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Variability in the Specific Interactions of H and L Chains of γG-Globulins*

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ABSTRACT: The specificity of noncovalent interactions between human γG-globulin H- and L-polypeptide chains was investigated. A previous study demonstrated that specificity exists in these interactions in that the heavy (H) and light (L) chains of a human G-myeloma protein tend to reassociate even if other free L chains are present [Grey, H. M., and Mannik, M. (1965), *J. Exptl. Med. 122*, 619]. The current study extends these observations and shows that the preferential recombination of autologous H and L chains, if present, occurs even at 80-fold molar excess of heterologous myeloma protein L chains. On the other hand, normal γG-globulin L chains contain populations of molecules that can replace randomly the autologous L chains of myeloma proteins from recombining with their H chains.

The percentage of such molecules varied from 23 to 73%, depending on the myeloma protein used. Natural γG-globulin molecules that contain one κ and one λ chain are not encountered. *In vitro*, such molecules form readily between a given H chain and a pair of κ and λ chains.

The noncovalent interactions between the heavy (H) and light (L) chains[1] of γG-globulins are sufficient to maintain the integrity of these four-chain molecules in neutral weak salt solutions so that they continue to function as specific antibody molecules and display many of the physiochemical characteristics of the native molecules (Edelman *et al.*, 1963; Olins and Edelman, 1964; Roholt *et al.*, 1964; Metzger and Mannik, 1964; Fougereau and Edelman, 1964). Specificity exists in these noncovalent bonds in that the H chains of a homogeneous γG-globulin, either from a myeloma protein or from specific antibody, tend to recombine with their own L chains even when L chains from other homogeneous γG-globulins are present (Grey and Mannik, 1965; Roholt *et al.*, 1965). The previous study, utilizing human G-myeloma proteins as models of homogeneous populations of molecules, showed considerable variation among L chains of various myeloma proteins in their capacity to replace autologous L chains from their interaction with H chains (Grey and Mannik, 1965). These and other observations stimulated more detailed studies of the competitive reactions with particular emphasis on the heterogeneous population of normal γG-globulin L chains which should contain molecules that are capable of replacing at random the autologous L chains. The data presented below show that the specificity of noncovalent interactions between autologous H and L chains of G-myeloma proteins, if present, persists in large molar excess of heterologous L chains. Furthermore, normal γG L chains contain molecules capable of replacing at random the autologous L chains from recombining with their H chains. Deductions from the data suggest that the variable portion of L chains participates in the noncovalent interactions with H chains.

Materials and Methods

Isolation of γG-Globulins. Myeloma proteins were isolated and immunochemically characterized as previously described (Grey and Mannik, 1965). Normal pooled γG-globulin was isolated from fraction II (Lederle Laboratories, lot no. C-803) by DEAE-cellulose chromatography. The protein concentrations in all experiments were determined by the method of Lowry *et al.* (1951).

Preparation of H and L Chains. The H and L chains from the isolated proteins were prepared by the methods

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[1] The nomenclature for immunoglobulins and their polypeptide chains is that recommended by a conference on immunoglobulins sponsored by the World Health Organization, *Bull. World Health Organ. 30*, 447 (1964).

of Fleischman *et al.* (1962) with the modifications already described (Grey and Mannik, 1965), except that in the current study 0.5 M propionic acid was employed to dissociate the H and L chains after reduction and alkylation of disulfide bonds. The same concentration of propionic acid was used for gel filtration to achieve separation of the L and H chains. The latter were again eluted in two protein peaks (Grey and Mannik, 1965), the first peak was termed H^1 and the second H^2. If the yield of H^2 was small, all heavy chains were pooled and termed H chains.

Iodination of Proteins. Only L chains were trace labeled with either ^{131}I or ^{125}I, employing carrier-free and reducing agent free solutions of isotopes, according to the methods of Helmkamp *et al.* (1960).

Starch Gel Electrophoresis. Starch gel electrophoresis in alkaline pH and 8 M urea was performed by methods of Cohen and Porter (1964) on the iodinated L chain preparations except that 0.1 M sodium borate buffer, pH 8.0, was used in the gels and trays. Under these conditions multiple banding of iodinated normal γG L chains was observed, they were numbered beginning at the anode end of their distribution from 1 to 10. The L chains from myeloma proteins were given numbers according to their positions in relation to the bands of normal γG L chains.

Recombination Procedure. The appropriate mixtures of H and L chains were made while these proteins were dissolved in 0.5 M propionic acid, this and subsequent procedures up to analysis on sucrose density gradients were carried out at 4°. The total protein concentration in each mixture was maintained at less than 1.0 mg/ml. The mixtures of polypeptide chains were taken through successive steps of dialyses while in Visking dialysis membranes (flat width $^{23}/_{32}$ in.) to remove the dissociating conditions. The outside dialysis fluid was in 200–500-fold excess. Steps of dialysis were as follows: (1) distilled water, (2) 0.01 M sodium acetate buffer, pH 5.5, and (3) 0.01 M sodium acetate buffer, 0.15 M NaCl, pH 5.5. Each step was carried out for 16–24 hr.

