NEUTRON SMALL-ANGLE SCATTERING STUDIES OF THE GENERAL STRUCTURE OF THE IMMUNOGLOBULIN G MOLECULE

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1. Introduction

Complete information about three dimensional structure of biopolymers can be obtained only by high resolution X-ray or neutron crystallography. As a rule intact immunoglobulin molecules do not crystallize, probably due to the relatively independent motion of their subunits [1]. Up to now only three intact myeloma immunoglobulins G have been crystallized, but in two a deletion in the hinge region of the molecule was found [2]. In recent X-ray crystallographic studies at high resolution only a Fab fragment [3] or L chain dimers [4] were used.

Methods based on small-angle scattering of X-rays (SASX) and neutrons (SASN) are also potential sources of information on the general structure of biopolymer molecules. Detailed investigations of intact antibody molecules using SASX were recently made by Pilz et al. [5]. Neutron small-angle scattering method is very promising, as a significant difference between the neutron scattering amplitudes of hydrogen and deuterium nuclei permits one to apply the wide range contrast variation by changing the heavy water content of the solution.

In the present communication the first data on general immunoglobulin structure obtained by SASN are reported.

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2. Theory

The intensity of SASN can be expressed by the following formula [6]:

$$I(\kappa) \sim (\sum_i a_i - \rho_s V_x)^2 \left| F(\kappa) \right|^2 \quad (1)$$

where κ is the value of the transferred momentum, a_i is the coherent scattering amplitude of the i-th atom of the particle, ρ_s is the scattering power of 1 cm^3 of the solvent, V_x is the volume of the particle under investigation and $|F(\kappa)|^2$ describes the form and internal structure of the molecule.

If κR is $\lesssim 1.5$, where R is the characteristic dimension of the particle, then the form factor can be expressed as [7]

$$\left| F(\kappa) \right|^2 \sim e^{-\frac{1}{3}\kappa^2 R_g^2} \quad (2)$$

where

$$R_g^2 = \frac{\int_{V_x} \rho(\bar{r}) \cdot \bar{r}^2 \cdot d^3\bar{r}}{\int_{V_x} \rho(\bar{r}) \cdot d^3\bar{r}} \quad (3)$$

R_g is the squared radius of gyration of the particle.

If any of the dimensions of the molecule is much more extended than those of the two others, one has

$$\left| F(\kappa^2) \right| \sim \frac{1}{\kappa} e^{-\frac{1}{2}\kappa^2 R_q^2} \quad (4)$$

where R_q is the so-called radius of gyration of the cross section of the particle [5].

From the first parenthesis on the right hand side of formula (1) it follows that the quantity $\sqrt{I(\kappa = 0)}$ is a linear function of the contrast (i.e. $\rho_{particle} - \rho_{solvent}$). When the contrast becomes equal to zero the following relations are valid:

$$\sum_i a_i = \rho_s V_x \tag{5}$$

i.e.

$$V_x = \frac{\sum_i a_i}{\rho_s}$$

Consequently, a knowledge of the chemical composition and formula (5) permit the volume of the molecule to be determined.

If the density of scattering power is distributed not uniformly inside the particle, the radii of gyration and the radii of gyration of the cross section do change with change of the contrast $\bar{\rho}$.

$$R^2 = A + \frac{B}{\bar{\rho}^{\infty}} + \frac{C}{\bar{\rho}^2} \tag{6}$$

As was shown by Stuhrmann [6], the intensity depends on the contrast in the following way:

$$I(\kappa) = \bar{\rho}^2 I_c(\kappa) + \bar{\rho} I_{cs}(\kappa) + I_s(\kappa) \tag{7}$$

Here $I_c(\kappa)$ depends only on the shape of the particle, $I_s(\kappa)$ corresponds to its internal structure and $I_{cs}(\kappa)$ is the cross term. It is clear that if one measures $I(\kappa)$ at least at three different contrasts, the three basic functions become determinable.

If a particle consists of two subunits, the corresponding radii of gyration follow the relation [8]

$$R^2 = f_1 \cdot R_1^2 + f_2 \cdot R_2^2 + f_1 \cdot f_2 \cdot a^2 \tag{8}$$

where R and R_1, R_2 are the radii of gyration of the whole particle and the two subunits, respectively. 'a' is the distance between the centre of inertia of the two subunits, and f_1 and f_2 are the relative scattering power dependent on the volume and density of scattering power:

$$f_1 = 1 - f_2 = \frac{\bar{\rho}_1 \cdot V_1}{\bar{\rho}_1 V_1 + \bar{\rho}_2 V_2} \tag{9}$$

3. Materials and methods

Human pooled IgG, myeloma IgGk and their papain Fab and Fc fragments were isolated as described in [1]. Dilute solutions (1–3 w%) of proteins at six different H_2O/D_2O concentrations were used for measurements which were made in the Max von Laue-Paul Langevin Institute in Grenoble using the D11 facility [10].

