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Functional SAXS study of haemocyanin dioxygen-carrier protein[1]

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Abstract

The effects of conformational rearrangements on dioxygen binding to molluscan haemocyanins have been investigated by small-angle X-ray scattering (SAXS). The SAXS patterns of the oxygenated and deoxygenated forms of *Octopus vulgaris* haemocyanin are significantly different; whereas the patterns of the two forms of *Rapana thomasiana* haemocyanin are almost superimposable. A program has been developed, based on the differences in molecular dimensions, in order to simulate the effects observed in the investigation.

Keywords: Small angle X-ray scattering; Haemocyanins; Conformational rearrangement

1. Introduction

Haemocyanins (Hcs) are oligomeric proteins of high molecular weight found in the haemolymph of several species belonging to the Phyla of Mollusca and Arthropoda. The biological role of these proteins consists in the transport-storage of molecular dioxygen. The binding site contains a couple of copper ions directly bound to the protein imidazole side-chains. The reversible dioxygen binding occurs via electron transfer from the two cuprous ions to the dioxygen, which in turn is bound as peroxide to the dinuclear cupric site. The proteins from the two phyla are very similar in physiological behaviour as well as in spectroscopic and biochemical properties, but differ greatly in quaternary structure and copper-to-protein ratio. The MW of the minimal functional unit capable of binding one dioxygen molecule is about 50 kDa in Mollusca and 75 kDa in Arthropoda. The quaternary structure of arthropod Hcs can be considered as the result of further polymerisation of a 16 S unit, which in turn consists of a trigonal antiprism built up from six kidney-shaped 75 kDa functional subunits. Higher aggregates of arthropod Hcs are dimers (24 S), tetramers (37 S) and octamers (62 S) of the 16 S unit. The basic unit of molluscan Hcs is formed from dioxygen-binding functional subunits of about 50 kDa each, covalently linked to form an 11 S structural subunit whose size and structure are still controversial. Further polymerisation of this basic unit gives a hollow cylinder of 350 Å diameter with a five- or tenfold symmetry axis [1].

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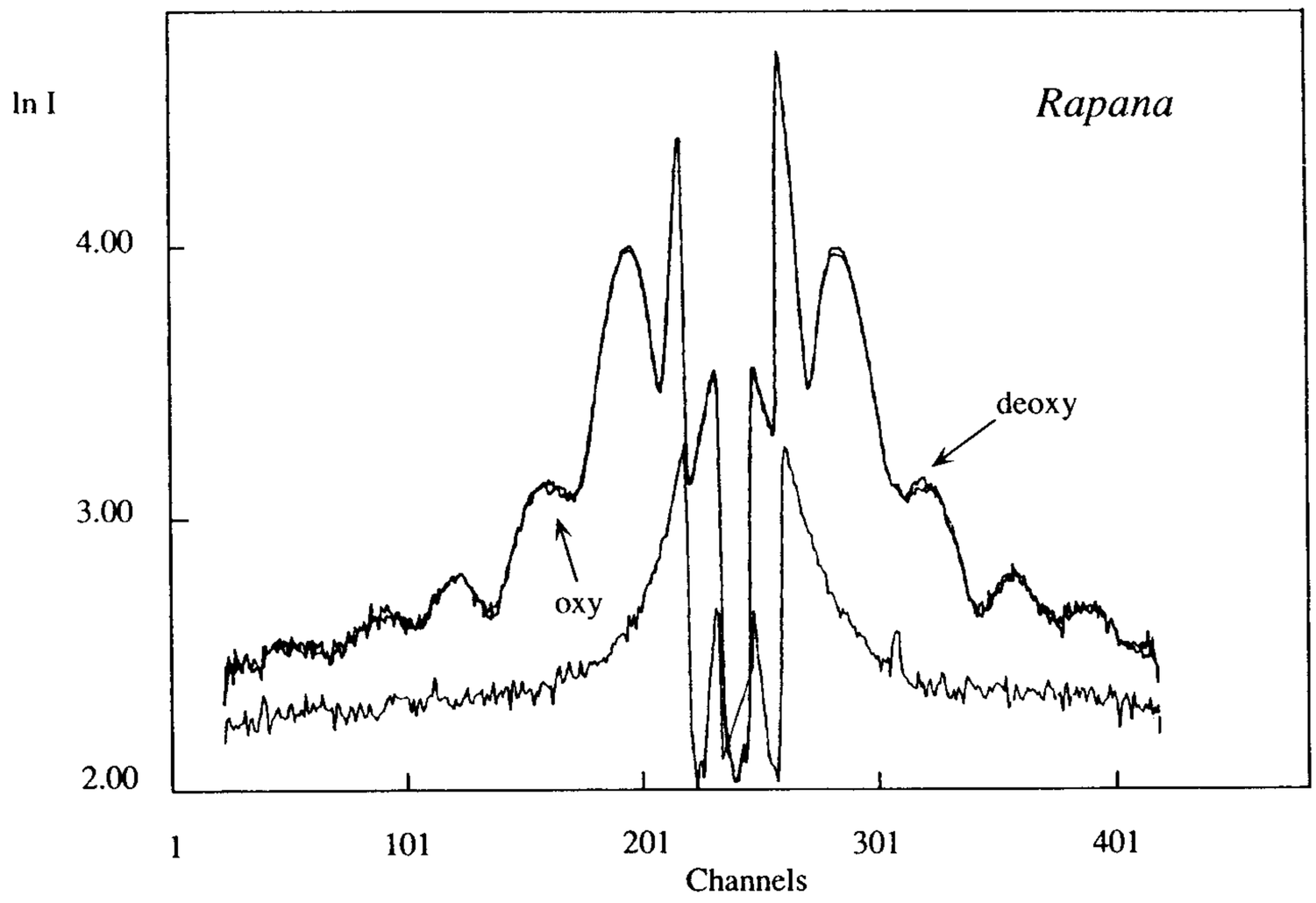