Analysis of Recombined Mixtures. The solutions of reformed molecules contained both free L chains, 7S molecules, and heavier aggregates. For analysis these species of molecules were best separated by sucrose density-gradient ultracentrifugation, but gel filtration could be used equally well. Linear sucrose gradients were formed from 5 to 15% sucrose in 0.01 M sodium acetate, 0.15 M NaCl, pH 5.5, with total gradient volume of 4.80 ml; 0.20-ml aliquots of material were applied. Centrifugation was performed at 38,000 rpm for 16 hr in Beckman-Spinco L or L2 centrifuges. The gradients were harvested through a punctured hole at the bottom of the tube. At times the linearity of gradients was checked by determining the concentration of sucrose by refractometry.

Subsequently the ^{125}I and ^{131}I radioactivity was determined in each gradient fraction. The activity of both isotopes in each fraction was plotted against the per cent of gradient volume, 100% representing 5.0 ml and the top of the gradients (see Figure 2). The position of the 7S peak was very reproducible from experiment to experiment under standardized conditions and therefore the addition of another marker was not necessary. From such plots (see Figure 2) the amount of radioactivity was determined in material sedimenting faster than 7 S, 7S peak, and free L chains. Absorption to the wall of the cellulose nitrate tube was 2–3% total radioactivity and was not included in further calculations; however, the material pelleted at the bottom of the tube was included among the material sedimenting faster than 7 S. From the total radioactivity in each peak and the specific activity of each L-chain preparation the amount of L chains in each molecular species was determined.

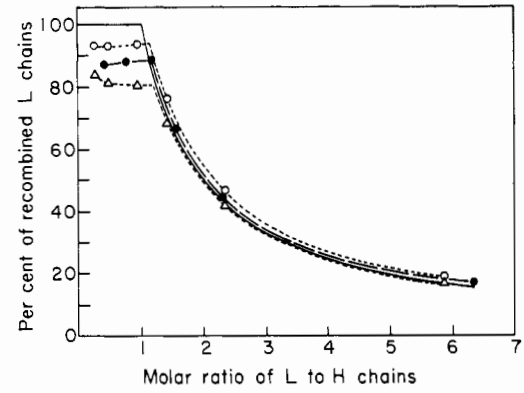

FIGURE 1: Results of recombining H and L chains at various molar ratios. The solid line without points indicates the calculated per cent of recombined L chains for formation of four-chain molecules with two H and two L chains at varying molar ratios of these polypeptide chains. The lines with points indicate the observed per cent of recombined L chains for the following mixtures: ●———●, Pa H^2 + Pa L; ○-----○, Ge H^2 + Ge L; and △-----△, Ge H^2 + Ne L.

Results

Recombination of H and L Chains at Varying Molar Ratios. Initially experiments were carried out to examine the influence of varying molar ratio of H and L chains on recombination and to ascertain that formation of four-chain molecules occurs in high molar excess of L chains. For this purpose H and L chains were isolated from myeloma proteins Ge, Ne, and Pa. The L chains of these proteins were trace labeled with ^{125}I or ^{131}I. To 300-μg aliquots of Pa H^2 chains were added Pa L chains, labeled with ^{131}I, in increasing amounts from 50 to 900 μg. Recombination was allowed to occur and analysis was performed as described. Similar experiments were performed with Ge H^2 plus Ge L ^{131}I and Ge H^2 plus Ne L ^{125}I chains. In all experiments a peak of radioactivity sedimented in the gradient as 7S protein. From the plots of radioactivity distribution on the density gradients the amount of radioactivity in 7S peak was determined. The per cent

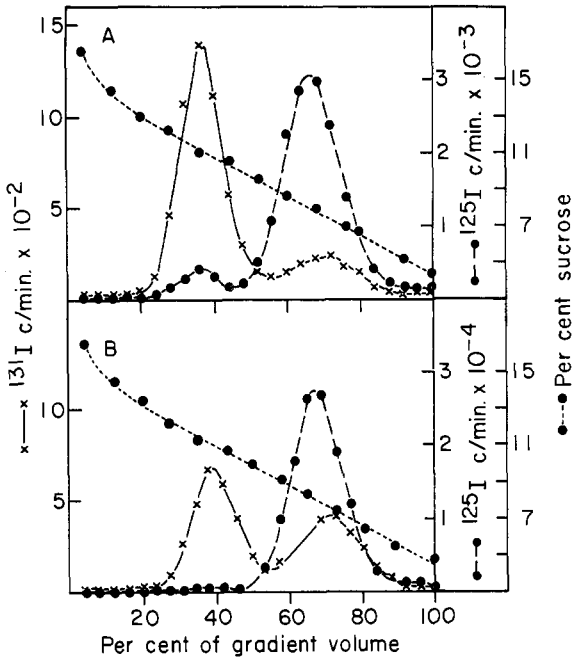

FIGURE 2: Sucrose density-gradient ultracentrifugation pattern of autologous H and L chains recombined in the presence of excess heterologous L chains. Gradients of 5–15% sucrose were utilized. The top of the gradient is represented by 100% on the right. In A and B the following chains were recombined: Gr H^2, Gr L ^{131}I, and Ne L^{125}. In A the Ne L ^{125}I chains were in twofold and in B in 20-fold molar excess of the autologous L chains.