4. Results

Typical distributions of scattered intensity are presented in figs.1 and 2. The data were analysed by the least squares method by formulae (2) and (4). The results partly given in table 1 were submitted to secondary analysis to determine the volume of the molecule and to find the dependence of the radii of gyration of the cross-section on the contrast (fig.3) as well as to compute the three basic functions (fig.4). In the procedure of computation, corrections for the extinction effect and for the H–D exchange effect were made. The last term was estimated by a direct

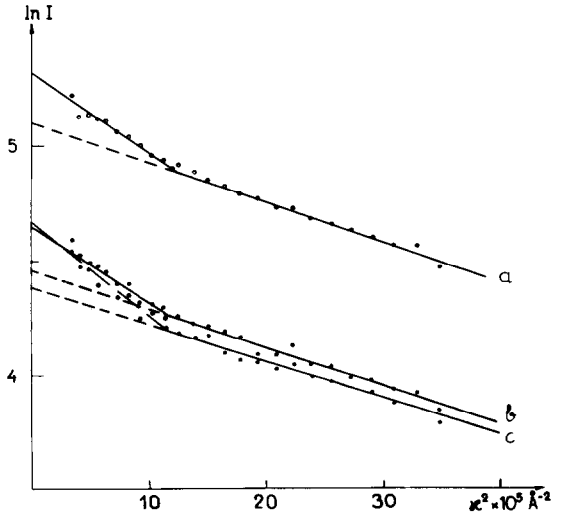


Fig.1. The Guinier plot of the IgG small angle scattering spectra: a – 3 w.% IgG in H_2O; b – 1.5 w.% IgG in H_2O; c – 1 w.% in D_2O.

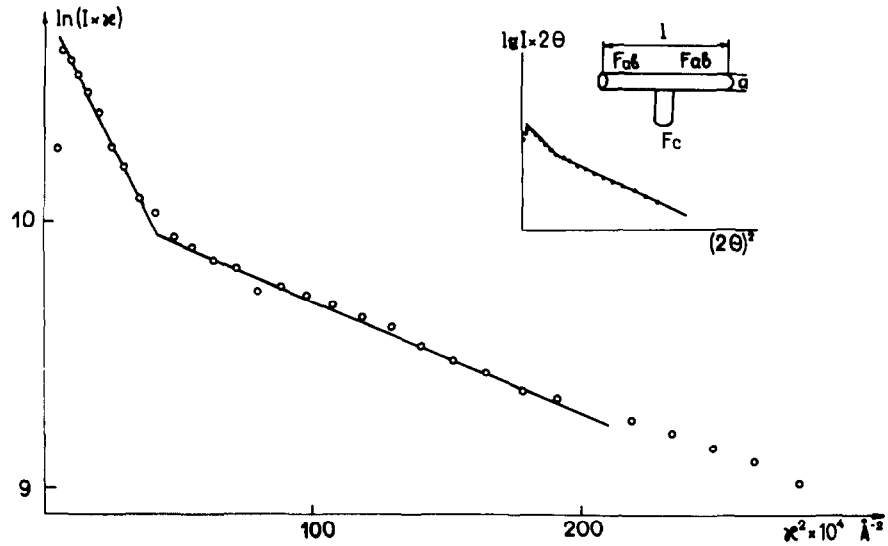


Fig.2. Typical cross section curve of the IgG molecule. The inserted picture corresponds to the cross section curve obtained in ref. [5], and schematic view of the T-shape model.

