ACTIVITY OF DISSOCIATED AND REASSOCIATED 19S ANTI-γ-GLOBULINS*

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(Received for publication, August 6, 1964)

It has been known for some time that 19S macroglobulins (1) and 19S class antibodies (2) are readily reduced by sulfhydryl compounds into smaller units. In most reported experiments with 19S antibodies this treatment has resulted in the complete loss of biological activity. However, some experiments to the contrary have been reported (3). Previous studies from this laboratory (4, 5) demonstrated that certain 19S anti-γ-globulins regained their activity on oxidative reassociation following dissociation with sulfhydryl compounds. Although no activity was demonstrated in the dissociated fragments, these experiments raised such a possibility. Isliker and associates have presented evidence that following treatment with $NaBH_4$-mixed molecules of high molecular weight isoagglutinins could be formed (6) and have recently reported evidence for activity in smaller units (7).

The present experiments were undertaken to extend the previous observations on the effect of reduction and reassociation on the biological activity of 19S antibodies. The anti-γ-globulins from human sera were chosen for these experiments because of their ready isolation in workable quantities. The large amount of evidence available that they represent antibodies to γ-globulin has been cited previously (8). The results obtained clearly indicate the presence of certain activity in the reduced and blocked dissociated fragments of these 19S proteins and the return of the original type of activity following reassociation.

Materials and Methods

The 19S anti-γ-globulins were isolated from the sera of two patients (Do. and Od.) having high titers in the various reactions for the detection of rheumatoid factors. These sera also contained significant amounts of intermediate γ-globulin complexes (9). Both patients had

* Aided by a grant from the National Foundation and by grant No. AM 03555 from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, United States Public Health Service.

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severe rheumatoid arthritis. The 19S anti-γ-globulins were initially precipitated from the sera with other euglobulins by dilution with fifteen volumes of cold distilled water. After standing in the cold overnight, the precipitates were washed twice with small volumes of cold distilled water and dissolved in a volume of saline equal to that of the serum utilized. The 19S materials were separated from the other components of the euglobulins by gel filtration on sephadex G-200 (Pharmacia, Upsala, Sweden). Gel filtration was performed as described by Flodin and Killander (10) except that 0.1 M sodium acetate buffer, pH 4.1, was utilized in place of the pH 8.0 buffer. Immediately prior to gel filtration, the euglobulin solution was equilibrated by dialysis with the pH 4.1 buffer. Any insoluble materials were removed by centrifuging. Column fractions containing the 19S γ-globulins were pooled, dialyzed against pH 7.5 sodium phosphate buffer, 0.2 ionic strength, and concentrated by ultrafiltration to a protein concentration of 4 to 6 mg/ml.

The 19S anti-γ-globulin preparations were reduced by dialysis against large volumes of 0.1 M ethyl mercaptan or mercaptoethanol (K and K Laboratories, Plainview, New York or Eastman Organic Chemicals, Rochester, New York) prepared in pH 7.5 sodium phosphate buffer, 0.2 ionic strength. Dialysis was allowed to proceed for 3 hours at room temperature with stirring of the ethyl mercaptan solution. At the end of the dialysis period the dissociated samples were either alkylated in order to prevent spontaneous reassociation or reassociated. Alkylation was effected by dialysis against a large volume of 0.02 M monoiodoacetamide (K and K Laboratories) prepared in the pH 7.5 buffer. Dialysis was allowed to proceed for 4 hours at room temperature with stirring of the iodoacetamide solution. The reduced and alkylated samples were finally dialyzed against pH 7.5 phosphate buffer in the cold overnight. The term "dissociated" will be used to designate samples which have been reduced and alkylated as described. Reassociation was effected by removal of the ethyl mercaptan by dialysis. The samples were dialyzed for 4 hours at room temperature against several changes of pH 7.5 phosphate buffer, with stirring of the buffer solution, then against the buffer overnight in the cold. The reassociated samples were essentially free of the odor of the mercaptan.

Ultracentrifuge analysis was carried out in a Spinco model E ultracentrifuge, using double sector 12 mm cells. Plate measurements were made according to the procedures described by Trautman (11). In correcting the sedimentation coefficients to the $S_{20,\,w}$ value, the partial specific volumes were assumed to be 0.73.