Fig. 1. SAXS patterns of *Rapana* Hc in the oxygenated (lower trace) and deoxygenated (upper trace) form. The featureless trace at the bottom represents the buffer scattering pattern.

The binding of dioxygen to Hcs generally involves site–site interactions, resulting in conformational changes associated with the process. We performed SAXS measurements on the deoxygenated and oxygenated forms of two Hcs from Mollusca, *Octopus vulgaris* and *Rapana thomasiana*, with the aim of detecting differences in the shape and dimensions of the molecule in the two functional states.

2. Experimental

Native Hcs were prepared according to standard procedures [2]. The Hcs were saturated with oxygen at atmospheric oxygen pressure. The buffers used were 50 mM Tris–HCl pH 7.5 containing 40 mM $CaCl_2$ with *Octopus* Hc, 50 mM imidazole–propionic acid pH 7.5 containing 0.5 mM $ZnCl_2$ with *Rapana* Hc. The samples were deoxygenated by addition of an aliquot of 200 mM sodium sulphite solution in the same buffer (final concentration 5 mM) in the presence of catalytic amounts of copper sulphate. Measurements were performed at different protein concentrations from 1.0 mg ml^{-1} up to 80 mg ml^{-1}. The reproducibility of the effects observed was further checked by replicate measurements.

The SAXS measurements were performed at the D24 station high-flux beamline of the LURE facility with the standard set-up. The wavelength of the high monochromatic ($\Delta\lambda/\lambda = 10^{-3}$) X-ray beam was 1.488 Å. The data were collected following the high-statistic procedure with a logging time $\Delta t = 400$ s. The scattered intensities were recorded on a position-sensitive proportional detector 2988 mm far from the sample, which allows angular resolutions $\Delta s = 9.760 \times 10^{-5}$ Å^{-1}($s = 2\sin(\theta)/\lambda$). Further details are given in [3]. The Fourier transforms of the hollow cylinder model were generated using home-made software.

3. Results and Discussion

A typical SAXS pattern of *Rapana* Hc is shown in Fig. 1 where several maxima are evident (the beam stop not perfectly centred on the primary light beam cut the scattering intensity asymmetrically). The shape of the scattering curve is not significantly affected by the protein concentration. The oxy- and deoxy-forms do not show significant differences, the scattering patterns being almost superimposable. Fig. 2 reports the results of the

