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Chemical Basis for Antibody Diversity and Specificity

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Antibodies are a class of proteins containing an enormous number of chemically similar, but biologically distinct, molecules. There are several types, the commonest of which (IgG) contains molecules with four polypeptide chains, two heavy (H) chains and two light (L) chains. The chemical basis for the diversity of antibodies lies in differences in the amino acid sequences of these chains. These differences are confined to limited regions of the chains, the constant portions being presumably responsible for the over-all chemical similarity between all IgG molecules. The specificity for a particular antigen is present in the isolated H chain of an antibody, but full expression of the affinity for the antigen is attained only when the specific H chain is combined with an L chain derived from an antibody of like specificity. A similar requirement has been found for the conformational integrity of all IgG molecules, as seen by optical rotatory dispersion. The results indicate that IgG molecules that have been reconstituted from separated H and L chains return to the exact native conformation only if each H chain is associated with the same L chain with which it was associated in the native state. The conclusion is that there exists a single unique H chain *and* a single unique L chain for each distinct IgG molecule. What biological advantage may reside in this duplicate specificity is not known.

One of the most exciting problems in protein chemistry today is that of achieving an understanding of the biosynthesis and mode of action of antibodies.[1] Antibodies belong to the class of proteins known as the *immunoglobulins*. There are several types, but most of the basic problems can be considered in terms of a single type, type G, which, at least in man, is the most prevalent one. Molecules belonging to this type are generally designated IgG.

Biologically, one is impressed by the diversity of antibodies and by their specificity. An individual must be capable of synthesizing many thousands of distinct IgG molecules, each with a specific binding site directed toward a different antigen. Even a preparation of antibodies against a single antigen consists of several distinct IgG molecules, for binding studies invariably show that antibodies represent a population of molecules that possess a range of binding constants for the particular antigen against which they are directed.

Chemically, on the other hand, it is the similarity between antibodies that is most striking. The entire population of IgG molecules shows far fewer gross chemical differences than are found among enzymes, for example. Even enzymes that are closely related in function, such as those that catalyze the hydrolysis of peptide bonds, possess a far wider range of chemical properties than can be found among IgG molecules.

The gross structure of IgG is shown in Figure 1.[2] Each molecule consists of two symmetrical halves, joined by a disulfide bond. Each half-molecule consists of two polypeptide chains, one heavy (H) and one light (L), joined by a disulfide bond. Each half-molecule contains a binding site for the antigen, present in a globular ordered region composed of approximately half an H chain plus the entire L chain. A small central portion of the H chain, including the disulfide bond that joins the two halves, appears not to be folded into a compact structure. It is readily attacked by a variety of proteolytic enzymes, so that the globular portions containing the binding sites are easily separated from the rest of the molecule in the form of *Fab fragments*. These fragments retain their original structure, specificity, and binding constant for antigen within the accuracy of measurement.[3] The lower halves of the H chains of IgG combine to form another globular region (*Fc fragment*) which is not involved in the determination of antibody specificity.

The exposed central portion of the H chains serves not only as a convenient locus for fragmentation. It also has a biological function in that it provides a link between the two binding sites that keeps them some dis-

(1) For a recent review of many aspects of this problem see "Nobel Symposium 3: Gamma Globulins," J. Killander, Ed., Almquist and Wiksell, Stockholm, and Interscience Publishers, Inc., New York, N. Y., 1967.

(2) M. E. Noelken, C. A. Nelson, C. E. Buckley III, and C. Tanford, *J. Biol. Chem.*, **240**, 218 (1965).

(3) A. Nisonoff, F. C., Wissler, and D. L. Woernley, *Arch. Biochem. Biophys.*, **88**, 241 (1960).

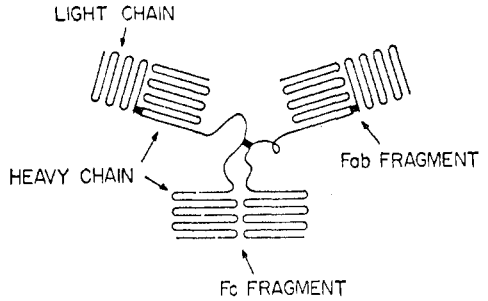

Figure 1. Schematic representation of the gross structure of IgG molecules. The three short heavy lines represent interchain disulfide bonds. The molecular weight of IgG is about 150,000. The molecular weights of the two chains are 24,000 and about 50,000, respectively.

tance apart and is sufficiently flexible so that this distance is variable,[2,4] an advantageous arrangement for a molecule that acts as a scavenger for undesirable macromolecules by cross-linking and precipitation.

