# SPECIFICITY OF RECOMBINATION OF H AND L CHAINS FROM HUMAN γG-MYELOMA PROTEINS*

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A considerable body of evidence indicates that γG-globulin[1] molecules are composed of two H polypeptide chains and two L polypeptide chains and that these chains are linked together by disulfide bonds and non-covalent interactions (2–6). The non-covalent interactions alone are sufficient to hold the four chain molecules together and provide them with specific antibody function (3). Furthermore, 7S molecules can be reformed from dissociated H and L chains by removal of conditions interrupting the non-covalent interactions. The reformed molecules resemble the unaltered γG-molecules in many physicochemical parameters even when the interchain disulfide bonds have not been reestablished by oxidation (7–10).

Normal γG-globulin is a chemically heterogeneous population of molecules as manifested by antigenic characteristics, electrophoretic mobility, genetic markers, chemical analysis, and antibody specificity. The present study was undertaken to determine whether structural differences among γG-molecules lend any specificity to the non-covalent interactions between individual H and L chains. If specificity exists in the H and L chain interaction, this would be difficult to ascertain by investigations in which comparisons are made between populations of γG-globulin which contain a broad spectrum of H and L chains since one population would contain many molecules whose structure is similar to that of the other population. However, such specificity would be evident in experiments comparing relative affinities between homogeneous populations of H and L chains. Purified antibodies would be ideal for this investigation, but isolated antibodies thus far obtainable are rather heterogeneous (11–13). For this reason γG-myeloma proteins were used to study the specificity of the non-covalent interactions between homogeneous populations of H and L chains.

Evidence will be presented that the interactions between H and L chains are specific in that the polypeptide chains derived from a myeloma protein recom-

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[1] The nomenclature used is that which was recommended by a conference on human immunoglobulins sponsored by the World Health Organization (1).

bined preferentially as compared with recombinations using chains derived from two different proteins.

## *Materials and Methods*

*Isolation of Myeloma Proteins.*—Serum was obtained from a number of patients with an established diagnosis of myltiple myeloma. The myeloma proteins were isolated by preparative electrophoresis on starch medium (14) and further purified, if necessary, by ion exchange chromatography on DEAE-cellulose columns (15). The protein concentration of these and other preparations was determined by the Folin procedure (16). Immunochemical characterization of the isolated proteins was carried out as previously described (17, 18).

*Iodination of Proteins.*—The iodination of isolated myeloma proteins was carried out according to the methods of Helmkamp *et al.* (19) with $I^{125}$ or $I^{131}$. Proteins were labeled with one mole of iodine per mole of protein. If iodinated H chains were desired, then the myeloma protein was iodinated prior to reduction and alkylation. Labeled L chains were obtained either from labeled whole molecules or the isolated L chains were labeled after dialysis against distilled water and borate buffer pH 8.0.

*Preparation of H and L Chains.*—The reduction and alkylation were performed with minor modifications according to the methods of Fleischman, Pain, and Porter (3). The myeloma protein solutions at a concentration of 10 mg per ml were rendered 0.1 M with 2-mercaptoethanol. After 1 hour at room temperature and 15 minutes in an ice bath the free sulfhydryl groups were blocked with a 10 per cent excess of sodium iodoacetate or iodoacetamide. In any given experiment all proteins were alkylated with the same reagent. Subsequently, the preparations were dialysed first against cold 0.15 M NaCl followed by dialysis against 1.0 M propionic acid at 4°C. The reduced, alkylated, and dissociated chains were separated by gel filtration according to the methods of Fleischman *et al.* (4), except sephadex G-100 was employed in columns of 3 × 70 cm, equilibrated with 1.0 M propionic acid. Appropriate fractions of the effluent were pooled for H and L chains. The H chains were concentrated by pressure dialysis through Visking membranes (wall thickness 0.0020 inches) at 4°C and the L chains were concentrated at the same temperature by negative pressure through collodion bags (Schleicher and Schuell, Keene, New Hampshire).

