X-RAY SMALL-ANGLE SCATTERING ON SOLUBLE ANTIGEN–ANTIBODY COMPLEXES

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1. Introduction

By ultracentrifugal and electrophoretical studies Singer and Campbell [1] have provided data on the composition and conformation of soluble antigen–antibody complexes formed in excess antigen. They found that with a large antigen excess, a complex 'A complex' is formed composed of two antigens bound by one antibody molecule (Ag_2 Ab) which are arranged in a linear array. Their findings concerning the composition of the A complex were confirmed by a number of investigations [2, 3]. Electron microscopic studies by Feinstein and Rowe [4] and Valentine [5] have shown DNP labelled ferritin molecules bridged by anti-DNP antibodies, the latter having a more or less flexible Y-shaped structure. Unfortunately the distances between the ferritin molecules varied within too large a range for precise dimensions of the complexes to be given. All electron micrographs revealed markedly smaller dimensions for the complexes than expected from the hydrodynamic [6] and X-ray data [7] available for γG immunoglobulin. These discrepancies might be ascribed partially to the difficulties in the staining method [8] and to total dehydration in the vacuum necessary for electron microscopy. However it is now well established that the antigens within the A complex are bound on the extreme ends of the respective F(ab) parts of the antibody molecule.

The present paper is a report of our attempts to obtain additional data on the conformation of the A complex with the aid of the small angle X-ray scattering method, which in the past has contributed information on the structure of immunoglobulin [7, 9, 10]. The work was performed on the system of serum albumin and its specific antibodies with a large antigen excess (eighty fold equivalence). From a comparison of our experimental results and those obtained by calculating a series of different models, we were able to show that the A complex must have a rather extended structure, and that the time average probability of an inflexion to angles less than 120° between the F(ab) parts of the antibody molecule is very low.

2. Materials and methods

2.1. *Serum albumin*

Serum albumin was obtained from pooled porcine sera by ammonium sulfate precipitation. The following preparative zone electrophoresis [11] and gel chromatography (Sephadex G 100) yielded a highly purified product. Double radial immunodiffusion tests and polyacrylamide gel electrophoresis of this

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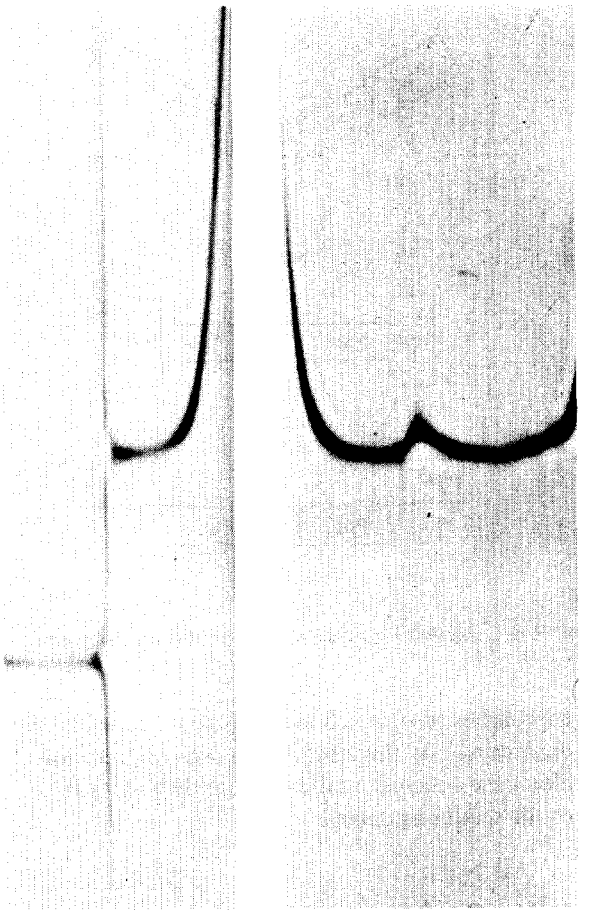

Fig. 1. Ultracentrifuge diagram of reaction mixture containing A complex and excess antigen. Sedimentation proceeds from left to right.

product showed a higher degree of purity than commercially available crystalline BSA preparations. The absorbance coefficient has been determined by dry weight:

$A_{280\ nm}^{10\ mg/ml} = 6.72$ (0.2 M tris-HCl buffer pH 8.6; 22°C).

