RECOVERY OF ANTIBODY ACTIVITY FROM INACTIVE HYBRIDS OF H AND L CHAINS*

By O. A. ROHOLT, PH.D., G. RADZIMSKI, AND D. PRESSMAN, PH.D.

(From the Department of Biochemistry Research, Roswell Park Memorial Institute,‡ Buffalo, New York)

(Received for publication 12 September 1966)

Globulin molecules can be reconstituted from the separated heavy (H) and light (L) chains of specifically purified antihapten antibody (1, 2). When the chains are derived from the same rabbit, antibody activity is recovered (1, 3, 4). Hybrid molecules of chains from different rabbits have been found to show only questionable activity (5, 6). Since the chains in hybrids are not linked by covalent bonds, we have now investigated their stability toward dissociation. We found that the hybrids in a mixture consisting of one hybrid having H chains from the anti-*p*-azobenzoate (anti-Xp)[1] antibody of one rabbit and L chains from the anti-*p*-azobenzenearsonate (anti-Rp)[1] antibody of a second rabbit and a second hybrid of the opposite combination are so stable at pH 8 and at 5°C that even after 2 wk only low residual binding activity toward either specific hapten was shown. Exposure of the mixture to 1 M propionic acid followed by buffer resulted in a major recovery of binding activity toward haptens specific for anti-Xp and for anti-Rp. These results show that no exchange occurred between the hybrids at pH 8, but that exchange did occur on exposure to propionic acid and with a preferential recombination of the chains to give effective antibody sites.

Materials and Methods

Preparation of Specific Antibody γG-Immunoglobulin.—Specifically purified anti-*p*-azobenzoate and anti-*p*-azo benzenearsonate antibody were prepared from the serum of individual rabbits (Nos. 3351 and 3287) after repeated injections with Xp–bovine γ-globulin and Rp–bovine γ-globulin conjugates, respectively (7).

* Presented at the Annual Meeting of the American Association of Immunologists, Atlantic City, N.J., April 1966. Supported in part by the United States Public Health Service, Grant AI03962 from the National Institute of Allergy and Infectious Diseases.

‡A unit of the N.Y. State Department of Health.

[1] The following abbreviations are used: The 7S γ-globulin is designated by IgG or γG-immunoglobulin as suggested by the memorandum on "Nomenclature for Human Immunoglobulins." 1964. *Bull. World Health Organ.* **30**:447. Specifically purified *antibody* is designated as IgG_{Ab}(anti-Rp) and IgG_{Ab} (anti-Xp). Rp stands for *p*-azobenzenearsonate; Xp stands for *p*-azobenzoate. Anti-Rp and anti-Xp refer to the corresponding antibody or serum.

The specific antihapten antibody was isolated from the immune serum by means of a solid absorbent in the manner previously reported (8).

Preparation of Heavy and Light Polypeptide Chains from Specific Antibody.—The heavy and light chains were prepared from the specific antihapten antibody by reduction with 0.2 M mercaptoethanol and alkylation using an equivalent of iodoacetamide and separating the chains on Sephadex G-100 equilibrated with 1 M propionic acid (8).

Determination of Hapten-Binding Activity by Equilibrium Dialysis.— The binding of ^{125}I-labeled *p*-iodobenzoate and *p*-iodobenzenearsonate was measured by the method of equilibrium dialysis as previously described (9).

RESULTS

Preparation of Heavy and Light Chain Recombinants.—The heavy chains were eluted from Sephadex G-100 as two peaks, as reported previously (6, 8), but they were pooled in a single fraction for use here. Heavy chains prepared from IgG_{Ab} (anti-Rp)[1] and from IgG_{Ab} (anti-Xp[1] were individually mixed in 1 M propionic acid with individual portions of the light chains from the two antibody preparations to give four mixtures, viz. two antibody recombinants, H(Xp)L(Xp) and H(Rp)L(Rp), and two hybrid recombinants, H(Xp)L(Rp) and H(Rp)L(Xp). The H and L chains were mixed in proportion to their relative molar absorbance at 280 mμ as found for the H chain and L chain recovered after passage through Sephadex G-100.[2]

