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Large-Angle X-ray Diffuse Scattering, a New Method for Investigating Changes in the Conformation of Globular Proteins in Solutions

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A comparative analysis of the sperm-whale myoglobin structure in the crystal and in solution has been carried out with the technique previously formulated by the authors which uses the large-angle X-ray diffuse scattering for investigating the protein structure in solution. A 'modified cube method', correctly taking into account the cavities within the protein molecule accessible to the solvent, is proposed for an accurate estimate of the solvent influence and for calculation of the scattering intensities. A comparison of the theoretical myoglobin scattering curves with the experimental scattering curve of this protein obtained by Stuhrmann shows rather noticeable quantitative divergences, which can be eliminated by a small increase in the distance between the 'hairpin' *GH* and the other part of the protein molecule. It is also shown that several other physically reasonable shifts of helices (or groups of helices) do not lead to an agreement between the experimental and theoretical scattering indicatrices.

Introduction

Considerable broadening of the experimental possibilities of X-ray and neutron scattering has greatly stimulated the further development of the theory of diffuse scattering by biopolymer solutions at all experimentally accessible angles. This is also true for the so-called 'large-angle' scattering region – that is, for angles from several degrees to $\sim 25\,^{\circ}$C. When it turned out that indicatrices of large-angle scattering from globular proteins with tertiary and quaternary structure have a high sensitivity to the type of polypeptide chain packing involved (Fedorov, Kröber, Damaschun & Ruckpaul, 1976) the question arose: how can this be used for investigating the internal structure of globular proteins? From this point of view we have analyzed in detail the structural possibilities of the large-angle scattering for a number of proteins (Fedorov & Denesyuk, 1977) and it has been shown that the method is sensitive to small *local* as well as *large-block* rearrangements in the protein structure. As a result, the most efficient approach in application of this method has been formulated. It suggests that the large-angle scattering should analyze not the protein structure itself, but the change (or lack of change) which can take place either in the molecule at the transition from crystal to solution or during the functioning of the protein. The investigation should be based on (*a*) the known protein structure in the crystal; (*b*) the scattering indicatrix calculated from this structure; (*c*) the experimental scattering curve for the protein in solution. In the case of a divergence between the calculated and measured curves the protein structure should be modified according to physical and functional considerations in order to obtain the best agreement between the experimental curve and that calculated anew.

In the present paper this method is applied to a comparison of the sperm-whale myoglobin structure (Mb–SW) in the crystal and in solution. This protein has been already studied in solution in different states (see, for example, Kirste & Stuhrmann, 1967; Stuhrmann, 1970; Beeman, 1967; Ibel & Stuhrmann, 1975) by small-angle and large-angle X-ray and neutron scattering, and reliable X-ray scattering curves have been obtained over a wide range of scattering angles. In 1972, for the first time a reasonable agreement between the theory and experiment was demonstrated by comparison of the Mb–SW experimental scattering curves with those calculated by the 'cube method' (Fedorov, Ptitsyn & Voronin, 1972). This gave evidence in favour of the similarity between the Mb–SW structure in the crystal and in solution. At the same time in the region of angles corresponding to Bragg distances of 12–40 Å there was a quantitative divergence between these curves, and the question remained unsettled whether this was connected with experimental errors and (or) incorrectness of the theory or whether this was due to a real rather small difference between the Mb–SW structures in the crystal and in solution.

To answer this question with the help of large-angle diffuse scattering, which is a differential method when used as described, it is necessary to have the most reliable experimental and theoretical scattering indicatrices. The scattering indicatrix of Mb–SW in an aqueous solvent, obtained by Stuhrmann (1975) and corrected for collimation, was used as the experimental curve. To obtain the most reliable theoretical scattering curve of Mb–SW a new 'modified cube method' was developed using the main principles of the earlier 'cube method' (Fedorov, Ptitsyn & Voronin, 1974*a*,*b*) and taking into account its shortcomings of the 'second order', which are important for a strict quantitative comparison of experimental and theoretical scattering indicatrices.

