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New Insights into Multiple Coagulation Factor Deficiency from the Solution Structure of Human MCFD2

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Human MCFD2 (multiple coagulation factor deficiency 2) is a 16-kDa protein known to participate in transport of the glycosylated human coagulation factors V and VIII along the secretory pathway. Mutations in MCFD2 or in its binding partner, the membrane-bound transporter ERGIC (endoplasmic reticulum–Golgi intermediate compartment)-53, cause a mild form of inherited hemophilia known as combined deficiency of factors V and VIII (F5F8D). While ERGIC-53 is known to be a lectin-type mannose binding protein, the role of MCFD2 in the secretory pathway is comparatively unclear. MCFD2 has been shown to bind both ERGIC-53 and the blood coagulation factors, but little is known about the binding sites or the true function of the protein. In order to facilitate understanding of the function of MCFD2 and the mechanism by which mutations in the protein cause F5F8D, we have determined the structure of human MCFD2 in solution by NMR. Our results show the folding of MCFD2 to be dependent on availability of calcium ions. The protein, which is disordered in the apo state, folds upon binding of Ca^{2+} to the two EF-hand motifs of its C-terminus, while retaining some localized disorder in the N-terminus. NMR studies on two disease-causing mutant variants of MCFD2 show both to be predominantly disordered, even in the presence of calcium ions. These results provide an explanation for the previously observed calcium dependence of the MCFD2–ERGIC-53 interaction and, furthermore, clarify the means by which mutations in this protein result in inefficient secretion of blood coagulation factors V and VIII.

Keywords: coagulation factor deficiency; secretory pathway; NMR; calcium binding; allosteric regulation

Introduction

Combined deficiency of coagulation factors V and VIII (F5F8D) is an inherited bleeding disorder characterized by reduced plasma concentrations of blood coagulation factors V and VIII, with both typically in the region of 5–30% of normal levels. F5F8D, which was first characterized by Oeri in 1954, is known to be an autosomal recessive inherited condition, and genetic analysis by Zhang *et al.* localized the disease-causing mutations to two proteins: ERGIC (endoplasmic reticulum–Golgi intermediate compartment)-53 (also known as LMAN1) and MCFD2 (multiple coagulation factor deficiency 2).[1]

ERGIC-53, where the majority of the mutations occur, is a 53-kDa type I transmembrane protein and has been shown by biochemical analysis to belong to a

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Abbreviations used: MCFD, multiple coagulation factor deficiency; ERGIC, endoplasmic reticulum–Golgi intermediate compartment; F5F8D, combined deficiency of factors V and VIII; ER, endoplasmic reticulum; CRD, carbohydrate recognition domain; PDB, Protein Data Bank; HSQC, heteronuclear single quantum coherence; Mes, 4-morpholineethanesulfonic acid; NOE, nuclear Overhauser enhancement; SA, simulated annealing; NOESY, NOE spectroscopy.

group of specific cargo receptors in the endoplasmic reticulum (ER) and ERGIC that facilitate an early step of the mammalian secretory pathway.[2] The receptor is thought to bind correctly folded glycosylated cargo proteins, including FV and FVIII, in the ER, recruiting the cargo for package into COPII-coated vesicles and transport first to the ERGIC and then to the Golgi.[3–5] This predicted role of ERGIC-53 was confirmed by a high-resolution X-ray structure of the N-terminal carbohydrate recognition domain (CRD),[6] which revealed a lectin-type fold consistent with its role in binding glycoproteins. The CRD comprises the largest domain of the ERGIC-53 protein, with the remainder composed of a stalk domain, a transmembrane helix, and a short cytoplasmic tail.[7] The stalk domain, which is predicted to be a coiled-coil structure, contains two cysteines thought to mediate oligomerization of the protein through disulfide interactions,[7,8] while the cytoplasmic tail includes a retrieval sequence to bring the transporter back to the ER after release of its cargo in the ERGIC.[9] At least 32 different F5F8D-causing mutations have been located in the ERGIC-53 gene, all of which are null mutants except for 1 that disrupts disulfide bond formation required for oligomerization.[10–15]