TABLE I: Characteristics of G-Myeloma Proteins Used for Recombination.

Myeloma Protein	H-Chain Group	L-Chain Type	Comparative Yield of H^1 and H^2 Chain	Mobility of L Chains[a]	Bence Jones Proteinuria
Co	We	K	$H^1 > H^2$	—	+
Ge	Ge	K	$H^1 < H^2$	8, 9, *10*	−
Gr	We	K	$H^1 < H^2$	6, *7*, 8	+
Ke	We	K	$H^1 > H^2$	*5*, *6*, *7*	+
Ne	Ne	L	$H^1 > H^2$	*3*, 4, 5	+
Pa	We	K	$H^1 = H^2$	3, *4*, 5	−
Ro	Ge	K	$H^1 > H^2$	—	+
Sz	We	L	$H^1 = H^2$	*6*, *7*	+
Vi	Vi	L	$H^1 < H^2$	*4*, *5*	+
We	We	K	$H^1 < H^2$	4, *5*, 6	+

[a] Number indicates the relative electrophoretic mobility of L chains in alkaline urea gel. See text for full explanation. The major component or components are in italics.

of radioactivity in this peak in relation to total radioactivity of L chains was calculated. These values were plotted against the calculated molar ratio of L:H chains present in the mixture prior to recombination, using the molecular weights 53,000 for H chains and 23,000 for L chains (Small and Lamm, 1966). The results are given in Figure 1. These results clearly show that the experimentally obtained values very closely resemble calculated values for formation of four-chain molecules with two H and two L chains.

If a significant number of molecules composed of chains other than two H chains and two L chains would have been formed, they would have sedimented at higher or lower values than 7 S or the curve representing the per cent of total L chains in 7S molecules would have deviated appreciably from the calculated theoretical values. In H chain molar excess 100% of L chains do not appear in 7S peak partially because 5–10% of the L chains were sedimented faster than 7S molecules, most likely owing to incorporation of some L

