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

(Extended Abstract only) By I. PILZ, *Institut für Physikalische Chemie, Universität, A-8010 Graz, Heinrichstrasse 28, Austria*, O. KRATKY, *Institut für Röntgenfeinstrukturforschung, A-8010 Graz, Steyrergasse 17, Austria* and A. LICHT and M. SELA, *Weizmann Institute of Science, Department of Chemical Immunology, Rehovot, Israel*

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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')<sub>2</sub>, 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% of 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  $8 \times 10^6$ , radius of gyration 12.1 nm, maximum diameter 36 nm, thickness 4.5 nm, volume 1800 nm<sup>3</sup>). 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 $\alpha$ -Crystallin of Calf Eye Lens

(Extended Abstract only) By J. BERGER, *Department of Structure Biology, Biocenter Basel, Switzerland* and R. J. SIEZEN,\* *Department of Biochemistry, University of Nijmegen, The Netherlands*

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$\alpha$ -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  $\alpha$ -crystallin from calf eye

lens.  $\alpha$ -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

\* Present address: Australian National University, PO Box 334, Canberra, ACT 2601, Australia.