The three interchain disulfide bonds shown in Figure 1 represent only a fraction of the molecule's total disulfide links. There are two intrachain disulfide links within each L chain, and about seven within each H chain. It is interesting that the three interchain disulfide bonds are in exposed positions and do not make a significant contribution to the binding forces between the chains. These disulfide bonds can be reduced to thiol groups and then protected by carboxymethylation without disruption of the over-all structure.

Chemical Basis for the Diversity. For many years there was debate concerning the chemical basis for the differences between antibodies. One school of thought believed that H and L chains of identical amino acid sequences could generate a variety of specific antibodies. This possibility is tenable if there are many alternative folded conformations (of the Fab region of the molecule) of comparable free energy and if there is a mechanism in the antibody-producing cell by which a choice among these conformations could be made on the basis of ability to react with a particular antigen. The second school of thought, relying on the principle deduced from studies of other proteins, that the laws of physical chemistry dictate one unique preferred conformation for each amino acid sequence, held that any difference between two IgG molecules must be ascribable to a difference in the amino acid sequence of the H chain or the L chain or both.

This question has been resolved in favor of the second alternative, *i.e.*, the principle of a one-to-one correspondence between amino acid sequence and conformation (and, therefore, biological activity) applies to the immunoglobulins. This conclusion was first established[5-7] by showing that specific antibodies or Fab fragments could spontaneously recover their specific biological activity after being converted to linear random coils, a state in which no ordered structures remain and in which the amino acid sequences of the constituent H and L chains constitute the only information that the molecule retains. This completely denatured state is attained by the action of concentrated guanidine hydrochloride to disrupt noncovalent interactions and by reaction with a reducing agent to rupture intrachain as well as interchain SS bonds. Removal of the denaturing agent and establishment of conditions under which disulfide bonds could re-form would not have been sufficient for spontaneous regeneration of specific activity if the specific activity resided in only one of many alternative folded states, a choice among which required the presence of the antigen. In fact, spontaneous recovery of activity was obtained in the absence of any external directive force. In a control experiment,[6] IgG molecules without initial activity toward a particular antigen could not be induced to acquire such activity by denaturation and reduction, followed by refolding in the presence of a large excess of the antigen itself.

A more direct proof for the existence of a unique amino sequence for each distinct antibody comes from sequence determinations themselves. It has not been possible to study the amino acid sequences of antibody molecules *per se*, because, as was noted earlier, even antibody preparations of high specificity consist of a mixture of several different IgG molecules. Chemically pure IgG molecules can be obtained, however, from individuals with multiple myelomatosis, a cancerous condition characterized by proliferation of a single type of IgG-producing cell. L chains from more than 20 individual myeloma IgG (human and mice) have been partially or completely sequenced.[8] *No two of them have the same sequence.* There is every reason to believe that a similar result applies to myeloma H chains, though only fragmentary data are yet available.[9]

Myeloma proteins are assumed to represent prototypes of all IgG molecules, even though no specific antibody action has been identified with most myeloma proteins examined so far, and these results are regarded as the strongest possible evidence in support of the suggestion that diversity in amino acid sequence is indeed the basis for functional diversity in all IgG molecules.

The sequences of the L chains reported so far have been found to have a most remarkable feature.[8] The sequence variability is contained entirely in the amino-terminal half of the chain. Apart from sequence differences that have no connection with specificity for antigen, the other half of the chain has an invariant amino acid sequence in each species. Even within the variable half, the substitutions appear to be concentrated in certain regions, as is illustrated by Figure 2. It is anticipated that similar regularity will exist in the H chain.[9]

The existence of this regularity in the location of amino acid substitutions has created great interest

(4) R. C. Valentine and N. M., Green, *J. Mol. Biol.*, **27**, 615 (1967).
(5) E. Haber, *Proc. Natl. Acad. Sci. U. S.*, **52**, 1099 (1964).
(6) P. L. Whitney and C. Tanford, *ibid.*, **53**, 524 (1965).
(7) M. H. Freedman and M. Sela, *J. Biol. Chem.*, **241**, 2383 (1966).
(8) References to actual sequences are given by O. Smithies, *Science*, **157**, 267 (1967), and in ref 1.
(9) R. R. Porter, *Biochem. J.*, **105**, 417 (1967).