*Procedure for Recombination.*—Aliquots of the appropriate mixtures of H and L chains were taken while still in the presence of 1.0 M propionic acid. All these and subsequent steps were carried out at 4°C until the analysis by density gradient ultracentrifugation. Subsequent to the mixing of the H and L chains the following steps of dialysis were carried out using collodion bags for dialysis membranes: twice against 0.01 M sodium acetate buffer, pH 5.0 and twice against 0.01 M sodium acetate buffer, pH 5.0 rendered 0.15 M in respect to NaCl. Each step of dialysis was carried out for 16 to 20 hours using a 100- to 200-fold excess of the outer solvent. An alternative but equally effective procedure was to first dialyse the H and L chains separately against 0.1 M propionic acid and then to prepare the appropriate H and L chain mixtures in this solvent. Subsequently they were dialysed in collodion bags against 100- to 200-fold excess of distilled water, followed by 0.01 M tris-HCl buffer, pH 8.0. The total protein concentration of the mixtures of H and L chains during the dialysis steps was between 0.5 to 1.0 mg per ml.

*Density Gradient Ultracentrifugation.*—Aliquots of the dialysed recombined mixtures were subjected to linear sucrose density gradient ultracentrifugation (20) to separate the free L chains, recombined 7S molecules, and possible heavier aggregates as previously described (10). The sucrose solutions were made in 0.01 M sodium acetate buffer, pH 5.0, rendered 0.15 M with NaCl or in 0.01 M tris-HCl buffer pH 8.0, depending on which procedure of dialysis was used.

*Analysis of results.*—Following the density gradient ultracentrifugation and determina-

tion of $I^{125}$ and $I^{131}$ in each fraction of the gradient, the radioactivity of each isotope was plotted against the per cent of gradient volume, 100 per cent representing 5.0 ml and the top of the gradient (see Fig. 1). The position of the 7S peak was very reproducible from experiment to experiment and at frequent intervals was checked by the presence of a 6.1 S alkaline phosphatase marker (7). From the plot obtained in this manner and as illustrated in Fig. 1, the per cent of radioactivity of $I^{125}$ or $I^{131}$ under the heavier aggregates, 7S peak, and under the L chain peak could be calculated.

## RESULTS

*Interaction of H and L Chains of γG-Myeloma Proteins.*—Upon separation of the H and L chains on sephadex G-100 different elution patterns were obtained.

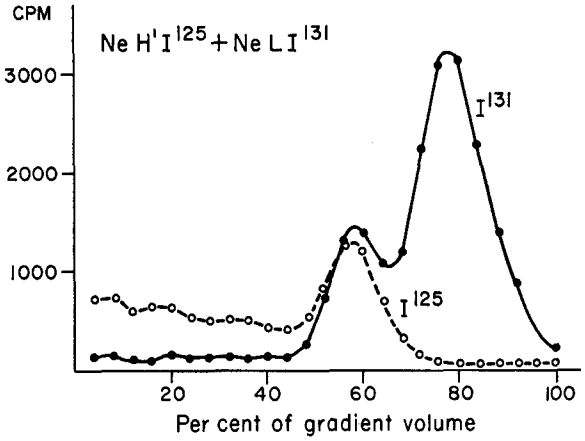



FIG. 1. Sucrose density gradient ultracentrifugation pattern of labeled recombined H and L chains of Ne myeloma protein. Gradient of 5 to 20 per cent sucrose was utilized. In this and subsequent illustrations of density gradients the top of the gradient is represented by 100 per cent of gradient volume on the right.