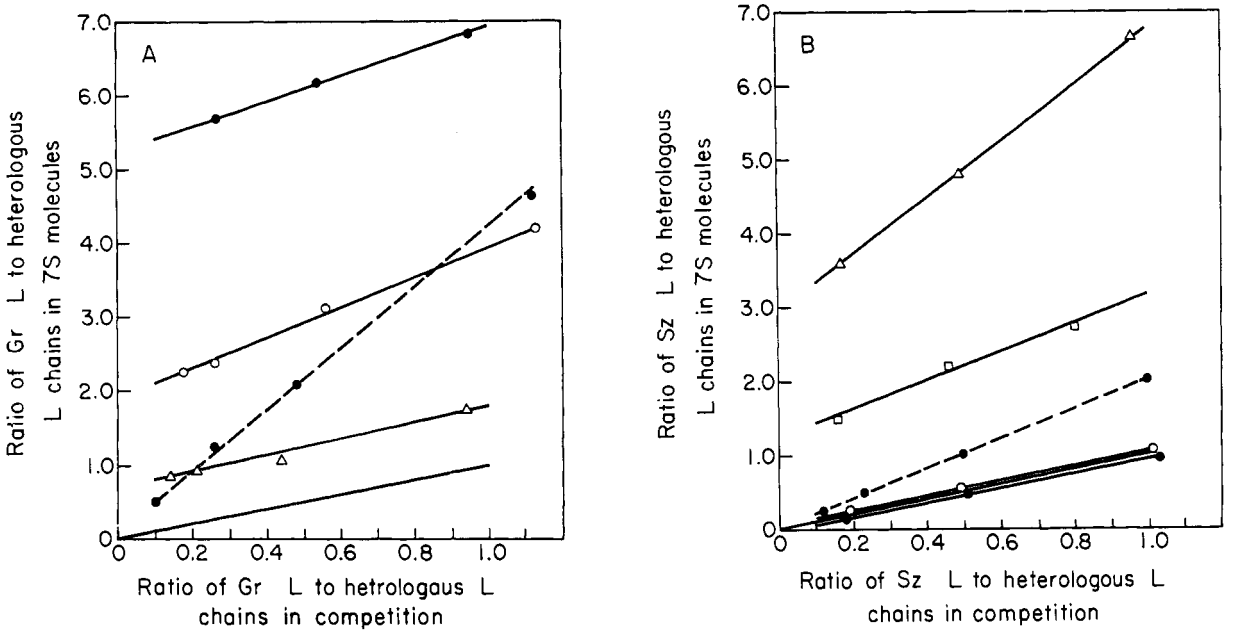

FIGURE 3: Influence of increasing excess of heterologous L chains on the recombination of autologous H and L chains. The ratio of autologous to heterologous L chains in recombined 7S molecules is plotted against the ratio of these chains in competition for H chains. The solid line without points indicates the calculated ratio of autologous to heterologous L chains in 7S molecules for random association of these L chains with given H chains at increasing excess of heterologous L chains. The lines with experimental points give the results for recombination of Gr H^2 and L chains in presence of increasing excess of heterologous L chains (A) and for recombination of Sz H^2 and L chains in the presence of increasing excess of heterologous L chains (B). ●——●, Gr H^2 + Gr L, Ne L. ○——○, Gr H^2 + Gr L, Pa L. △——△, Gr H^2 + Gr L, Ge L. ●------●, Gr H^2 + Gr L, γG L. ●——●, Sz H^2 + Sz L, Vi L. ○——○, Sz H^2 + Sz L, We L. △——△, Sz H^2 + Sz L, Ke L. □——□, Sz H^2 + Sz L, Ge L. ●------●, Sz H^2 + Sz L, γG L.

chains into H-chain aggregates. In experiments with Ge H^2 and Ne L ^{125}I up to 10% of Ne L chains failed to recombine at all with excess Ge H^2 chains.

Recombination of H and L Chains in Presence of Excess Heterologous Myeloma Protein L Chains. A series of experiments were performed to determine if an excess of heterologous L chains are capable of replacing the autologous L chains from preferential recombination with their H chains. Heavy and light chains were isolated from ten G-myeloma proteins, listed with their characteristics in Table I. Light chains were labeled with either ^{131}I or ^{125}I. Subsequently mixtures of H chains and L chains in 0.5 M propionic acid were made consisting of 300 μg of H chains, 150 μg of autologous labeled L chains, and 150 μg or more of heterologous L chains labeled with the other isotope of iodine. Recombination and analysis on sucrose density-gradient ultracentrifugation were carried out as outlined above. Two typical density-gradient runs are depicted in Figure 2.

From the distribution of radioactivity in the sucrose density gradients and specific activity of each of the L-chain preparations the amount of autologous and heterologous L chains in the 7S peak was determined. Thus the molar ratio of L chains in reformed molecules as well as in the entire mixture was determined. Initially it was ascertained that the ratio of the two sets of L chains did not change more than ±1–2% from the time the mixtures were made to the end of analysis on density gradients. Since there was no preferential loss of the reformed molecules or free L chains during the steps of recombination, the ratio of the two sets of L chains in the initial mixture and the same ratio in the reformed 7S molecules became a convenient measure to express the presence or absence of preferential recombination of autologous H and L chains. This was a practical method since working with small volumes total recovery of the specimens was not essential and losses due to adherances on glass surfaces and dialysis membranes were unavoidable. The total recovery of materials was around 80%.

The molar ratio of autologous and heterologous L chains in reformed molecules was determined for at least three points, *i.e.*, equimolar amounts of the two L chains, twofold molar excess, and eight- to tenfold molar excess of the heterologous L chains. The resulting values were plotted against the ratio of competing L chains in the original mixture. This is illustrated for a series of experiments for two proteins, Sz and Gr, in Figure 3. If recombination occurs completely at random between the H chains and the autologous and heterologous L chains, then the competing ratio at equimolar amounts of L chains is 1.0 and also the ratio in reformed 7S molecules would be 1.0. If the heterologous L chains

TABLE II: Summary of Competitive Recombination of H Chains with Autologous and Heterologous Myeloma Protein L Chains.

H Chain and Autologous L Chain	Heterologous L Chains Added in Equimolar and Tenfold Molar Excess to Autologous L Chains; Ratio of Autologous:Heterologous L Chains in Recombined 7S Molecules is Given below Symbols Identifying These Proteins[a]									
	1:1	1:10	1:1	1:10	1:1	1:10	1:1	1:10	1:1	1:10
	Pa L		Gr L		Ne L		Ro L		γG L	
Ge H^2 + Ge L	12.4	3.0	5.6	2.1	4.0	2.0	1.60	0.30	2.3	0.25
	Ne L		Pa L		Ge L				γG L	
	6.9	5.4	3.9	2.1	1.75	0.80			4.2	0.43
Gr H^2 + Gr L	6.8	5.1	3.8	2.2						
	Pa L		Ge L		Vi L				γG L	
Ke H + Ke L	3.6	2.9	2.2	0.80	1.70	1.40			3.8	0.40
	Ne L		Gr L		Ge L				γG L	
Pa H^2 + Pa L	7.5	3.1	2.2	0.50	1.1	0.15			2.3	0.25
	Ke L		Ge L		We L		Vi L		γG L	
Sz H^2 + Sz L	6.8	3.3	3.2	1.5	1.05	0.15	0.95	0.08	2.05	0.20
	Sz L		Pa L		Ke L		Co L		γG L	
Vi H^2 + Vi L	7.6	1.6	5.6	1.6	2.4	0.50	1.5	0.20	1.4	0.15
	Ke L		Co L		Sz L				γG L	
We H^2 + We L	4.8	1.1	1.4	0.35	2.0	—			2.25	0.25