The mixtures were then dialyzed against 40 volumes of cold water overnight followed by pervaporations in the cold alternated with dialyses against cold 0.01 M propionic acid. When the volume was reduced to an estimated protein concentration of about 2.5 mg/ml, the solutions were dialyzed against two changes of saline during 24 hr followed by two changes of borate buffer of pH 8 (10) during 24 hr. There was very little precipitate in the bags; the contents were removed from the bags and centrifuged, and the protein concentrations were determined from the absorbance at 280 mμ. The recoveries of protein ranged from 92 to 103%. The concentrations of the protein in the hybrid solutions was adjusted to 2 mg/ml and, in the antibody recombinant solutions, to 1 mg/ml.

In order to determine the extent to which exchange might occur between the chains in a mixture of the hybrids, several mixtures were made. 2 ml portions of each of the two hybrid solutions were mixed and stored in the refrigerator on day 0. 1 ml portions of both hybrid solutions were also mixed after 5, 11, and 13 days and stored in the refrigerator.

On the 11th day, 0.4–0.5 ml portions of the day 0 mixture were placed in each of four dialysis bags and, during the next 2 days, were dialyzed against cold saline followed by two changes of 250 ml of cold 1 M propionic acid, then against a large volume of cold water, and finally against several changes of cold pH 8 borate buffer. The hybrid mixture in the bags had thus been exposed to cold borate buffer for 11 days followed by exposure to cold 1 M propionic acid for about 16 hr, and had then been returned to borate buffer preparatory to hapten-binding activity measurement.

Hapten-Binding Activity.—On the 13th day, each of the hybrid mixtures which had been stored at pH 8 in the cold for varying periods was distributed among four dialysis bags (0.4–0.5 ml each). Similar bags were prepared containing untreated anti-Rp antibody, untreated anti-Xp antibody, each of the two hybrid solutions, and also each of the two antibody recombinants.

Two bags of each set were then placed in a common solution of ^{125}I-labeled *p*-iodobenzoate,

[2] For the anti-Xp antibody preparation this ratio for the relative molar absorbance at 280 mμ of the heavy to light chains was 68:32 and for the anti-Rp antibody it was 70:30. The antibody recombinants were mixed in these respective ratios; the hybrids were mixed in the ration of 70:30.

and two bags from each set in ^{125}I-labeled *p*-iodobenzenearsonate, and the hapten-binding activity was determined for each preparation by equilibrium dialysis.

The results are shown in Table I.

The chains comprising the hybrids in the mixture did not exchange at pH 8 even after nearly 2 wk [preparations (*g–i*)], since the binding activity of the mixture was only equal to the sum of the binding activities of the individual hydrids alone [preparations (*e*) and (*f*)]. However, exposure of the hybrid mix-

TABLE I

Binding Activity of Hybrids of H and L Chains

Preparation	Protein concn	Relative binding activity	
		p-Iodobenzoate	*p*-Iodobenzenearsonate
	mg/ml		
Intact antibodies			
(*a*) XpAb	1.0	100	2
(*b*) RpAb	"	1	100
Antibody recombinants			
(*c*) H(Rp)L(Rp)	"	2	88
(*d*) H(Xp)L(Xp)	"	68	6
Hybrids			
(*e*) H(Rp)L(Xp)	"	8	4
(*f*) H(Xp)L(Rp)	"	6	7
Mixtures of hybrids*			
(*g*) 0 days at pH 8 as a mixture	2.0	14	13
(*h*) 2 " " " " " " "	"	10	15
(*i*) 8 " " " " " " "	"	15	17
(*j*) 11 " " " " " " " , then 1 M propionic acid	"	55	51
(*k*) 13 days at pH 8 as a mixture	"	12	19