Method for calculating scattering indicatrices

It is known that the main difficulty in calculating indicatrices of large-angle scattering is to take correctly into account the solvent influence, and this requires the description of the macromolecule volume inaccessible to the solvent. In the 'cube method' the macromolecule is mentally placed in a parallelepiped, which is subdivided into several small cubes (with edges of 1–1·5 Å) and each cube is examined in order to decide whether it pertains to the macromolecule or to the solvent. When all the cubes have been considered, it is possible to represent the outline and the volume of the dissolved particle with good accuracy. The mathematical background of this method is described in detail by Fedorov, Ptitsyn & Voronin (1974*a*,*b*).

The main shortcoming of the 'cube method' consists in a rather rough account of the internal cavities accessible to the solvent. In the majority of cases these cavities are automatically taken into account in evaluating whether a given cube pertains to the molecule or to the solvent, but in a number of cases the method *overestimates* the number of

cavities accessible to the solvent. In fact, let us presume that in the molecule there is a long narrow cleft (say 2 Å wide) along which the number of cubes increases. According to our method all the cubes entering the cleft will be attributed to the solvent since the edge length is 1–1·5 Å, while no water molecule can enter this cleft owing to its insufficient width. As a result of this shortcoming the profile of large-angle scattering indicatrices is slightly dependent on the mutual orientation of the molecule and the superposed network of cubes. Thus, Fig. 1(*a*) represents the scattering curves of lysozyme (hen egg) calculated by the 'cube method' in the initial coordinate system and in a coordinate system considerably 'turned' relative to the initial one. Coordinates of lysozyme non-hydrogen atoms were taken from the paper by Imoto, Johnson, North, Phillips & Rupley (1972).

To overcome this shortcoming the 'modified cube method' uses another way to evaluate whether each cube belongs to the molecule or to the solvent. The macromolecule is also inscribed in a parallelepiped which is subdivided into separate cubes of edge R (we used $R = 1{\cdot}3$ Å). The modified procedure consists in choosing cubes *not belonging* to the macromolecule and the choice is made not for separate cubes but for the whole group roughly modelling the water molecule. The proposed algorithm is as follows: if the distance from the centre of the considered cube to the nearest atom of the particle is greater than the value $R + B$ (B is the parameter regulating the overall molecule volume), such a cube is considered as a potential centre of the water molecule and not only this cube but also all the neighbouring cubes along the three coordinate axes (7 cubes altogether) are excluded from the macromolecule volume. Thus all the narrow clefts and cavities inaccessible to the solvent automatically belong to the macromolecule. Then the adjacent cubes are joined in parallelepipeds and the intensity of scattering is calculated in the same way as in the case of the usual 'cube method': scattering amplitudes of the macromolecule and the solvent displaced are calculated proceeding from the system of cubes and then the squared difference of these amplitudes – the intensity of scattering at a fixed point of the reciprocal space – is averaged over all the points of the reciprocal space equidistant from the origin of coordinates of this space. Like the earlier one (Fedorov, Ptitsyn & Voronin, 1974*a*,*b*), the present method is based on the non-specific account of the solvent influence, *i.e.* we replace the real solvent by a uniform structureless continuum. Such a replacement is justified by theoretical investigations (Fedorov, 1967) as well as by direct calculations showing that taking account of the oxygen atoms of the 'bound' water [in particular, of the three 'bound buried' molecules in lysozyme (Imoto *et al.*, 1972)] does not lead to noticeable deformations of the scattering curve (Timchenko, 1978).

Fig. 1(*b*) represents lysozyme scattering curves calculated by the 'modified cube method' in the same initial and the same 'turned' system of coordinates as in Fig. 1(*a*). It is seen that the divergence between the scattering indicatrices is considerably smaller. To attain a practically complete coincidence of the scattering curves at different turns of the macromolecule it is necessary to average, in addition, the scattering intensities at different shifts (along coordinate axes) of the cube network relative to the macromolecule. It should be noted that this method of evaluating the macromolecule shape and volume in solution is physically similar to Lee & Richards's method (1971). In order to test our method we calculated several cuts of sperm-whale myoglobin and it turned out that the internal cavities determined by our method are similar to those determined by Lee & Richards. Thus, for example, the sperm-whale myoglobin cut at $x = 18$ Å leads to two cavities coinciding with cavities D and J in Lee & Richards's nomenclature.