In certain proteins the H chains were eluted as two incompletely resolved peaks. These were arbitrarily termed and separated into $H^1$ and $H^2$ peaks; the $H^1$ peak appeared first and probably consisted of dimers and heavier polymers of H chains (4). In other proteins, however, only the polymeric $H^1$ peak was obtained on gel filtration. The yield of L chains from all myeloma proteins was 33 to 35 per cent of total protein by the Folin protein determination. Further studies on the differences of $H^1$ and $H^2$ peaks in recombination with L chains will be reported separately (21). The only important point to be made in connection with the current report is that similar to the H peak of rabbit γ-globulin (10) the $H^1$ peak of γG-myeloma proteins was less effective than the $H^2$ peak in recombining with L chains.

In experiments where the H chains were labeled with $I^{125}$ and L chains with $I^{131}$, both labels clearly appeared in the recombined molecules sedimenting upon

sucrose density gradient ultracentrifugation as 7S molecules (see Fig. 1). From such experiments the per cent of L chains in the 7S peak and in the more slowly sedimenting free L chain peak was calculated as well as the per cent of H chains in the 7S peak and in heavier aggregates. From the specific activities of the individual chains the composition of the 7S molecules was calculated; the per cent of L chains approximated the per cent of L chains obtained on gel filtration, indicating that four chain molecules were reformed.

Subsequent recombinations were performed between $I^{125}$-labeled H chains from individual γG-myeloma proteins and the $I^{131}$-labeled L chains from the same or different γG-proteins using the dialysis procedure with sodium acetate

TABLE I

*Recombination of γG-Myeloma Protein H Chains with Autologous and Heterologous L Chains*

| H and L chains used for recombination | L chains in 7S peak |
|---|---|
| | *per cent* |
| Sp $H^2$ + Sp L | 54.7 |
| Sp $H^2$ + Ge L | 38.1 |
| Sp $H^2$ + Ne L | 16.2 |
| Ro $H^1$ + Ro L | 17.3 |
| Ro $H^1$ + Bl L | 9.5 |
| Ro $H^1$ + Sp L | 7.7 |
| Ro $H^1$ + Ne L | 1.9 |
| Ku $H^2$ + Ku L | 60.0 |
| Ku $H^2$ + Ge L | 59.8 |
| Ku $H^2$ + Sp L | 54.3 |
| Ku $H^2$ + Ne L | 46.2 |

buffers. The ratio of H and L chains was 1:1 on the basis of protein concentration, thus providing a better than 2-fold molar excess of L chains and allowing optimal incorporation of H chains into 7S molecules. From the distribution of labeled L chains in the heavier aggregates, the 7S peak, and the L chain peak the percentage of L chains in the 7S peak was calculated. Results of such experiments are given in Table I for three different H chains. Sp $H^2$ chains recombined best with the autologous L chains and a decrease in the effectiveness of recombination was observed with heterologous L chains. Similar decrease of recombination with heterologous L chains was noted with Ro $H^1$ chains (this protein gave primarily an $H^1$ peak on gel filtration), in spite of the fact that the yield of recombined molecules was poor as reflected by the low per cent of L chains incorporated into the 7S peak. The H chains in this experiment formed aggregates that contained only small amounts of L chains and sedimented faster

than 7S. In contrast to the first two H chain preparations, Ku $H^2$ chains were able to recombine with two other L chains to the same extent as with the autologous L chains.

