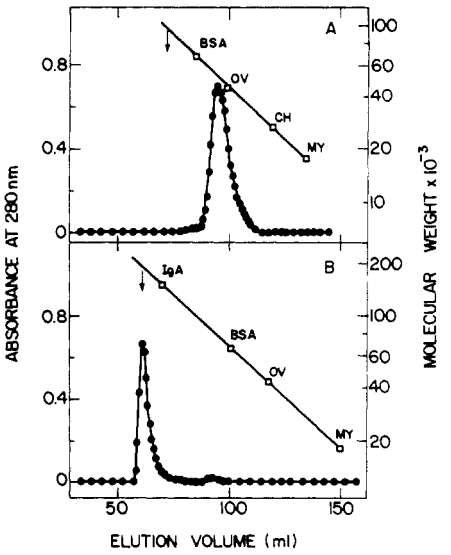

FIGURE 4: Sizing of M-315 L chain (A) and H chain (B) in PBS by gel filtration. The columns (1.5 × 95 cm) that contained Sephadex G-100 in A and Sephadex G-150 in B were equilibrated and developed with the pH 7.4 buffer. Molecular weight standards are: MY, myoglobin (17 200); CH, chymotrypsinogen A (25 700); OV, ovalbumin (43 000); BSA, bovine serum albumin (67 000); IgA, M-315 IgA monomer (153 000). Arrows indicate void volume of the columns determined with blue dextran.

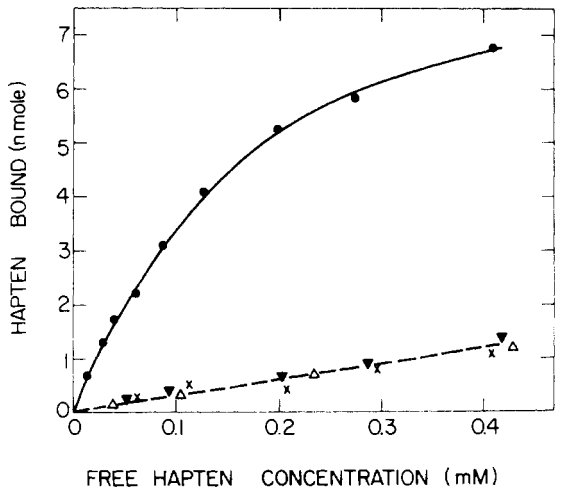

FIGURE 5: Binding of [^{3}H]DNP-Lys by Ig chains determined by equilibrium dialysis at 4 °C. Protein solutions were in 0.075 ml of the pH 7.4 buffer. Protein concentration (and amount in the sample) are: (●) M-315 L chain 2.4 mg/ml (3.9 nmol of dimer); (X) and (▼) two batches of M-315 H chain each at 2.6 mg/ml (3.6 nmol of monomer); (△), M-321 L chain, 2.6 mg/ml (4 nmol of dimer).

is probably due to nonspecific binding occurring at the high concentrations of ligand and proteins employed. The corrected binding data of M-315 L chain (obtained by subtraction of the nonspecific binding, Figure 5), gave a linear Scatchard plot (Scatchard, 1949). The intrinsic association constant (K_A), determined from the slope, was 6.3×10^3 M^{-1}. The number of ligand molecules bound at saturation (determined from abscissa intercept) was 1.94 for 45 260-dalton L chain (Figure 6). The binding experiments were performed in the pH 7.4 buffer. Since the M-315 L chain is in the form of dimer in this buffer (Figure 4A), these results imply that the M-315 L-chain dimer has two binding sites for DNP-Lys. Slope of the logarithmic Sips plot (Sips, 1948) gave a heterogeneity index of 0.98 (Figure 7), thus showing that the two binding sites of the

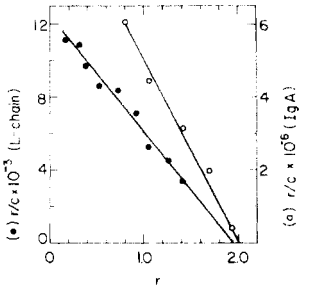

FIGURE 6: Scatchard's plot for binding [^{3}H]DNP-Lys by M-315 L chain (●) and M-315 IgA (○) in the pH 7.4 buffer at 4 °C. r is moles of hapten bound per L-chain dimer (45 260) or per IgA monomer (153 000) at the free hapten concentration (c).