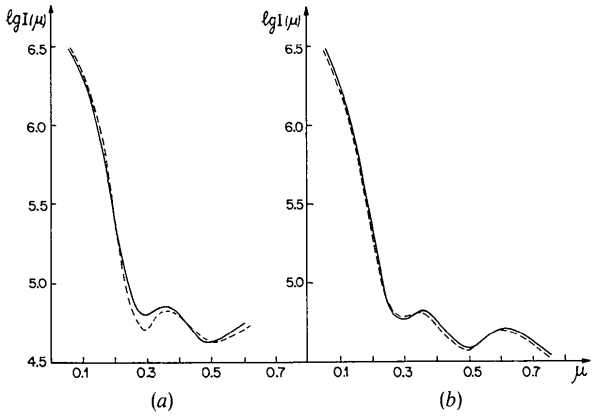

Fig. 1. Theoretical scattering indicatrices of hen-egg lysozyme; coordinates of atoms are written in the initial coordinate system (——) and in a system of coordinates considerably turned relative to the initial one (- - -). Calculation was done by the 'cube method' (*a*) and by the 'modified cube method' (*b*).

Analysis of structural changes in sperm-whale myoglobin in solution in comparison with the crystal

On the basis of the 'modified cube method' the scattering indicatrix of Mb–SW was calculated in an aqueous medium and averaged in addition for five scattering curves with the

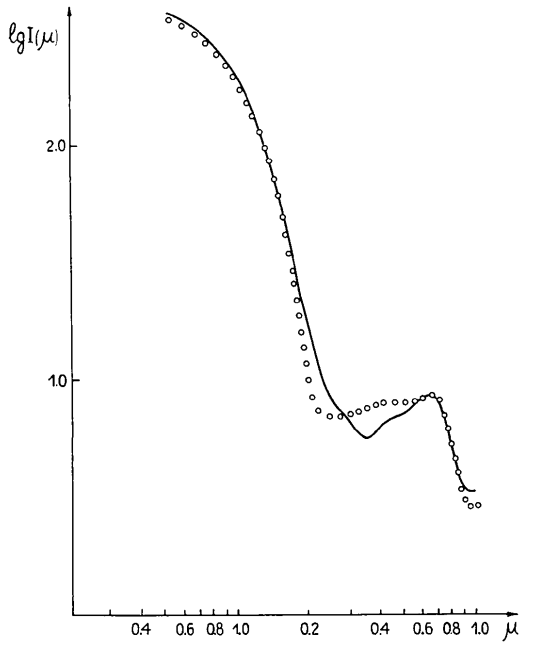

Fig. 2. Experimental scattering curve of Mb–SW obtained by Stuhrmann (1975) (OOO) and the theoretical scattering indicatrix of the same protein calculated by the 'modified cube method' (——). The curves pertain to the aqueous solvent.

shift of the cube network relative to the protein molecule taken into account (the first curve is the main one, the next three curves were obtained by independent shifts of the cube network by $\frac{1}{2}R$ along the three coordinate axes and the fifth was obtained by a simultaneous shift of the cube network by $\frac{1}{2}R$ along three coordinate axes). The parameter B was chosen to be equal to 1·17 Å and with $R = 1{\cdot}3$ Å the Mb–SW volume V was obtained as ≈ 22200 Å^3, which agrees well with the data from neutron scattering. Coordinates of non-hydrogen atoms were taken from the paper by Watson (1969).

Fig. 2 represents a comparison of the Mb–SW scattering indicatrix calculated in this way with the experimental curve obtained by Stuhrmann (1975). It is seen that divergences between the calculated and measured curves not only remain but are even somewhat increased in comparison with the Mb–SW scattering curves obtained under the same conditions by the 'cube method' (Fedorov, Ptitsyn & Voronin, 1974).