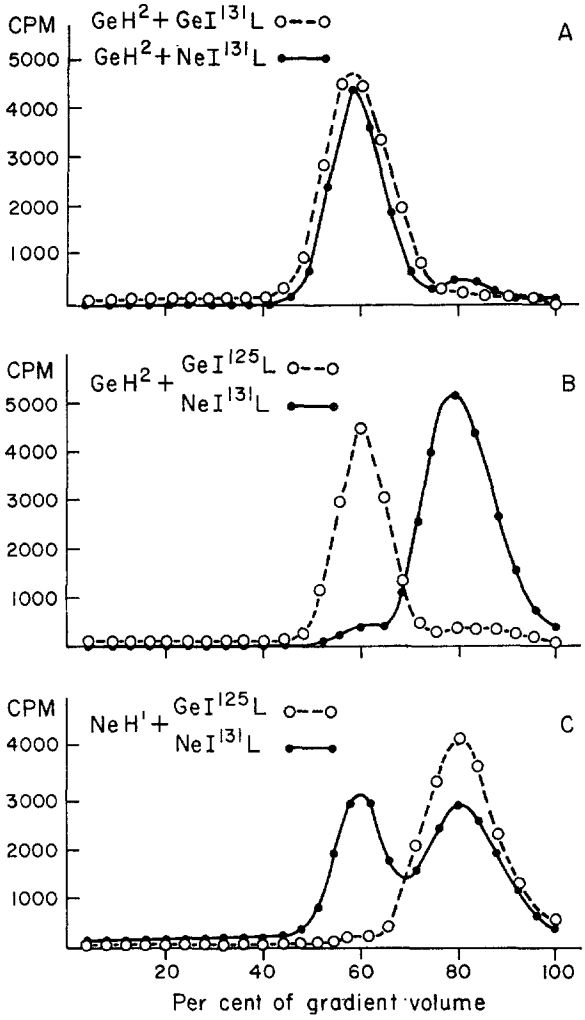
The above experiments demonstrate that the recombination of isolated H and L chains from γG-myeloma proteins occurs and in some instances there is increased affinity between the H and L chains from the same protein. It was thought, however, that if there was a general affinity between H and L chains, then the specificity of the non-covalent interactions between the polypeptide chains of one γG-myeloma protein might be masked by this general affinity of H and L chains. This difficulty might be avoided in a situation where the H chains have a choice of competitively recombining with L chains from the same protein or L chains from another protein.

The following experiments with two γG-myeloma proteins serve to illustrate the significance of competitive recombination.

Unlabeled Ge $H^2$ chains, Ge L chains labeled with $I^{125}$ (designated Ge L $I^{125}$), and Ge L chains labeled with $I^{131}$ (designated Ge L $I^{131}$) were prepared. Similarly unlabeled Ne $H^1$ chains and Ne L chain labeled with $I^{131}$ (designated Ne L $I^{131}$) were prepared. Recombination was performed with 500 μg of H chain preparation and 250 μg of each L chain preparation (all dissolved in 1.0 M propionic acid) in the following manner:

1. Ge $H^2$ + Ge L $I^{131}$
2. Ge $H^2$ + Ne L $I^{131}$
3. Ge $H^2$ + Ge L $I^{125}$, Ne L $I^{131}$
4. Ne $H^1$ + Ne L $I^{131}$
5. Ne $H^1$ + Ge L $I^{131}$
6. Ne $H^1$ + Ne L $I^{131}$, Ge L $I^{125}$

In mixtures (3) and (6) the amount of H chain preparation was identical to other mixtures, but 250 μg of both L chains were present. This provided a slight molar excess of each L chain. Subsequently the mixtures were carried through the steps of dialysis using the weak sodium acetate buffers to remove dissociating conditions as outlined under methods. The results of sucrose gradient ultracentrifugation analyses of the recombined materials are illustrated in Figs. 2 *a* to 2*c*. Good recombination occurred between Ge $H^2$ and Ge L chains as well as between Ge $H^2$ and Ne L chains, as shown in Fig. 2 *a*. However, when Ge $H^2$ chains had an equal opportunity to recombine with Ge L or Ne L chains, they preferentially recombined with their own L chains as illustrated in Fig. 2*b*. The same was observed with Ne $H^1$ chains, in that in competitive recombination they preferentially recombined with Ne L chains as illustrated in Fig. 2 *c*. The percentage of the L chains incorporated into 7S material in each of the 6 mixtures is given in Table II. From the percentage of L chains in the 7S peak the ratio of autologous L chains to heterologous L chains was determined and expressed in the right hand column. For the Ge $H^2$ chains in mixtures (1) and (2) in non-competitive recombination the ratio between autologous and heterologous L chains is 1.08, but the same ratio in a competitive recombination is 21.




