RECONSTITUTION OF ANTIPHAGE ANTIBODIES FROM L AND H POLYPEPTIDE CHAINS AND THE FORMATION OF INTERSPECIES MOLECULAR HYBRIDS*

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Admixture of the separated and relatively inactive L and H polypeptide chains of guinea pigs 7S antiphage and antihapten antibodies (1) has been found to result in reconstitution of the capacity to combine specifically with antigen. The activity was associated with reconstituted 7S molecules which had molecular weights of 160,000 and an arrangement of chains similar to that of native 7S γ-globulin (1–4). Greater reconstitution of activity resulted if the H and L chains of the original antibody were recombined, than if only one of the chain types of the original antibody was combined with the complementary chain type from an unrelated antibody or from non-specific γ-globulin. Confirmation of these results has recently been obtained with rabbit antihapten antibodies (5). Most of these experiments were performed with purified antibodies obtained from several animals of the same species.

The present report extends these observations in two directions. Data were obtained on the reconstitution of antiphage antibodies from individual sheep and guinea pigs. In addition, since 7S molecules could be formed from H or L chains from one animal species and the complementary chains from another species (2), the question of whether activity to a phage antigen could be regenerated in such hybrid molecules was investigated.

Materials and Methods

All of the procedures have been described in detail elsewhere (1, 2). A general description will be given here to facilitate the account of the present experiments.

Immunization of Sheep.—One sheep received 5 mg of f1 phage and 5 mg of f2 phage dispersed in Freund's complete adjuvant, intradermally in two separate areas. A booster injection of 10 mg of each phage in the absence of adjuvant was given intramuscularly 1 month later. The animal was bled 2 weeks after this injection, and then at different periods of time, according to need. A second booster injection of 10 mg of each phage was given 6 months later.

Isolation of γ-Globulin.—7S γ-globulin was isolated by zone electrophoresis in starch from immune sera after absorption with the antigens used to immunize the animal. This preparation is termed residual γ-globulin. In a few instances, sheep 7S γ-globulin was isolated by zone electrophoresis in starch from serum obtained before immunization was started.

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Purification and Assay Methods.—7S antibodies to f1 and f2 bacteriophages were purified from the sera of single sheep and guinea pigs (1). The single antibody preparations were reduced and alkylated in the absence of urea (6) and the L and H polypeptide chains were separated by gel filtration in 0.5 N propionic acid (7). Aliquots of the appropriate chain fractions were then mixed in 0.5 N propionic acid and dialyzed against neutral aqueous buffers. The mixtures and individual control fractions were concentrated and tested for phage-neutralizing capacity (1) according to the equation $-\ln (p/po) = Kct$, where p/po is the fraction of plaques remaining after time t and c is the antibody concentration (8). Results were calculated in terms of K, the first order rate constant for neutralization, expressed in dimensions of minutes^{-1} A^{-1} where A is the absorbancy at 280 mμ of the fraction tested.

To demonstrate the formation of interspecies molecular hybrids, the H and L chains of different animal origin were labeled with I^{131} and I^{125} isotopes, and the appropriate mixtures of chains were subjected to density gradient centrifugation in sucrose. The procedures have been described in detail (2).

Conventions.—The designations of chain mixtures and specificities are the same as those used previously. Some examples follow: Ab(f1), antibody to f1 phage; H(f1), heavy chains from Ab(f1); L(f1), light chains from Ab(f1); H(f1) + L(f1), reconstituted mixture of complementary chains of Ab(f1); H(γ), heavy chains of nonspecific γ-globulin; shp H(f1) + gp L(f1), hybrid mixture of H chains of sheep antibody to f1 phage with L chains of guinea pig antibody to f1 phage.

In the isotopic labeling experiments the appropriate isotope is indicated: H_I^{125}; L_I^{131}.

RESULTS

Mixtures of Chains from Antibodies of Single Animals.—The data on phage-neutralizing activity of the mixtures of H and L chains of anti-f1 antibodies from a single sheep are presented in Table I. Reconstitution of activity in mixtures of the chains of the antibody was compared with that of hybrid mixtures of antibody chains and chains of γ-globulin from the same animal. All experiments were performed on two separate mixtures prepared with different mass ratios of H chains to L chains.