While the role of ERGIC-53 in F5F8D is relatively clear, at least on a superficial level, less is known about the action of MCFD2. The first link between MCFD2 and the secretory pathway was established when it was identified as the location of F5F8D-causing mutations in the approximately 30% of patients with a normal ERGIC-53 gene.[1] MCFD2 is a small soluble protein of 16 kDa with two predicted calcium-binding EF-hand motifs in the C-terminal region, and homologues of the MCFD2 gene have been found in a range of eukaryotes including rat, cow, and several species of fish. Human MCFD2 and ERGIC-53 are known to form a stable, Ca^{2+}-dependent complex with 1:1 stoichiometry,[16,17] and its interaction with factor VIII has been observed by cross-linking experiments in cells.[1] However, at present, little structural information on the MCFD2/ERGIC-53 complex is available. While several proteins, to date, have been identified as cargo of ERGIC-53 (FV, FVIII, cathepsin C, cathepsin Z, nicastrin, and α1-antitrypsin),[18–21] MCFD2 is only known to be required for transport of the blood coagulation factors,[22] suggesting a possible role for MCFD2 as a specific recruitment factor for this subset of ERGIC-53 cargo proteins. Interestingly, an MCFD2 mutant that fails to coimmunoprecipitate with ERGIC-53 has been shown to retain the ability to interact with FVIII,[23] implying that the interaction between MCFD2 and the coagulation factors may be independent of MCFD2/ERGIC-53 binding. Unlike ERGIC-53, MCFD2 does not include the Phe–Phe (FF) motif required for COPII binding or the Lys–Lys (KK) motif that functions as an ER retrieval signal, suggesting that correct localization of MCFD2 is reliant on its interaction with ERGIC-53.[1]

To date, 15 mutations in MCFD2 have been characterized in patients with F5F8D.[1,12,15,24,25] Nine of the mutations disrupt the open reading frame, while the others are substitution mutants, one of which creates a stop codon at position 122, effectively deleting the last three amino acids of the protein.[25] Two of the substitution mutations are located in the second EF-hand motif, and both of these have been shown to abolish the interaction between MCFD2 and ERGIC-53 *in vivo*.[1] It has been suggested that this implies Ca^{2+} binding of MCFD2 to be a prerequisite for formation of the transporter complex, but no structural evidence is currently available to prove this theory.

The ERGIC-53/MCFD2 complex is an extensively studied mammalian cargo receptor, yet the mechanism by which it functions remains poorly understood. At this point, we cannot define how the two proteins interact or how the complex recognizes,

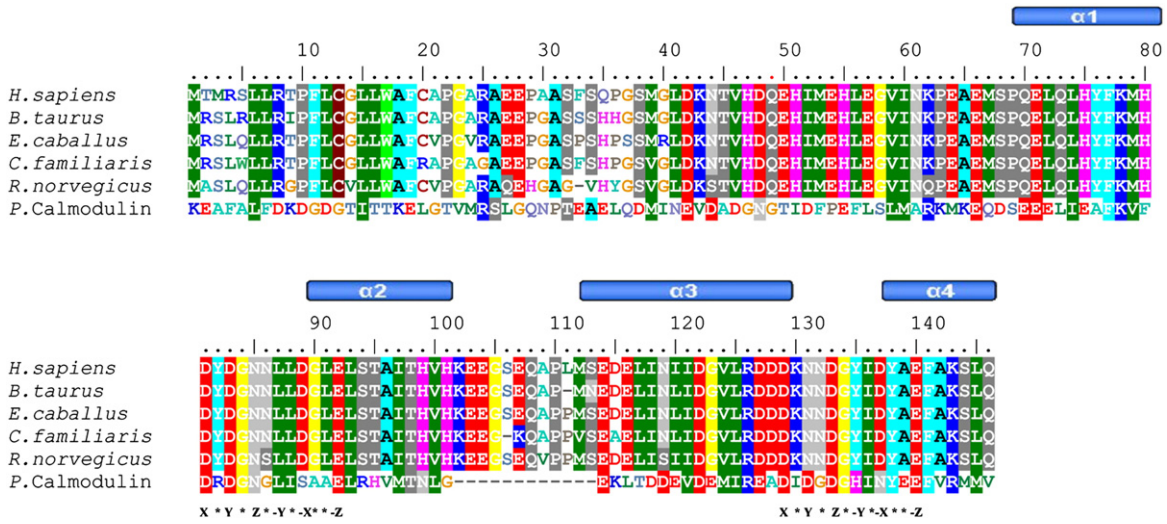

Fig. 1. Alignment of the sequence of human MCFD2 with the putative MCFD2 proteins identified in other species. Also shown is *Paramecium* calmodulin, the highest identity protein of known structure. Helical secondary structure is indicated by cylinders above the alignment, and the conserved EF-hand loop motifs are identified below.