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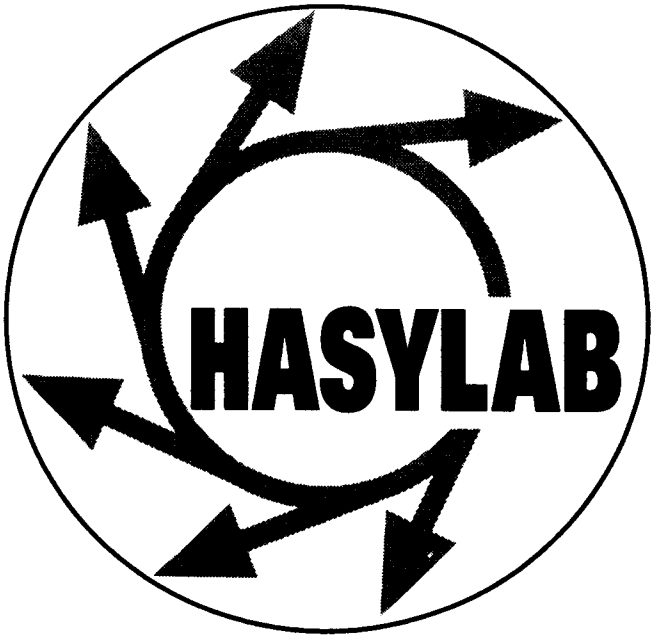

X-ray small angle scattering of the glycoprotein Gp80 (Clusterin)

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The Gp80 glycoprotein complex (Clusterin) [1,2] is a large protein (M= 80,000) that is present in body fluids of vertrebrates, e.g. man. It can aggregate cells and modifies the immunologic system by binding of the complement factors C5b-9. Some of the Clusterin is located in high density lipoprotein particles (HDL) as apolipoprotein J. The Gp80 consists of two protein subunits (M= 45,000 and 35,000), about 40% of the particle mass consists of oligosaccharide.
We have isolated and partially characterized the Gp80 glycoprotein from epithelium (MDCK) cells [1]. The gene was characterized and sequenced. The Gp80 producing cells were grown in cell cultures. The Clusterin was isolated from the medium and purified by ion exchange chromatography.
The structure of Clusterin was investigated the very first time by X-ray small angle scattering of the protein in solution of the detergent taurodesoxycholate (TDOC) at the A1-beamline of HASYLAB and the EMBL beamline. For the experiments radiation of 0.15 nm wavelength was used. The detector-sample distances were 1.9 m and 3.7 m. In earlier studies we have shown that TDOC is sufficient for scattering experiments with membrane proteins [3] because of its very small micelle size [4]. The residual micelle scattering was eliminated by subtracting the scattering of a protein-free detergent buffer with nearly contrast-matching glycerol content.

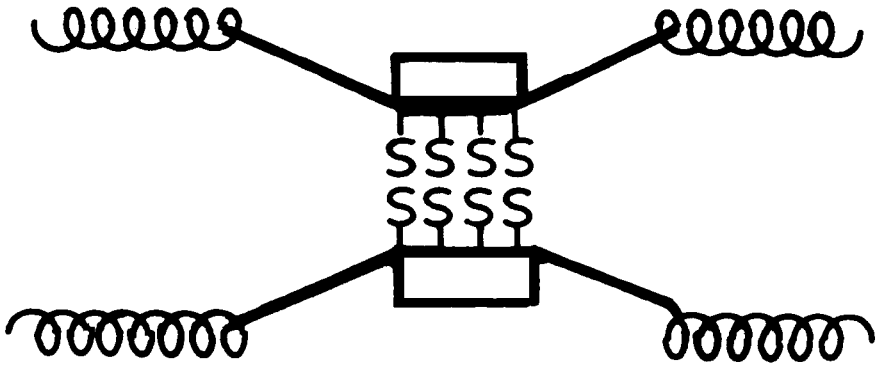

Fig.: Tentative model of Gp80 structure. The four amphipatic "legs" of the X-shaped molecule are possibly in contact with the membrane.

The scattering profile of Gp80 in presence of TDOC showed no evidence for any protein aggregates. The Guinier extrapolation of the profile yielded a radius of gyration of R_g= 27 nm. The radius of gyration corresponds with a sphere of 50,000 g/mol molecular mass, which is in good aggreement with the mass of the protein entity according to the gene sequence (51,000). The scattering is now interpreted by molecular models consisting of cubes according to the FVM-method [5]. The present, tentative view of the Gp80 molecule is an X-like structure (fig.). The four "legs" of the protein consist of amphipatic helices, according to the gene sequence, which are possibly in contact with the membrane surface.

1) Urban, J.; Parczyk, K.; Leutz, A.; Kayne, M.; Kondor-Koch, C. (1987) J.Cell.Biol. 105, 2735-2743
2) Jenne, D.; Tschopp, J. (1992) Trends in Biol. Sci. 17, 154-159
3) Nawroth, T.; Conrad, H.; Vienken, J.; Dose, K. (1983) Hoppe Seyler's Z.Physiol.Chem. 364, 923-931
4) Conrad, H.; Dose, K.; Nawroth, T. (1989) Physica 156 B, 474-476
5) Nawroth, T. (1989) Physica 156 B, 493-495