In accord with previous findings on guinea pig antibodies (1), the activity of separated chains was considerably lower than that of whole antibodies. Mixtures of the chains of f1 antibodies showed a fourfold potentiation at both mixing ratios, and the K values were approximately 20 per cent of those obtained with the native antibodies. Mixtures of H(f1) chains with L(γ) chains from residual γ-globulin showed a slight inhibition of activity. On the other hand, H(γ) + L(f1) mixtures showed definite potentiation, although the level of activity was low. It should be stressed that the native residual γ-globulin possessed slight neutralizing activity. Nonetheless, the activity in H(γ) + L(f1) mixtures was greater than would be expected if the activities of the chains were merely additive.

In another experiment, the activities were measured in the nine possible mixtures of chains of antibodies to f1 and f2 phage and of γ-globulin, all obtained from the same individual sheep (Table II). Chains from antibodies of the same specificity showed excellent potentiation, twentyfold for H(f1) + L(f1)

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and sixfold for H(f2) + L(f2). Each antibody preparation showed slight activity against the unrelated phage. Ab(f2) had 0.03 per cent of the activity of Ab(f1) when tested with f1 phage and Ab(f1) had 1.5 per cent of the activity of Ab(f2) against f2 phage. These values probably reflect small amounts of contaminating antibodies in each preparation. γ-Globulin had negligible activity in both systems.

TABLE I

Neutralization of f1 Phage by Mixtures of Polypeptide Chains of Sheep Anti-f1 Antibodies and Residual γ-Globulin Obtained from a Single Animal*

Mass Ratio H/L in mixture	Sample	K‡	$K_{calculated}$§	$\frac{K}{K}$ calculated	$\left(\frac{K}{K_{Ab(f1)}}\right) \times 100$
	Ab(f1)	2800			100.0
	H(f1)	150			5.4
	L(f1)	9.4			0.33
	γ-globulin	15			
	H(γ)	0.56			
	L(γ)	0‖			
2.5/1	H(f1) + L(f1)	440	110	4.	15.7
	H(f1) + L(γ)	65	107	0.6	2.3
	H(γ) + L(f1)	11.9	3	4.	0.425
	H(γ) + L(γ)	0	—	—	—
5/1	H(f1) + L(f1)	520	127	4.10	19.5
	H(f1) + L(γ)	42	125	0.3	1.5
	H(γ) + L(f1)	4.7	1.7	2.50	0.17
	H(γ) + L(γ)	0	—	—	—

* Electrophoretically isolated after absorption of immune serum with f1 phage.

‡ From $-\ln p/po = Kct$ where p/po is the fraction of original plaques remaining at time t in minutes and c is protein concentration of fraction tested in absorbancy units.

§ Calculated from the amounts and residual activities of the chain fractions present in the mixtures.

‖ No neutralization observed at $c = 10^{-3}$($p/po = 0.90$ at $c = 10^{-3}$ would give $K = 0.3$)

H(f1) chains mixed with L(γ) or L(f2) chains showed approximately threefold potentiation of anti-f1 activity. This should be compared with the twentyfold potentiation of H(f1) + L(f1) mixtures. As in the previous experiment, L(f1) chains showed potentiation of activity to f1 phage when mixed with H(γ) or H(f2) chains.

In contrast, the H(f2) chains showed only slight potentiation when mixed with L(f1) or L(γ) chains, and the activity of L(f2) chains was not potentiated when they were mixed with H(γ) or H(f1) chains.

Experiments on anti-f2 antibodies and γ-globulins of a single guinea pig

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(Table III) confirmed previous results (1) and yielded data similar to those observed with sheep antibodies from single animals. Of particular interest was the observation that L(f2) chains showed enhanced neutralization of f2 phage when mixed with H(γ) chains. This was not observed in the previous experiments when chains isolated from different guinea pigs were used (1).

TABLE II

Antiphage Activity in Mixtures of Polypeptide Chains of Two Non-Cross-Reacting Antibodies and γ-Globulin Obtained from a Single Sheep

Sample	K (per min. per unit absorbancy at 280 mμ)	
	f1 neutralization	f2 neutralization
Ab(f1)	3700	14.0
H(f1)	51.6	0.28
L(f1)	4.64	0.30
Ab(f2)	12.5	500.0
H(f2)	1.13	11.0
L(f2)	0.63	0.65
γ*	0.06	1.3
H(γ)	0.81	0
L(γ)	0.53	0
H(f1) + L(f1)	610 (28)‡	0 (0.45)
H(f1) + L(f2)	81 (26)	0.31
H(f1) + L(γ)	68.2 (26)	0.06
H(f2) + L(f2)	0.52	33.0 (5.8)
H(f2) + L(f1)	9.58 (2.8)	6.5 (5.6)
H(f2) + L(γ)	1.09	7.9 (5.5)
H(γ) + L(γ)	0.60	0
H(γ) + L(f1)	3.80 (2.7)	0
H(γ) + L(f2)	0.50	0.16 (0.32)

* γ-Globulin was prepared by zone electrophoresis on starch from serum of the same sheep obtained prior to immunization.

