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On the Conformation of Antibodies in the Presence and Absence of Antigen (Small-Angle X-ray Studies)*

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The conformations of different IgG antibodies were studied before and after interaction with antigen (hapten). In every case a strong change of the conformation was observed. Binding of hapten caused a decrease of the radius of gyration by 2 to 8% and a decrease of the volume by 3 to 10%, depending on the degree of saturation with hapten. Two IgG antibodies (*anti*-poly-D-alanyl) were split by enzymes into fragments which contain one binding site (Fab') and two binding sites $(Fab')_2$, respectively, for hapten. No changes of conformation were observed with these fragments upon the interaction with hapten. These findings lead to the conclusion that the conformation change does not take place within the area of the combining site but relatively far away, at the area of the hinge region and/or the Fc-fragment.

To prove this assumption the hinge region was modified by splitting disulfide bonds by reduction and alkylation. Small-angle X-ray measurements were performed on the free antibody, the antibody saturated with hapten, the reduced, alkylated antibody and the reduced, alkylated antibody after saturation with hapten. The free antibody showed the usual change in conformation upon interaction with hapten indicated by a decrease of the radius of gyration by 7% (from 6·50 nm to 6·10 nm) and a decrease of the maximum diameter by 1·5 nm. This effect was clearly diminished when the antibody was reduced and alkylated before saturation with hapten. The decrease of the radius of gyration was only 2·5%, that of the maximum diameter about 0·5 nm.

These findings confirm the conclusion that the conformation change takes place within the area of the hinge region and/or the Fc fragment.

Besides the IgG antibodies, an IgM antibody was also studied both in the absence and in the presence of the corresponding hapten. This molecule could be best described by the model of a flat star (molecular weight 8×10^6, radius of gyration 12·1 nm, maximum diameter 36 nm, thickness 4·5 nm, volume 1800 nm^3). No comparable change in conformation upon interaction with hapten could be observed. Only a shift of the subsidiary maxima indicates a change of the substructure.

The work about the IgM antibodies has been submitted for publication in the *European Journal of Biochemistry*; the studies on the intact and modified IgG antibodies in the presence and absence of hapten will be published in *Biochemistry*.

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Small-Angle X-ray Scattering Study on α-Crystallin of Calf Eye Lens

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α-Crystallin is one of the main structural proteins of the mammalian eye lens. The biochemistry of structural proteins of the mammalian eye lens has been reviewed by, for example, Harding & Dilley (1976). This work reports X-ray scattering studies on the native α-crystallin from calf eye lens. α-Crystallin was prepared according to Hoenders & van Kamp (1972). It was concentrated by dissolving an ultracentrifuge pellet. Two different types of camera were used: a Kratky camera operated at a Philips PW 1130 generator, copper tube, and a laboratory-constructed double-focusing camera (Elliott generator GX 6).

Nine different concentrations of solutions were measured with the Kratky camera (concentration range from 4 to

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35 mg/ml; 0·1*M* tris-buffer pH 7·3 at 5 °C). By means of the point-focusing camera a pellet was investigated (camera length 15 cm).

Background subtraction was achieved by measuring the buffer in the same capillary and subtracting the recorded intensity from the data obtained from the solutions.

Deconvolution of the diffraction pattern obtained with the Kratky camera was done iteratively (Glatter, 1973) and by means of an indirect Fourier transformation (Glatter, 1977).

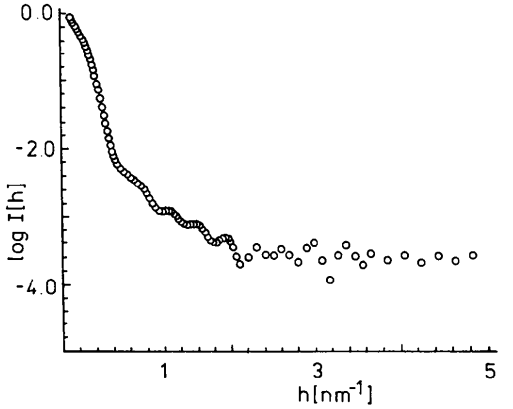

Fig. 1. Semilogarithmic plot of the diffraction pattern of α-crystallin; Kratky camera; concentration = 29 mg/ml; $h = (2\pi/\lambda) \sin \theta$.

