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Annual Report II

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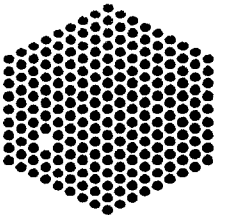
Contributions with the MPSD group :



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Evidence for carbohydrate mediated molecular interaction of the glycoprotein clusterin.

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Clusterin (gp80) is a large glycoprotein of 80,000 mass, which is present in the blood and other fluids of mammals, e.g. man. This multifunctional protein modifies the surface of cells by binding and interacts with components of the immunologic system and receptors. It consists of a larger protein entity (50,000 mass), which is assembled from two subunits linked by disulfide bonds, and a slighly smaller carbohydrate part (30,000 mass). Due to its content of four amphipatic protein helices, clusterin is capable of binding to membranes. It can thus act as membrane peripheral protein.

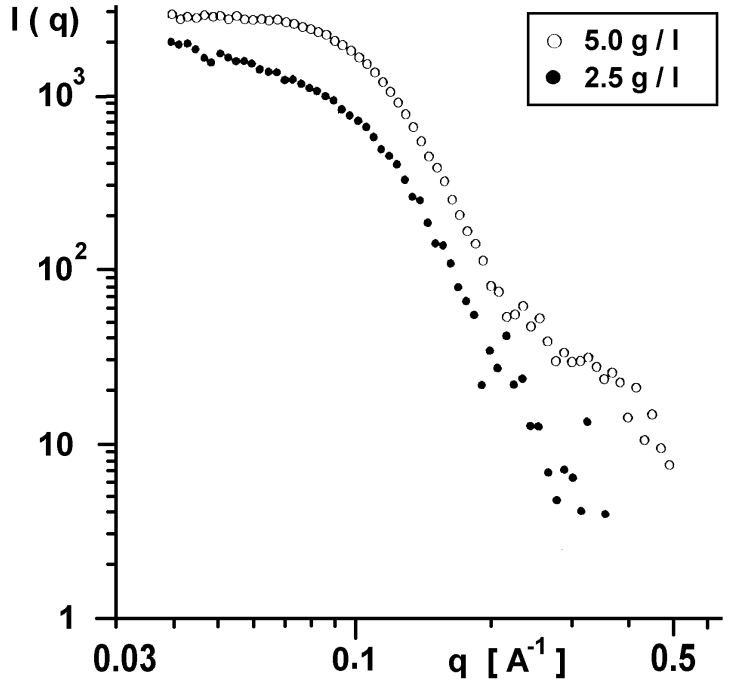

Fig.1: X-ray small angle scattering of the glycoprotein clusterin in weakly buffered detergent solution (TDOC).

We have investigated the structure of purified glycoprotein clusterin (gp80) from MDCK cell cultures 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 protein was subjected to the experiment as component of a weakly buffered detergent solution (5 mM taurodesoxycolate, TDOC, 50 mM Tris-buffer, pH7.5; 10% glycerol) at 4°C. The weak scattering signal of the small TDOC-micelles [1] was estimated with protein-free samples and subtracted.

The scattering profile of clusterin consists of a central signal and characteristic weak side maxima, resulting from the internal structure of the complex (fig.1). Surprisingly the scattering depended strongly on the protein concentration. Above a concentration of 2.5 g/l the profiles showed an interparticle-like scattering effect. In this case an extra-signal yielded a flat scattering profile at very small scattering vector, whereas no evidence for a formation of compact aggregates was found. The evaluation of the X-ray scattering profiles with Guinier plots (Fig.2) resulted in radii of gyration of R_g = 2,5 nm to 1.9 nm by increasing the concentration from 1 to 5 g/l. The effect was furthermore investigated by neutron scattering at the KFA Jülich (IFF / KWS-II).

As this effect was observed in a concentration range, which was more than a magnitude of order smaller than the concentration where an interparticle effect is expected with respect to the molecular mass (c > 20 g/l at 80.000 mass), we concluded that the extra-signal in the scattering profile results from a specific interparticle interaction. Due to the X-ray scattering, the interaction consists of a flexible link. As such an effect is unknown for soluble as well as for membrane proteins, it must depend on the high and specific glycosylation of clusterin. Possibly, this is the structural basis of finding and binding glyco-ligands to clusterin, e.g. glycoprotein.

1) Conrad, H.; Dose, K.; Nawroth, T. (1989) Physica B 156, 474-476

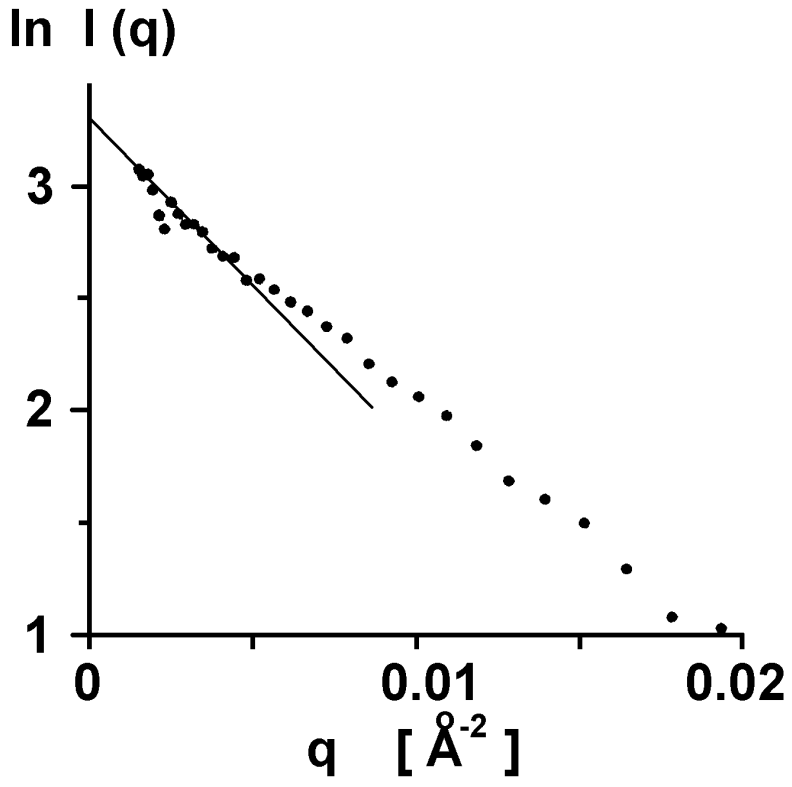

Fig.2: Guinier plot of the X-ray small angle scattering of clusterin from MDCK cell cultures in weakly buffered detergent solution (2.5 g/l protein; 3h).