‡ The numbers within the parentheses represent the activities of the chain mixtures expected if no potentiation occurred, calculated on the basis of the content and activities of both L and H chains. In the original mixtures the mass ratio H/L was 1/1.

Formation of Molecular Hybrids between the Chains of Antibodies and γ-Globulins of Two Different Animal Species.—It has been shown that H and L polypeptide chains of human γ-globulin would form 7S molecules with the complementary chains of rabbit γ-globulin (2). This finding suggests that the structural

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features of the chains responsible for the complementary interaction may be general to all 7S γ-globulins. In the present study, attempts were made to form molecular hybrids between the γ-globulins of sheep and guinea pigs. Efforts were also made to determine whether interspecies molecular hybrids of chains from antibodies to f1 phage would be more active than the intraspecies hybrids formed with chains of anti-f1 antibodies and those of non-specific γ-globulins.

Hybrid formation between the L chains of guinea pig and the H chains of sheep γ-globulins is demonstrated in Fig. 1. Mixtures of complementary chains

TABLE III

Neutralization of f2 Phage by Mixtures of Chains from Anti-f2 Antibodies and Normal γ-Globulin Obtained from a Single Guinea Pig

Sample	K (per min. per unit absorbancy at 280 mμ)
Ab(f2)	1230
Ab(f2), RAP*	31.4
H(f2)	5.70
L(f2)	1.45
γ-Globulin	0.05
γ-Globulin, RAP	0.03
H(γ)	0.02
L(γ)	0.07
H(f2) + L(f2)	16.8 (3.6)‡
H(f2) + L(γ)	2.73 (2.9)
H(γ) + L(f2)	2.51 (.72)
H(γ) + L(γ)	0.05

* RAP, reduced and alkylated preparations that had been exposed to 0.5 N propionic acid.

‡ The numbers given in parentheses are the expected activities of the mixtures calculated on the basis of their chain content. In the original mixtures the H/L mass ratio was 1/1.

labeled with different iodine isotopes were subjected to density gradient centrifugation. The H chain fraction migrated in the 6S region as shown by coincidence of the peak with that of alkaline phosphatase activity. L chains moved in the 3S to 4S region. The mixture shp H_I^{125} + gp L_I^{131} showed a peak in the 7S region and the H/L mass ratio in the peak was 2.75/1. This is consistent with a 1/1 molar ratio of the chains and indicated effective reconstitution. Reconstitution was less effective in mixtures of guinea pig H chains and sheep L chains (Fig. 2) and the H/L ratio was 4.2/1 indicating an excess of H chains in the reconstituted product.

The H and L chains of anti-f1 antibodies and residual γ-globulins of both species were used to test for reconstitution of activity (Table IV). As before, mixtures of antibody chains from a single animal showed effective reconstitu-

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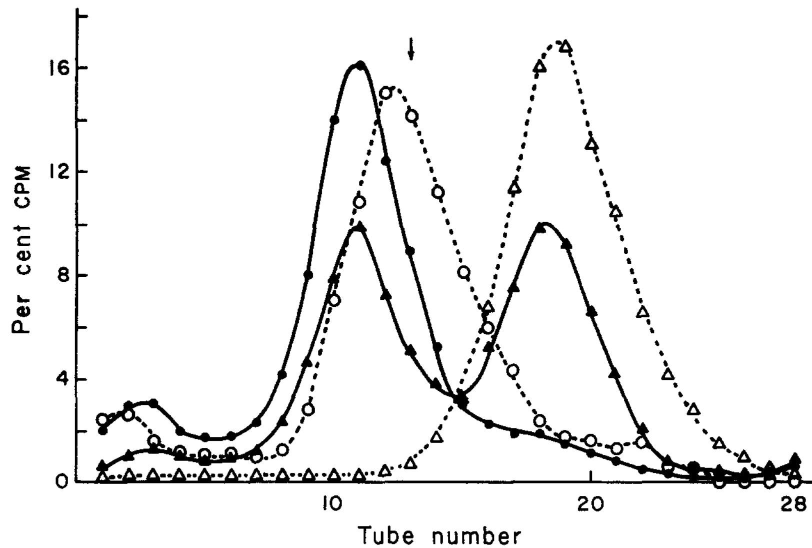

FIG. 1. Ultracentrifugation in sucrose density gradients of hybrid mixtures of H and L chains from γ-globulins of different animal species. Sheep H chains labeled with I^{125} (—●—●—) reconstituted with guinea pig L chains labeled with I^{131} (—▲—▲—) compared with H chain fraction (---O---O---) and L chains (---△---△---). Sedimentation proceeded from right to left. ↓, position of the peak of activity of alkaline phosphatase (s_{20}~ 6S) added to the mixtures prior to layering over the gradient. Per cent CPM, per cent of total counts per minute recovered from gradient.

