Hamburger Synchrotronstrahlungslabor HASYLAB
am Deutschen Elektronen-Synchrotron DESY

Jahresbericht 1999

Contributions of the MPSD group :

Part1, 865-866 : Nawroth et al. : XANES and EXAFS of Metalloproteins

Part1, 979-980 : Gebhardt et al. : pH dependent SAXS of Hemocyanin

Structural changes of the oxygen transport protein Hemocyanin detected by pH dependent USAXS

R. Gebhardt, I. Lauer, T. Nawroth, H. Decker[1], G. Goerigk[2], G. v. Krosigk[3]

Inst. f. Biochemie, Membrane Protein Structure Dynamics Group MPSD, Universität, D-55099 Mainz
[1]Inst. f. Molekulare Biophysik, Fachbereich Biologie, Universität, Welderweg 26, D-55128 Mainz
[2]Forschungszentrum FZ Jülich, IFF, Postfach 1913, D-2425 Jülich
[3]DESY - HASYLAB, Notkestrasse 85, D-22603 Hamburg
http://www.MPSD.de

Hemocyanin is the oxygen transport protein in the blood of atropods and several other organisms. It is a large oligomeric metalprotein, which bears two copper atoms in each protein subunit (70,000 mass). The investigated Hemocyanin from the spider *Eurypelma californicum* is a 24-mer of 1,700,000 mass which shows a molecular regulation by allosteric conformational changes. As the human Haemoglobin, an important functional regulation of Hemocyanin depends on pH, which results in the release of tightly bound oxygen in tissue under stress, e.g. after exhaustive work.

We investigated the pH-dependence of the structure of Hemocyanin from *Eurypelma californicum* in solution (10g/l in 0,1 M Tris/HCl-MES buffer, 10 mM $CaCl_2$, 10 mM $MgCl_2$). The ultra small angle scattering was observed at q = 0.003 - 0.033 $Å^{-1}$ at the USAX-beamline (BW4; wiggler) with a novel flat window flow-through cell [1], whereas the small angle scattering was investigated at q = 0.010 - 0.6 $Å^{-1}$ at the JUSIFA SAXS-beamline (B1; bending magment) at HASYLAB ($q = (4\pi/\lambda)\sin\theta$; θ is the half scattering vector) with the flow-through capillary equipment as described for XANES, EXAFS and SAXS / ASAXS experiments in this issue [2]. The flat window flow-through cell was equipped with two biocompatible 15 µm Nalophan windows (unstretched polyester), which was a gift of the Kalle AG, Wiesbaden (Dr. J. Coudandin). The estimation of the scattering profiles took 20 min for the USAXS range (flux 10^{10} ph/s in 1x1 mm, d = 12.7 m) and 2 + 4 h for the SAXS range (flux 10^8 ph/s in 1x0.6 mm; d = 3.618 and 0.918 m).