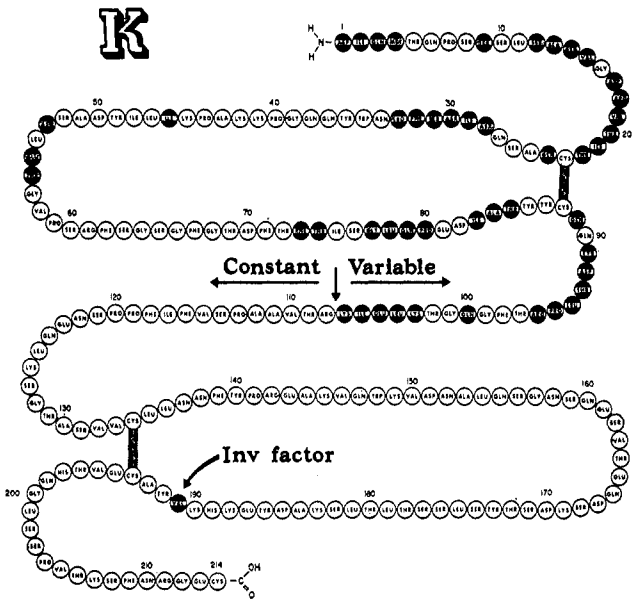

Figure 2. Amino acid sequences of human L chains (type κ) from patients with multiple myeloma. White circles represent positions where all κ chains investigated so far have been found to be invariant. Black circles represent variable positions, and the amino acids named for those positions refer to the κ chain from a particular patient. The variability at position 191 is not related to antibody specificity, but determines whether the protein is of genetic type Inv (a+) or Inv (b+). The former has leucine at this position, the latter has valine. (This figure is taken from a review by F. W. Putnam (ref 1, p 45).)

among geneticists and immunologists, and numerous speculative proposals have appeared to suggest how it might come about. Most such proposals have one common feature: that the number of intrinsic structural genes which specify sequences of H and L chains in the nuclei of undifferentiated cells is far smaller than the number of possible sequences that may be generated by antibody-producing cells. Several simple mechanisms for multiplying the number of possible sequences have been based on the possibility of somatic crossing-over within genes during cell division. Edelman and Gally,[10] for example, have proposed that precursor cells contain a series of tandem genes resulting from successive duplications from an ancestral gene. Mutations have occurred during evolution to alter the individual genes in the tandem series, but substantial similarities remain. These similarities make the occurrence of somatic crossing-over a much more probable event than it usually is, and thereby lead to the possible existence of a very large variety of structural genes in the creation of immunologically competent cells from precursor cells. An unknown mechanism must limit the occurrence of prolific crossing-over to the initial creation of immunologically competent cells. The proliferation of the latter occurs without change in the type of antibody being produced.

Edelman and Gally[11] have also suggested that the number of H and L chains with different sequences that are needed to generate antibody diversity is not necessarily as large as the number of possible antibodies. For example, if it is possible to generate 10^4 possible H-chain sequences and 10^4 possible L-chain sequences, and if the two sequences in an antibody-producing cell are selected independently, there is a potential for producing 10^8 distinct antibodies provided that there are no restrictions as to the choice of partners in the assembly of an IgG molecule.

Localization of the Binding Site. The active site of an antibody molecule is located in the Fab portion of the molecule (Figure 1). This represents a moiety of mol wt 50,000, containing all of an L chain and about half of an H chain. Only a portion of so large a molecular entity is likely to be involved in forming the actual binding site for the antigen, and it is therefore desirable to localize this site to a more limited region of the Fab piece of the protein. One obvious limitation is suggested by the sequence studies summarized by Figure 2. If the L chain forms part of the binding site, only the amino-terminal half is likely to be involved; no specificity could be generated by the half of the L chain that has an invariant amino acid sequence.

