STUDIES ON THE MECHANISM OF SOLUBILIZATION OF IMMUNE PRECIPITATES BY SERUM*

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Immune precipitates formed by bovine serum albumin (BSA) and antibodies to BSA dissolve rapidly when incubated in fresh serum (1). The solubilized complexes are aggregates of antigen (Ag), antibody (Ab), and complement (C) components (C3). The involvement of C in this activity of serum (complex-release activity or CRA) was postulated because treatments that destroyed C in serum also removed CRA. The present paper further clarifies the mechanism of CRA. It will be shown that (a) CRA of serum is observed with many Ag-Ab systems and therefore appears to be a general phenomenon. (b) The rate of solubilization of complexes varies in different Ag-Ab systems and is strongly influenced by the affinity of Ab for Ag in the immune precipitate. (c) CRA is a function of the properdin pathway of C activation. (d) C-mediated CRA does not involve proteolysis of the Ab, and (e) CRA can also be achieved by the specific binding of a large peptide onto the Fab or Fc portions of the Ab that is part of the immune precipitate. Based on these results we propose that CRA is caused by the intercalation of C fragments (C3b, C4b) into the lattice formed by Ag and Ab.

Materials and Methods

Diluents. CRA assays were carried out in phosphate-buffered saline (PBS, Grand Island Biological Co., Grand Island, N. Y.), pH 7.4, which contains Ca++ (7 × 10^-4 M) and Mg++ (5 × 10^-4 M).

Antigens and Antibodies. Pneumococcal polysaccharide type VII (S VII) was obtained as described by Tyler and Heidelberger (2) and was a gift from Dr. M. Heidelberger. Dextran was kindly supplied by Dr. M. Leon. Ovomucoid (OVM) and ovalbumin (OA) were purchased from Sigma Chemical Co., St. Louis, Mo. Bovine gamma globulin (BGG) and bovine serum albumin (BSA, 2 × crystallized) were purchased from Pentex Inc., Kankakee, Ill. BSA was purified on Sephadex G-200 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) to remove aggregates. BSA and BGG were conjugated with 2,4-dinitrophenol (DNP), as described in (3) by reaction of 1-fluoro-2,4-dinitrobenzene and dioxane under alkaline conditions, followed by extensive dialysis. Protein concentrations were determined by Kjeldahl analysis, and the degree of substitution was calculated by absorbancy at 360 nm. DNP-BSA and DNP-BGG had 32 and 57 DNP mol per mol of carrier protein. Normal rabbit immunoglobulin (IgG) was purchased from Miles Laboratories, Kankakee, Ill. and further purified on a DEAE-cellulose column. Normal sheep IgG and guinea pig IgG (γ-2)

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Abbreviations used in this paper: BGG, bovine gamma globulin; BSA, bovine serum albumin; CFA, complete Freund's adjuvant; CRA, complex release activity; DNP, 2,4-dinitrophenol; OA, ovalbumin; OVM, ovomucoid; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate.
were prepared by passage through DEAE-cellulose columns after precipitation from serum in 40% \((NH_4)_2SO_4\). Purified mouse myeloma protein (MOPC 104E) of the IgM class, with specificity for dextran, was purchased from Litton Bionetics, Inc., Kensington, Md. A globulin fraction from a rabbit antiserum to pneumococcus S VII was given to us by Dr. M. Heidelberger. Antibodies to OVM were raised in CBA/J mice (The Jackson Laboratories, Bar Harbor, Maine) by injection of 250 \(\mu g\) of OVM in complete Freund's adjuvant (CFA, Difco Laboratories, Detroit, Mich.) into the foot pads (total volume injected 0.10 ml) and bleeding 4 wk later. Mouse and rabbit antibodies to BSA and rabbit antisera to mouse Ig were obtained as previously described (1, 4). Rabbit antisera to F(ab')\(_2\) fragments of guinea pig IgG (y2) and to DNP-BGG were obtained by injection of 1 mg of purified protein emulsified in CFA into the footpads (total volume injected 1 ml) and bleeding 2-12 wk later. Sheep antisera against the Fab fragment of rabbit IgG was obtained by Dr. M. Lamm by injection of purified Fab (5) emulsified in CFA. Sheep antiserum to rabbit IgG containing a high concentration of antibodies to the Fc portion of the rabbit IgG was a gift from Dr. G. J. Thorbecke. It was extensively adsorbed with F(ab')\(_2\) fragments of rabbit Ig conjugated to Sepharose beads activated with CNBr (6). Ig fractions of both sheep antisera (anti-Fab, anti-Fc) were prepared as described above, and their specificities were verified by immunoelectrophoresis against a papain digest of rabbit IgG. In addition the sheep antirabbit Fab gave a reaction of identity by double diffusion in agar gels between rabbit F(ab')\(_2\) of IgG and native IgG. The sheep antirabbit Fc did not precipitate rabbit F(ab')\(_2\) in liquid or gelled media. The antibody concentration of all antisera was determined by quantitative precipitation (7) as described in (8).

