Immune Reactions in Polysaccharide Media

THE COMPOSITION OF THE ANTIGEN-ANTIBODY COMPLEXES IN THE PRECIPITIN REACTION

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The precipitin reaction is enhanced in the presence of polysaccharides (Hellsing, 1966). This reaction has now been studied in detail with labelled antigen (^{125}I -labelled human serum albumin) and antibody (^{131}I -labelled rabbit anti-albumin immunoglobulin G). The relative proportions of antigen and antibody in the precipitates are unchanged by the addition of dextran in spite of the increased precipitation. The ratio of antibody to antigen in the soluble immune complexes decreases with increasing polysaccharide concentration. This can be interpreted as a decrease in the aggregate size of the complexes. At the same time the amount of free antigen in the solution increases. The results are consistent with a decrease in solubility, primarily of the large immune aggregates, together with a shift in the equilibrium between small and large complexes. The effect is in accord with a steric-exclusion phenomenon.

Neutral and acid polysaccharides enhance the precipitin reaction (Hellsing, 1966, 1969a). Increased precipitation occurs in the region of antigen excess and at the same time there is a decrease in the concentration of soluble antigen-antibody complexes in the supernatant fluids. The mechanism of enhancement has now been investigated in more detail by the use of ^{125}I -labelled human serum albumin and ^{131}I -labelled specific anti-albumin antibodies. By this technique it was possible to determine the influence of dextran on the composition of the precipitates and the antigen-antibody complexes that remain in solution.

MATERIALS AND METHODS

All experiments were carried out at 4° in 0.05 M-phosphate buffer (9.8 mM- K_2HPO_4 -40.2 mM- NaH_2PO_4), pH 7.4, containing 0.1 M-NaCl unless otherwise stated.

Antigen. The solutions of ^{125}I -labelled purified human serum albumin (5–80 $\mu\text{g.}/100\mu\text{l.}$) were the same as those used before (Hellsing, 1969a).

Antibodies. Immuno-electrophoretically pure human serum albumin (1.5 g.; Behringwerke A.-G., Marburg/Lahn, Germany) was coupled to 15 g. of *p*-aminobenzylcellulose (Cellex PAB; BioRad Laboratories, Richmond, Calif., U.S.A.) as described by Campbell, Leuscher & Lerman (1951). The material, equilibrated with phosphate buffer, was packed in a 2 cm. $\times$ 28 cm. column, which was operated by reverse flow. Anti-albumin serum prepared in rabbits (Hellsing, 1966) was applied in 25–60 ml. batches containing 15–60 mg. of antibody to the column. The column was eluted with phosphate buffer until the eluate, monitored

by a Uvicord absorptiometer (LKB Produkter, Stockholm, Sweden), was free from u.v.-absorbing material. The adsorbed antibodies were then dissociated in two steps by using 0.1 M-glycine-HCl buffers containing 0.1 M-NaCl of pH 3.06 and 2.40 respectively. The two fractions were collected in 1 M-tris-HCl, pH 8.0, to maintain the pH of the solution above 7. Only fractions obtained after dissociation at pH 2.4 were used in this as well as in the previous investigation (Hellsing, 1969a). On immunoelectrophoresis against sheep anti-(whole rabbit serum) (Hellsing, 1969a) only one band corresponding to IgG* could be detected. The antibody fraction was iodinated with ^{131}I and treated as described by Hellsing (1969a) to yield a product of specific radioactivity about 20 $\mu\text{C}/\text{mg.}$ at a concentration of 0.60 mg./ml. About 95% of the labelled antibody fraction reacted with albumin in a gel-electrophoretic analysis (Fig. 1); similar reactivities for immunosorbent-purified antibodies have been reported by others, who have used free electrophoresis (Singer, Fothergill & Shainoff, 1960; Behrens, Inman & Vannier, 1967).

Polysaccharide. Dextran 80 (molecular weight 80000) was kindly supplied by Pharmacia AB, Uppsala, Sweden.