[a] If the recombination of autologous and heterologous L chains occurs completely at random, then at equimolar concentration of competing L chains the recombined 7S molecules contain these chains in a ratio 1.0. At tenfold molar excess of heterologous L chains the recombined 7S molecules contain the chains at a ratio of 0.1 (ratio of autologous:heterologous L chains).

are in twofold excess, then at random recombination the ratio of autologous:heterologous L chains in 7S molecules would be 0.5. At tenfold excess of the heterologous L chains the ratio in reformed molecules of autologous:heterologous L chains would be 0.1. In both illustrations the theoretical values for random recombination are depicted in the solid line without points. In an infinite excess of heterologous L chains the recombined molecules would contain no autologous L chains and the line would run through the zero point in the plot, as illustrated in Figure 3.

As illustrated in Figure 3, Gr L chains preferentially recombine with Gr H^2 chains when in competition with Ne L, Pa L, or Ge L chains, but to a variable degree with each of the heterologous L chains. This preferential recombination persists in tenfold molar excess of the heterologous L chains, but in each instance becomes less pronounced than at equimolar ratio of these chains. With Gr H^2 and L chains the three myeloma protein L chains tested are unable to replace the Gr L chains at random. However, in similar experiments with Sz H^2 and L chains the Vi L and We L chains are able to replace the autologous Sz L chains completely at random from recombining with Sz H^2 chains for all the points tested. Light chains from Ke and Ge are unable to displace the autologous Sz L chains at random.

Similar sets of experiments were performed for seven myeloma proteins. The results are expressed in abbreviated form in Table II. For each set of experiments the molar ratio of autologous to heterologous L chains in the 7S molecules is given for equimolar amounts and for tenfold molar excess of heterologous L chains. The ratio of tenfold molar excess of heterologous L chains was either obtained from experimental values or extrapolated from experimental values as seen in Figure 3 by extending the straight line connecting the experimental points. These values for the experiments with Gr and Sz polypeptide chains were obtained from the plots illustrated in Figure 3A, B. Reproducibility of the results was checked and is indicated in Table II for recombination with H and L chains of Gr.

Table II illustrates the variability of the capacity of heterologous L chains to replace any given autologous chains. Only some heterologous L chains recombine at random with the H chains in the presence of autologous L chains. Examples of such experiments are Sz H^2 chains competing for Sz L and Vi L chains and Sz H^2 chains competing for Sz L and We L chains. Another similar example is Pa H^2 competing for Pa L and Ge L. Interestingly, however, when Vi H^2 chains compete for Vi L and Sz L, the autologous L chains preferentially recombine with the H chains. Similarly, Ge H^2 chains competing for Ge L and Pa L chains leads to preferential recombination of the autologous chains. Vi L and We L are not capable of invariably binding with high affinity with H chains as experiments with other H chains show.

As previously observed (Grey and Mannik, 1965) and corroborated by other workers (Gordon and Cohen, 1966), the H-chain subgroup of a myeloma protein, the L-chain type, and electrophoretic mobility of L chains in alkaline urea starch gels are not of essential importance in preferential recombination of H and L chains. Similarly the presence or absence of Bence Jones proteinuria in patients producing these proteins and the relative amounts of H^1 and H^2 peaks upon gel filtration have no correlation with the variability in preferential recombination of autologous H and L chains. These conclusions are evident by comparing the characteristics of the proteins in Table I with the tabulated results of recombination in Table II.

In the experiments just described the heterologous L chains were present in up to tenfold excess and the preferential recombination, if present, persists. For this reason experiments were performed with higher excess of heterologous L chains. In such experiments the routine analysis on sucrose density gradients became difficult since the heterologous free L chains are present in large amount and result in more diffusion. Thus the relatively small percentage of these L chains under the 7S peak is difficult to determine and large errors are possible as can be seen from the curves in Figure 2B. Therefore, these experiments were analyzed only in respect to the percentage of autologous L chains in 7S and L-chain peaks. The results of such experiments are illustrated in Figure 4. At 20-fold excess of Ne L chains 55% of Gr L chains still recombine with Gr H^2 chains. At 80-fold excess of Pa L chains 40% of Gr L chains recombine with Gr H^2 chains. If recombination would have been a random phenomenon, then only 1.23% of Gr L chains would recombine with Gr H^2 chains in this experiment.