**Preparation of Fab, Fab', and F(ab')\(_2\)** Fab fragments of normal or immune sheep Ig and of rabbit IgG were obtained as in reference 9, except that after digestion with papain the fragments were separated from undigested material by chromatography on Sephadex G-200 columns. F(ab')\(_2\) fragments were also prepared as in reference 9. Fab' fragments of rabbit antibodies to mouse Ig were prepared by reduction of F(ab')\(_2\) with mercaptoethylamine hydrochloride (9) and further purified by chromatography on Sephadex G-100 columns. Two protein peaks were eluted, one containing Fab' and the other nonreduced F(ab')\(_2\).

**Determination of the Binding Affinity (\(K_0\)) of Rabbit Anti-DNP Antibodies.** Sera were obtained 2 wk and 12 wk after the immunization of rabbits with DNP-BGG. The IgG fractions were prepared, and the concentration of antibodies to DNP was determined by specific precipitation with DNP-BSA. Affinities (\(K_0\)) were estimated by the method of Stupp et al. (10) with [\(^3\)HIDNP-e-aminocaproic acid (synthesized by Dr. J. Quagliata) as hapten.

**Iodination of Proteins.** Antibodies and protein antigens were iodinated by the chloramine T method (11), as described in reference 12. The specific activity of labeled proteins was about 2 \(\times\) 10^6 cpm/\(\mu\)g. Labeled proteins were frozen at \(-20^\circ\)C and used within 5 wk.

**Complement Components and C-Deficient Sera.** Human C2, C3, and guinea pig C4 were bought from Cordis Laboratories, Miami, Fla. Factor B of the properdin pathway, prepared as in reference 13, was a gift from Dr. O. Götte and it showed a single band by acrylamide gel electrophoresis. Human C2- and C3-deficient sera were gifts from Dr. N. K. Day, and C4-deficient guinea pigs were obtained from Dr. M. Frank. Sera to be used as a source of C were kept at \(-70^\circ\)C.

**Assay for CRA.** Based on data from quantitative precipitation curves, immune precipitates were prepared at different Ag-Ab ratios with \(^{125}\)I-labeled antigen or \(^{125}\)I-labeled IgG fraction of the specific antiserum, washed with PBS, resuspended in PBS, and finely divided by passage through a 27-gauge needle. Immediately before incubation at 37°C, the precipitates were added to reaction mixtures containing serum diluted with PBS. The final vol of the mixtures varied from 200-400 \(\mu\)l. Samples (50 \(\mu\)l) were withdrawn at certain time intervals and placed in 10 \(\times\) 75-mm test tubes which contained between 0.25-1 ml of an ice-cold suspension of 0.05% sheep erythrocytes in 0.15 \(M\) NaCl (the erythrocytes were added as a marker to facilitate decantation). After centrifugation at 1200 \(g\) at 4°C for 10 min, the tubes were decanted, and both pellets and supernatant fluids were counted in a gamma counter. Results were generally expressed as percent solubilization (100 \(\times\) cpm in supernatant fluids/total cpm). Controls consisted of the same complexes incubated in serum pretreated with zymosan (1) or serum heated at 56°C for 30 min.