2.2. *Rabbit antiserum albumin 7 S antibodies*

Immunization of rabbits was performed by multiple site intramuscular injections of porcine serum albumin incorporated in incomplete Freunds Adjuvans. The antibody titer of the pooled antisera was determined by quantitative precipitin tests according to Kabat [12]. The isolation of the specific antibodies was carried out by maximum precipitation with porcine serum albumin, centrifugation and washing of the precipitate with phosphate buffered saline at 4°C. The precipitate was dissolved in 0.1 M acetic acid and fractionated on Sephadex G 100. The sedimentation velocity of the antibody fraction obtained was exactly the same ($s_{c,\ app} = 4.9$ S) as that found for a highly purified human 7 S γG immunoglobulin under the same conditions (Beckman ultracentrifuge model E, AnD rotor, 60,000 rpm, $c = 0.6\%$ (w/v), 0.1 M acetic acid, 20.0°C). For concentration determination, a value $A_{280\ nm}^{10\ mg/ml} = 15.0$ (0.1 M acetic acid as solvent) [13] was used.

2.3. A *complex*

A 1.26% (w/v) solution of specific antibody in 0.1 M acetic acid was added with stirring to a 9.7% (w/v) solution of porcine serum albumin in phosphate buffered saline (pH 7) to give an antigen excess of eighty fold equivalence (calculated on the basis of a molecular weight ratio of Ag:Ab = 1:2.3 and a molecular equivalence ratio of Ag:Ab = 1:3). This mixture was exhaustively dialyzed against phosphate buffered saline (containing 0.1% sodium azide as a preservative). No precipitate was formed. A 1:3 dilution of this solution was run in the analytical ultracentrifuge, at 60,000 rpm and 20.0°C using Schlieren optics. Fig. 1 shows a photograph of this run, taken 42 min after reaching maximum speed. The apparent sedimentation velocity of the faster moving component was 8.4 S, that is in close agreement to the values found by Singer and Campbell [1] for the A complex. The slower moving peak could clearly be ascribed to the excess antigen as was shown by reference runs with porcine serum albumin under the same conditions ($s_{c,\ app} = 4.0$ S). No further component could be seen, within detectable limits.

This preparation was used for X-ray measurements.

2.4. *X-ray measurements*

The X-ray small-angle scattering camera used in this study was the same type as described elsewhere [14, 15] equipped with an electronically programmable step scanning device [16]. During measurements the solutions were kept in a thermoelectrically cooled cuvette [17] at a constant temperature of 4°C. Monochromatization [18] and elimination of

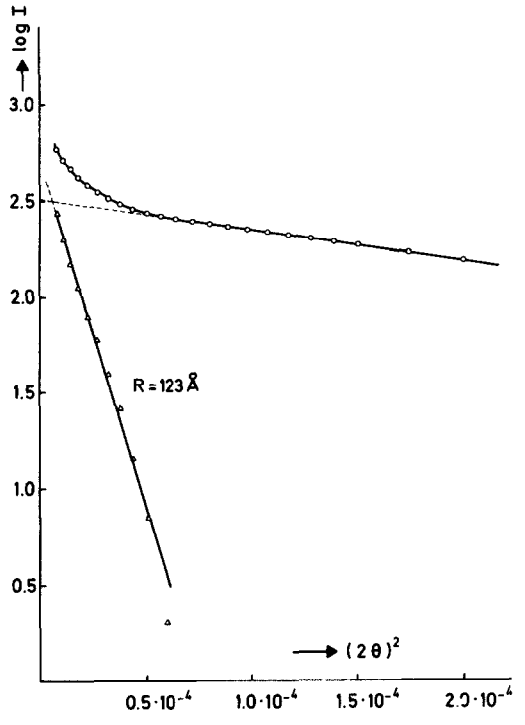

Fig. 2. Experimental scattering curve (○) and scattering curve of the A complex (△). The dotted line shows the scattering curve of the excess antigen that has been subtracted from the sum curve.

collimation errors caused by the line shaped primary beam [19] were achieved by computerized calculations [20].

For both dilutions studied, three scattering curves were measured: a) the curve of the solution containing the A complex and the excess of antigen; b) the scattering curve of the antigen; and c) the background scattering of the buffer solution, which was subtracted from each scattering curve. For every point on the curves, 8×10^4 pulses were counted.