The next important stage consists of determination of specific structural changes occurring in Mb–SW in conformity with the experimental scattering curve of this protein. If we assume that the protein structure somewhat 'loosens' at transition from crystal to solution, it is possible, proceeding from the number of hydrophobic contacts between single helices and groups of helices, to evaluate, from the location of these groups in the protein globule and their steric possibilities what regions of the polypeptide chain are the most susceptible of a certain mobility relative to the main bulk of the protein globule. An analysis of the Mb–SW structure has shown that these can be terminal helices A and H, 'hairpin' GH, the group of helices CD, D and the group of helices F, G, H, though in the latter case there must be a certain additional stabilization of this group owing to the contact of the helix F through the haem with the other part of the protein.

To localize structural changes in Mb–SW and to prove their correlation with the changes of the scattering indicatrix we largely used the method of large-angle diffuse scattering described above. First of all we analyzed the possibility of the terminal helices A and H shifting relative to the main bulk of the molecule. With the help of the rotation matrix these helices were turned by 10° (helix A) and by 5° (helix H) around axes indicated in Fig. 3.* For each modified protein structure the diffuse-scattering indicatrix was calculated by the 'modified cube method' and then compared with the experimental scattering curve of Mb–SW. The results of this comparison are given in Fig. 3. It is seen that none of the calculated curves agrees with the experimental one though these indicatrices are closer to the experimental curve than is the original one. Then the shifts of the groups of helices FGH and of the groups CD, D were analyzed. Fig. 4 represents the rotation axes of these groups and the results of the calculation of the large-angle scattering indicatrix for each protein structural modification obtained. As follows from the same figure, in these cases the calculated curves are rather different from the experimental one. However it should be noted that the modification by the shift of groups CD, D leads to noticeable changes of the initial theoretical curve only in a narrow interval of scattering angles ($\mu = 0{\cdot}30 - 0{\cdot}45$ Å^{-1}) and thus can be 'invisible' against the background of other structural changes in the protein.

* Deflection angles were chosen so that the maximum shifts of atoms did not surpass 2 Å.

In the third group of structural changes a shift of the hairpin GH was considered. We turned this group of helices 'from both sides' (Fig. 5): in one case the rotation axis was chosen in the region of connection of this 'hairpin' to the helix F and in the other in the region of its contact with the helix A. It turned out that in both cases the scattering curves of structures modified in this way give virtually complete agreement between the theory and experiment over a wide range of scattering angles. The latter fact is, of course, essential for evaluation of the curve coincidence and therefore we cannot prefer modification I to modification II. In fact, both theoretical curves describe well enough the experimental curve, and deviations of the curve at modification II have only a local character, which seems unimportant when the possible error of the experimental curve is taken into account.

Discussion

The above consideration suggests that the divergence between large-angle scattering indicatrices of Mb–SW in the crystal and in solution can be explained by a structural change (occurring when the protein passes from crystal to solution) resulting in a small (up to 2 Å) increase of the distance between the 'hairpin' GH, and the other part of the