Density gradient ultracentrifugation was performed according to the general methods previously described (12). A continuous gradient of 10 to 40 per cent sucrose prepared in 0.9 per cent sodium chloride was utilized. After centrifugation serial fractions were collected from the bottom of the tube. In certain experiments samples from various levels of the gradient were taken by means of capillary pipettes from the top of the tube.

Agglutination tests with group O Rh-positive cells coated with various incomplete anti-Rh antibodies were made by the slide technic as described elsewhere (13). Dilutions were prepared using pH 7.5 sodium phosphate buffer, 0.2 ionic strength. Reactivity with aggregated-γ-globulin was determined by precipitin formation in capillary tubes (14) or by precipitin curve analysis (15). Protein concentrations were estimated by the Folin-Ciocalteu method (16) using fraction II as the standard.

RESULTS

Isolation and Properties of the 19S anti-γ-globulins.—The euglobulin precipitate from either the Od. or Do. serum contained moderate or large amounts of intermediate γ-globulin complexes and 7S γ-globulin in addition to the 19S anti-γ-globulins. Gel filtration of the euglobulins from serum Do. on sephadex

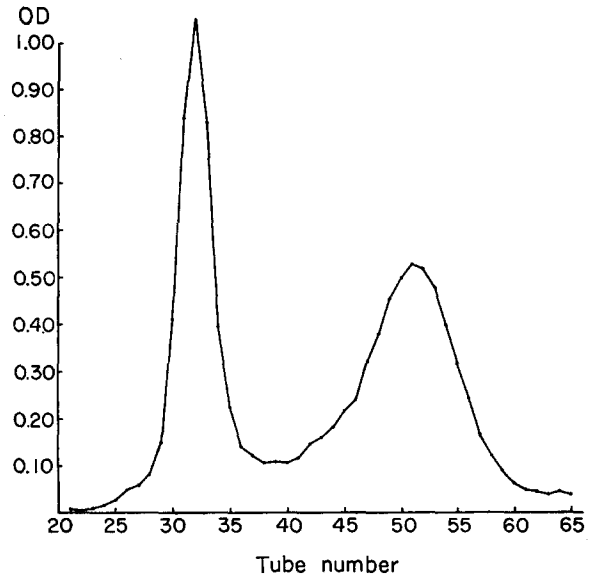

FIG. 1 *a*

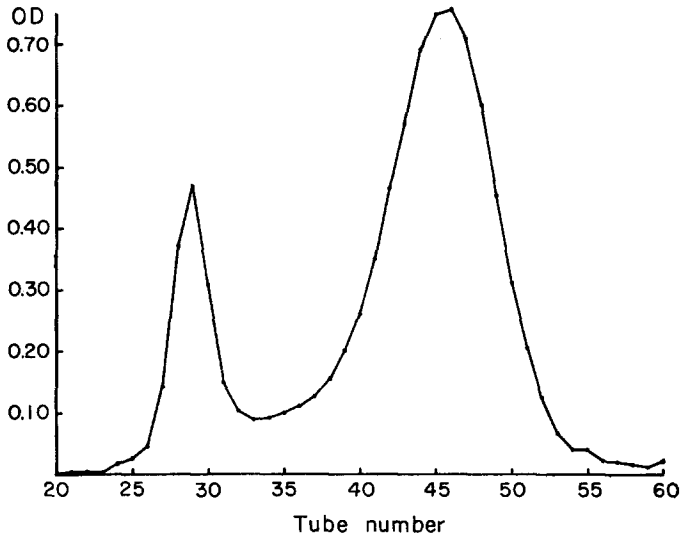

FIG. 1 *b*

FIGS. 1 *a* and 1 *b*. Distribution of components obtained by gel filtration on sephadex G-200 at pH 4.1 of the euglobulin fractions of sera Od. (Fig. 1 *a*) and Do. (Fig. 1 *b*).

G-200 using pH 8.0 tris-NaCl buffer (10) did not result in a separation of the 19S anti-γ-globulins from the intermediate complexes. However, the use of pH 4.1 sodium acetate buffer, 0.1 M, permitted the separation of the 19S materials from the intermediate complexes since at this acid pH the intermediate complexes dissociate into their 7S components (9). Examples of the separations obtained from the euglobulins from sera Od. and Do. are shown in Figs. 1 a and 1 b. The first peak contained the 19S anti-γ-globulins. The second peak contained the components of the intermediate complexes and any 7S γ-globulins present in the euglobulin.