For the solubilization of immune precipitates by Fab fragments, the same general procedure was used, except that the reaction mixture (150 \(\mu\)l) contained Fab fragments instead of serum, 2 \(\times\) 10^-3 M recrystallized iodoacetamide (Schwarz/Mann, Orangeburg, N. Y.), and 0.5 mg OA to reduce nonspecific binding of proteins to the glass tube.
TABLE I

Antigen-Antibody Complexes Solubilized by Serum

| Antigen                  | Species of origin of antibody          |
|--------------------------|----------------------------------------|
| BSA                      | Rabbit, mouse (7S)                     |
| Pneumococcal S VII       | Rabbit (7S)                            |
| OVM                      | Mouse (7S)                             |
| Guinea pig F(ab')<sub>2</sub> of IgG<sub>2</sub> | Rabbit (7S)                           |
| Dextran                  | Mouse (MOPC-104E) (19S)               |
| DNP-BSA                  | Rabbit (7S)                            |

Immune precipitates were prepared in Ab excess, at equivalence, and in Ag excess. Various amounts of these precipitates were treated with serum. In each case, CRA was detected. The rates of solubilization, however, were considerably different. For example, 150 µl fresh rabbit serum solubilized 5.2 µg of an immune precipitate containing anti-S VII and S VII within 4 min and 0.75 µg of an immune precipitate containing anti-DNP and DNP-BSA only after 50 min. Both precipitates were prepared at equivalence.

**Acrylamide Gel Electrophoresis.** Samples of solubilized products were either treated with 1% sodium dodecyl sulfate (SDS) for 1 h at 60°C or denatured and reduced by incubation in 1% SDS, 5% 2-mercaptoethanol, and 0.5 M urea in 0.1 M Tris buffer, pH 8.3, for 1 h at 60°C. Analysis of these samples by acrylamide gel electrophoresis was as described by Shapiro et al. (14), except that the gels were removed from the glass tubes, frozen with dry ice, and cut into 1-mm fractions with a Mickle slicer. The radioactivity in each fraction and in the uncut end of the gel were counted on a gamma counter.

**Results**

**Solubilization of Ag-Ab Complexes by Serum (CRA) is a General Phenomenon.** CRA was initially observed with immune precipitates of BSA and mouse or rabbit anti-BSA (1). Listed in Table I are other immune precipitates which are solubilized by serum. This serum and the antibody in the immune precipitate do not have to originate from the same species of animal. Specific precipitates, prepared with mouse antibodies to BSA and BSA, are solubilized at different rates by pooled serum from different species. In general, we found that CRA of rat > human > mouse > guinea pig > rabbit serum with precipitates prepared with mouse antibodies.

**Effect of the Affinity of Antibody on CRA.** Although solubilization occurred in all Ag-Ab systems tested, its rate varied considerably even when the same source of C was used. For example, in rabbit serum, precipitates of rabbit anti-S VII and S VII are more rapidly solubilized than identical amounts of rabbit anti-BSA and BSA. Furthermore, for a given amount of serum, there is a maximum quantity of immune precipitate which can be solubilized, and this varies for different Ag-Ab systems. We calculated that the maximum amount of precipitate that can be solubilized by 0.4 ml of a one-half dilution of rabbit serum contains about 1.8 µg rabbit (IgG) anti-BSA. Under the same conditions, more than 50 µg rabbit (IgG) anti-S VII can be released from immune precipitates.

One of the parameters that affects and may explain these differences is the affinity of the Ab for the Ag in the precipitate. In Fig. 1 we compare CRA among complexes prepared with anti-DNP antibodies of different affinities for the
Solubilization of Immune Precipitates by Serum

Fig. 1. Effect of affinity of antibody on CRA. Immune precipitates prepared at various Ab/Ag ratios were prepared with \(^{125}\)I-labeled DNP-BSA and purified rabbit IgG fractions that contained either high (\(K_o = 10^8\) liters/mol) or low (\(K_o = 2 \times 10^7\) liters/mol) affinity antibodies to DNP. Aliquots containing 0.6 µg of complexed Ab were incubated at 37°C with human serum (100 µl) in a total volume of 300 µl.

Hapten and show that rates of solubilization are considerably higher for the antibodies of lower affinity at all Ag-Ab ratios tested.

