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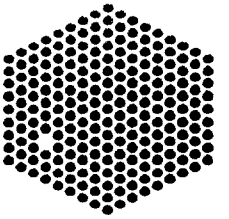

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Structural dynamics of the oxygen transporting metallo-protein Hemocyanin.

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In Arthropodes, e.g. spiders, the functions of red blood cells of mammals and some other blood components are localized in a large protein complex, which is a solute of the cell-free haemolymph: hemocyanin. These huge protein complexes are a hetero-oligomers of 6, 12, 24 or 48 subunits of 72 kDa molecular mass (fig.1). The complex is capable of at least two functions: i) oxygen transport, and ii) tyrosinase enzymic activity. Due to its universal functionality, Hemocyanin provides up to 80% of the total protein content of blood in Arthropods.

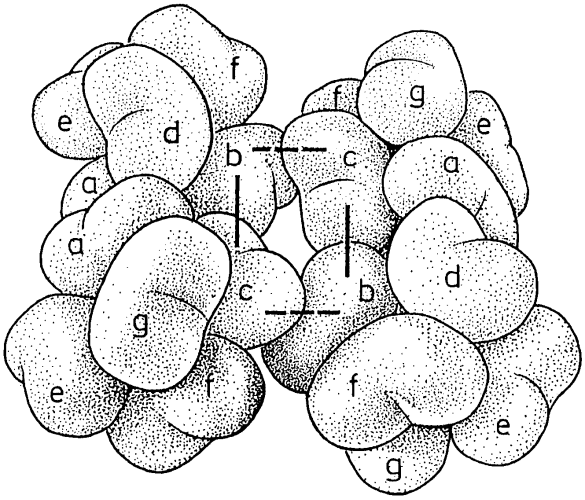

Fig.1: Proposed structure of a 2 x 12 - meric hemocyanin. Each subunit contains a copper-center [1].

The highly cooperative oxygen transport by Hemocyanin is mediated by a metal center in each subunit. In contrast to haemoglobin, this is not an iron (haem), but a binuclear copper center (Cu_2O_2). Hemocyanin shows a molecular regulation due to the variety of metabolic situations in Arthropods, e.g. the oxygen pressure in legs and body, probably by structural rearrangements.

We have investigated the structure of 12-meric hemocyanin from lobster (molecular mass 864,000) in aqueous solution by X-ray small angle scattering at the JUSIFA camera at the beamline B1 at DESY / HASYLAB, Hamburg. The solution was irradiated at 4°C in a quartz flow-through capillary using a 0.9 x 1.1 mm^2 beam of 8 keV (1.5 Å) photons. Scattering profiles of protein solution (6 g/l) and buffer were taken at 0.9 and 3.6 m distance from the sample using a 2D-detector (256 x 256 pixel) in 3 h for each specimen.

The scattering profile of free hemocyanin in the presence of oxygen (250 µM) consists of a strong central signal and characteristic weak side maxima, resulting from the assembly of the protein subunits separated by solvent filled clefts (fig.2). The evaluation of the profile resulted in a radius of gyration of R_g = 6.78 nm, which represents the extension of the complex. The averaged subunit distance of d_s = 6.3

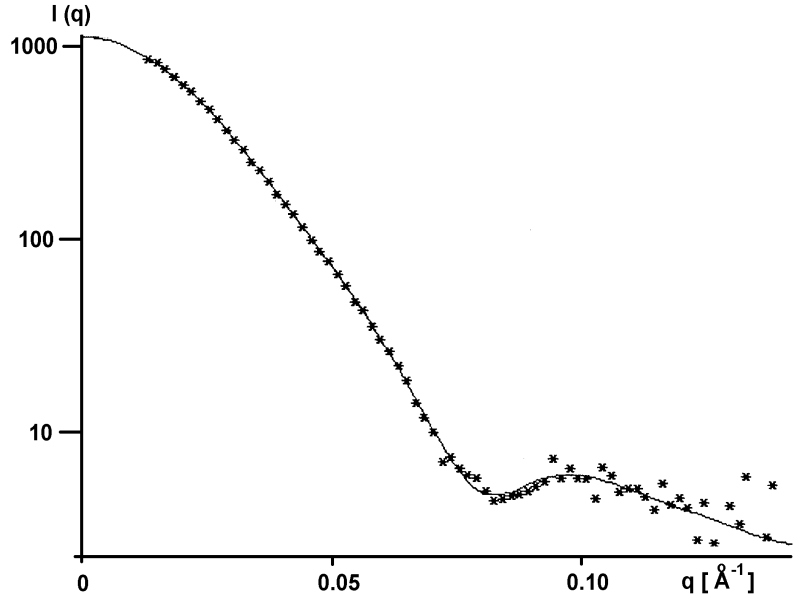

Fig.2: X-ray small angle scattering of 12-meric hemocyanin from lobster (6 g/l; 2 x 3h).

nm^{-1} was obtained from the position of the broad side maxima q_S according to Bragg's law ($d_S = 2\pi / q_S$). The distance distribution function (fig.3) was obtained by indirect fourier transformation. The profile indicated the maximal dimension inside the molecule of r_{max} = 24 nm.

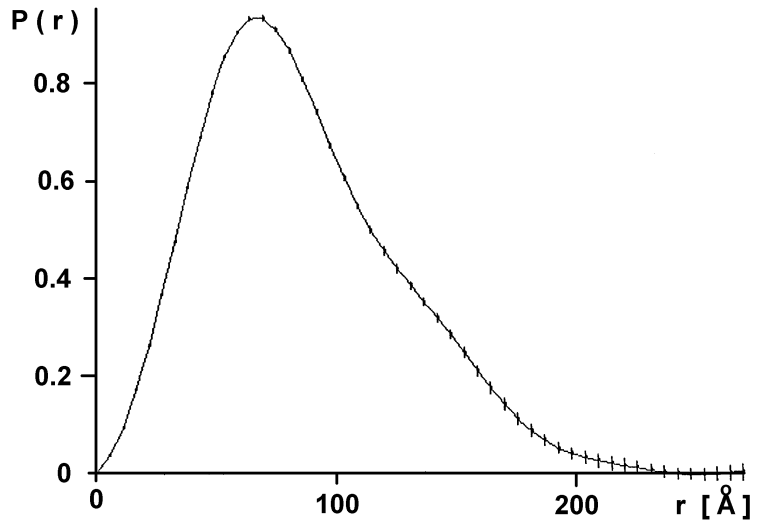

Fig.3: The distance distribution function of hemocyanin indicates a maximal dimension of 24 nm.

In the presence of the metabolic ligand lactate (20 mM), which is an regulative effector due to functional studies, a shrinking of the hemocyanin molecule was observed. The radius of gyration of the Haemocyanin-lactate complex was R_g = 6.5 nm. The position of the side maxima shifted scarcely after addition of the effector. The shape of the broad side maximum at 1 nm^{-1}, which is suggested to be heterogenous, became narrower. These results indicate a structural flexibility of the Hemocyanin complex. This special case of allostery is obviously mediated by movements of subunits. A description, which allows the calculation of the resulting modulation of the biological function, has been given with the "nesting model" of structural rearrangements.

1) Decker, H.; Hartmann, H.; Sterner, R.; Schwarz, E.; Pilz, I. (1996) FEBS Lett. 393, 226-230