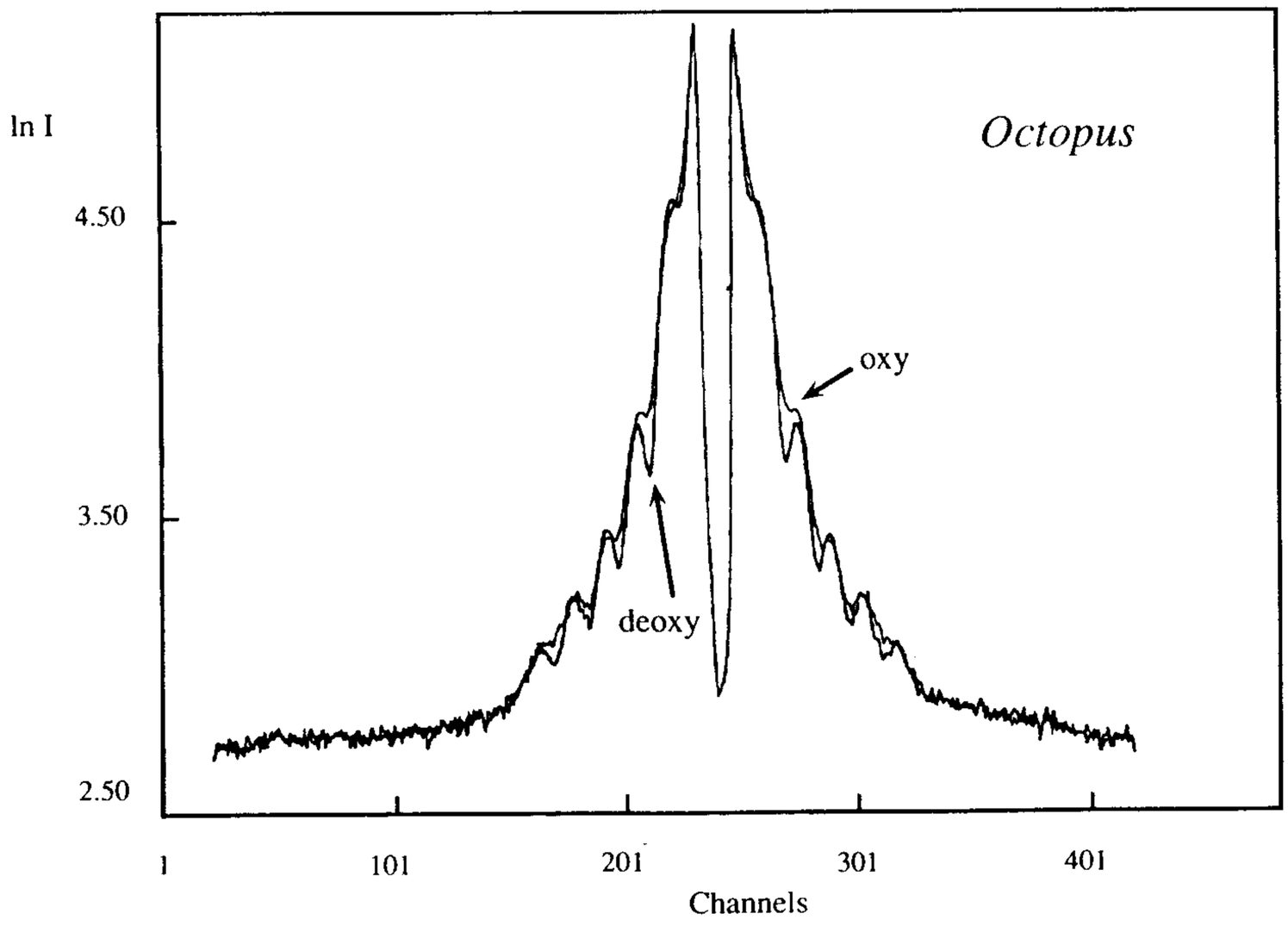

Fig. 2. SAXS patterns of *Octopus* Hc in the oxygenated (upper trace) and deoxygenated (lower trace) form.

same measurement with *Octopus* Hc: in contrast to *Rapana* Hc, striking differences are evident on passing from the oxygenated to the deoxygenated form, but no concentration effects are observed for either. In order to interpret the structural basis of such SAXS differences, we are developing a simulation program to generate the scattering function on the basis of the shape and of the dimensional parameters of a given structure. The shape of the Hc molecule used in our simulations was taken