Solubilization is C Mediated. The involvement of C in the process of solubilization was previously suggested because CRA was inhibited at 0°C, in the presence of chelating agents, and by pretreatment of the serum with zymosan or cobra venom factor (1). Direct evidence for the participation of C is shown in Figs. 2-4. CRA of human serum is destroyed at 50°C for 20 min. Total restoration of activity is achieved by the addition of physiological concentrations of purified factor B (Fig. 2).

Also, CRA was studied in a human serum lacking C3, but containing close to normal concentrations of all other C components of the classical pathway (15). It failed to solubilize immune precipitates even when undiluted, although normal human serum rapidly solubilized the same complexes when diluted one-twentieth. The addition of purified human C3 to the deficient serum partially
restored CRA and the rate of solubilization was dependent on the quantity of C3 added. Human C3, in the absence of serum, failed to solubilize these complexes at all concentrations tested (Fig. 3).

Involvement of the classical pathway of C activation in the release process was demonstrated by the addition of purified C2 to the serum of a patient who was homozygous for C2 deficiency (Fig. 4) or of C4 to the sera of C4-deficient guinea pigs (not shown). Although CRA was detected in the deficient sera, addition of the missing C components led to enhanced CRA in each instance.

_Solubilization Does Not Involve Extensive Proteolysis of Ab in the Precipi-

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**Fig. 2.** Solubilization is dependent on factor B. In a reaction mixture of 200 μl, fresh human serum (50 μl), which had been heated at 50°C for 20 min, failed to solubilize 0.35 μg immune precipitates prepared at equivalence with rabbit 125I-labeled anti-BSA and BSA. After supplementation with 15 μg purified human factor B, the rate of solubilization was nearly the same as that of the tube containing unheated serum. Purified factor B (15 μg), by itself, failed to solubilize complexes.
FIG. 3. CRA is dependent on the concentration of C3. Human C3-deficient serum (15) failed to solubilize immune precipitates at all dilutions tested unless C3 was added to the reaction mixtures (final vol 200 μl) which contained: 0.05 μg 131I-labeled rabbit anti-BSA precipitated with BSA in Ab excess, normal or C3-deficient human serum, and various amounts of C3 (1,000 CH₅₀ U/ml). C3 alone at all concentrations or heated human serum did not solubilize immune precipitates.

FIG. 4. Solubilization is enhanced by C2. Human C2-deficient serum solubilized immune precipitates in reaction mixtures (final volume 200 μl) which contained 2.8 μg rabbit anti-BSA complexed with 131I-labeled BSA in Ag excess. Solubilization was enhanced when human C2 (75 CH₅₀ U) was added. Solubilization did not occur in normal or C2-deficient human serum heated at 56°C for 30 min and supplemented with different amounts of C2.
tates. One explanation for the phenomenon of solubilization is that proteolytic activity is generated during C activation leading either to the fragmentation of the Ab and formation of nonprecipitating monovalent Fab-like fragments or to the alteration of the combining site. By acrylamide gel electrophoresis, however, we did not detect fragments of Ab among the solubilized products which had been treated with sodium dodecyl sulfate (Fig. 5 a), but did consistently find labeled material of high molecular weight in such preparations and not in the controls. This material is probably aggregated Ab and/or Ab molecules complexed with C components. It is unlikely that this material of high molecular weight is fragmented Ab which became aggregated under the conditions used to dissociate Ag from Ab, because after complete reduction and alkylation of the solubilized Ab, no fragments of labeled H or L chains were detected by acrylamide gel electrophoresis (Fig. 5 b).

Additional evidence against the hypothesis that a simple enzymatic reaction determines the solubilization of complexes is shown in Fig. 6. Various amounts of immune precipitate were added to a constant volume of a dilution of rabbit serum, and the rates of solubilization were determined. After subtracting backgrounds, we calculated the absolute amounts of antibody that were solubilized in the presence of excess immune precipitate after different periods of incubation. In several runs we found that the maximum amounts of solubilized complexes generated by a constant amount of serum were independent of the initial concentrations of immune precipitate. For example, in the experiment shown, 2.8, 5.6, and 11.2 μg of BSA-precipitated rabbit antibody to BSA were incubated at 37°C with 400 μl of a one-half dilution of rabbit serum. After the reaction reached completion, about 61, 32, and 13% of the antibody had been released, that is 1.7, 1.8, and 1.5 μg, respectively. Furthermore, the amount of released antibody did not increase by prolonging incubation at 37°C. These results are compatible with solubilization as a result of a stoichiometric reaction, not an enzymatic degradation.