* The theoretical value for a mixture of 1 mg each of (*e*) and (*f*) is 14 for binding of *p*-iodobenzoate and 11 for binding of *p*-iodobenzenearsonate.

ture to 1 M propionic acid and return to borate buffer of pH 8 [preparation (*j*)] led to a significant increase in the binding of both haptens. In the case of the Xp system, the acid-treated hybrid mixture [preparation (*j*)] showed a recovery of 79% of the activity of that of the sum of the binding activities of the two individual antibody recombinants [preparations (*c*) and (*d*)] and, in the Rp system, 54%. Thus, the hybrids must have not dissociated in the borate buffer, but exposure to the propionic acid solution did lead to their dissociation and the separated heavy and light chains recombined while the pH was returned to 8 by dialysis. This recombination favored interaction between heavy and light

chains which gave molecules similar to the antibody molecules from which they were originally derived, i.e. molecules with effective sites.

DISCUSSION

The fact that hybrid molecules are formed between heavy and light chains of IgG means that all the heavy chains have common or cross-reactive structural features (1). Likewise all light chains of IgG have portions with common or cross-reactive structural features. It is these two sets of common regions that are involved in the association of the heavy and light chains of the IgG. Indeed, these common regions are of broad enough specificity that heavy chains from one species will combine with light chains of another species to form interspecies IgG hybrids as shown by Fougereau et al. (5).

Superimposed on this general heavy and light chain interaction, however, is another, even more specific interaction which is the basis for the recovery of hapten-binding activity, as described here, from the related inactive hybrids, H(Rp)L(Rp) and H(Rp)L(Xp), through a preferential recombination of those heavy and light chains that give molecules of good binding activity.

Although the binding between the chains forming the hybrids is so strong that essentially no exchange takes place in neutral solution under the conditions used here, there is a preferential recombination of chains after they have been dissociated in propionic acid and allowed to recombine competitively. This indicates that in a population of light and heavy chains, certain light and heavy chains, i.e. those which preferentially recombine to give effective sites, have, in the region of association, structures that are more or less specific for each other and are responsible for the combination of those pairs of heavy and light chains that give effective binding sites. These structures contribute the additional energy that enables the correct combination of heavy and light chains to take place.

The preferential combination of heavy and light chains as superimposed on the more general specific combination is depicted in Fig. 1.

The first and second diagrams show reduced and alkylated anti-Xp and anti-Rp molecules. The third shows the hybrid in which the heavy and light chains are held together by the structures on the common region, which are effective in the formation of stabile inactive hybrids.

Additional distinctive structures are depicted which are important in contributing the additional energy required for holding together the correct chains forming the effective sites.

There must exist such specific regions even in antibodies directed against the same hapten group. This follows from our observation that the chains from antibody molecules from one rabbit do not necessarily cross-combine to form effective sites with chains from antibody molecules directed against the same hapten group, but from another rabbit (6). The chains do show a preferential combination to form effective sites rather than ineffective sites.

Even for a single rabbit there must exist such distinctive regions which are different between antibody molecules, since there is a preferential combination of those heavy and light chains of globulin which give effective antibody sites (8). This must involve a specific interaction between appropriate chains. Ineffective sites are formed when an H or L chain from active antibody is permitted to combine with the other chain of inactive residual globulin (the IgG remaining after antibody has been removed) in the absence of the other chain from the antibody. This combination to form an ineffective hybrid must be due to the common features on each chain.