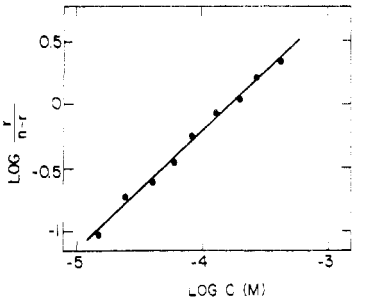

FIGURE 7: Sips plot for binding [^{3}H]DNP-Lys by M-315 L chain in the pH 7.4 buffer at 4°C. Heterogeneity index (slope) is 0.98.

L-chain dimer are homogeneous with respect to affinity for the ligand.

As a control we measured the binding of DNP-Lys to the M-315 IgA monomer. The binding parameters determined ($K_A = 5.4 \times 10^6$ M^{-1}, $n = 2$, Figure 6) agreed with the published values (Underdown et al., 1971; Bridges and Little, 1971).

Discussion

The results of this investigation show that the L chain, but not the H chain, obtained from the M-315 IgA bind the DNP ligand. The K_A values for binding DNP-Lys by the L chain and intact IgA are 6.3×10^3 and 5.4×10^6 M^{-1}, respectively. Thus, in terms of K_A, the affinity of the L chain is three orders of magnitude lower than the affinity of the intact IgA molecule. It would be an oversimplification, and probably erroneous, to conclude from these data that the L chain comprises only 0.1% of the binding site in the IgA. It seems that more relevant information on the contribution of the L chain may be obtained by expressing the interactions in terms of the unitary free energy of binding (Utsumi and Karush, 1964; Schechter, 1970; Painter et al., 1972), calculated for 4 °C (in kcal mol^{-1}) from the equation: $\Delta F_u = -1.27 \log K_A - 2.23$ (Utsumi and Karush, 1964). Thus, by comparing the binding of series of inhibitors to papain with crystallographic models of papain–inhibitor complexes, it was found that increased contact between the inhibitor and the active site correlated well with increased binding expressed in terms of ΔF_u (Berger and Schechter, 1970; Berger et al., 1971). It seems reasonable to assume that the same correlation holds true for antibodies (Schechter, 1970). For example, the K_A (and ΔF_u) values for binding alaninamide and trialaninamide to anti-polyalnyl antibodies are 1.3×10^3 M^{-1} ($\Delta F_u = -6.1$) and 5.4×10^6 M^{-1} ($\Delta F_u = -10.7$), respectively (Schechter, 1971). The ratios between these ligands are 4150 in terms of K_A, and 1.8

in terms of ΔF_u. When stretched, the lengths of alaninamide and trialaninamide are about 4 and 11 Å, respectively. Evidently, the increased contact with the binding site resulting from elongation of alaninamide to trialaninamide is not reflected by the ratio of K_A since this would have indicated binding site 16 600 Å (4150 × 4) in length. On the other hand, by using the ratio of ΔF_u we obtain a more realistic estimate (although not precise) of the change in contact areas between ligand and combining site, associated with changes in strength of interaction. Coming back to our system, the ΔF_u values for binding DNP-Lys to the M-315 L chain and IgA (at 4 °C) are -7.1 and -10.8 kcal mol^{-1}. That is, interaction with the L chain brings out 66% (7.1/10.8) of the binding energy in the site of the intact IgA. In other words, it seems more justified to estimate that about two-thirds (estimated from ΔF_u ratio), and not 0.1% (estimate from K_A ratio), of the binding site of the intact IgA is retained in the free L chain.