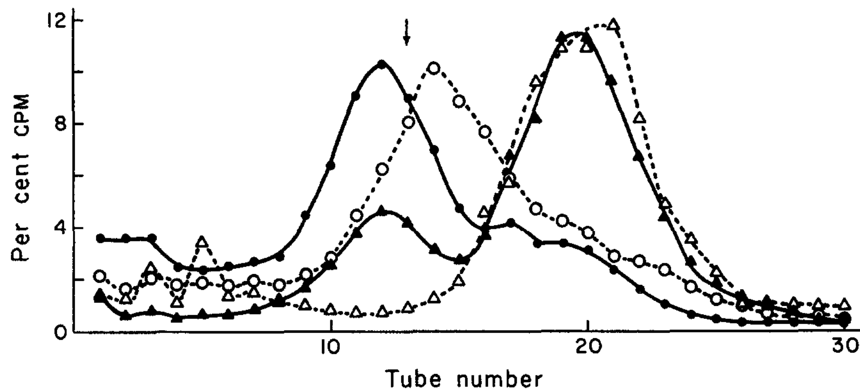

FIG. 2. Ultracentrifugation in sucrose density gradients of hybrid mixtures of H and L chains from γ-globulins of different animal species. Guinea pig H chains labeled with I^{131} (—●—●—) reconstituted with sheep L chains labeled with I^{125} (—▲—▲—) compared with H chain fraction (---O---O---) and L chains (---△---△---). Sedimentation proceeded from right to left. ↓, position of alkaline phosphatase peak. Per cent CPM, per cent of total counts per minute recovered from gradient.

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tion of activity. When mixed with H(f1) chains of one species, the L(f1) and L(γ) chains of the other species showed no better potentiation of activity than the L(γ) chains of homologous species origin. Interspecies crosses of H(γ)

TABLE IV

Neutralization of f1 Phage by Mixtures of Polypeptide Chains from Anti-f1 Antibodies and Residual γ-Globulins Obtained from Sheep and Guinea Pig

Sample*	K	$K_{calculated}$‡	$\frac{K}{K_{calculated}}$
shpAb(f1)	820	—	—
gpAb(f1)	135	—	—
shpH(f1)	35	—	—
shpL(f1)	5.2	—	—
gpH(f1)	7.2	—	—
gpL(f1)	2.8	—	—
shp(γ)	4	—	—
gp(γ)	0	—	—
shpH(f1) + shpL(f1)	65	27.3	2.4
shpH(f1) + shpL(γ)	35	26.0	1.3
shpH(f1) + gpL(f1)	42	26.7	1.6
shpH(f1) + gpL(γ)	35	26.0	1.3
gpH(f1) + gpL(f1)	49.0	6.1	8.0
gpH(f1) + gpL(γ)	4.1	5.4	.86
gpH(f1) + shpL(f1)	11.4	6.7	1.65
gpH(f1) + shpL(γ)	8.3	7.0	1.18
shpH(γ) + shpL(f1)	§	—	—
shpH(γ) + gpL(f1)	0.5	0.7	0.7
gpH(γ) + gpL(f1)	0.6	0.7	0.85
gpH(γ) + shpL(f1)	1.7	1.3	1.30

* shp(γ), sheep γ-globulin prepared by starch block electrophoresis from absorbed serum of the same sheep. Isolated chains from sheep γ-globulin showed no neutralization at $c = 10^{-3}$. The guinea pig antibodies were obtained from three animals. The guinea pig γ-globulin was prepared by electrophoresis on starch block from one of these sera after absorption with f1 phage.

‡ Calculated from amounts and residual activities of the chain fractions present in the mixtures. In the original mixtures the H/L mass ratio was 3/1.

§ See Table I.

chains and L(f1) chains showed no enhancement of activity. Generally similar results were obtained using anti-f2 antibodies (Table V). In the latter case, however, sheep L chains showed a slight potentiation of the activity of guinea pig H(f2) chains, as did guinea pig L chains isolated from γ-globulins. In both

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of the experiments on the activity of interspecies hybrids, the preparations of chains of sheep antibodies showed lesser degrees of potentiation than observed in earlier experiments. Such variability has been observed before (1); its origins remain to be clarified.