It would be helpful if the binding site could be localized to one of the constituent polypeptide chains, and binding studies have been carried out with separate chains with this objective in mind. The first step in the experimental procedure for chain separation[12] is to break interchain disulfide bonds by reduction, under conditions where intrachain links are not exposed and therefore unreactive. The resulting thiol groups can be protected from further reaction by carboxymethylation. Since the disulfide bonds do not make a significant contribution to the binding forces between chains, this reduction *per se* does not separate the chains, nor does carboxymethylation of the thiol groups prevent subsequent recombination of the chains after they have been separated by other means.[13]

The native structure of IgG is unstable at low pH. It has, however, been established that low pH alone is not sufficient for separation of H and L chains. It has been found that 1 *M* propionic acid is a suitable medium for chain separation[12] and does not lead to any irreversible changes in the chains themselves.

Studies of the separated chains have been hampered to some extent by the tendency of the H chains to aggregate with each other at neutral pH and to precipitate from solution when L chains are not present. This problem has been overcome by attaching short lengths of poly-DL-alanine to some of the exposed lysyl amino groups of the parent protein.[14] Because they consist of mixtures of D and L amino acids, these short polypeptide chains adopt no ordered structure but extend

(10) G. M. Edelman and J. A. Gally, *Proc. Natl. Acad. Sci. U. S.*, **57**, 353 (1967).

(11) G. M. Edelman and J. A. Gally, *ibid.*, **51**, 846 (1964).

(12) J. B. Fleischman, R. Pain, and R. R. Porter, *Arch. Biochem. Biophys. Suppl.*, 1, 174 (1962).

(13) Here and subsequently, we shall use the terms "H chain" and "L chain" to refer to chains derived from reduced and carboxymethylated IgG. When reference is made to recombined chains or reconstituted IgG molecules, it is to be understood that the thiol groups derived from the original interchain disulfide bonds remain carboxymethylated, and that the disulfide bridges are not re-formed.

(14) S. Fuchs and M. Sela, *J. Biol. Chem.*, **240**, 3558 (1965).

Table I

Association Constants for the Reaction of Anti-DNP[a] with Specific Hapten[b]

	Binding constant, l./mole × 10^{-6}
Intact antibody	370
Specific H chain[c] alone	6
Specific H + nonspecific L	15
Specific H + specific L	260

[a] Antibody prepared against dinitrophenyl (DNP) derivative of carrier protein. Binding constants were measured by determining extent of reaction against DNP-lysine. [b] Data of Haber and Richards.[16] [c] Chains derived from poly-DL-alanylated antibody were used.

outward from the surface of the molecule. They appear to have no effect whatever on the conformation of the rest of the molecule.[15] They do not affect the ability to recreate this conformation spontaneously after denaturation and rupture of intrachain disulfide bonds,[7] presumably because the modification has affected only those amino groups that are at the surface of the native molecule and play no role in the internal folding. For the same reason, poly-DL-alanylation does not interfere with separation and recombination of H and L chains. The modification does, however, prevent the aggregation of separated H chains, and H chains derived from poly-DL-alanylated IgG can therefore be studied under conditions where unmodified H chains would precipitate from solution.

Typical results of antibody activity measurements[16] are given in Table I. They show that isolated H chain is capable of binding antigen, albeit with a binding constant that is nearly two orders of magnitude smaller than the binding constant of the native antibody binding site. Similar results have been obtained in several laboratories[17-21] with a variety of antibodies: the binding constant of the H chain for antigen is always 20- to 1000-fold smaller than the binding constant of the native H–L pair.[22]

When isolated L chains from specific antibodies have been studied, they have usually been reported as having no detectable affinity for the specific antigen, either alone or in combination with nonspecific H chains. One recent study[24] has demonstrated existence of affinity between isolated L chains and specific antigen, but the binding constant found was four to five orders of magnitude lower than that of the native antibody.

The results of Table I, and similar results for other antibodies,[17,18,20,25,26] show that IgG molecules reconstituted by combination of specific H chains with nonspecific L chains have a binding constant for antigen that is not appreciably higher than that of the specific H chain alone. When the specific L chain is used, on the other hand, marked enhancement occurs. The affinity for antigen does not, however, ever quite match that of the native antibody. This is very likely a consequence of the heterogeneity of antibody preparations. The separated H and L chains derived even from a highly purified antibody represent heterogeneous populations from the chemical point of view. In the recombination, individual H chains will not necessarily become paired with the same L chains with which they were originally associated. The results suggest that a unique L chain may be required to elicit maximal affinity from a given H chain.