FIGS. 2 *a* to 2 *c*. Sucrose density gradient ultracentrifugation patterns illustrating the non-competitive and competitive recombination of H and L chains. Gradient of 5 to 20 per cent sucrose was utilized.

FIG. 2 *a*. Patterns of two separate experiments are superimposed to show the degree of non-competitive recombination of Ge $H^2$ chains with either Ge L or Ne L chains.

FIG. 2 *b*. A competitive recombination experiment which illustrates the preferential recombination of Ge $H^2$ chains with Ge L chains.

FIG. 2 *c*. A competitive recombination experiment which illustrates the preferential recombination of Ne $H^1$ chain with Ne L chains.

Similarly for Ne $H^1$ chains this ratio in non-competitive recombination is 1.33 and for competitive recombination 13.3.

The above experiments clearly show the advantage of competitive recombination in bringing out the autologous specificity of the non-covalent interactions between the H and L chains of these γG-myeloma proteins. Therefore in subsequent experiments the H chains of individual proteins were presented with L chains from the same protein as well as with one of several heterologous L chains.

*Competitive Recombination of H Chains with Autologous and Heterologous L Chains.*—In order to determine whether the specificity of recombination was a generalized phenomenon or limited to only certain combinations of L and H chains, five different H chains representative of the γ-chain subgroups (18, 22)

TABLE II

*Comparison of Non-Competitive and Competitive Recombination of H and L Chains*

| H and L chains used for recombination | L chains in 7S peak | Ratio of autologous to heterologous L chains in 7S peak |
|---|---|---|
| | *per cent* | |
| Ge $H^2$ + Ge L | 92.3 | 1.08 |
| Ge $H^2$ + Ne L | 85.3 | |
| Ge $H^2$ + Ge L, Ne L | Ge 84.0; Ne 4.0 | 21.0 |
| Ne $H^1$ + Ne L | 39.6 | 1.33 |
| Ne $H^1$ + Ge L | 29.8 | |
| Ne $H^1$ + Ne L, Ge L | Ne 40.0; Ge 3.0 | 13.3 |

* See text for ratio of H and L chains.

were used to test whether there was a higher affinity for the autologous L chains when placed in competition with a variety of heterologous L chains derived from proteins representative of the major light and heavy chain groups. The data are presented in Table III and are expressed as the ratio of the percentage of autologous to heterologous light chains found in the 7S peak of the sucrose density gradient as discussed for Table II. The most striking feature is the high degree of specificity that all five H chain preparations exhibit for the autologous L chains. There was no tendency for heterologous L chains derived from myeloma proteins similar in antigenic structure to the autologous polypeptide chains to compete more favorably than antigenically different heterologous chains. Thus the L chains of the protein Ke whose H and L chains were in the We and K groups respectively did not compete any more favorably for Gr H chains, than L chains derived from proteins of other antigenic groups.

It was found, however, that a few heterologous L chains consistently competed either very well or very poorly with any of the autologous combinations

TABLE III

*Competitive Recombination of γG-Myeloma Protein H Chains with Autologous and Heterologous L Chains*