TABLE V

Neutralization of f2 Phage by Mixtures of Chains from Anti-f2 Antibodies and Residual γ-Globulins Obtained from Sheep and Guinea Pigs

Sample*	K	$K_{calculated}$‡	$\frac{K}{K_{calculated}}$
shpAb(f2)	2030	—	—
gpAb(f2)	517	—	—
shp(γ)	0	—	—
gp(γ)	0	—	—
shpH(f2)	108	—	—
shpL(f2)	14	—	—
gpH(f2)	15	—	—
gpL(f2)	0.4	—	—
shpH(f2) + shpL(f2)	164	84	2.0
shpH(f2) + shpL(γ)	96	81	1.16
shpH(f2) + gpL(f2)	106	81	1.3
shpH(f2) + gpL(γ)	67	81	.83
gpH(f2) + gpL(f2)	98	11	8.9
gpH(f2) + gpL(γ)	37	11	3.3
gpH(f2) + shpL(f2)	30	14	2.14
gpH(f2) + shpL(γ)	40	11	3.6

* shp(γ), sheep γ-globulin prepared by starch block electrophoresis from absorbed serum of the same sheep. Isolated chains from sheep γ-globulin had showed no neutralizing effect at $c = 10^{-3}$. The guinea pig antibodies were obtained from two animals. The γ-globulin was prepared by electrophoresis on starch block from one of the sera after absorption with f2 phage.

‡ Calculated from amounts and residual activities of chain fractions present in the mixtures. In the original mixtures the H/L mass ratio was 3/1.

DISCUSSION

The reconstitution of 7S molecules from separated L and H polypeptide chains makes it possible to test a number of hypotheses on the origin of the specificity of antibodies. The present studies confirm previous conclusions that both L and H chains are required for complete binding activity of antibodies. Additional information has been obtained on the relative contributions of each type of chain.

In all of the experiments, reconstitution of activity was greatest when both

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the L and the H chains were obtained from the same antibody preparation. Lesser degrees of reconstitution resulted from mixing H chains of the antibody with L chains of an unrelated antibody or γ-globulin. In certain cases, hybrid mixtures formed from L chains of the original antibody with H chains of residual γ-globulin from the same animal also showed potentiation of activity, although the level of activity was relatively low. This effect was not observed in experiments using antibody chains isolated from several animals (1).

All of the present experiments were performed with antibodies from individual animals. Comparison of the present data on antibodies from single guinea pigs with those obtained using several animals (1) showed little or no difference in the degree of reconstitution. Detailed comparisons are hampered by variability in the K values of different antibody preparations, as well as by variations in the degree of reconstitution from experiment to experiment. Although the causes of these variations have not been ascertained, the qualitative conclusions concerning chain interaction and reconstitution of activity are supported without exception by the data on all of the preparations.

Despite the fact that interspecies hybrids were readily formed, activity was not greatly potentiated even when chains of antibodies directed to the same antigen were employed. As in the previously reported cross of rabbit and human chains (2), the results on the formation of hybrid 7S molecules were not entirely symmetrical. Sheep H chains showed better complementation with guinea pig L chains than in the reverse cross. The slight enhancement of activity was similar in both cases, however.

It would be of considerable interest to examine the antibodies of two closely related species in the same fashion. On genetic grounds, it would be expected that H and L chains have evolved together as a complementing system similar to those described in microorganisms (9). Considered in these terms, it is perhaps not surprising that chains of different species might fail to complement each other to form an active site even though hybrid molecules are formed (10, 11). The results described here are compatible with the hypothesis that, in a given animal, interaction of polypeptide chains having different amino acid sequences and behaving as products of an intercistronic complementing system may generate a large number of active sites of different specificities.

SUMMARY

Reconstitution of phage-neutralizing activity was observed after admixture of separated L and H polypeptide chains of purified antibodies obtained from single sheep or guinea pigs and directed against f1 and f2 phages. Hybrid mixtures of complementary chains of antiphage antibodies and γ-globulins of the same animal showed less activity than the homologous mixtures of chains from antibodies of the same specificity. 7S interspecies molecular hybrids could be formed between L or H chains of sheep γ-globulins and the complementary

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chains of guinea pig γ-globulins. In contrast to the results obtained within a single animal species, mixtures of L or H chains of antiphage antibodies from one species with the complementary chains of antibodies to the same phage from the other species showed slight or negligible potentiation of neutralizing activity.

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