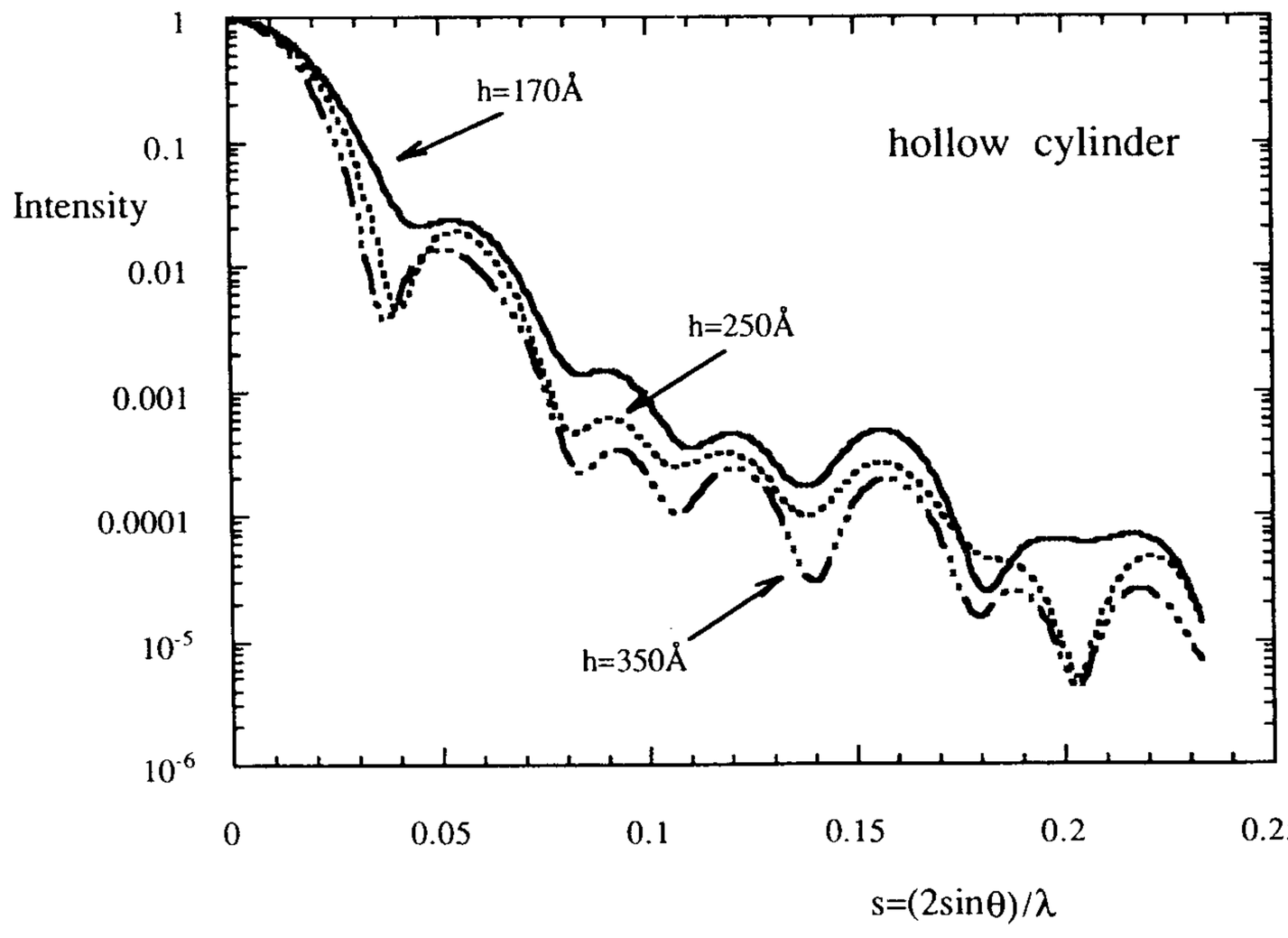

Fig. 3. Theoretical scattering curves of a hollow cylinder; the internal (100 Å) and external (350 Å) diameters are taken as constants; the cylinder length as a variable.

from electron microscopy images, which show hollow cylinders for both *Octopus* and *Rapana* Hcs. For the dimensions we have taken the internal (100 Å) and external (350 Å) diameters as constants and the length of the cylinder as a variable. As shown in Fig. 3, the relative minima increase with increasing cylinder length.

The preliminary results obtained with the simulation program are consistent with the hypothesis that the differences observed in the SAXS curves for *Octopus* oxy- and deoxy-Hcs are related to a rearrangement of the mass distribution that involves changes in the ratio between the two main dimensions (external diameter and length). In this context, the lack of effects found for the oxygen binding process of *Rapana* Hc can be interpreted as due to internal compensation of the conformational effects, related to the cooperativity of the oxygen binding, which leaves the main dimensions of the molecule almost unchanged. In order to check the correlation between cooperativity and oxygen-related SAXS effects, we plan to perform further experiments in the range of pH where Hcs show Bohr effects, which result in changes in oxygen affinity and cooperativity.

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References

[1] B. Salvato and M. Beltramini, Life Chem. Rep., 8 (1990) 1.
[2] G. Preaux and C. Gielens, R. Lontie (Ed.), in Copper Proteins and Copper Enzymes, Vol. II, (CRC Press Inc., Boca Raton, 1984), p. 159.
[3] M. Beltramini, E. Borghi, P. Di Muro, A. Ghiretti Magaldi, A. La Monaca, B. Salvato, C. Santini and G. Tognon, J. Phys. IV Colloq. C8 (Suppl. JP I) 3 (1993) 249.