Immunological techniques. These, including the procedure for the precipitin test, have been described in detail before (Hellsing, 1966, 1969a). Separation of free and antibody-bound albumin was performed only by the immunoelectrophoretic technique. Under these conditions only a few per cent of the ^{131}I radioactivity were found in that part of the gel containing free albumin.

Radioactivity. This was measured as described earlier (Bill & Hellsing, 1965; Hellsing, 1969a). The radioactivity values (c.p.m.) were recalculated as moles of protein by use of the original specific labelling of the materials, the known

*Abbreviation: IgG, immunoglobulin G.

Fig. 1. Determination of the reactivity of the antibodies with gel electrophoresis. A routine precipitin test (Hellsing, 1969a) was carried out with the isotope-labelled antibody fraction (60 μ g.) and unlabelled albumin purified as described by Hellsing (1966). The supernatant solution at large antigen excess (210 μ g. of albumin) was subjected to agarose-gel electrophoresis in phosphate buffer (Hellsing, 1966). The gel was cut, perpendicular to the direction of migration, into 2mm.-broad strips and the radioactivity in each strip was determined. The migration of the antibody in antigen excess (○) is compared in the diagram with the migration of the pure antibody (×). From the shapes of the curves it may be concluded that in the presence of antigen about 95% of the material has moved as an essentially symmetric peak towards the anode.

half-lives of the isotopes and the molecular weights of human serum albumin (69000; Schultze & Heremans, 1966) and rabbit IgG (145000; Marler, Nelson & Tanford, 1964).

RESULTS

The increased immune precipitation caused by dextran followed the same qualitative pattern as described before regardless of which immune component was measured in the precipitate (Figs. 2a and 2b). The points of maximal precipitation of ^{125}I and ^{131}I did not, however, coincide exactly. In the absence of dextran maximal precipitation of antigen was obtained by the addition of 10 μ g. of albumin, maximal precipitation of antibody with 8 μ g. and maximal total precipitation (on a weight basis) with 9 μ g. The corresponding values in the presence of 80 mg. of dextran/ml. were 16 μ g., 13 μ g. and 14 μ g. of added albumin respectively. The maximum amount of antibody precipitated was 78–82% of the total.

The ratio of antibody to antigen in the precipitate varied with the amount of antigen added in the precipitin reaction (Fig. 3a), but was not affected by the addition of the polysaccharide. In the region of antibody excess it increased to more than 4 antibody molecules/albumin molecule compared

Fig. 2. Influence of dextran on the precipitin reaction. Samples (60 μ g.) of ^{131}I -labelled antibody were mixed with increasing amounts of ^{125}I -labelled albumin in the presence of Dextran 80. The radioactivity in the precipitate was plotted versus the amount of albumin added. (a) ^{125}I ; (b) ^{131}I . The following dextran concentrations were used: ×, none; ○ 10 mg./ml.; ●, 20 mg./ml.; △, 40 mg./ml.; ▲, 80 mg./ml.

with the maximal precipitation point where there were 2:8. The molar composition of the precipitate at large antigen excess is also shown in Table 1. The total amount of precipitate increased markedly on addition of dextran, but the molecular ratio of antibody to antigen was constant at approx. 2:1.

The concentration of free and antibody-bound albumin in the supernatant fluids in the region of antigen excess was determined by immunoelectrophoresis. At the same time it was possible to study the concentration of soluble antibodies that could be recovered quantitatively (94–108%) in the antigen-antibody complex fraction.

The distribution of albumin and anti-albumin in the different soluble fractions at antigen excess is shown in Table 1. In the soluble immune complex fraction, the total amount of both antigen and antibody decreased on addition of dextran. There was, furthermore, a change in the ratio of antibody to antigen. In the example described the ratio

decreased from 0.88 in the absence of dextran to 0.61 in the presence of dextran (80 mg./ml.) That the ratio of antibody to antigen decreased throughout the precipitin series when the polysaccharide was added is demonstrated in Fig. 3(b).