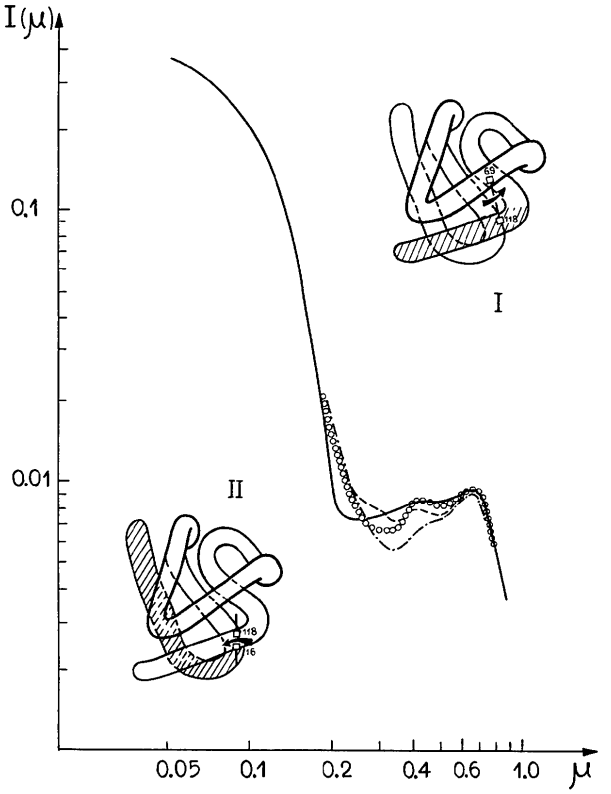

Fig. 3. Theoretical scattering curves of Mb–SW and its structural modifications. The Mb–SW structure in crystal —·—·—; ··· the helix A rotated around the indicated axis by 10° (modification I); --- the helix H rotated around the indicated axis by 5° (modification II); —— experimental scattering curve of Mb–SW (Stuhrmann, 1975). Here and in Figs. 4 and 5 numbers are given for the amino acids through which passes the rotation axis of the hatched part of the molecule.

protein molecule. This 'detachment' can proceed from the helix *F* side and (or) from the helix *A* side. Changes connected with the modification of the region *CD* and helix *D* affect the scattering indicatrix little, and therefore mobility of the regions *CD* and *D* can be neither assumed nor rejected.

It has been already suggested [in particular, proceeding from the data of X-ray diffuse scattering (Stuhrmann, 1973)] that the Mb–SW structure slightly changes ('loosens') at the protein transition from crystal into solution. However, only in this paper have these changes been structurally interpreted. It is important to note that these changes are rather small (up to 2 Å) and it seems that they cannot be localized by any other physical method. In fact, such investigations require a method which gives direct *structural* information, which 'sees' changes over a rather *wide* range of distances – from a few ångstroms up to ten ångstroms, and which is sensitive to small structural rearrangements. No physical method except X-ray diffuse scattering satisfies all these requirements. For example, optical methods are rather sensitive to small-scale distances and give important information on the protein secondary structure, but they do not 'see' large-scale rearrangements. Our large-angle technique satisfies all the requirements mentioned and therefore is unique for solving these problems. This method can play an important role in the determination and testing of the structural principles of protein functioning in solution and therefore is quite promising for further studies of protein structure.

The authors express their sincere gratitude to Professor H. B. Stuhrmann for supplying the experimental X-ray scattering curve of sperm-whale myoglobin.

References

BEEMAN, W. W. (1967). *Small-Angle X-ray Scattering*, edited by H. BRUMBERGER, pp. 197–211. New York, London, Paris: Gordon and Breach.

FEDOROV, B. A. (1967). *Itogi Nauki Techn.: Mol. Biol.* **8**, (1), 6–69.

FEDOROV, B. A. & DENESYUK, A. I. (1977). *Acta Cryst.* B**33**, 3198–3204.

FEDOROV, B. A., KRÖBER, R., DAMASCHUN, G. & RUCKPAUL, K. (1976). *FEBS Lett.* **65**, 92–95.

FEDOROV, B. A., PTITSYN, O. B. & VORONIN, L. A. (1972). *FEBS Lett.* **28**, 188–190.

FEDOROV, B. A., PTITSYN, O. B. & VORONIN, L. A. (1974*a*). *J. Appl. Cryst.* **7**, 181–186.

FEDOROV, B. A., PTITSYN, O. B. & VORONIN, L. A. (1974*b*). *Molekul. Biol. (Moscow)*, **8**, 693–702.

IBEL, K. & STUHRMANN, H. B. (1975). *J. Mol. Biol.* **93**, 255–265.

IMOTO, T., JOHNSON, L. N., NORTH, A. C. T., PHILLIPS, D. & RUPLEY, J. A. (1972). *The Enzymes*, edited by P. BOYER, 3rd ed., Vol. 7, pp. 665–868. New York and London: Academic Press.