TABLE I

Activity and Sedimentation Characteristics of Representative 19S γ-Globulin Preparations and the Corresponding Dissociated and Reassociated Preparations

Patient	Sample	Precipitation with aggregated γ-globulin*	Agglutination of red cells coated with incomplete anti-Rh antibodies‡	Sedimentation characteristics	
				Principal component(s)	Minor component(s)
				$S_{20,w}$	$S_{20,w}$
Do.	The original 19S γ-globulin	++++	1/20480	18.6	7, 29
	Dissociated	0	1/20	6.6	19
	Reassociated	+++	1/1280	7.3, 14.9	11, 17, 19
Od.	The original 19S γ-globulin	++++	1/81920	17.5	26
	Dissociated	0	1/5120	6.8	
	Reassociated	++++	1/20480	15.7, 17.5	7

* The strengths of precipitation of the undiluted samples with aggregated γ-globulin in the capillary tube test are listed.

‡ The agglutination titers with red cells coated with anti-Rh Ripley are recorded.

Some of the ultracentrifuge characteristics and activities of representative 19S anti-γ-globulin preparations are listed in Table I. The ultracentrifuge pattern of the 19S anti-γ-globulin preparation from patient Od. is also illustrated in Fig. 2. In addition to the 19S component, only a small quantity of faster sedimenting material was present in this preparation. No materials sedimenting at a rate slower than the 19S component were detected. Similarly, the 19S anti-γ-globulin preparation from patient Do. contained a small quantity of material sedimenting faster than the 19S component. However, the Do. anti-γ-globulin preparation differed from the Od. preparation in that it contained a small quantity of slow sedimenting material. On reaction with aggregated γ-globulin in a capillary tube, the 19S anti-γ-globulin preparations from both patients gave very heavy precipitates. The precipitin curve given

by the preparation from patient Od. and aggregated γ-globulin is given in Fig. 3. The preparations from both patients also gave very high agglutination titers with group O Rh-positive red cells coated with incomplete anti-Rh antibodies.

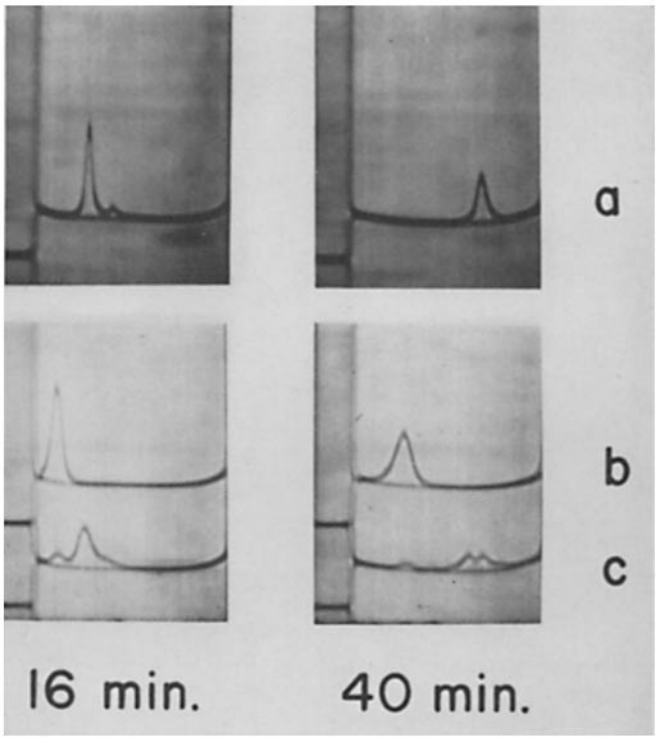

FIG. 2. Ultracentrifuge patterns of a 19S anti-γ-globulin preparation from patient Od. (*a*) and the corresponding dissociated (*b*) and reassociated (*c*) samples. The photographs were taken after 16 and 40 minutes at 52,640 RPM using a phase plate angle of 60 degrees.

Activity of the Dissociated Anti-γ-Globulins.—Included in Table I are the ultracentrifuge characteristics and activities of the same anti-γ-globulin preparations after reduction by ethyl mercaptan and alkylation by iodoacetamide. Reduction of the anti-γ-globulin preparations from either patient resulted in the expected dissociation to 7S subunits. The sedimentation pattern given by the dissociated anti-γ-globulin preparation from patient Od. is illustrated in Fig. 2. No materials sedimenting faster than 7S were detected in the dissociated Od. preparations. A relatively small quantity of 19S material usually remained in the dissociated Do. preparations.