Recombination of Myeloma Protein H and L Chains in the Presence of Excess Normal γG L Chains. Experiments were performed with seven myeloma protein H- and L-chain pairs where the heterologous L chains were replaced with normal γG L chains, labeled with the isotope of iodine different from that on the autologous L chains. Recombination and analysis of results was carried out as before. Figure 3 shows the results of adding increasing amounts of γG L chains to Gr H and Gr L chains and to Sz H and Sz L chains, respectively. In both instances at equimolar amounts of autologous L chains and γG L chains preferential recombination of autologous L chains occurs. However, with increasing amounts of γG L chains progressively more autologous L chains are replaced by the γG L chains, approaching random recombination of a portion of γG L and autologous L chains with the myeloma protein H chains. The straight lines connecting the observed points for these experiments in Figure 3 approach the theoretical line for random recombination of two L chains with the H chains at increasing excess of γG L chains. This phenomenon occurs with each of seven H and L chain pairs tested. The data are presented in Table II for equimolar and tenfold molar excess of γG L chains in respect to autologous

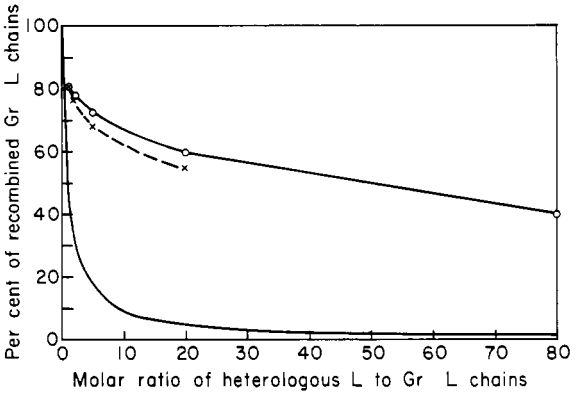

FIGURE 4: Results of recombining autologous H and L chains in the presence of large molar excess of heterologous L chains. The solid line without points indicates the calculated per cent of recombined autologous L chains for random recombination of autologous and heterologous L chains with Gr H^2 chains in increasing molar excess of heterologous L chains. ×------×, indicated the per cent of recombined Gr L chains in the mixture Gr H^2, Gr L, and Ne L with increasing molar excess of Ne L chains. o——o, indicated the per cent of recombined Gr L chains in the mixture Gr H^2, Gr L, and Pa L with increasing molar excess of Pa L chains.

L chains. For each of these experiments the per cent of γG L chains was determined that is capable of recombining with the given myeloma protein H chains at random with the autologous L chains. These values are given in Table III. These calculated values range

TABLE III: Capacity of γG L Chains to Replace at Random Autologous Myeloma Protein L Chains from Recombining with Their H Chains.

H Chains from G-Myeloma Protein	% γG L Chains That Replace at Random the Autologous L Chains from Interacting with Their H Chains
Gr	23
Ke	24
Pa	43
We	44
Ge	44
Sz	48
Vi	73

from 23 to 73%.

Formation of 7S Molecules with One κ and One λ Chain. Data in a preceding section and previously

TABLE IV: Formation of Hybrid γG-Globulin Molecules with One κ and One λ Chain.

Recombination Mixture	Molar Ratio of We L:Sz L in Isolated 7S Molecules	Antiserum Added	Radioactivity Complexed with Antiserum			
			Expected (%)		Observed (%)	
			^{131}I	^{125}I	^{131}I	^{125}I
Sz H^2, Sz L ^{125}I, We L ^{131}I	1.03	anti-κ	100	51	96	54
		anti-λ	49	100	47	96
We H^2, Sz L ^{125}I, We L ^{131}I	2.00	anti-κ	100	67	99	68
		anti-λ	33	100	22	88

published studies (Grey and Mannik, 1965; Gordon and Cohen, 1966) demonstrate that the antigenic type of L chains (either κ or λ) has no relationship to the preferential recombination of autologous L chains. For example, in certain instances κ chains replace at random the autologous λ chains in recombination with H chains and vice versa. Therefore, experiments were performed to test if 7S molecules are formed containing one κ and one λ chain.