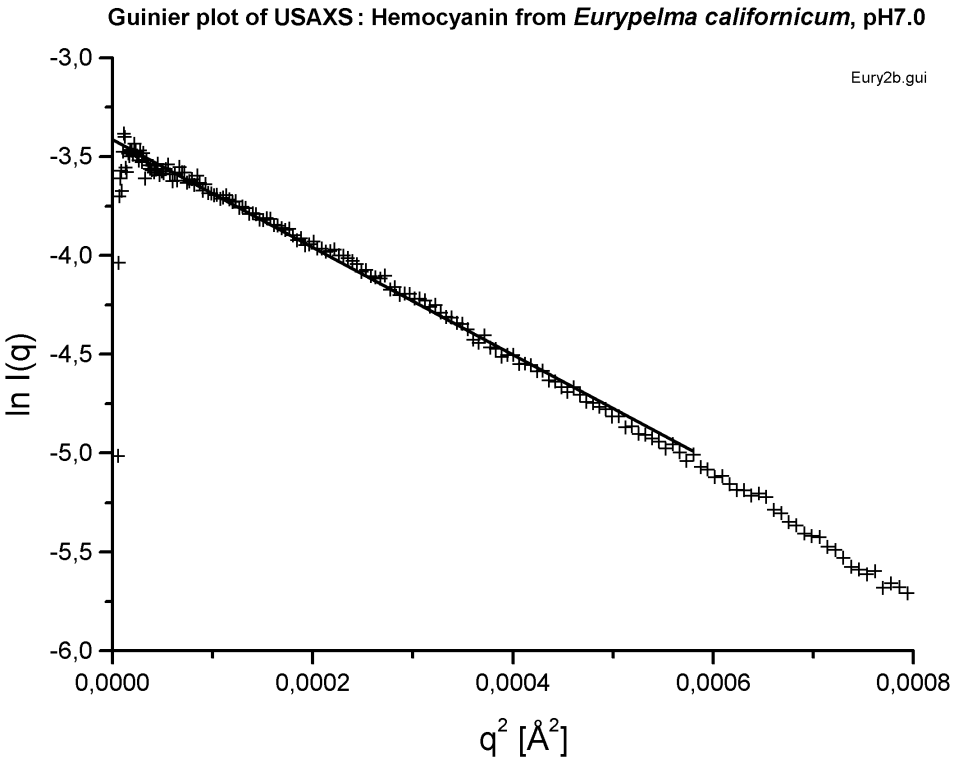

Figure 1: The Guinier representation of USAXS of Hemocyanin from *Eurypelma californicum* (10g/l) yields at pH7.0 an apparent radius of gyration of R_g = 90.31 ± 0.31 Å (q (fit) = 0.00336 - 0.0225 $Å^{-1}$).

The ultra small angle scattering of Hemocyanin is shown in fig.1 as Guinier plot. The fit in the region q = 0.00336 - 0.0225 $Å^{-1}$ yielded an apparent radius of gyration of R_g = 90.31 ± 0.31 Å. The linear USAXS range shows that the enzyme was at c = 10 g/l free of aggregates, which may appear at higher concentration.

The investigation of the pH dependence of the protein structure yielded the profile shown in fig.2. For the experiment a single sample was repetitively investigated by SAXS after stepwise addition of MES-buffer (The Tris-MES system allows a simple pH-adjustment by mixing of 2 buffer solutions). The result depicts that the Hemocyanin from Eurypelma appears in several structural modifications, which differ in the expansion of the molecule. The probable explanation of subunit displacements in and between the oligomeric protein complex (24-mer) has to be tested by molecular modeling after further estimation of the pH dependent SAXS. The size changes correspond to changes in the biological function: The Hill plot of the oxygen binding function indicates changes in the subunit cooperativity in that ranges, where the size depends on the pH of the solution.

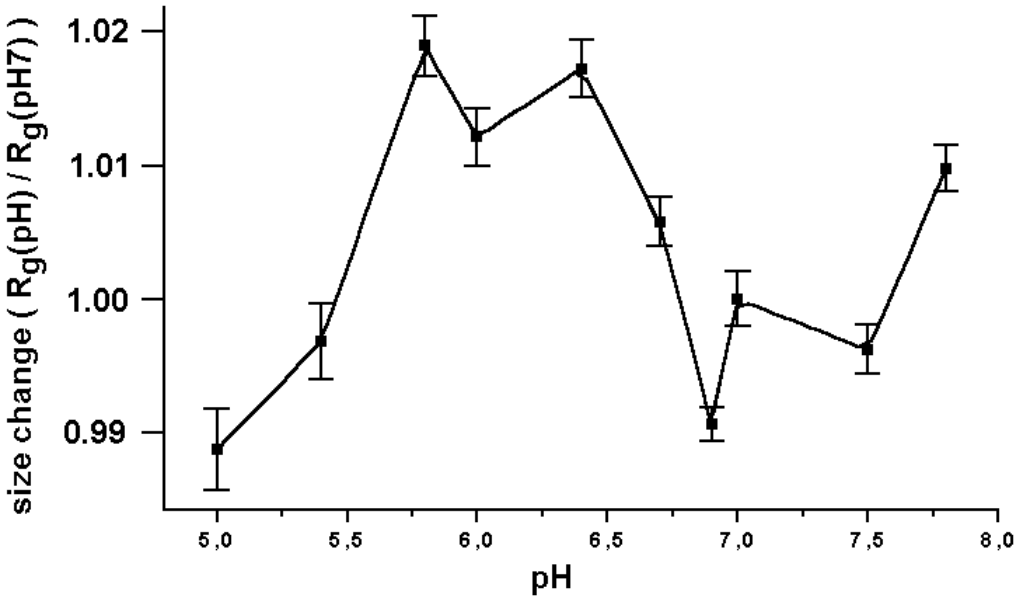

Figure 2: Structural changes of Hemocyanin from *Eurypelma californicum* (10g/l) upon pH-variation as estimated at the USAX beamline. The pH-dependent changes in the expansion of the molecule correspond to transitions in the biological function, i.e. changes in the subunit cooperativity for oxygen binding.

Addendum : The pH-values given in Fig.2 are apparent pH's at pH < 8 obtained with a reference buffer. The true pH-vaule with the protein solution varies from 8.0 (right) to about 6.5 (left).

References

[1] T. Nawroth, I. Lauer, K. Zwicker, H. Hartmann, H. Decker, M. Rössle, H. Heumann, G. Goerigk, G. v. Krosigk, HASYLAB Annual report 1, 921 (1998)

[2] T. Nawroth, R. Gebhardt, H. Decker, G. Goerigk, HASYLAB Annual report, this issue (1999) "XANES and EXAFS of Metalloproteins by subtraction of true reference spectra obtained with a flow throuh cell"