The dissociated preparations from both patients failed to precipitate ag-

gregated γ-globulin. The precipitin curve given by a dissociated Od. preparation and aggregated γ-globulin is shown in Fig. 3. The dissociated Do. preparations also gave as routine only insignificant agglutination titers with group O Rh-positive red cells coated with incomplete anti-Rh antibodies. However, the dissociated Od. preparations consistently gave significant agglutination titers with red cells coated with incomplete anti-Rh Ripley. The dissociated Od. preparations also agglutinated red cells coated with other incomplete anti-Rh antibodies. The agglutination of the Ripley-coated cells by dissociated Od. anti–γ-globulin was not due to a specificity for the cells employed. The same group O cells, treated in the same manner as the Ripley-coated cells

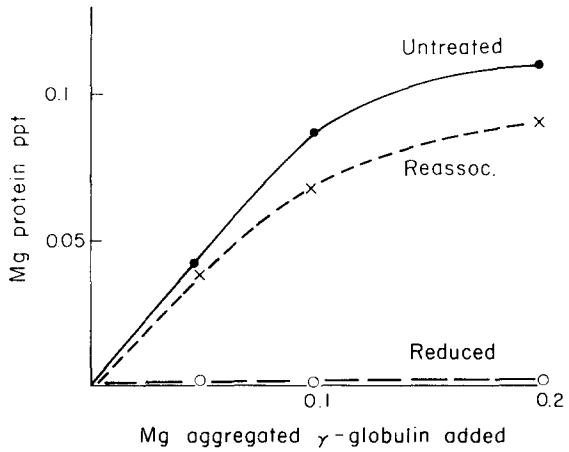

FIG. 3. Precipitin curves with aggregated γ-globulin given by dissociated (reduced), reassociated (reassoc.), and the original (untreated) 19S anti–γ-globulin from patient Od. Each point represents 0.06 mg of sample.

except that the incomplete anti-Rh antibody was omitted, were not agglutinated by dissociated Od. anti–γ-globulin. The activities toward aggregated γ-globulin and Ripley-coated cells of samples of either the Do. or Od. preparations when tested immediately following reduction were similar to those of the reduced and alkylated preparations described above.

Further studies of the agglutination reactions of the dissociated Od. anti–γ-globulin with red cells coated with incomplete anti-Rh antibodies, indicated considerable differences from the agglutination of the original 19S preparations. Most striking was a strong inhibition by Fr II γ-globulin, which was not evident in the original material. This is shown in Table II for a 1/320 dilution of the dissociated anti–γ-globulin; however, it was also evident at other dilutions. The original undissociated material showed very little effect of Fr II even at 1 mg/ml concentration at any dilution. Various myeloma proteins

also inhibited the dissociated system, but to various degrees which correlated with the H chain subgroup of the myeloma protein. The specificity of the dissociated system was also evident in its selective reaction with different anti-Rh antibody coats as compared to the parent 19S anti-γ-globulin.

The failure to detect any 19S material in the dissociated Od. preparation on ultracentrifuge analysis suggested that the anti-γ-globulin activity was associated with the 7S components. Density gradient analysis of the Od. anti-γ-globulin before and after dissociation demonstrated that this activity was indeed associated with the 7S components of the dissociated preparation and that prior to reduction the activity was present only in the 19S proteins. Examples of the density gradient fractionations are given in Fig. 4. In some ex-

TABLE II

Inhibition by Fraction II and Various 7S Type Myeloma Proteins of the Agglutination of Red Cells Coated with Incomplete Anti-Rh Antibodies (Ripley) Produced by Reduced and Alkylated 19S Anti-γ-Globulin from Serum Od

Inhibitor	Concentration of inhibitor added (mg/ml)					
	1.0	0.5	0.25	0.125	0.06	0.03
Fraction II	0	0	0	Tr.	1	2
Fe	0	0	0	0	1	3
Cu	0	0	0	0	1	2
Ne	0	Tr.	1	2	3	3
Saline control	3	3	3	3	3	3

The degree of agglutination for a 1:320 dilution of the dissociated preparation in the presence of the indicated inhibitor is given.