For this purpose the polypeptide chains of Sz (type L) and We (type K) were utilized because the light chains from these proteins randomly recombine with Sz H chains. A slight molar excess of both types of light chains were added to the H chains in the following mixtures: Sz H^2 + Sz L ^{125}I and We L ^{131}I; We H^2 + Sz L ^{125}I and We L ^{131}I. From these recombined mixtures 7S molecules were isolated by gel filtration over a Sephadex G-200 column equilibrated with 0.2 M sodium borate buffer, 0.15 M NaCl, pH 8.0. In the isolated 7S material the molar ratio of We L: Sz L was calculated from the total radioactivity and the specific activities of each of the L chains. Subsequently antisera specific to κ and λ light chains were added to aliquots of each of the 7S protein preparations in antibody excess. After 48-hr incubation at 4° the precipitates were separated by centrifugation and washed. The amount of radioactivity was assayed in precipitates and supernatants. From the latter 0.2-ml aliquots were then analyzed on density gradients of 10–30% sucrose by ultracentrifugation to determine what per cent of molecules were complexed with antibody but had failed to precipitate and what per cent of the 7S molecules remained unbound to the specific antibodies. From these values calculations were made for the total per cent of ^{125}I and ^{131}I radioactivity that was complexed with specific antibodies to κ and λ chains. The results are given in Table IV. The addition of an antiserum to κ chains to the 7S molecules isolated from Sz H^2 + Sz L ^{125}I, We L ^{131}I should complex 100% of ^{131}I radioactivity, any ^{125}I radioactivity complexed would be due to hybrid molecules. Similarly, any ^{131}I radioactivity that is complexed to antibodies upon the addition of an antiserum to λ chains would be due to hybrid molecules. As seen from Table IV the observed values are close to expected values for random formation of hybrid molecules considering that in the isolated 7S molecules the ratio of We L:Sz L chains was 1.03. Similar experiments were carried out on the 7S molecules isolated from We H^2 + Sz L ^{125}I, We L ^{131}I. Again the addition of an antiserum to κ chains yielded results that would be expected on random hybrid formation. Upon the addition of an antiserum to λ chains, only 88% of type L material (labeled with ^{125}I) was complexed with antibodies. At the same time 22% of ^{131}I labeled type-K proteins were complexed with antibody. Clearly, hybrid molecules are present but somewhat less than expected on random formation of such molecules. Allowing for 88% efficiency of the antiserum to λ chains in this experiment, the theoretical expected value would be 29% in contrast to 22% observed value.

Discussion

The initial experiments in this study demonstrate that 7S molecules are formed from a mixture of H and L chains with the latter in considerable excess. The reformed molecules contain a proportion of the excess L chains consistent with the formation of four-chain molecules. This occurs with autologous L chains as well as with L chains from another myeloma protein. On the other hand, in H chain excess not all the available L chains are incorporated into 7S molecules (see Figure 1); this is partially owing to formation of heavier aggregates composed of H chains and a few L chains (M. Mannik, unpublished observations). As already pointed out, the reformed molecules without interchain disulfide bonds resemble the native molecules in many physical and chemical parameters and they also have the same catabolic fate *in vivo* as unaltered molecules of γG-globulin (Cohen and Mannik, 1966).

In competitive recombination studies certain heterologous myeloma protein L chains are able to replace at random the autologous L chains from interacting with their H chains. In many instances, however, the autologous L chains preferentially recombine with their own H chains as previously observed (Grey and Mannik, 1965). This preferential recombination is variable among the proteins tested. Nevertheless, it persists even up to 80-fold excess of the heterologous L chains. The degree of preferential

recombination decreases with increased excess of heterologous L chains. Light-chain heterogeneity or contamination with other L chains is not the explanation for this phenomenon since at marked excess of such L chains the degree of preferential recombination should decrease and rapidly approach a random phenomenon because of increasing amount of contaminants. Furthermore, since the amount of H chains remains constant in presence of increasing amount of heterologous L chains, the decrease of preferential recombination can not be attributed to H chain heterogeneity. Recombination of heterologous H and L chains leads to formation of four-chain 7S molecules (Figure 1); the noncovalent bonds in this interaction clearly must have less free energy of binding than the formation of autologous H- and L-chain bonding. A large excess of heterologous L chains seems to be capable of replacing more and more autologous L chains. This must take place prior to the completion of the noncovalent bonds since incubation of full molecules held together by noncovalent interactions with labeled autologous L chains in 0.01 M sodium acetate, 0.15 M NaCl, pH 5.5, will not allow incorporation of labeled L chains into the full molecules (M. Mannik, unpublished observations).

In this investigation as well as in previous studies (Grey and Mannik, 1965; Gordon and Cohen, 1966) the capacity of heterologous L chains to replace the autologous L chains was not related to the antigenic structure or the electrophoretic mobility of the parent proteins, or to the banding of isolated L chains in alkaline urea gel electrophoresis. Heterogeneity of L chains was present on alkaline urea starch gel electrophoresis similar to observations by others (Cohen and Porter, 1964; Terry *et al.*, 1966); iodination could have altered the electrophoretic mobility of L chains (Hughes-Jones, 1965).

