Jahresbericht 1997

Annual Report I

Hamburger Synchrotronstrahlungslabor
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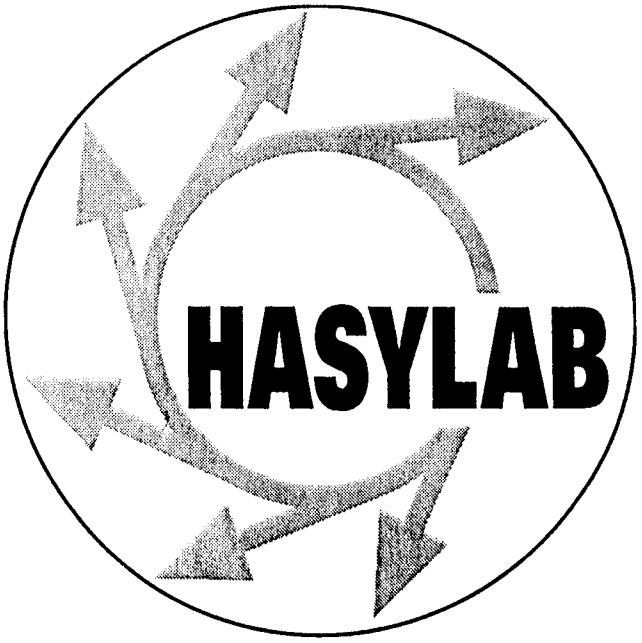

SAXS Measurements of Keyhole Limpet Hemocyanin (KLH1) in the Didecameric Form

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Hemocyanins are large oxygen transport proteins in the hemolymph of molluscs and arthropods. Two classes of hemocyanins are known which are different in structure. While hemocyanins in arthropodes consist of complexes that exhibit 6-meric multiples of 72 kDa subunits, the molluscan counterparts are ring shaped molecules exhibiting 10 subunits of enormous size (350-450kDa). In the case of the hemocyanin from the keyhole limpet (*megathura crenulata)* two different forms with 8 (KLH1) or 7 (KLH2) oxygen-binding domains are known. Depending on the pH of the solution the decameric rings complex to multidecameres.

In order to reveal the quaternary structure of the di-decameric form of KLH1 SAXS measurements were performed on the JUSIFA device at the beamline B1 / HASYLAB Hamburg. Due to the enormous size of the molecule only the longest (3618mm) of four possible distances of the 2D-detector was chosen for these experiments. The protein solution (pH 7,4) was kept in a 1mm quartz flow-through capillary which was irradiated by a 10000eV (1.25A) beam at a temperature of 19°C. Measurements with a total time of 180min were taken for the protein under oxygenating conditions and buffer, respectively.

The pronounced periodic maxima and minima of the scattering curve indicate a particle shape similar to a hollow cylinder. However as a comparison of our data with theoretical scattering curves shows, the interference maxima are not as pronounced as they should be for a pure hollow cylinder (not shown). Preliminary simulations yield a possible model for the molecular shape which consists of a hollow cylinder with two torus shaped collars located in the inner part of the ring (Fig.1).

Fig 1: Schematic view of the model

The suggested model corresponds to previous findings for the single-decameric KLH1 by similar measurements [1]. The didecamer appears to consist of to decameric building blocks turned by 180°. These results are in qualitative agreement with those obtained for a similar hemocyanin (*Helix pomatia*) [2]. However, in comparison with known electron-microscopic images [3][4], our model is smaller by about 15%.

Thorough investigations of the data lead us to the assumption that these deviations are mainly due to some difficulties in the application of the JUSIFA device for the structure analysis of proteins as large as molluscan hemocyanins. In our case the major problems were the

inaccessibility of the very small angle range (Guinier region) and the typically very low scattering intensities of proteins which made perturbative effects of the device crucial. To overcome these limiting factors additional measurements at the USAXS device at the HASYLAB and neutron scattering experiments are planned.

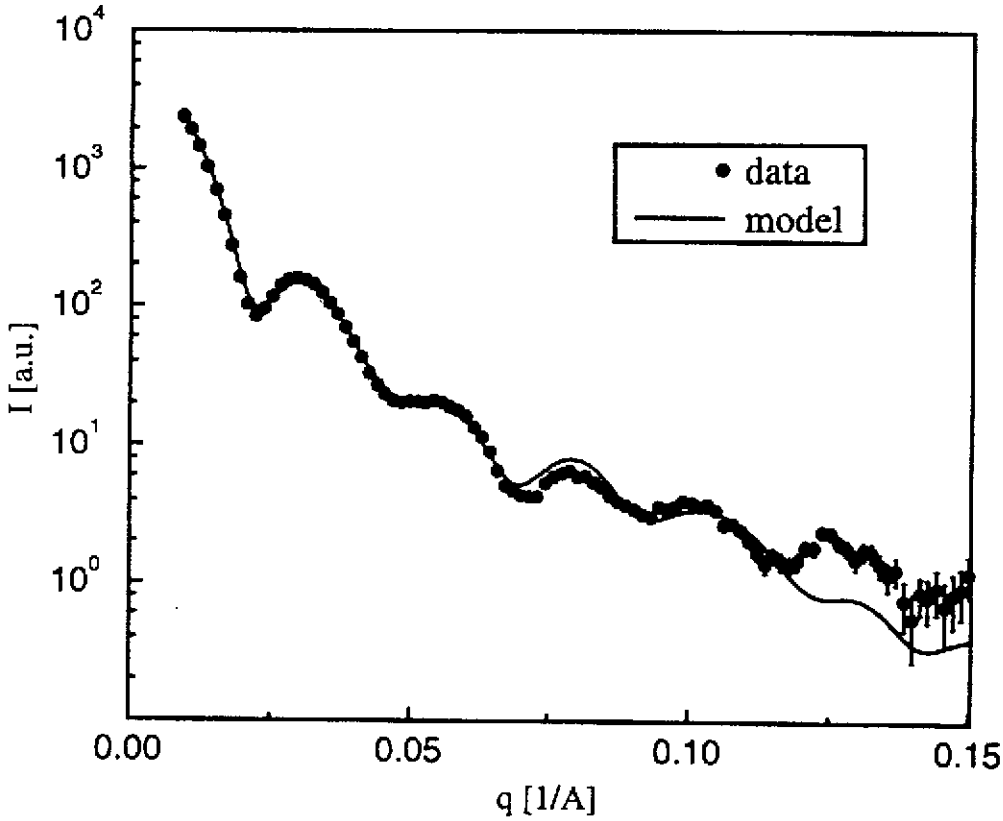

Fig.2: Small angle x-ray scattering data of didecameric KLH1 and theoretical scattering curve from the suggested model

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We like to acknowledge J.Markl, Universität Mainz, for the donation of the KLH. This work was supported by NMFZ.