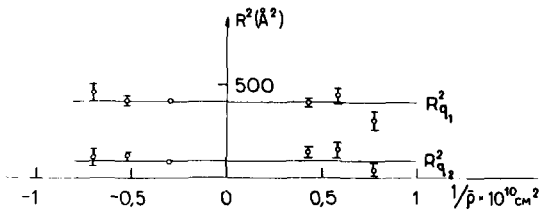
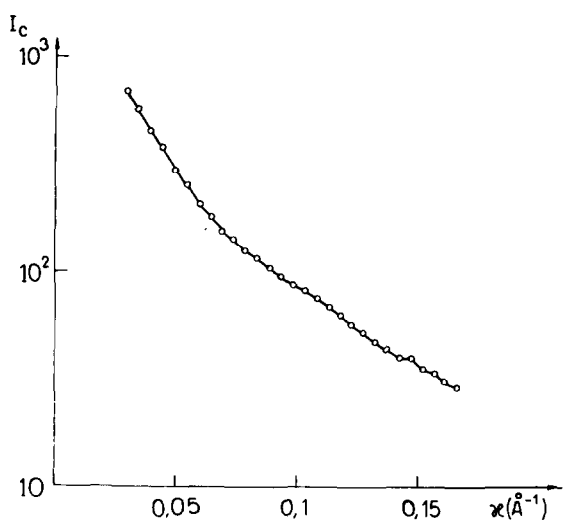


Fig.3. Squared radii of gyration of cross section as a function of the reciprocal value of the excess scattering power.

Table 1
Parameters derived from scattering curves[a]

	Radius of gyration, R_g (Å)	Radius of gyration of cross sections, R_q (Å)
Fab	32.5 ± 0.3 (32.0)	11.0 ± 0.7 (15.0)
Fc	32.8 ± 0.5 (33.1)	12.8 ± 1.8 (16.6)
IgG	72.4 ± 1.3 (75.8)	21.3 ± 1.2 (21.9)[b]
		12.1 ± 1.4 (14.6)[c]

[a] In parenthesis the data taken from [5].
[b] R_{q_1}
[c] R_{q_2}



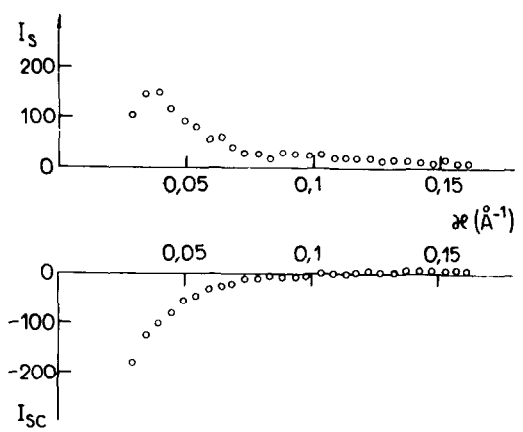


Fig.4. The three basic functions of the IgG molecule. (a) The shape function I_c. (b) The function of the internal structure, I_s (upper plot) and the interference curve (lower curve).

count of the N–D and O–H bonds in the IgG molecule. At the same time we assumed that the exchange equilibrium state corresponds to the actual ratio of H_2O/D_2O concentration in the solvent.

5. Discussion

The steeper parts of the curves in fig.1 were interpreted as due to IgG dimers. Using this assumption the radius of gyration of IgG monomer was obtained from the scattering curve (fig.1) with the help of the least squares method considering the experimental curve as a result of superposition of scattering curves of two independent particles. The value of this quantity differs slightly from that of ref. [5] (see the table).

The shapes of the cross section curves are very similar to those obtained by Pilz et al. [5] (fig.2). According to these authors the two straightline sections may be explained by the existence of two parts of different thickness in the IgG molecule (fig.1). Moreover, from the similarity of R_q (Fab) and R_{q_2} (IgG) values one can conclude that Fab fragments in the IgG molecule are connected end to end.

To determine the volume of the IgG molecule formula (5) was used. The concentration of D_2O at which the square root of intensity at zero momentum transfer becomes zero is equal to 41% which corresponds to $\rho_s = 2.28 \times 10^{-10}$ cm^2. The sum $\sum a_i$ was computed from the primary structure of IgG [9] by direct summation. The chemical formula of IgG turns out to be $C_{6662}H_{10282}N_{1786}O_{2178}S_{50}$ and 2747 of the hydrogen atoms seem to be exchangeable. The volume (V) is computed as 202 000 ± 14 000 Å^3, which is somewhat smaller than the corresponding results from X-ray measurements.

Figure 3 shows that the $R^2_{q_1}$ and $R^2_{q_2}$ values only slightly depend on the contrast $\bar{\rho}$. This means that on average there are no great inhomogeneities in scattering density along the cross section of the molecule, neither in the Fab fragment region nor in the Fc region.