**Immune Precipitates are Solubilized by Specific Fab Fragments.** It was previously reported (1) that Fab fragments of rabbit antimouse Ig rapidly solubilized immune precipitates of BSA and mouse anti-BSA. This implies that the binding of a large peptide to Ig molecules is sufficient to disrupt the Ag-Ab lattice, thus increasing the solubility of the immune complexes. This finding led to the hypothesis that solubilization of immune precipitates by C might occur by a similar process. In this case, fragments of C components, such as C3b, would intercalate into the lattice.

We show here that (a) a large peptide that is believed to intercalate into the lattice must be monovalent for solubilization of immune precipitates to occur. This was clearly shown when the effects of Fab' and F(ab')2 on immune precipitates were compared. Various amounts of Fab', and F(ab')2 were mixed with washed precipitates of mouse antibodies to BSA (2 μg) and 125I-BSA, incubated at 37°C for 30 min, and centrifuged. Fab' and Fab solubilized the immune precipitates with almost the same efficiency (Table II). Not only did F(ab')2 fail to solubilize the immune precipitates, but it decreased their solubility to levels below that of the controls.
The peptide that intercalates into the lattice can solubilize immune precipitates by binding either to the Fab or the Fc portions of the Ig in the precipitates. As shown in Fig. 7, a precipitate of rabbit anti-S VII and S VII can be solubilized by Fab of sheep antirabbit Fab or Fc. About 3 μg of either type
of specific Fab were required to solubilize about 50% of the precipitates containing 0.6 μg antibody. This is a ratio of about 15 molecules of Fab per molecule of Ab. The specificity of the Fab reagents is demonstrated in Fig. 8. Normal rabbit IgG inhibited solubilization by the Fab fragments of both anti-Fab and anti-Fc, but F(ab')2 fragments of nonspecific rabbit IgG inhibited only the solubilization by Fab anti-Fab.

Discussion

We show here that solubilization of Ag-Ab aggregates by fresh serum is a general phenomenon mediated by the C cascade. The species of origin of the Ab in the complexes and of the serum used as a source of C can be different. Ag-Ab complexes prepared with rabbit or mouse Ab are solubilized by mouse, rat, rabbit, guinea pig, or human C, which suggests that both the C activation sites and the targets of the reaction within the complexes may be similar among
FIG. 6. Effect of concentration of immune precipitates and time of incubation at 37°C on C-mediated CRA. Various concentrations of immune precipitates consisting of 125I-labeled rabbit anti-BSA and BSA at equivalence were treated with 200 µl fresh or heated (56°C for 30 min) rabbit serum in a reaction mixture of 400 µl. After incubation at 37°C, samples (50 µl) were withdrawn at intervals, placed in tubes (10 x 75 mm) that contained 0.25 ml ice-cold 0.05% SRBC, and centrifuged. Note that although the rates of solubilization are different, the maximum amount of precipitate that can be solubilized by 200 µl rabbit serum under these conditions contains about 1.7 µg of Ab.

### TABLE II

| Nature of fragments of rabbit antimouse Ig | Amount of specific fragment µg | Solubilization % |
|-------------------------------------------|-------------------------------|------------------|
| Fab                                       | 1.0                           | 10.1             |
|                                           | 2.5                           | 45.5             |
|                                           | 5.0                           | 90.4             |
|                                           | 10.0                          | 93.2             |
| Fab'                                      | 1.0                           | 15.5             |
|                                           | 2.5                           | 78.4             |
|                                           | 5.0                           | 93.4             |
|                                           | 10.0                          | 93.8             |
| F(ab')2                                    | 1.0                           | 2.1              |
|                                           | 2.5                           | 2.4              |
|                                           | 5.0                           | 2.3              |
|                                           | 10.0                          | 2.7              |
| Control                                   | -                             | 7.8 ± 0.9*       |

* Mean ± SD.
different species and were well preserved during evolution. The absolute amount of complexes that can be solubilized by C varies widely in different Ag-Ab systems. The explanation for these differences is not clear, but one factor is the affinity of the antibody for Ag in the precipitate. Precipitates made with antibodies to DNP of higher affinity for the hapten required a higher concentration of C and a longer period of incubation at 37°C.

