SPERM WHALE MYOGLOBIN STRUCTURE IN SOLUTION DIFFERS FROM ITS STRUCTURE IN CRYSTAL BY A SHIFT OF THE 'HAIRPIN' GH

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1. Introduction

The new method described in [1] and used in previous communications [2–3] for the study of structural re-arrangements in globular proteins by large-angle X-ray diffuse scattering is applied in this paper to analysis of concrete structural changes in sperm whale myoglobin on its transition from crystal to solution. This protein has been already studied in solution in different states (e.g. [4–7]) by small-angle and large-angle X-ray and neutron scattering and reliable X-ray scattering curves have been obtained over a wide interval of scattering angles. In 1972 for the first time a reasonable agreement between the theory and experiment was demonstrated by a comparison of the Mb-SW experimental scattering curves with those calculated using the 'cube method' [8]. This provided evidence in favour of the similarity of the Mb-SW structure in 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 due to experimental errors and/or incorrectness of the theory or to a real, rather small difference in the Mb-SW structures in crystal and in solution.

2. Calculation method and results

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 [9] 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 [10] using the main principles of the earlier 'cube method' [11] and taking into account its shortcomings of the 'second order' which are important for a strict quantitative comparison of experimental and theoretical scattering indicatrices.

On the basis of the 'modified cube method' the scattering indicatrix of Mb-SW was calculated in an aqueous medium. It is represented in fig.1 in comparison with the experimental curve obtained [9]. It turned out that divergences between the calculated and measured curves not only remained but even somewhat increased in comparison with the Mb-SW scattering curves obtained in the same conditions by the 'cube method' [8]. This suggests that there is a certain difference between the Mb-SW structure in crystal and in solution.

The next important stage consists in determination of concrete 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 to evaluate, proceeding from the quantity of hydrophobic contacts between single helices and groups of helices, 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. Appropriate analysis of the Mb-SW structure

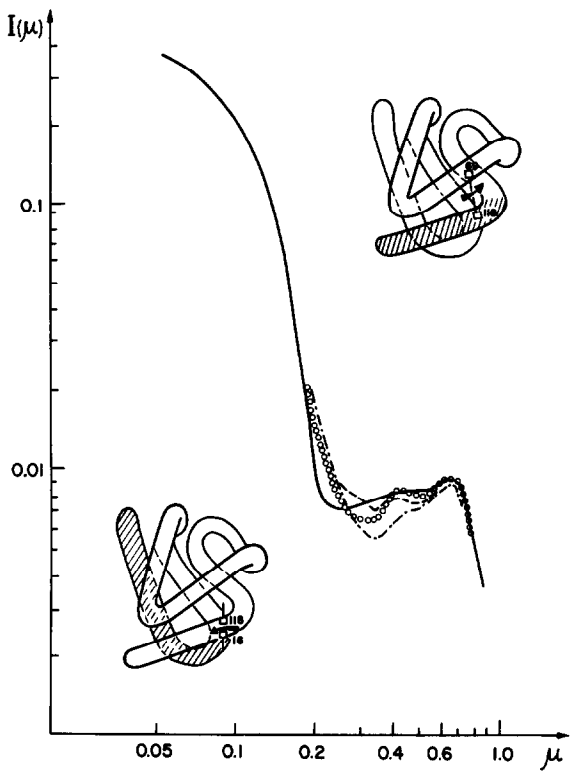

Fig.1. Theoretical scattering curves of Mb-SW and its structural modifications. Mb-SW structure in crystal (–·–·–); (o o o) helix A turned around the indicated axis by 10° (modification I); (– – –) helix H turned around the indicated axis by 5° (modification II); (——) experimental scattering curve of Mb-SW [8]. Here and in figs 2 and 3 numbers are given for the amino acids through which the rotation axis of the hatched part of the molecule passes.