| Autologous chains used for recombination* | Competing heterologous L chains‡ | Ratio of autologous to heterologous L chains in 7S peak |
|---|---|---|
| Gr H (We) GrL (K) | Li (γA, K) | 6.8 |
| " " | Ke (We, K) | 3.9 |
| " " | Met (We, L) | 3.3 |
| " " | Da (γA, L) | 3.1 |
| " " | Ro (Ge, K) | 1.6 |
| " " | Ku (We, L) | 1.5 |
| " " | Fe (Vi, K) | 0.98 |
| Ke H (We) Ke L (K) | Mer (We, K) | 6.5 |
| " " | Di (γM, L) | 6.4 |
| " " | Ge (Ge, K) | 5.5 |
| " " | Ne (Ne, L) | 4.5 |
| " " | Ku (We, L) | 4.0 |
| " " | Sz (We, L) | 2.4 |
| " " | Met (We, L) | 2.4 |
| " " | Gr (We, K) | 2.1 |
| " " | Am (γA, L) | 2.1 |
| " " | Ro (Ge, K) | 2.0 |
| " " | Fe (Vi, K) | 0.48 |
| Mu H (We) Mu L (K) | Ku (We, L) | 10.4 |
| " " | Ne (Ne, L) | 8.4 |
| " " | Gr (We, K) | 4.3 |
| " " | Di (γM, L) | 4.3 |
| " " | Ke (We, K) | 3.4 |
| " " | Ge (Ge, K) | 2.9 |
| " " | Sz (We, L) | 1.8 |
| " " | Met (We, L) | 1.3 |
| Ka H (Ne) Ka L (L) | Ne (Ne, L) | 5.6 |
| " " | Di (γM, L) | 2.9 |
| " " | Am (γA, L) | 2.7 |
| " " | Ge (Ge, K) | 2.6 |
| " " | Sz (We, L) | 2.1 |
| " " | Met (We, L) | 1.9 |
| " " | Mer (We, K) | 1.3 |
| " " | Ro (Ge, K) | 0.41 |
| Ge H (Ge) Ge L (K) | Ne (Ne, L) | 21.0 |
| " " | Mer (We, K) | 3.1 |
| " " | Ro (We, K) | 1.1 |
| " " | Fe (Vi, K) | 0.59 |

* The antigenic subgroup of H chains is given in parenthesis (either We, Ge, Ne, or Vi), and the L chain type is given in parenthesis (either K or L).

‡ In the parenthesis for each L chain preparation is given the H chain subgroup of the protein from which the L chains were derived or the class of immunoglobulin if the protein was other than a γG-protein; this is followed by the antigenic type of L chain.

tested. Thus, Fe L chains combined better than the autologous L chains in the three experiments in which these L chains were used. Also Ro L chains competed consistently well with the autologous L chains and in one experiment the Ro L chains combined with the H chains preferentially to the autologous Ka L chains. On the other hand, Ne L chains consistently combined relatively poorly when compared to the autologous and other heterologous L chains. However, these L chains still retained the capacity to combine highly specifically with their own H chains as shown in Fig. 2. The explanation for the generally high affinity of Ro and Fe L chains for all heavy chains is not apparent and is under further investigation.

TABLE IV

*Competitive Recombination of γG-Myeloma Protein H Chains with Heterologous L Chains*

| H chain preparation* | Competing L chains and their per cent in 7S peak* |
|---|---|
| Gr (We, K) | Ge (Ge, K) 50 Sz (We, L) 56 |
| " | Ge (Ge, K) 55 Ke (We, K) 57 |
| " | Ku (We, L) 41 Di (γM, L) 60 |
| " | Ke (We, K) 45 Li (γA, K) 42 |

* In the parenthesis for each polypeptide chain the H chain subgroup of the protein is given if it belongs to the γG-class or the class of immunoglobulin if the protein is other than a γG-protein; this is followed by the antigenic type of L chain.

*Competitive Recombination of H Chains with Two Heterologous L Chains.*—The possibility arose that the high degree of specificity for autologous recombination would obscure any other specificity which was related to the antigenic structure of the competing heterologous polypeptide chains. For this reason competition experiments were performed in which both L chains were derived from proteins heterologous to the protein from which the H chains were derived. Combinations were chosen to test the possibility that the H chain antigenic structure may influence recombination with L chains so that the L chains obtained from a myeloma protein with the same H chain characteristics would combine preferentially over L chains derived from a protein of dissimilar H chain characteristics. Table IV illustrates these experiments in terms of the per cent of the competing L chains that sedimented in the 7S peak of the sucrose density gradient. The H chains were derived from Gr protein, which has We group H chains and type K light chains. There was no trend for these H chains to bind preferentially with L chains derived from proteins with similar antigenic characteristics. The lack of preferential recombination of chains derived from antigenically related proteins extended to L chains derived from proteins of different immunoglobulin classes in that L chains derived from γA- and γM-proteins

competed favorably for H chains of γG-proteins with L chains derived from γG-proteins.