Conformational Change in the Combination of H and L Chains.[15] In order to find a possible structural explanation for the binding studies discussed above, we have carried out conformational studies of native IgG molecules, isolated chains, and recombined H–L pairs. Relying on the similarity in behavior of all IgG molecules, we have not used antibodies to carry out these studies but have employed a human myeloma protein as a prototype *homogeneous* IgG and nonspecific serum IgG as a prototype *heterogeneous* sample, consisting of a mixture of diverse molecules with many different kinds of H and L chains. It should be recalled that chemically homogeneous antibody preparations are not available; antibody preparations such as were used to obtain the results of Table I presumably occupy an intermediate position between myeloma proteins and nonspecific serum IgG with respect to heterogeneity.

We have used the optical rotatory dispersion of IgG in the 220–300-mμ region as an indicator of conformational integrity. All native IgG molecules appear to have a nearly identical ORD curve in this wavelength region,[15,27] typified by the one shown for nonspecific rabbit IgG in Figure 3. The principal features are: (1) the mean residue rotation is remarkably small throughout the wavelength range;[28] (2) there is a double trough, with minima near 225 and 230 mμ; and (3)

(15) K. J. Dorrington, M. H. Zarlengo, and C. Tanford, *Proc. Natl. Acad. Sci. U. S.*, **58**, 996 (1967).

(16) E. Haber and F. F. Richards, *Proc. Roy. Soc.* (London), **B166**, 176 (1966).

(17) G. M. Edelman, D. E. Olins, J. A. Gally, and N. D. Zinder, *Proc. Natl. Acad. Sci U. S.*, **50**, 753 (1963).

(18) M. Fougerau, D. E. Olins, and G. M. Edelman, *J. Exptl. Med.*, **120**, 349 (1964).

(19) S. Utsumi and F. Karusch, *Biochemistry*, **3**, 1329 (1964).

(20) M. Lamm, V. Nussenzweig, and B. Benacerraf, *Immunology*, **10**, 309 (1966).

(21) R. R. Porter and R. C. Weir, *J. Cellular Physiol. Suppl.*, **1**, 51 (1966).

(22) Utsumi and Karusch[19] have pointed out that relative binding constants are somewhat misleading measures of the affinity for antigen. In terms of the results of Table I, for example, the binding constant of 6×10^6 l./mole between H chains and antigen still represents a very sizable affinity. In terms of the *unitary* free energy[23] of complex formation, the results of Table I give −14.0 kcal/mole for the native antibody and −11.5 kcal/mole for the isolated H chain.

(23) The unitary free energy is the standard free energy, corrected for the loss in entropy that opposes the combination of two molecules to form a complex.

(24) T. J. Yoo, O. A. Roholt, and D. Pressman, *Science*, **157**, 707 (1967).

(25) O. Roholt, K. Onoue, and D. Pressman, *Proc. Natl. Acad. Sci. U. S.*, **51**, 173 (1964).

(26) R. Hong and A. Nisonoff, *J. Immunol.*, **96**, 622 (1966).

(27) L. A. Steiner and S. Lowey, *J. Biol. Chem.*, **241**, 231 (1966).

(28) The mean residue rotation at the minimum near 230 mμ is −1200 deg cm/dmole. Synthetic polypeptides in the α-helical conformation and in the β conformation also have minima near 230 mμ, but the $[m']$ values for a typical polypeptide (poly-L-lysine) are[29] −15,000 and −6000, respectively. Even randomly coiled polypeptides, which do not reach a minimum until 205 mμ, still have more negative rotations near 230 mμ than native IgG. For randomly coiled poly-L-lysine,[30] $[m']$ is −2300 at 230 mμ.

(29) N. Greenfield, B. Davidson, and G. D. Fasman, *Biochemistry*, **6**, 1630 (1967).