A weak dependence of $I_s(\kappa)$ on κ and its small value would be additional evidence for the lack of any inhomogeneity. In fig.4 one can see all three basic functions. It is seen that $I_s(\kappa)$ at high κ values has a low-lying and flat shape. The significant peak at the beginning of the curve can be explained by either the existence of dimers or by the type of packing of the

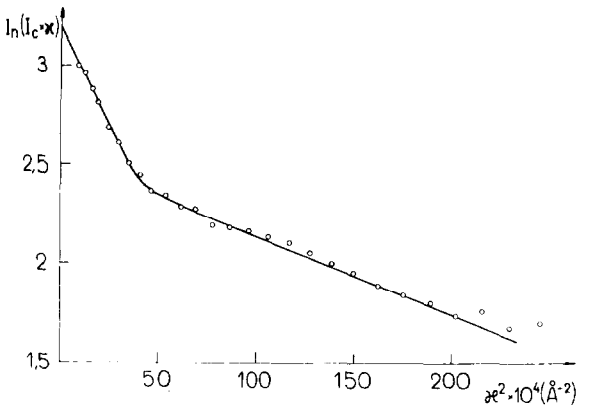


Fig.5. The cross section curve evaluated from the shape function I_c.

three fragments in the IgG molecule. The shape function $I_c(\kappa)$ retains the characteristic feature of the scattering curve observed at high values (fig.5).

Using formula (8) a simple relation may be derived through the measured values of radii of gyration for the connection between the distances of centres of inertia of $F(ab)_2$ and Fc fragments ('a') and of the distance of the two Fab fragments ('b'):

$$b = 2\cdot\sqrt{\frac{R^2_{IgG}}{f_{F(ab)_2}} - \frac{f_{F_c}}{f_{F(ab)_2}} R^2_{Fc} - R^2_{Fab} - f_{Fc}\cdot a^2} \qquad (10)$$

This relation describes the geometrical positions of the centres of inertia of Fab fragments (see continuous curve in fig.6). In fig.6 are presented three possible relative situations of IgG fragments for which dimensions are taken from ref. [5]. The fragments are situated in a common plane in such a way that the axes of the equivalent cylinders and the longest diameter of their elliptical bases are in this same plane.

In the first case (I), 'b' was evaluated from the measured value of the radius of gyration of the $F(ab)_2$ fragment [5]. Case II corresponds to the Y-shape of IgG molecule observed in the electron microscope [11] and with X-ray structure investigation [12]. Finally, case III represents the T-shape model proposed by Pilz et al. [5]. Naturally, any intermediate situation is also permitted by the set of our experimental data. In order to choose the real

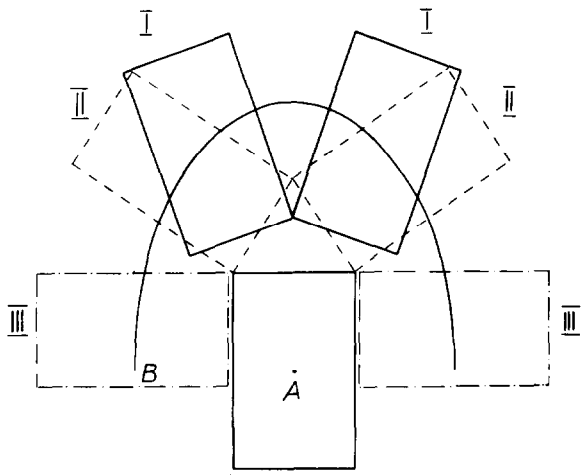


Fig.6. Three possible configurations of the IgG molecule: I – radius of gyration of $F(ab)_2$ fragment is equal to 53 Å [5]; II – Y-shape model; III – T-shape model. A – The centre of gravity of the Fc fragment. B – The curve of the geometrical places of the centre of inertia of Fab fragments.

configuration some additional information has to be involved. Namely, on one hand it seems to be necessary to measure the distance between the ends of Fab fragments with the help of labelled antigens using the small angle neutron scattering method, and on the other hand to compute scattering curves for different models of the IgG molecule. Because each of these procedures are unambigous one may hope that they together will provide the solution to the problem.

6. Conclusions

In general, all the parameters of the neutron scattering spectra coincide with those obtained by SASX, the only exception being the volume of the molecule. As far as the two different values of the volume are concerned, we prefer the one obtained by neutron scattering. With X-ray measurements the volume may be determined by integration of the weighted scattering curve. However, this curve is measured only over a finite κ range. The correction for the unknown part of the spectrum was effected rather approximately by linking the experimental curve with its estimated asymptotic tail. The volume obtained in our work involves no 'swelling' effect in solution. A simple computation based on measured parameters of Fab and Fc fragments given in the previous section leaves unanswered the question about the real configuration of IgG fragments. In order to find an exact model, both neutron experiment and model computations are under preparation.

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