Previous data on the binding of homologous antigen by the isolated L chains were not sufficiently quantitative due to low affinity, and (or) because the antibodies investigated were heterogeneous since they were elicited by injection of antigen. Goodman and Donch (1965) showed phage neutralization by specific L chain, which was far less efficient than the specific H chain. Painter et al. (1972) investigated the isolated chains of rabbit antibodies to DNP. The K_A for binding DNP-Lys by the specific L chain was low ($2–5 \times 10^2$ M^{-1}), and it was not possible to determine with accuracy the valency of the L chain. Nakamura et al. (1973) studied rabbit antinaphthalene sulfonate antibodies. They found specific binding of hapten by the isolated L chain ($K_A < 10^2$ M^{-1}) which was at least 10 000 folds lower than binding by the H chain ($K_A > 10^6$ M^{-1}), and no attempt was made to calculate the valency of the L chain. Recently it was shown that crystals of L-chain dimers contain a "cleft" between the variable domains (Edmundson et al., 1974; Epp et al., 1974) which is similar in overall structure to the binding site in Fab fragments (Segal et al., 1974; Poljak, 1975). A variety of ligands were bound inside the "cleft" by soaking the L-chain crystals in appropriate solutions for several days (Edmundson et al., 1974). These findings have indicated that the L-chain dimer may serve as a model for primitive antibody (Edmundson et al., 1974; Epp et al., 1974). In the present study binding of DNP-Lys by the isolated M-315 L chain was significantly higher than nonspecific binding by the unrelated M-321 L chain (Figure 5). The data (Figure 6) permit reliable determination of two binding sites for M-315 L chain dimer, that bind DNP-Lys with K_A of 6.3×10^3 M^{-1}.

The observation of stronger binding of ligand by L chain than by H chain (Figure 5) has not been previously reported (see above). We would like, however, to recall the reservation that these results could have originated from the fact that the M-315 H chain was in the form of aggregates, whereas the M-315 L chain was in the form of a dimer (Figure 4). Yet, it seems unlikely that aggregation per se would have decreased possible binding activity by the H chain to undetectable level (Figure 5). Utsumi and Karush (1964) reported that 87% of the affinity found in the intact antibody is retained in the isolated H chains which were considerably aggregated. Also aggregations of M-315 IgA increase, rather than decrease, binding of the protein to DNP-Lys-Sepharose (Goetzl and Metzger, 1970a).

The M-315 IgA and L chain provide a unique system for analyzing in details to what extent structural features of the binding site in the intact Ig are conserved in the isolated chain. This is because both proteins are uniform in structure, amino acid sequences of the L chain (Dugan et al., 1973) and of the variable region of the H chain (Francis et al., 1974) are known, these proteins bind the same ligand in a fashion characteristic for homogeneous binding sites (Figures 6 and 7), and the binding site of the M-315 IgA has been extensively studied. For example, it was found that in the intact M-315 IgA the *m*-nitrobenzenediazonium salt (Goetzl and Metzger, 1970b) and the bromoacetyl derivative of DNP-ethylenediamine reacted exclusively with Tyr-34 in the L chain, while the bromoacetyl derivative of DNP-Lys reacted exclusively with Lys-54 in the H chain (Haimovich et al., 1972). It would be interesting to study the reactivity of the above reagents toward the isolated L chain, to find out whether Tyr-34 will be also labeled in the pure L-chain dimer, and to explore the possibility of labeling amino acid residues from the constant region of the L chain. The binding site of M-315 IgA was mapped by measuring its interaction (K_A) with series of DNP-containing ligands (Haselkorn et al., 1974). It seems desirable to measure the interaction of these ligands with the M-315 L chain, and to compare the two sets of K_A values. These and other experiments may provide information on whether contact residues of the binding site undergo conformational change when the isolated chains associate to form the intact 7S molecule, and whether the binding site in the isolated chain is constructed for interaction with a distinct portion of the ligand.

It has been reported that the free M-315 L chain does not bind DNP-Lys as judged by fluorescence quenching (Bridges and Little, 1971). The apparent discrepancy with our data is explained by the fact that in the above study the reactants (L chain and DNP-Lys) were tested at concentrations which were lower by two orders of magnitude than required to achieve significant binding.

In view of the results reported here it seems desirable to renew the search for antibody activity in free L chains mainly in mice, and to use immunoadsorbents. This is because previous studies on isolated L chains were conducted on preparations obtained from horse or rabbit antibodies, and immunoadsorbents have been rarely used (reviewed by Porter and Weir, 1966; Nakamura et al., 1973).

The biological significance of binding activity in the isolated L chains is not clear. We would like to recall, however, that free L chains circulate in the blood of man and mice bearing myeloma tumors, the L chains constitute the pathological deposits in certain types of amyloidosis (Terry et al., 1973), and among the conflicting evidence about the nature of the antigen receptor of T lymphocyte there are experiments suggesting the presence of L chain, but not of H chain, on the surface of T lymphocytes (Rouse and Warner, 1972).