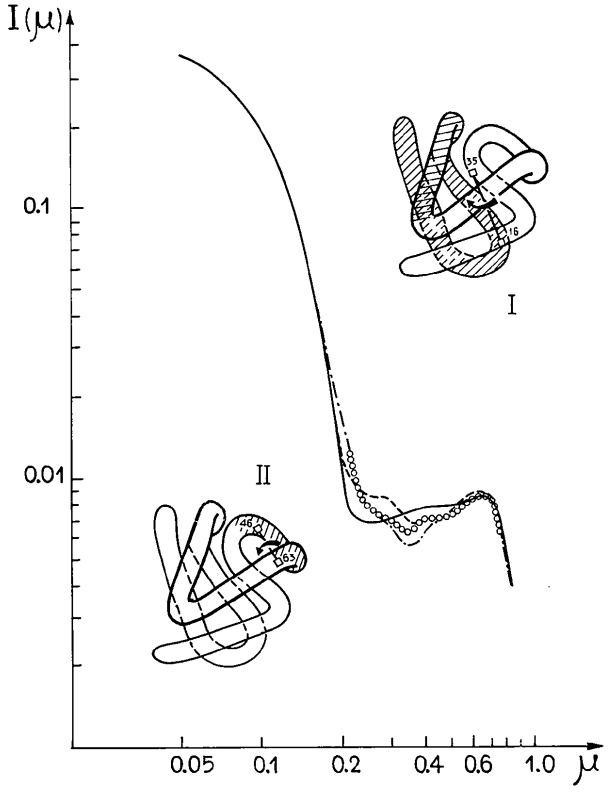

Fig. 4. Theoretical scattering curves of Mb–SW and of its structural modifications. The Mb–SW structure in crystal –·–·–; --- the group of helices *FGH* is turned by 5° around the indicated axis (modification I); ··· the region *CD* and the helix *G* are turned by 10° around the indicated axis (modification II); —— the experimental scattering curve of Mb–SW (Stuhrmann, 1975).

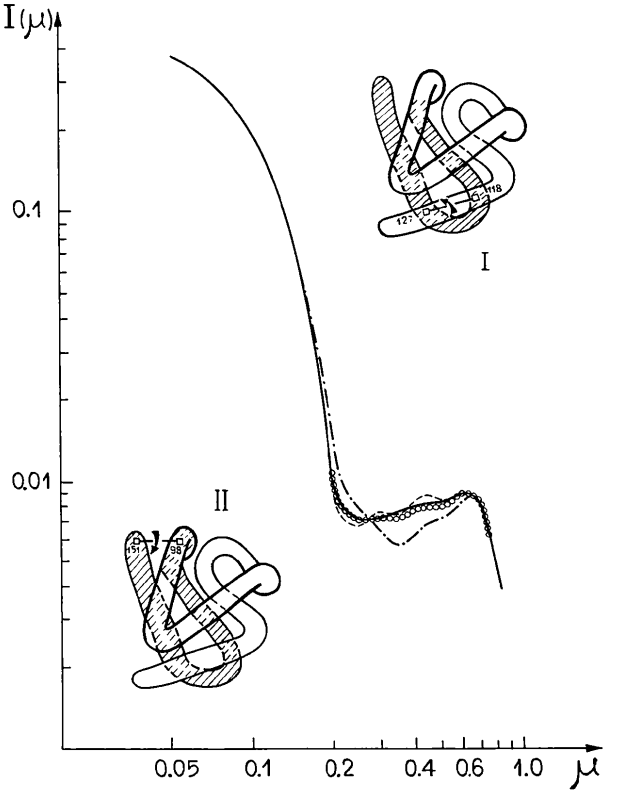

Fig. 5. Theoretical scattering curves of Mb–SW and of its structural modifications. The Mb–SW structure in crystal · · ·: the 'hairpin' *GF* is turned by 5° around the indicated axes; ··· modification I; --- modification II; —— the experimental scattering curve of Mb–SW (Stuhrmann, 1975).

KIRSTE, R. G. & STUHRMANN, H. B. (1967). *Z. Phys. Chem. (Frankfurt)*, **56**, 338–341.
LEE, B. & RICHARDS, F. M. (1971). *J. Mol. Biol.* **55**, 379–400.
STUHRMANN, H. B. (1970). *Z. Phys. Chem. (Frankfurt)*, **72**, 185–198.
STUHRMANN, H. B. (1973). *J. Mol. Biol.* **77**, 363–369.
STUHRMANN, H. B. (1975). Private communication.
TIMCHENKO, A. A. (1978). To be submitted to *Kristallografiya*.
WATSON, H. C. (1969). *Prog. Stereochem.* **4**, 299–333.

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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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