## DISCUSSION

The present report demonstrates that the polypeptide chains derived from myeloma proteins recombined to form four chain molecules sedimenting as 7S proteins whether the polypeptide chains were derived from a single myeloma protein or whether the recombination took place between chains of different proteins. When L chains from the autologous and heterologous proteins were compared in terms of the degree of recombination obtained with H chains derived from a single source, only a slight trend was demonstrable toward preferential recombination of the H and L chains from the same protein (Table I, Fig. 2 *a*). On the other hand, when experiments were performed so that autologous and heterologous L chains were present together in excess, thereby setting up a competition for the H chains present in the mixture, then a striking preference for the autologous recombination was observed. This suggested that the forces responsible for the H-L interaction are such that sufficient free energy of binding is present between heterologous chains so that in the absence of other competing chains of higher affinity there is little difference in the degree of recombination between the autologous and heterologous chains. However, the competition experiments demonstrated that the autologous L chains have a higher binding affinity for the H chains than heterologous L chains.[2]

L chains from two γG-myeloma proteins were found to have "non-specifically" high affinity for H chains even in the presence of the autologous L chains. At present there is no explanation for this, but it is possible that under the conditions used for recombination these L chains had a relatively large number of reactive groups on their surface which allowed them to combine with several H chains with relatively high affinity.

As pointed out earlier the H chains of some γG-myeloma proteins were difficult to recombine with any L chain preparations. The suggestion that these were H chain polymers is based on the fact that undissociated L chains could not account for the heavier $H^1$ peak since the yield of L chains is not diminished in such proteins and undissociated L chains could have been present only in small quantities as judged by immunochemical testing with anti-L chain antisera. A possible explanation for obtaining poor recombination with the H chains that tend to polymerize is that in the polymerized H chains the region important for binding of L chains may be unavailable for the H-L interaction.

Beyond the individual specificity of autologous chain interaction no evidence was obtained to indicate that the antigenic structure of either the H or L chains

[2] Dr. S. Cohen, at the 1965 Antibody workshop, presented data which also demonstrated preferential recombination of autologous chains. His experiments were non-competitive in nature and separation of free and bound L chains was performed by gel filtration.

was important in determining the degree of recombination of different chains. This is not altogether unexpected since the antigenic determinants responsible for the subgroups of H chains are primarily located on the Fc fragment, an area of the molecule not involved in the H and L chain interaction (18, 22). Furthermore, myeloma proteins of the different H chain subgroups are encountered with either type K or type L light chains (18). Another parameter of L chain structure which did not appear to correlate with the degree of recombination was the mobility of the L chains in alkaline urea gel (21, 23) in that if the autologous L chain had a slow mobility, there was no indication that other L chains of similar mobility bound preferentially to those of fast mobility.