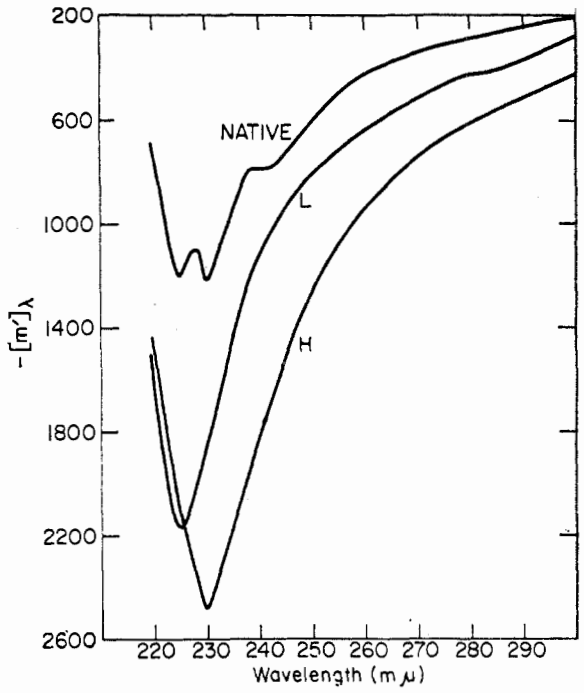

Figure 3. ORD curves of native nonspecific rabbit IgG and of the separated H and L chains derived from it.

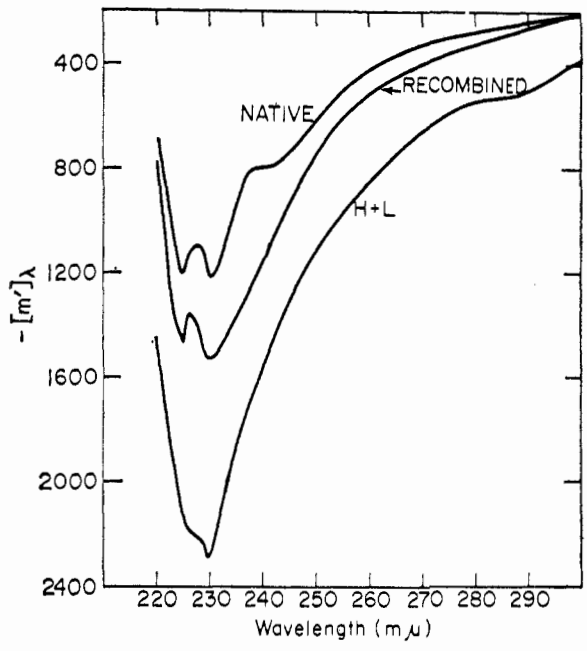

Figure 4. ORD curves of native nonspecific rabbit IgG, of an equimolar mixture of separated H and L chains (calculated from the results of Figure 3), and of the protein obtained on recombination of the chains.

there is a small Cotton effect centered near 240 mμ. The over-all pattern is unique to IgG. ORD curves of other classes of immunoglobulins are somewhat similar, but there is no resemblance to the ORD curves of any other known proteins. The ORD curves are not affected, qualitatively or quantitatively, by poly-DL-alanylation.

Our present knowledge of the origin of optical rotation does not enable us to relate the ORD curve of Figure 3 to possible structural features of a protein molecule.[30] The 240-mμ Cotton effect cannot even be assigned to a particular chromophore.[31] It is possible, however, by making similar studies with separated Fab and Fc fragments to assign the observed effects to the Fab or Fc regions of the molecule. Such studies[27] show that the 225-mμ trough and the 240-mμ Cotton effect originate from the Fab region, while the 230-mμ trough originates from the Fc region.

Figure 3 shows the ORD curves of the separated H and L chains derived from rabbit IgG, as well as those of the native protein, examined under essentially identical conditions. The ORD spectra of the isolated chains clearly do not display the characteristic features of the native protein. The specific rotation is much more negative, and the 240-mμ Cotton effect has disappeared. The two troughs persist, that assigned to the Fc portion being found in the H chain and that assigned to the Fab portion being in the L chain. The composite curve in the region of the two troughs, calculated for an equimolar mixture of H and L chains, is very different, however, from the double minimum seen in the native protein, as is shown by Figure 4. Similar results were obtained for human nonspecific IgG and for human myeloma IgG.

The most interesting results were obtained when we attempted to reconstitute the native conformation from the separated chains. The results for nonspecific rabbit IgG are shown in Figure 4. The native conformation was clearly not reestablished entirely; in particular, the 240-mμ Cotton effect is missing. Again, nonspecific human IgG gave essentially the same result. On the other hand, when these experiments were repeated with human myeloma IgG, full recovery of the native conformation was obtained, as shown in Figure 5.