periments with the dissociated Od. preparation the activity recovered from the gradient was less than that which would have been expected from the activity of the sample placed over the gradient. The reason for this apparent loss of activity has not been established. However, in every instance, the only activity recovered was associated with the 7S materials. Small amounts of apparent activity were also noted in the fractions from the 7S region of the gradients on which the original Od. anti-γ-globulin preparations were run. This was only found when drops were collected from the bottom of the tubes and was due to a trailing phenomenon which occurred on collection. As noted previously (5) high concentrations of 19S material leave residual material at the bottom of the tube and frequently contaminate upper fractions. It is therefore important to always carry out experiments where samples from the various regions of the gradient are collected from the top by capillary tubes. In several experiments, fractions from the 7S and 19S regions were collected from the top of gradients on which untreated Od. anti-γ-globulin and dissociated Od.

anti-γ-globulin were run. Significant agglutination was only obtained in the upper fractions in the case of the dissociated anti-γ-globulin preparations and not for the undissociated material.

Fig. 5 illustrates the results of experiments on a different preparation of isolated Od. anti-γ-globulin. In this case the activities recovered from the

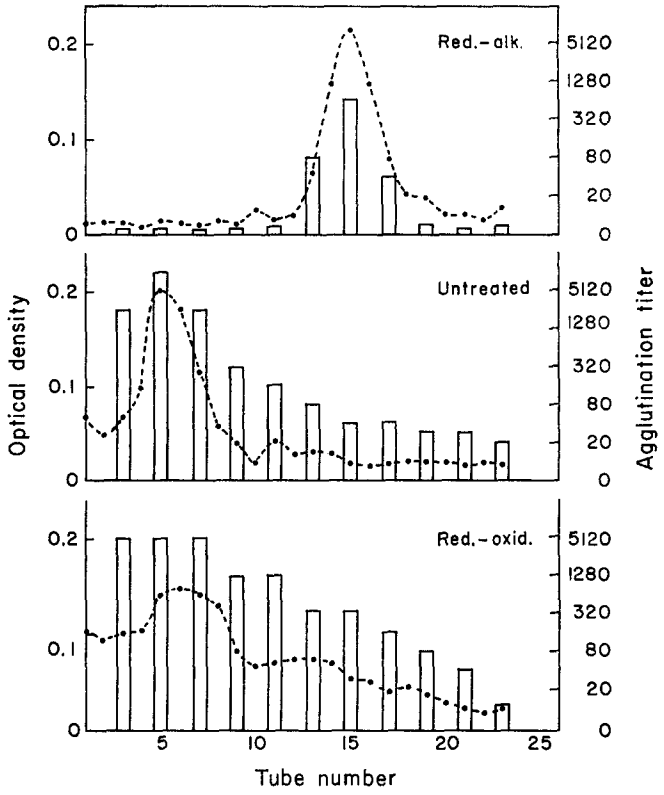

FIG. 4. Density gradient ultracentrifugation experiments showing the distribution of agglutinating activity against red cells coated with incomplete anti-Rh antibodies (Ripley) for an untreated 19S anti-γ-globulin from patient Od. and the corresponding dissociated (red.-alk.) and reassociated (red.-oxid.) samples.

gradient fractions were relatively low. However, the activity was entirely in the low molecular weight fractions and differed distinctly in distribution from the original material. The agglutination titer of the dissociated sample applied to the gradient was 1:5120 and that of the original material was 1:81,920. Also shown is the pattern obtained on a reduced sample which was not blocked with iodoacetamide and only partially reassociated on dialysis against buffer. Here two peaks of activity are evident; considerable activity was found in the

upper fractions. The agglutination titer of this sample before fractionation was 1:5120.

Direct evidence for the reaction of the 7S subunits of the 19S anti-γ-globulin preparations with human 7S γ-globulin was obtained by ultracentrifuge studies. The addition of human 7S γ-globulin (fraction II) to the dissociated anti-γ-globulin from either patient Od. or Do. at pH 7.5 resulted in the formation of a significant quantity of complex sedimenting at approximately 10S (Figs.