Solubilization by C is mediated through the properdin pathway. It proceeds in the absence of Ca++ (1), but not in the absence of Mg++, C3, factor B (this paper), properdin, or factor D (Takahashi, Brade, and Nussenzweig, in preparation). The enhancement of CRA by the addition of Ca++ (1), or of C2 and C4 to C2- and C4-deficient sera can be explained by the generation of C3b through the classical pathway and its positive feedback effect on the properdin pathway (16). The possibility, however, that CRA can also be mediated directly through the classical pathway, without participation of the properdin pathway, cannot be ruled out.

The mechanism of solubilization does not appear to involve degradation of the
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Fig. 8. The precipitates described above were mixed with 15 μg (3 × 10⁻¹⁰ mol) of Fab fragments of sheep anti-rabbit Fab or sheep anti-rabbit Fc. Various concentrations of purified rabbit IgG or purified rabbit Fab(Fab')₂ were added to these mixtures that were incubated for 2.5 h at 25°C and treated as described above. Addition of Fab(Fab')₂ inhibited the activity of Fab anti-Fab, but not of Fab anti-Fc. Both activities were inhibited by rabbit IgG. Symbols: (Δ—Δ) Fab anti-Fab (solubilizing agent) and rabbit Fab(Fab')₂ (inhibitor); (■—■) Fab anti-Fab (solubilizing agent) and rabbit F(ab')₂ (inhibitor); (▪—▪) Fab anti-Fc (solubilizing agent) and rabbit IgG (inhibitor).

complexed antibody. By acrylamide gel electrophoresis we failed to detect small molecular weight products of Ag among solubilized complexes.

An alternative explanation for the phenomenon of solubilization is that it follows the incorporation of fragments of C components, such as C3b and C4b, into the Ag-Ab lattice. These high mol wt peptides (175,000 and 200,000, respectively) are generated by enzymatic cleavage of C3 and C4. C3b and C4b have labile "nonspecific" combining sites, perhaps of a hydrophobic nature, for constituents of the cell membrane and also for immunoglobulin (17-19). It is generally thought that the state of activation of C3b and C4b is of extremely
short duration and that, consequently, activated C3b and C4b bind only to acceptor sites in molecules that are in close proximity. Otherwise they become inactive. According to the intercalation hypothesis the binding of these peptides on to the Ag-Ab aggregates would inhibit lattice formation and reverse precipitation.

The main arguments to support the intercalation hypothesis are: (a) solubilization appears to be a stoichiometric reaction and not an enzymatic process. The maximum amount of complexes that can be solubilized by individual sera is independent of the initial concentration of complexes, cannot be increased by prolonged incubation, and correlates well with the concentration of C3 in the serum (Czop, J. K., A. Ferreira, and V. Nussenzweig, manuscript in preparation); (b) C3 and C4 (Takahashi, M., and V. Nussenzweig, unpublished observation) fragments are found on solubilized complexes (1); and (c) immune precipitates are solubilized by Fab fragments specific for the Ig in the precipitates. The possibility that this key finding is an artefact due to the persistence of papain adsorbed on the Fab fragments is very unlikely because as shown here, Fab-mediated CRA proceeds rapidly in the presence of $2 \times 10^{-3}$ M iodoacetamide. Moreover, Fab' fragments, but not F(ab')$_2$ fragments of rabbit antimouse Ig, solubilize immune precipitates as effectively as Fab fragments. Even if pepsin was present and active at pH 7.4 only in the preparations of Fab' and not in those of F(ab')$_2$, the resulting product would be F(ab')$_2$ fragments and no solubilization should have occurred.