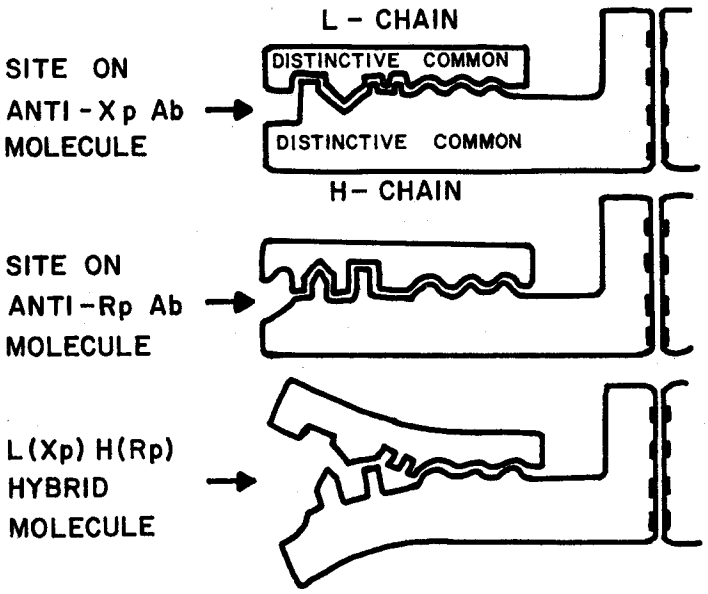

FIG. 1. Specificity in the combination of H and L chains.

Both the common and the distinctive features on the heavy chains exist on the Fd part of the heavy chains, since preferential recombination to give active sites has also been observed between light chains and the Fd fragment (11).

A structural basis for this preferential recombination has been developing through reports of studies of light and heavy chains of IgG molecules. The amino acid sequences of several light chain preparations show that the residues at the carboxyl-terminal end of the polypeptide chain, consisting of about one-half of the total residues, have a common sequence almost without exception (12, 13). It would thus appear that the structural features of the light chain responsible for hybridization in general are probably associated with this common sequence.

On the other hand, the amino-terminal half of the polypeptide chain varies

in sequence from light chain to light chain in such a way that this sequence is distinctive for the individual or cell type from which it is derived. This distinctive part, which is probably involved in the antibody specificity, is apparently also involved in the structural features important for the specificity of the interaction between the chains. The involvement of the distinctive part could occur by direct contact with the heavy chain or by a conformational effect on some part of the common sequence portion of the light chain which then makes contact with the heavy chain.

An analgous situation appears to exist in the case of the heavy chains. In this case, the common sequence appears to include all of the Fc portion of the chains and a part of the Fd fragment. Frangione and Franklin (14) have shown that Fd fragments from different human heavy chains show several common tryptic peptides and some peptides which vary from individual to individual. This distinctive sequence of the Fd fragment, like that of the light chain, comprises the amino-terminal end of the fragment chain and is involved in antibody specificity, although not necessarily through the contribution of contact residues to the site. The heavy chain is known to contribute contact amino acids to the site (15). In an analogous manner this distinctive region appears to be involved in the specific combination of chains, either by direct contact or by effect on the conformation of the common sequence region of the Fd fragment.

A similar preferential combination of H and L chains from globulin molecules has been reported by Grey and Mannik (16) in the case of human myeloma proteins. They observed the preferential combination of H chains of a particular myeloma protein with L chains from the same myeloma protein rather than with L chains of other myeloma proteins. This preferential combination would appear to depend on the same type of structural features.

SUMMARY

Hybrid IgG molecules were prepared from the heavy and light chains of specifically purified antibody against two different haptens. One hybrid consisted of the H chains from the anti-*p*-azobenzoate antibody from one rabbit and L chains from the anti-*p*-azobenzenearsonate antibody from the second rabbit, and the second hybrid consisted of the opposite combination, light chains from the first rabbit and heavy chains from the second. The two hybrids were mixed at pH 8 and were found to be so stable in the mixture that even after 2 wk at 5°C there was still only low hapten-binding activity toward *p*-iodobenzoate and *p*-iodobenzenearsonate. However, exposure of the mixture of hybrids to 1 M propionic acid followed by buffer at pH 8 resulted in recovery of binding activity for *p*-iodobenzoate and *p*-iodobenzenearsonate. Thus no exchange occurred between the light and heavy chains of the hybrids in the buffer, but exchange did occur on exposure to propionic acid, and this exchange favored a preferential combination among the chains in such a manner that effective antibody sites resulted.

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