The concentration of free albumin in the supernatant fluids increased when dextran was present. This fact is best demonstrated at large antigen

excess (Table 1), since the more antigen added the greater the increase. The relative increase was not very large and this is the main reason why it had earlier been regarded as insignificant (Hellsing, 1966, 1969a). In absolute amounts it was, however, of the same order of magnitude as the increased amount of albumin in the precipitate. The increase in the amounts of free albumin and precipitated albumin corresponded to the decrease in albumin in the soluble complexes. In all the present experiments, as well as in subsequent work (Hellsing, 1969c,d), a tendency for an increase in free albumin on addition of the polymer was found.

DISCUSSION

In the present investigation it was considered advantageous to use specific antibodies. These were prepared by an immunosorbent technique involving acid dissociation at pH 2.4. The antibodies were then labelled with ^{131}I . Although the extreme conditions during the preparation and labelling may affect the conformation of the protein molecules, 80% of the antibody preparation could be precipitated with the antigen and it was about 95% reactive as measured by a simple electrophoretic method. This was considered satisfactory for the present purposes and indicates that no serious irreversible change had resulted. Further, the composition of the precipitate throughout the control precipitin series was in good agreement with that generally reported (Heidelberger, 1938). The molar ratio of antibody to antigen in the soluble immune complexes decreased with increasing amounts of antigen (Fig. 3b). This agrees with the established fact that in the region of antigen excess the soluble immune complexes exist in different sizes and that they decrease in size as more antigen is added finally reaching the composition: 1 antibody + 2 antigen molecules (Singer & Campbell, 1952). The larger the complex the higher is the ratio of antibody to antigen.

Fig. 3. Molar ratio of antibody to albumin in (a) the precipitate and (b) the immune complex fraction in the supernatant solutions taken from the experiments described in Fig. 2. The same symbols as in Fig. 2 were used.

Table 1. *Distribution of antigen and antibody in different fractions after mixing of 80 µg. of albumin and 60 µg. of anti-albumin in the precipitin experiments*

The results are expressed in moles $\times 10^{11}$.

Concn. of dextran (mg./ml.)	Precipitate			Supernatant			
	Albumin	Antibody	Antibody/albumin ratio	Free albumin	Immune complexes		
					Albumin	Antibody	Antibody/albumin ratio
0	0.6	1.2	2.1	76.0	38.1	33.4	0.88
10	2.3	5.0	2.1	76.8	35.5	28.7	0.81
20	3.6	8.1	2.2	79.9	30.1	23.8	0.79
40	7.5	15.8	2.1	81.8	26.7	20.3	0.76
80	9.0	19.2	2.1	82.2	22.6	13.7	0.61

The addition of dextran had no effect on the composition of the precipitate in spite of the increased precipitation (Fig. 3a and Table 1). This provides further confirmation of the assertion that the enhanced precipitation is not due to complex-formation between dextran and the proteins (Hellsing, 1969b), since it is unlikely that such complexes would have the same composition as regular immune complexes.

A definite change was, however, obtained in the composition of the soluble immune complexes on addition of the polysaccharide. The ratio of antibody to antigen decreased (Fig. 3b and Table 1) concomitantly with the decrease in the total amount of soluble complexes. The large complexes having a high ratio of antibody to antigen had thus selectively been removed from the solution. The composition of the remaining complexes (0.61; Table 1) at high dextran concentration was close to that for the theoretical minimum size (0.50). The change in composition of the soluble complexes was accompanied by a release of free albumin into the solution.

The above observations can be interpreted in terms of steric exclusion (Laurent, 1968). When an immune precipitate has been formed the supernatant solution is saturated with the largest soluble immune complexes. These exist in equilibrium with the free immune reactants and with a number of other complexes, which vary in size and composition. Addition of dextran to the supernatant solution will exclude the various components to different degrees from the solvent depending on their size, and the reaction equilibrium will change. The equilibrium will be displaced towards an increase in the relative concentration of small complexes and free immune reactants with a subsequent decrease in the relative concentration of large immune complexes. The experimental findings agree with these suppositions.

The composition of the precipitate is determined by the activity of the immune reactants in the supernatant solution. The similarity in composition of the precipitate in the absence and presence of dextran (Fig. 3a) indicates that the increase in activity coefficient caused by dextran is balanced by the decrease in concentration of immune reactants.

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