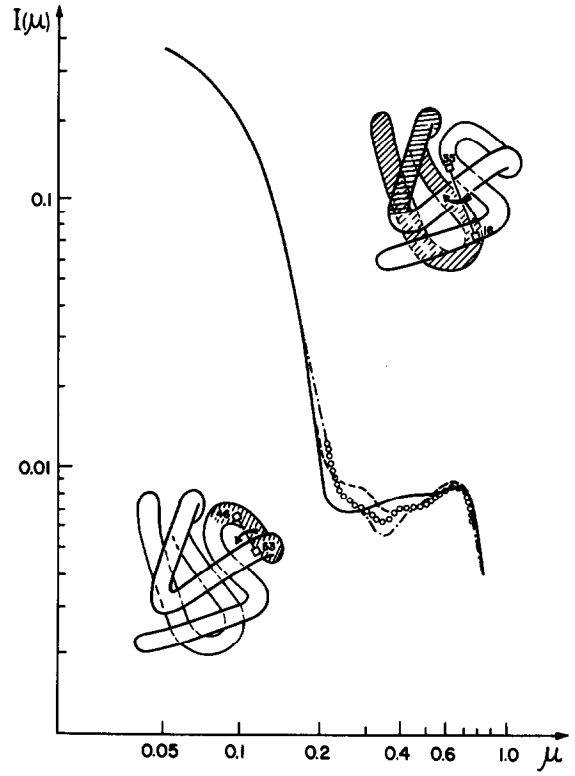

Fig.2. Theoretical scattering curves of Mb-SW and its structural modifications. Mb-SW structure in crystal (–·–·–); (– – –) the group of helices FGH is turned by 5° around the indicated axis (modification I); (o o o) the region CD and helix G are turned by 10° around the indicated axis (modification II); (———) experimental scattering curve of Mb-SW [8].

has shown that these can be the 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 there must be a certain additional stabilization of this group owing to the contact of 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 mainly used the described method of large-angle diffuse scattering. 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 rotation matrix these helices were turned by 10° (helix A) and by 5° (helix H) around axes indicated in fig.1.* 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.1. It is seen that none of the calculated curves agrees with the experimental one though these indicatrices are closer to the experimental curve in comparison to the original one. Then the shifts of the groups of helices FGH and of the group CD, D were analyzed. Figure 2 represents the rotation axes of these groups and the results of the calculation of the

* Here and further deflection angles were chosen so that the maximum shifts of atoms do not surpass 2 A

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 does not result in considerable changes of the initial theoretical curve and thus can be 'invisible' against the background of other structural changes in the protein.

In the third variant of structural changes a shift of the hairpin GH was considered. We turned this group of helices 'from both sides' (Fig.3): in one case the rotation axis was chosen in the region of the connection of this 'hairpin' with helix F and in the other, in the region of its contact with helix A. It turned out that in both cases the scattering curves of structures modified in this way give a virtually complete agreement between the theory and experiment.

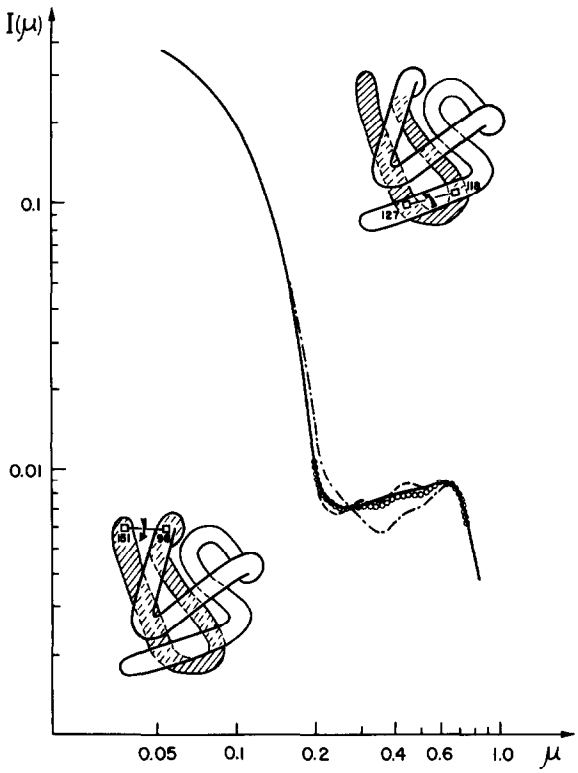

Fig.3. Theoretical scattering curves of Mb-SW and its structural modifications. Mb-SW structure in crystal (–·–·–); the 'hairpin' GH is turned by 5° around the indicated axes; (o o o) modification I; (– – –) modification II; (———) experimental scattering curve of Mb-SW [8].

3. Discussion

Thus the above consideration suggests that the divergence between large-angle scattering indicatrices of Mb-SW in 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 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 little the scattering indicatrix, therefore mobility of the regions CD and D can be neither observed nor rejected.

It has been already suggested (in particular, from the data of X-ray diffuse scattering [12]) that the Mb-SW structure slightly changes ('loosens') on transition of the protein from crystal into solution. However, only now are these changes interpreted structurally. 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 since such investigations require a method which would give direct structural information, would 'see' changes in a rather wide range of distances, from several Å to 10 Å and would be sensitive to small structural rearrangements. No physical method, except X-ray diffuse scattering, satisfies all these requirements.

Acknowledgements

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