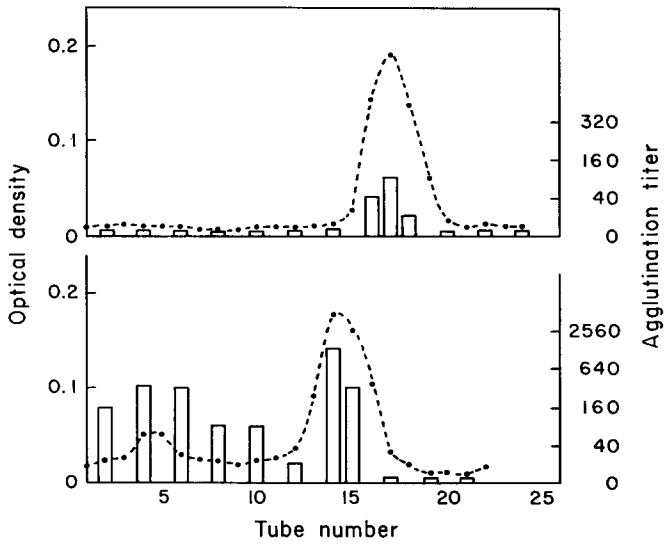

FIG. 5. Density gradient ultracentrifugation experiments showing the distribution of agglutinating activity against red cells coated with incomplete anti-Rh antibodies (Ripley) for a preparation of anti-γ-globulin from patient Od. after reduction and alkylation (upper section) and after reduction and partial reassociation (lower section).

6 *a* and 6 *b*). Control analyses of the fraction II and of the dissociated preparations before the addition of the fraction II clearly indicated that the complexes were not present in either before mixing. Complex formation was observed in all experiments with preparations of dissociated Od. and Do. although the amount of complex was somewhat variable. The Od. preparations consistently gave larger amounts of complex. Evidence was obtained that not all of the dissociated material complexed with Fr II, but in some experiments it exceeded 50 per cent.

Activity of the Reassociated Anti-γ-Globulins.—Also listed in Table I are the ultracentrifuge characteristics and activities of the same anti-γ-globulin proparations after reduction and reassociation.

Reassociation of reduced Do. anti-γ-globulin resulted in the return of its

capacity to precipitate aggregated γ-globulin. The reduced and reassociated Do. anti-γ-globulin preparations also gave agglutination titers with red cells coated with incomplete anti-Rh antibodies which were significantly higher than the titers given by the corresponding reduced and alkylated samples or samples tested immediately following reduction. On the other hand, the titers given by the reassociated Do. preparations were much less than those of the original anti-γ-globulin preparations. The failure to regain the initial activity may be due to the presence of inactive fragments from foreign 19S γ-globulins

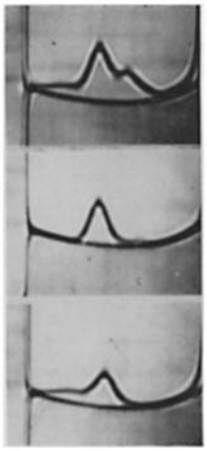

FIG. 6 *a*

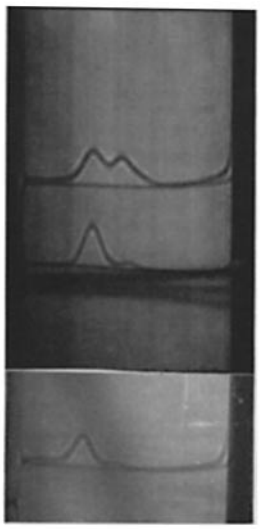

FIG. 6 *b*

FIGS. 6 *a* and 6 *b*. Ultracentrifuge patterns showing complexing of γ-globulin with reduced and alkylated anti-γ-globulins (Fig. 6 *a*, Do.; Fig. 6 *b*, Od.). The lower pattern in each case shows the reduced anti-γ-globulin; the middle pattern, Fr II γ-globulin; the upper pattern, the mixture of the two. The photographs were taken after 60 minutes at 52,640 RPM.

in the preparations. Previous studies from this laboratory (4, 5) demonstrated that the addition of reduced inactive fragments from heterologous 19S γ-globulin to a reduced anti-γ-globulin resulted in a significantly lower activity for aggregated γ-globulin on reassociation than the appropriate control reassociated in the absence of the reduced heterologous fragments. In the present study, similar observations were noted for reduced Do. anti-γ-globulin reassociated after the addition of an equal or twofold quantity of reduced foreign macroglobulin. Both the precipitin and agglutination activities were lower than those of controls carried through the same steps except for the addition of the reduced inactive macroglobulin. Treatment of the reduced and reassociated Do. anti-γ-globulins with iodoacetamide did not significantly alter their activity. This fact together with the observation that the activities of samples

when tested immediately following reduction were similar to those of samples tested after reduction and alkylation, indicates that the differences in activities between the dissociated and reassociated preparations were not due to the alkylation step.

