Characterization of the ABC-transporter PglK and its Complexes with Nanobodies using High-Mass Matrix-Assisted Laser Desorption/Ionization-Mass Spectrometry

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The structural investigation of membrane proteins is an important area of research due to their relevance in biochemical processes. These include the transport of ions and proteins, signaling, and cell-cell interactions. The characterization of the membrane proteins involved could lead to a better understanding of these processes, e.g., the development of more specific drugs or the prevention of drug resistance in cancer therapy (1).

Investigation of membrane proteins via mass spectrometric techniques (e.g. ESI MS) (2) often requires extensive sample preparation prior to analysis, due to high concentrations of salts and detergents in “protein-friendly” buffers. To minimize such sample preparation we apply matrix-assisted laser desorption ionization (MALDI) MS combined with a high-mass detector for accurate mass determination and characterization of membrane proteins and their complexes (3).

In this study we investigated a lipid-linked oligosaccharide flippase (PglK), known as an important biological actor for the translocation of lipid-linked oligosaccharides that serve as donors in N-linked protein glycosylation. PglK was in complex with different nanobodies, designed to stabilize its native structure, in order to obtain pure crystals for x-ray crystallography. We applied chemical cross-linking to investigate the stoichiometry of the nanobody-PglK complexes. Furthermore, we incubated ATP and ADP with the ABC-transporter to observe the effect of structural changes on nanobody-PglK interactions. Based on the different complex formation in the presence of nucleotides, we could locate the binding site of certain nanobodies. These results are in agreement with crystal structures obtained from PglK and NBs. In another experiment, we investigated different molar ratios of PglK and NBs to get insight into the binding behavior and classify the binding strength of the interacting proteins. These results could contribute to obtaining high quality crystals for x-ray experiments.

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