It is evident from these results that there is a striking parallel between attainment of the unique conformational features of IgG molecules in general and the attainment of maximal affinity for specific antigen of IgG antibodies. In both cases it appears to be essential that the H chain be combined with a complementary L chain, the same with which it was combined in the native state.

Another striking illustration of the same phenomenon[33] is provided by the results shown in Figure 6, which were obtained using nonspecific rabbit IgG. As was pointed out earlier, the interchain disulfide bonds of IgG are not significantly involved in the forces of attraction between the chains. In the experiments cited so far, these bonds were broken at the beginning of the experiment and never re-formed because they are not needed for recombination of H and L chains. In Figure 6 we have made use of the irrelevance of these disulfide bonds in another way: it is clear that if the bonds are not

(30) As footnote 29 shows, it can obviously not be considered as a mixture of the ORD curves for synthetic polypeptides in the α-helical, β, and randomly coiled conformations.

(31) Ribonuclease has a *positive* band in circular dichroism at 240 mμ, which has been suggested[32] as being due to a disulfide bond. The 240-mμ Cotton effect in Figure 3 is probably a *negative* one.

(32) S. Beychok, *Science*, **154**, 1288 (1966).

(33) Unpublished results of K. J. Dorrington.

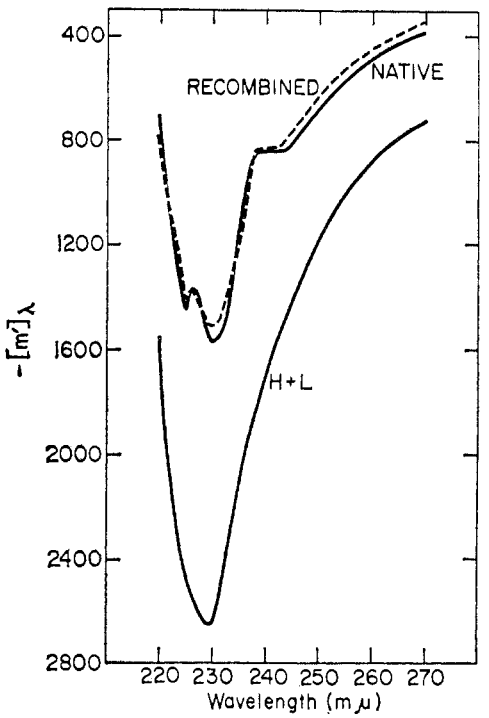

Figure 5. ORD curves obtained in the same way as those of Figure 4, but using a poly-DL-alanylated human IgG as starting material.

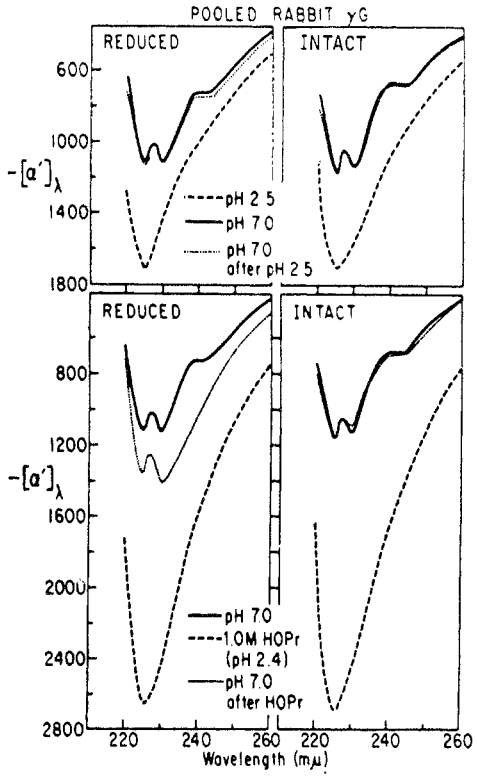

Figure 6. Comparison between nonspecific rabbit IgG with interchain disulfide bonds reduced (left half of figure) and nonspecific rabbit IgG with these bonds intact (right half of figure). Propionic acid (HOPr) is used to break the noncovalent bonds between H and L chains.

needed to bind H and L chains to each other, they should also not interfere with the dissociation of H and L chains from each other at contact points other than the disulfide link itself. This prediction is confirmed by the comparative data shown in the figure for parallel experiments, one set being conducted with nonspecific IgG with interchain disulfide bonds broken, the other with the same protein retaining all its disulfide bonds.