The ultracentrifuge pattern of the reduced and reassociated Do. anti-γ-globulin was complex. The principal components sedimented at 7S and 15S. Also present in smaller amounts were components sedimenting at 11S, 17S, and 19S. Density gradient analysis of reduced and reassociated Do. anti-γ-globulin demonstrated that the activity was associated with the faster sedimenting components. However, due to the limitations of the method, the exact sedimentation rate of the reassociated active molecules could not be determined. The activity of the original preparation was located in the 19S fractions.

Reassociation of the reduced Od. anti-γ-globulin resulted in the return of its capacity to precipitate aggregated γ-globulin. The precipitin curve given with the aggregated γ-globulin is shown in Fig. 3. The agglutination titers with red cells coated with incomplete anti-Rh antibodies of the reassociated Od. preparations were equal to or somewhat higher than those of the corresponding dissociated preparations. However, density gradient analysis showed that on reassociation, the activity usually shifted from the 7S components to the faster sedimenting components. Partial reassociation gave two peaks of activity as shown in Fig. 5. The ultracentrifuge pattern of reduced and reassociated Od. anti-γ-globulin was not as complex as that of the reassociated Do. preparation. An example is illustrated in Fig. 2. Two components about equal in size sedimenting at 16S and 18S and a small amount of 7S material were present. As was also observed with reassociated Do. preparations, treatment of reduced and reassociated Od. anti-γ-globulin with iodoacetamide did not alter its reaction with aggregated γ-globulin or red cells coated with incomplete anti-Rh antibodies.

DISCUSSION

The results of this study indicate that the 7S fragments produced from human 19S anti-γ-globulins by reduction retain the capacity to combine with 7S γ-globulin. The activities of the fragments made themselves evident in a number of ways. First, in the case of the fragments from one of the 19S anti-γ-globulins, the capacity to agglutinate red cells coated with incomplete anti-Rh antibody was partially retained. The inhibition of this agglutination by human fraction II and by various myeloma proteins of the 7S γ-globulin class further demonstrated that this activity represented a specificity for human 7S γ-globulin. Secondly, the direct reaction between the fragments from the 19S anti-γ-globulins and human 7S γ-globulin was demonstrated by analytical ultracentrifugation. This was evident for dissociated preparations from 19S anti-γ-globulins of both sera studied.

The exact reason for the agglutinating capacity of the dissociated fragments

is not clear. Certainly the specificity observed and its selective inhibition by certain 7S γ-globulins was quite different from the parent 19S protein. The possibility exists that these fragments are divalent, but it also seems possible that their simple attachment to the sensitized cells brought about agglutination. It is known that alteration in the medium will under certain circumstances bring about agglutination of such anti-Rh–coated cells even without a secondary antibody as used in the Coombs' test. The 10S complexes noted by analytical ultracentrifuge analysis of fragments and 7S γ-globulin were compatible with simple Ag-Ab units although the possibility of Ag_2-Ab units could not be ruled out. Further exact quantitative studies of these complexes and the complexes produced by the original macroglobulin with different types of 7S γ-globulin should prove of considerable interest. The failure of the fragments to precipitate aggregated γ-globulin might be cited against a divalent state since divalent rabbit 7S antibodies readily cause precipitation.

In experiments with anti-Rh antibodies isolated by chromatography on DEAE cellulose and believed to be of the 19S γ-globulin class, Chan and Deutsch (3) reported that agglutinating activity was destroyed by 2-mercaptoethanol and obtained evidence that the mercaptoethanol dissociated antibody was still capable of combining with the antigen. However, the authors failed to demonstrate antibody activity after mercaptoethanol treatment of the 19S antibody isolated by ultracentrifugation in a sucrose density gradient. The reason for the discrepancy between the two experiments was not determined. More recently, Jacot-Guillarmod and Isliker (7) have reported evidence for the production of active fragments by reduction with $NaBH_4$ of human 19S isoagglutinins and rabbit 19S anti-sheep red cell antibody.