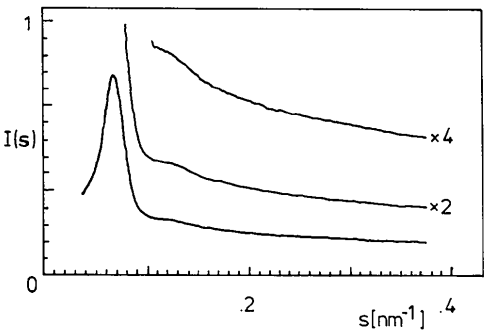

Fig. 2. Circularly integrated intensity of a pellet of α-crystallin; double focusing camera (15 cm camera length). Exposure time: 10 h. The film was the second one of a packet. The strong peak corresponds to a resolution of 15·0 nm. I = intensity; $s = \theta/\lambda$, θ = scattering angle, λ = wavelength = 0·154 nm.

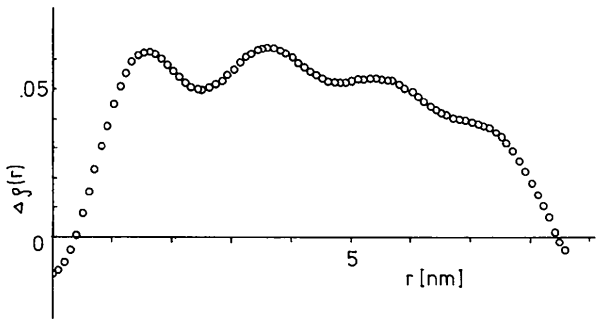

Fig. 3. Radial electron density distribution of α-crystallin. ϱ = electron density on an absolute scale (e Å^{-3}). $\Delta\varrho = \varrho_{solvent} - \varrho_{solution}$. Seven orders transformed, temperature factor 0·3.

The results of the two methods were the same. Fig. 1 shows the diffraction pattern in a semi-logarithmic plot.

The film of the point-focusing camera (exposure time 10 h) was scanned with an Optronics system (25 μm steps) and the recorded intensity was integrated circularly. Fig. 2 shows the integrated intensity. The strong reflection at $s = 0{\cdot}067$ nm^{-1} ($s = \theta/\lambda$, θ = scattering angle) corresponds to a resolution of 15·0 nm, the weak peak at $s = 0{\cdot}125$ nm^{-1} corresponds to the first subsidiary maximum of the Kratky data. Results from the solution measurements are listed in Table 1.

Table 1. *Molecular parameters of native α-crystallin at* 5 °C

Radius of gyration	6·17 nm
Molecular weight	840 000
Invariant volume	1660 nm^3
Water content (calculated from the invariant volume)	46%
Maximal distance within the particle	15·5–16·0 nm

If an ellipsoid of rotation is assumed, the axial ratio can be calculated from the radius of gyration and the maximal distance D_{max} within the molecule. This ratio is 1 : 1 : 1·08 for $D_{max} = 15{\cdot}5$ nm and 1 : 1 : 0·99 for $D_{max} = 16{\cdot}0$ nm. This and the position of the subsidiary maxima, which correspond to the position of the orders of a sphere of a radius of 7·8 nm, justify the evaluation of the data as a pattern of a particle of spherical density distribution. At low resolution the orders are very poor, which can be related to a slightly ellipsoidal shape or to a shape in which there is only a small amount of matter at the periphery.

We followed the method used by Anderegg (1967) and coworkers to determine the amplitudes. Alternating signs could be assumed, since neither did we use a buffer of high electron density nor is there lipid in the molecule. Furthermore, extensive model calculations showed that alternating signs, especially for our restricted number of orders, is absolutely justified. Seven orders were Fourier transformed. The termination effect was reduced by multiplying the values by a Gaussian function, which was 0·3 at the last point.

Fig. 3 shows the radial electron density distribution on an absolute scale. The lower density at higher radius corresponds to the 'smearing' of the first orders of the diffraction pattern (slightly ellipsoidal shape or less matter outside).

Since the particle consists of approximately 40 polypeptide chains, we assume that approximately 30 of these form the core of the particle while the others are distributed at the outside. The constant growth of the protein could proceed by the linking of polypeptide chains to the surface of the molecule.

This study together with a study on reassociated α-crystallin will be submitted to the *European Journal of Biochemistry*.

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