This study clearly shows that *in vitro* γG-globulin molecules can be formed that contain one κ- and one λ-polypeptide chain. Such molecules are not encountered *in vivo;* myeloma protein molecules as well as normal γG-globulins possess either two κ or two λ chains (Mannik and Kunkel, 1962; Mannik and Kunkel, 1963; Fahey, 1963a,b). Similar symmetry exists also in rabbit γG-globulins in respect to the allotypic markers present on light chains (Gilman *et al.*, 1964). However, *in vitro* functioning rabbit antibody molecules can be produced with light chains differing in these allotypic markers (Mannik and Metzger, 1965). On the basis of enzymatic cleavage and depolarization of fluorescence of rabbit γG-globulin, Noelken *et al.* (1965) have proposed that these molecules are composed of three relatively rigid structures that are linked by flexible portions of peptide chains. In this model the two Fab fragments and the Fc fragment constitute the rigid portions, linked by a flexible H-chain region that is subject to proteolysis by enzymes. In view of such a model for γG-globulin one would expect that the noncovalent bonds formed in one Fab fragment will have no direct influence on the similar bonds formed in the other Fab fragment. *In vivo* individual plasma cells produce either type K or type L immunoglobulins (Pernis and Chiappino, 1964; Bernier and Cebra, 1964). Since there are no structural restrictions to forming hybrid κ and λ molecules, the reasons for symmetrical molecules are to be sought among the mechanisms for control of immunoglobulin synthesis.

The current observations on recombination of H and L chains and the formation of mixed κ- and λ-chain molecules lend further support to the previously discussed possibility that the noncovalent bonds between the H and L chains are formed between the variable portions of these polypeptide chains. The carboxyl-terminal halves of type K Bence Jones proteins are identical, except for single amino acid differences that correlate with the Inv characteristics of these proteins, and they differ from the corresponding region of type L Bence Jones proteins (Hilschmann and Craig, 1965; Putnam and Easley, 1965; Titani *et al.*, 1966; Baglioni *et al.*, 1966). Furthermore, the carboxyl-terminal halves of these proteins contain one intrachain disulfide bond (Milstein, 1966). Thus many conformational variations are unlikely in the common regions of these molecules. In contrast, the same studies on the amino acid sequences show considerable variations in the amino-terminal halves of Bence Jones proteins. Two different light chains that at random combine with a given H chains should have similar conformations in the region responsible for the noncovalent bonds. Since the specificity of recombination of H and L chains is not related to the light chains being either κ or λ chains, then it appears reasonable to postulate that the amino-terminal variable portions of these polypeptide chains participate in the noncovalent bonds with the H chains. Some gross conformational similarities must exist in L chains since in the absence of autologous L chains the heterologous L chains recombine with the given H chains quite effectively (Grey and Mannik, 1965) and recombination occurs even between H and L chains from different species (Olins and Edelman, 1964). However, from functional point of view several studies show that for recovery of maximal antibody activity proper pairing of H and L chains is required (Roholt *et al.*, 1964; Metzger and Mannik, 1964; Roholt *et al.*, 1965; Hong and Nisonoff, 1966).

The data presented here show that γG L chains from a pool of normal γG-globulin contain a rather high percentage of L chains that are capable of randomly replacing the autologous G-myeloma protein L chains from recombining with their H chains. In all instances at equimolar concentration of autologous L and γG L chains, preferential recombination of autologous L and H chains occurs. However, with increasing excess of the γG L chains the results approach values of random recombination of the myeloma protein L chains and a proportion of the γG L chains with the given H chains. This observation suggests that the conformations of these L chains are similar so that there is nearly equal free energy of binding between certain γG L chains and myeloma protein H

chains and between the autologous H and L chains of myeloma proteins. In 6 out of 23 pairs (see Table II) the heterologous and autologous L chains approach random recombination with H chains. The finding was surprising that up to 73% of γG L chains can randomly replace some autologous myeloma L chains from combining with their H chains. Another possibility is that all γG L chains are able to replace the myeloma protein L chains, but their free energy of binding with H chains is only somewhat less then that of the autologous chain interactions. Thereby the increasing excess of γG L chains would be capable of replacing randomly the autologous L chains. If this is true, then obviously individual myeloma protein H chains behave differently toward the γG L chains.

Kotynek and Franěk (1965) observed that an excess of γG L chains added to isolated antibody H chains were capable of restoring more antigen-binding activity than the equimolar concentration of these chains. This observation suggests that the pool of γG L chains contains a population of molecules that in some way resemble antibody L chains and therefore are capable of restoring activity to the antibody H chains. Experiments with isolated antibodies to haptens show that specificity exists in the noncovalent bonds between certain antibody H and L chains (Roholt *et al.*, 1965). The current study from purely structural point of view suggests that a certain degree of variability exists in these interactions in that a limited variety of L chains may combine with the given H chains to produce active antibody molecules with the given specificity. It is not clear how the current observations relate to the observed heterogeneity of L chains in antibodies to relatively simple haptens (Choules and Singer, 1966), but since some variability in the H–L interaction seems permissible, a limited variety of L chains may interact with given H chains to produce antibodies specific to one hapten.

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