It appears then that the autologous specificity is a reflection of somewhat unique configurations of the respective L chains and the Fd fragments of the H chains which favor the autologous combination to occur in preference to other combinations thermodynamically less favorable. If the secondary and tertiary structure of rabbit antibodies is a function of primary amino acid sequence as recent studies indicate (24, 25) then it would follow that portions of the H and L chains of these myeloma proteins have amino acid sequences that differ from one to the other and result in the special configuration necessary for preferential recombination. With antibodies specific for individual myeloma proteins the individually specific antigenic determinants have been localized on the Fab fragments of these proteins, and these determinants may exist on the H chains, L chains or in some cases the individual specificity is demonstrable only when the H and L chains are combined (26). Furthermore, peptide mapping of Bence Jones proteins, L chains, and H chains derived from myeloma proteins indicates structural individuality in at least some parts of L chains and Fd fragments (27–29). Recent work by Hilschmann and Craig (30) indicates that in the case of type K light chains it is the amino-terminal half of the chain that is variable from one protein to another. This is probably true also of type L Bence Jones proteins and light chains since the carboxyl-terminal peptide is common to all type L proteins (27, 31) and many peptides vary from protein to protein as well. Therefore it appears likely that at least part of the amino terminal half of the L chains influences the non-covalent interactions between the H and L chains by providing the configuration of L chains that leads to preferential binding of the autologous chains. This maybe due to a direct contact between the amino-terminal half of the L chain and the H chain or the amino-terminal half of the L chain may indirectly influence the configuration of the carboxyl-terminal half of the chain that ends with the peptide which forms the disulfide bond with the H chain.

In a previous study with isolated rabbit antibodies to the 2,4-dinitrophenyl determinant it was shown by double isotope techniques that the antibody L chains and non-specific L chains recombined at random with the antibody H chains (10). The presence of the specific hapten during recombination resulted

in preferential recombination of antibody H and L chains. The antibody L chains in this previous investigation were heterogeneous on electrophoresis and resembled the L chain population of the non-specific γG-globulin. This fact may well account for the random recombination of antibody L chains and non-specific L chains with the antibody H chains in the absence of the antigen. Two possible mechanisms were offered for the selectivity brought out by the presence of antigen during recombination: (*a*) the antigen could complex with the antibody binding site on the H chain, and then the antigen-H chain complex would seek out the proper L chain because of a possible binding site on the L chain; and (*b*) the H chain could possess a region or site for binding with the L chain, and hapten complexed to the antibody-binding site could alter this "L chain–binding site" on the H chain in such a manner that recombination with the proper L chain would be thermodynamically favored. The current observations indicate that the latter mechanism has to be strongly considered in the previous results. The studies of Roholt *et al.* (32) with rabbit antibodies to two different haptens have suggested that preferential recombination of autologous chains can take place in the absence of hapten since in autologous recombination the recovered antibody activity was higher than in heterologous recombinations. The data presented here are in accord with this and clearly show that in competitive recombination the H and L chains derived from the same protein have a greater affinity for each other than for L chains derived from a different homogeneous protein.

The current observations lead to some interesting speculations on the mechanisms of antibody formation. Due to the specificity of H and L chain interaction it is possible that an antigen specifically induces the formation of only one of the polypeptide chains (either H or L) rather than both. The polypeptide chain that is specifically induced by the antigen would then selectively recombine with limited species of chains out of a large pool of chains due to the specificity of the non-covalent interactions described herein. This combination would then remove the selected chains from the pool and would release a feedback inhibition which would result in the continued synthesis of those species of chains.

## SUMMARY

Dissociated H and L chains of human γG-myeloma proteins were recombined by removal of conditions interrupting non-covalent interactions. In the process of recombination 7S molecules were formed. It was demonstrated that the H chains from individual γG-myeloma proteins recombine with their own L chains but also with L chains derived from other myeloma proteins. In some instances, however, the L chains from other myeloma proteins did not recombine as avidly with the H chains as the autologous L chains.

The specificity of the non-covalent interactions of H and L chains was par-

ticularly well brought out by competitive recombination experiments where an individual H chain had a choice of recombining with its own L chain or with the L chain obtained from another myeloma protein. In this manner a spectrum of affinities between individual H chains and several L chains was demonstrated. In the vast majority of recombinations there was a clearcut preference for recombination to take place between the H and L chains derived from the same protein. It is postulated that this specificity is related to differences in the primary structure, which cause differences in configuration of these homogeneous H and L chains and that these configurations then dictate on thermodynamic grounds the pairing of H chains with particular L chains.

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