Since the scattered intensities of two types of particles in dilute solution can be added together [21], the scattering curve for the A complex was obtained by subtracting the separately measured antigen scattering curve (at the same concentration as in the complex solution) from curve (a). This is shown in the Guinier plot (log I vs. $(2\theta)^2$) in fig. 2. It can be seen that for larger values of 2θ the scattering intensity of the larger A complex

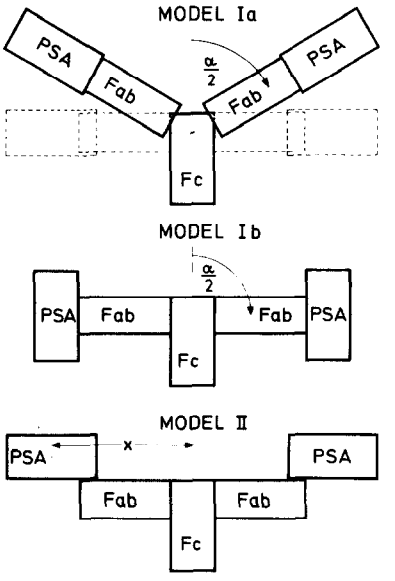

Fig. 3. Models for which the values of R are calculated.

approaches zero, which makes evaluation easier. The error caused by this assumption does not seriously affect the conclusions drawn from the inner part of the scattering curves.

3. Results and discussion

From the slope of the inner part of the resulting scattering curve in the log I vs. $(2\theta)^2$ plot, the radius of gyration can be derived according to Guinier and Fournet [22]. For the A complex, the radium of $R = 123 \pm 3$ Å.

From a molecular weight of 3×10^5 [1] and an average partial specific volume of 0.73 $cm^3\ g^{-1}$ the radius of gyration R_0 of the complex can be calculated as if it were a compact sphere. Thus a value R/R_0 of 3.6 is obtained. The value for γGl immunoglobulin Eu calculated on the basis of results of Pilz et al. [7] gives $R/R_0 = 2.7$.

If the radii of gyration for a series of different possible models for the A complex (fig. 3) are calculated, the values given in table 1 are obtained. The molecular parameters used for these calculations are shown in table 2. The dimensions for the antibody molecule were taken from the values of Pilz

Table 1
Results of the calculation for different values of $\frac{\alpha}{2}$ and x (fig. 3).

Model I a:	$\frac{\alpha}{2}$	30°	45°	60°	90°
	R (Å)	99	112	125	136
Model I b:	$\frac{\alpha}{2}$	30°	45°	60°	90°
	R (Å)	90	98	114	124
Model II :	x (Å)	75	100	125	150
	R (Å)	83	95	108	122

Table 2
Molecular parameters used for the calculation of R.

		Equivalent elliptic cylinder			
	$M \times 10^{-5}$	R (Å)	$2a$ (Å)	$2b$ (Å)	1 (Å)
Antibody	1.62	75.8	–	–	–
Fab	0.48	32.0	22	56	98
Fc	0.55	33.1	21	63	99
Antigen	0.70	33.0	38	50	100

et al. [7] and those for the antigen from our own measurements [23].

A comparison of the theoretical values thus obtained to the experimentally found radii of gyration indicates that the distance between the centers of gravity of the whole complex and the antigen molecules must be at least 150 Å. This allows, in model Ia (fig. 3), an inflexion of the antibody molecule up to a minimum of 120° between the F(ab) parts (Ia) or, with larger angles, for a partial overlap of molecules within the combining region. If the antigens are bound as shown in model Ib the calculation gives an experimental value only with an angle of 180°. In model II, which is based upon the assumption of a fixed 180° angle between F(ab) parts, a distance of 151 Å from the center of gravity of the antigen molecules to the vertical symmetry axis gives the experimental value of R.

These results lead to the conclusion that the antigen molecules in the case of the Ag_2Ab complex are bound to the extreme ends of a stretched antibody molecule. So far our findings are consistent with the model proposed by Green [8]. The overall dimensions of the complex, however, must be much greater than shown by their evaluation from electron micrographs. Our radius of gyration of 123 Å can be interpreted only by molecular dimensions previously found by Kratky et al. [9, 10] and Pilz et al. [7] on a human immunoglobulin G also using the X-ray small-angle technique.

As to the flexibility of the antibody molecule, we can exclude an inflexion between the F(ab) parts to angles lower than 120 degrees. This indicates that the binding of two rather heavy antigen molecules partially restricts segmental flexibility as observed by Yguerabide et al. [24] in the case of hapten labelled antibodies.

In this connection it will be of particular interest to study a variety of other species of antigen–antibody complexes or hapten labelled antibodies by the X-ray small-angle scattering method.

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