Figure 6 shows that exposure to pH 2.5 causes an identical conformational change, as reflected in the change in ORD, in both proteins. It is known that exposure to pH 2.5 does not disrupt the forces that hold the H chain to the L chain, for no separation of the reduced chains can be achieved under these conditions. The conformational change is therefore reversible both for the reduced and unreduced protein. No interchange of chains has been possible in the reduced protein.

Figure 6 shows that addition of 1 *M* propionic acid causes a substantially greater conformational change than exposure to pH 2.5. This is the medium that is used for separation of reduced H and L chains, *i.e.*, the noncovalent bonds between the chains are broken. The identity between the ORD curves for reduced and unreduced proteins indicates that the same structural change has occurred in both, and therefore that the noncovalent bonds are broken, and the chains effectively separated from each other, in the unreduced as well as in the reduced proteins. The only difference is that the chains in the unreduced protein are still held together by the interchain disulfide bond. This difference, however, is seen to have a profound effect on the reversibility of the conformational change. When the disulfide bonds are broken, and interchange of chains is possible, the original conformation is not recovered, as in Figure 4. When interchange is not possible, the conformational change is seen to be completely reversible. Clearly, regeneration of the native conformation is possible even for the heterogeneous nonspecific IgG provided that each H chain is forced to remain associated, in the final product, with its proper complementary L chain.

Why Are There Two Chains? To a chemist it may seem strange to seek a purpose for a structural feature such as the use of two polypeptide chains in the construction of the binding site of antibodies. To the biologist, however, such a question is logical. It is very likely that the primitive forerunner of present-day antibodies consisted of a single polypeptide chain. Chance mutations of all sorts must have occurred during the evolution of the modern vertebrates and are still occurring today. The fact that all known antibodies (including those that do not belong to the IgG type) make use of the H-chain–L-chain combination to create the biologically active site implies that this arrangement confers an advantage on the organism enabling it to survive in the natural selection process.

One possible advantage was suggested earlier. If there is independent selection of H and L chains for antibody formation, and if any H chain can combine with any L chain to form an antibody, then the number of distinct chain sequences for which potential genetic information must be provided is much smaller than the

number of distinct antibodies that the organism needs to be able to make.

The preceding experiments argue against this possibility. The failure to regain more than fractional specific antibody activity upon combination of a specific H chain with a nonspecific L chain *could* indeed be explained by supposing that such combination frequently leads to the formation of IgG molecules with antibody specificity for a different antigen. The failure to regenerate what seems to be a common structural feature of *all* IgG molecules cannot be explained in this way. It suggests that the combination of an H chain with an L chain other than the uniquely complementary one does not create a genuine IgG molecule at all. It indicates that each unique antibody requires a unique H chain *and* a unique L chain.

Another possible explanation that can presumably be discarded is that there is some analogy between H- and L-chain interaction and allosteric interactions in enzymes. There is no evidence to suggest that isolated H chains normally exist *in vivo*. There is no obvious reason why their diminished affinity for antigen would ever be a useful factor in the immunological reaction.

It is possible that the need for two chains is associated with the selection mechanism, *i.e.*, the mechanism whereby the antigen in the living system triggers the proliferation of cells that make specific antibodies against it. That mechanism itself, however, is still largely unknown.

It must be concluded therefore that the present state of knowledge does not provide any information regarding the biological advantage that is conferred upon the organism by the apparent requirement for duplicate specificity in the IgG molecule.

This paper has summarized chemical and physicochemical experiments that provide considerable insight into the structure of antibody molecules and into the way in which diversity in structure and function is achieved. The results seem to raise as many questions as they answer, and it is evident that more work is needed before we can achieve a true understanding of the intricate process that nature has evolved to provide immunity against infectious disease.

I wish to acknowledge my indebtedness to my coworkers who have participated in that portion of the work described that has originated from my laboratory. I also wish to express my appreciation to the National Institutes of Health, U. S. Public Health Service, for a research career award and for general research support.