Karolinska Institutet

**Department of Medical Biochemistry and Biophysics**

# Structural studies of the ERGIC-53/MCFD2 glycoprotein transport receptor complex

AKADEMISK AVHANDLING
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av


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## ABSTRACT

The secretory pathway defines and maintains the intracellular architecture of the eukaryotic cell. Proteins targeted to either the plasma membrane, the extracellular medium or to specific organelles within the cell are dependent on the correct transfer along this pathway. The importance of its function has become increasingly clear and several proteins in this pathway have been linked to human diseases. One disease that is genetically associated with transport processes in the early secretory pathway is the combined blood coagulation factor V and VIII deficiency (F5F8D). It has been established that F5F8D is caused by mutations in the genes that code for the membrane bound glycoprotein receptor ERGIC-53 or its co-receptor protein MCFD2. Together these two proteins form a calcium dependent complex with 1:1 stoichiometry that specifically interacts and assist transport of the glycosylated blood coagulation proteins FV and FVIII from the endoplasmic reticulum.

In this thesis, NMR-spectroscopy and X-ray crystallography have been applied in order to clarify the organisation of the ERGIC-53/MCFD2 glycoprotein transport receptor complex. The work resulted in the three-dimensional structure of MCFD2 in solution determined by NMR and the crystal structure of MCFD2 in complex with the carbohydrate recognition domain of ERGIC-53. These structures gave a first molecular view of the organisation of a cargo receptor complex in the early secretory pathway and revealed that MCFD2 undergoes significant conformational changes upon complex formation.

NMR and CD-spectroscopy analysis showed MCFD2 to be disordered in the absence of $Ca^{2+}$ ions, but to adopt a predominantly ordered structure upon binding $Ca^{2+}$ ions. Hence, these data suggest that calcium binding and consequent folding of MCFD2 to be the underlying mechanism for the previously observed calcium dependence of the MCFD2/ERGIC-53 interaction. Moreover, the consequences of all known F5F8D causing missense mutations found in MCFD2 could be explained at a molecular level. The results highlight the importance of intact calcium binding EF-hand motifs for the structural stability of MCFD2 and point toward disruption of the ERGIC-53/MCFD2 interaction as the underlying mechanism for these mutations in causing F5F8D.

Overall, this thesis work has provided new insights into the structural organisation of the ERGIC-53/MCFD2 transport receptor complex and highlighted the importance of calcium for the regulation of its function. By studying this complex the hope is that we have shed some light, not only on the mechanisms behind transport of FV and FVIII by ERGIC-53 and MCFD2, but also on general processes underlying the assisted glycoprotein transport in the early secretory pathway.


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