The results of the present study also demonstrate that the reduced and reassociated anti-γ-globulin preparations have antibody properties similar to those of the untreated preparations. The return on reassociation of precipitin activity for the reduced anti-γ-globulins and the return of agglutinating activity may be due to an increase in the valence accompanying reassociation. The possible alteration of the active site by the reduction process and the subsequent reversal of this alteration by oxidation to polymeric forms must be considered at least for some of the specificities in these anti-γ-globulin preparations. The important role of reformed disulfide bonds in the reassociated molecules was apparent from a variety of studies. Reduction with ethyl mercaptan again resulted in a loss of precipitin and the bulk of agglutinating activity with a fall in *s* rate. Attempts to dissociate the reassociated polymers with acid and urea were unsuccessful indicating that the units were indeed linked by reformed disulfide bonds.

In view of the reactivity of dissociated fragments and the reassociated preparations, it seems surprising that the loss of activity on reduction with sulfhydryl compounds can be successfully utilized as a test for 19S antibodies.

One explanation is that conditions which are widely used for such purposes, mercaptoethanol at concentrations of 0.1 M or greater and reduction periods of 16 hours or longer, result in the irreversible destruction of the active sites. It is interesting to note that under the conditions employed in the present study, mercaptoethanol gave activities for reduced and reassociated anti-γ-globulins which were similar to those obtained with the ethyl mercaptan. However, the number of experiments with mercaptoethanol is too small to permit a detailed comparison of the two reducing reagents. Another probable reason for the failure to see this more frequently may be due to the presence of a large quantity of inactive molecules in the usual antibody preparations. The preparations used in this study contained a relatively high percentage of active molecules. In the case of Od. absorption experiments with aggregated γ-globulin indicated that at least 75 per cent of the 19S preparation represented anti-γ-globulins. Therefore, on reassociation the chances for active fragments coming together are relatively great. It has been demonstrated in previous studies (4, 5) and confirmed in the present study that the addition of foreign fragments during the reassociation lowers the activity of the reassociated preparation. In certain experiments a gradual increase in the agglutinating activity of reassociated Do. preparations was observed over a period of several weeks. A similar gradual increase in activity was also observed for reduced and reassociated preparations in earlier experiments (5). This may have been due to further reassociation as ultracentrifuge analysis of reassociated preparations showed the presence of 7S material in addition to the faster sedimenting components. Other possibilities include rearrangement of the subunits to some optimal configuration or perhaps the slow restoration of the active site.

While the conditions employed in the present study gave satisfactory results, no systematic investigation of the conditions for the reduction and reassociation were undertaken. The use of acid buffer for the gel filtration on sephadex G-200 proved particularly useful in the separation of the 19S components from various other constituents of the euglobulins. The acid buffers caused dissociation of the various complexes of γ-globulin–anti-γ-globulin in these preparations which was essential for the isolation of 19S anti-γ-globulins free of inhibiting 7S material. It seems possible that previous failure to demonstrate activity for the dissociated fragments was due to small amounts of contaminating 7S γ-globulin. The marked inhibitory effect of small amounts of γ-globulin on the activity of the dissociated fragments noted in the present study makes this a likely possibility. The measurement of combining activity through complex formation in analytical ultracentrifuge experiments had not been utilized before. Previous studies (4, 5) had demonstrated the reassociation with return of activity for certain unusual anti-γ-globulins occurring in extreme amounts in macroglobulinemia sera. The present investigations indicate that similar results can be obtained with the common types of anti-γ-globulins provided these are isolated in a high state of purity.

SUMMARY

19S anti-γ-globulins were isolated in a high state of purity from the sera of two patients with rheumatoid arthritis. Following reduction with ethyl mercaptan and alkylation by iodoacetamide, fragments were produced which retained the capacity to combine with 7S γ-globulin.

The fragments from one of the 19S anti-γ-globulins agglutinated red cells coated with incomplete anti-Rh antibodies. This activity was shown by density gradient ultracentrifugation to be associated with low molecular weight fractions. The agglutination of the coated red cells by the fragments was strongly inhibited by normal and myeloma 7S γ-globulins and showed a greater specificity than the parent 19S material.

Analytical ultracentrifuge experiments demonstrated that the fragments from either of the 19S anti-γ-globulins formed complexes with 7S γ-globulin.

Reassociation of the dissociated fragments through reformation of disulfide bonds resulted in the formation of fast sedimenting molecules having properties similar to those of the untreated 19S material in respect to precipitation with aggregated γ-globulin and agglutination of coated red cells.

The authors wish to acknowledge the generosity of Dr. Israeli A. Jaffe for providing one of the sera (Od.) and Dr. Marion Waller for one of the anti-Rh antibody coats (Ripley).

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