Fab and Fab' fragments solubilized complexes at ratios of about three molecules of the monovalent reagent to one molecule of antibody in the precipitate. However, at all concentrations, F(ab')$_2$ decreased the solubility of the immune precipitates, probably because they further cross-linked the antibody in the precipitate. According to these findings, the binding of a large monovalent peptide onto the Fab or Fc portions of the antibody may alter its ability to precipitate in the presence of antigen. By analogy, if the intercalation hypothesis is correct, the C fragments which solubilize the complexes should be functionally monovalent. In contrast, C components such as Clq, which have multiple binding sites for Ig, should decrease the solubility of immune complexes.

How specific Fab anti-Ig (and perhaps C fragments) interfere with lattice formation and precipitation is not clear. The bulky peptides may sterically interfere with the primary binding of antibody to antigen, or induce conformational changes in the combining site, or diminish the flexibility of the Fab arms around the hinge region and thereby restrain the cross-linking of antigen molecules. The Fab fragments may also prevent lattice formation by inhibiting nonspecific Ig-Ig interactions which occur during specific precipitation. For example, Nisonoff and Pressman (20) acetylated specific antibodies to increase their negative charge. Antibodies, acetylated at low levels, did not precipitate with homologous antigens although the combining site was not affected. Apparently, the forces involved in Ag-Ab interaction were not strong enough to bring the Ag and the mutually repelling Ab molecules together. In contrast to acetylation, iodination of antibodies enhances precipitation apparently by increasing hydrophobic interactions between neighboring antibodies (21). It is possible that
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C3 fragments (and perhaps other C components) incorporate into the lattice during the release reaction and cause solubilization by similar mechanisms.

The finding that precipitates prepared with high-affinity antibodies are more difficult to solubilize by C activity demonstrates that primary Ag-Ab bonds influence the reaction. According to the intercalation hypothesis, the rate of solubilization will depend on the relative affinities of the C peptides for the Ab in the precipitate and of the Ab for the Ag. An alternative and less likely explanation for the marked effect of the binding affinity of Ab on CRA is that antibodies of lower affinity interact more efficiently with C to generate more effector peptides (22).

The results of these experiments may be relevant to immunopathology. Various biological and pathological processes are triggered by the Ag-Ab reaction, both when it occurs in the fluid phase and on the membrane of cells. It is likely that under normal circumstances Ag-Ab complexes which activate the C cascade through the properdin pathway are transformed into released complexes which contain C3 and C4 fragments. Since C affects the degree of aggregation, solubility, and composition of complexes, released complexes could have different biological and pathological activity from nonreleased complexes. Indeed, we found that released complexes have low binding affinity for the membranes of platelets and blood leukocytes (23, 24). Because Ag-Ab complexes (without C) mediate the release of inflammatory agents from cells, it is conceivable that the C system plays a hitherto unsuspected role in protecting the body from potentially damaging complexes. Furthermore, according to our results, immune complexes which are formed in vivo under conditions of C depletion would have a greater tendency to aggregate (25). This may increase their tendency to deposit in tissues. If correct, this hypothesis would have consequences for the therapy of diseases in which low levels of C are observed: in some circumstances the enhancement of serum levels of certain C components might be beneficial for the patient.

Summary

Antigen (Ag)-antibody (Ab) aggregates prepared with several different antigens are solubilized by fresh serum at 37°C (complex-release activity of serum or CRA). The rate of solubilization varies in different systems and is strongly influenced by the affinity of Ab for the Ag in the immune precipitate. With a given Ag-Ab precipitate, the maximum amount of complex that can be solubilized by individual sera is independent of the initial concentration of complexes and cannot be increased by prolonged incubation. CRA occurs in the absence of C2 and C4, but not in the absence of C3 and factor B of the properdin pathway. Addition of C2 to C2-deficient serum or C4 to C4-deficient serum enhances CRA. Solubilization does not involve extensive degradation of the complexed antibody, as might be detected by acrylamide gel electrophoresis of released antibody after reduction and alkylation to separate H and L chains. Immune precipitates can also be solubilized by incubation with monovalent fragments (Fab or Fab') of antibodies against determinants of the Ab molecules in the immune precipitate. In contrast, F(ab')2 fragments decrease the solubility of the
immune precipitates. In view of these findings, we propose that CRA is mediated by the binding of functionally monovalent C fragments (C3 and C4) onto Ab molecules in the precipitates.

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