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References

Berger, A., and Schechter, I. (1970), *Philos. Trans. R. Soc. London, Ser. B. 257,* 249.

Berger, A., Schechter, I., Benderly, H., and Kurn, N. (1971), in Tenth European Symposium on Peptides, Amsterdam, North-Holland Publishing Co., p 290.

Bray, G. A. (1960), *Anal. Biochem. 6,* 2682.

Bridges, S. H., and Little, J. R. (1971), *Biochemistry 10,* 2525.

Cuatrecasas, P. (1970), *J. Biol. Chem. 245,* 3059.

Dorrington, K. J., and Tanford, C. (1970), *Adv. Immunol. 12,* 333.

Dugan, E. S., Bradshaw, R. A., Simms, E. S., and Eisen, H.

N. (1973), *Biochemistry 12,* 5400.
Edmundson, A. B., Ely, K. R., Girling, R. L., Abola, E. E., Schiffer, M., Westholm, F. A., Fausch, M. D., and Deutsch, H. F. (1974), *Biochemistry 13,* 3816.
Eisen, H. N., Simms, E. S., and Potter, M. (1968), *Biochemistry 7,* 4126.
Epp, O., Colman, P., Fehlhammer, H., Bode, W., Schiffer, M., and Huber, R. (1974), *Eur. J. Biochem. 45,* 513.
Fleischman, J. B. (1966), *Annu. Rev. Biochem. 35,* 835.
Fleischman, J. B., Porter, R. R., and Press, E. M. (1963), *Biochem. J. 88,* 220.
Francis, S. H., Leslie, R. G. Q., Hood, L., and Eisen, H. N. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 1123.
Goetzl, E. J., and Metzger, H. (1970a), *Biochemistry 9,* 1267.
Goetzl, E. J., and Metzger, H. (1970b), *Biochemistry 9,* 3862.
Goodman, J. W., and Donch, J. J. (1965), *Immunochemistry 2,* 351.
Haimovich, J., Eisen, H. N., Hurwitz, E., and Givol, D. (1972), *Biochemistry 11,* 2389.
Haselkorn, D., Friedman, S., Givol, D., and Pecht, I. (1974), *Biochemistry 13,* 2210.
Inbar, D., Rotman, M., and Givol, D. (1971), *J. Biol. Chem. 246,* 6272.
Maizel, J. V. (1972), *Methods Virol. 5,* 179.
McKean, D., Potter, M., and Hood, L. (1973), *Biochemistry 12,* 749.
Nakamura, H., Grossberg, A. L., and Pressman, D. (1973), *Immunochemistry 10,* 485.
Painter, R. G., Sage, H. J., and Tanford, C. (1972), *Biochemistry 11,* 1327.
Poljak, R. J. (1975), *Nature (London) 256,* 373.
Porter, R. R., and Weir, R. C. (1966), *J. Cell. Physiol., Suppl. 1 67,* 51.
Potter, M. (1967), *Methods Cancer Res. 2,* 105.
Rouse, B. T., and Warner, N. L. (1972), *Cell. Immunol. 3,* 470.
Scatchard, G. (1949), *Ann. N. Y. Acad. Sci. 51,* 660.
Schechter, I. (1970), *Nature (London) 228,* 5272.
Schechter, I. (1971), *Ann. N.Y. Acad. Sci. 190,* 394.
Segal, D. M., Padlan, E. A., Cohen, G. H., Rudikoff, S., Potter, M., and Davies, D. R. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 4298.
Sips, R. (1948), *J. Chem. Phys. 10,* 490.
Terry, W. D., Page, D. L., Kimura, S., Isobe, T., Osserman, E. F., and Glenner, G. G. (1973), *J. Clin. Invest. 52,* 1276.
Underdown, B. J., Simms, E. S., and Eisen, H. N. (1971), *Biochemistry 10,* 4359.
Utsumi, S., and Karush, F. (1964), *Biochemistry 3,* 1329.
Yoo, T. J., Roholt, O. A., and Pressman, D. (1967), *Science 157,* 709.