Abstract

VWA domains are independent folding units with a length of about 200 amino acids that occur in many intra- and extracellular proteins where they mediate protein-protein interactions. A well conserved metal ion dependent adhesion site (MIDAS) is often involved in cation dependent ligand binding. The three-dimensional structures of several VWA domains were already solved, but no structure of a VWA domain of the collagen and matrilin phylogenetic branch is known. Matrilins and collagen VI are extracellular matrix proteins involved in the organization of pericellular networks and their VWA domains mediate interactions with other matrix molecules.

To solve the structure of a matrilin VWA domain a variety of constructs, expression conditions, and stabilizing agents were tested to increase the protein solubility. The matrilin-4 VWA2 domain was best soluble when carrying a One-STrEP-tag instead of a His-tag and in the presence of N-ethylmaleimide. The zebrafish matrilin-4 VWA2 domain showed a higher stability than any murine matrilin VWA domain. Eukaryotically expressed matrilin VWA domains were more stable and showed a different binding behaviour than the bacterially expressed domains, although the circular dichroism (CD) spectra were similar. It was still not possible to determine the structure of any matrilin VWA domain by NMR or crystallography. As an alternative, homology models were generated which will serve as a basis for future studies defining the binding surfaces.

The structure of matrilin VWA domains, that all contain a MIDAS motif, depends on divalent cation binding. CD-spectra indicated a stabilizing effect of Mg^{2+} und Mn^{2+} and a destabilizing effect of Ca^{2+} und Zn^{2+} on the thermal stabililty. In NMR-experiments no marked conformational change could be observed upon cation binding, as is the case for integrin I-domains. A destabilizing effect of Zn^{2+} was also seen for the collagen VI α3N5 domain which does not contain a MIDAS motif. This effect is therefore probably due to unspecific interactions. A binding to COMP could be seen for all matrilin VWA domains, except for matrilin-2 VWA2, in surface plasmon resonance studies. The matrilin-1 VWA2 domain had the highest association rate but dissociation rates were similar for all matrilin VWA domains.

The collagen VI α3N5 domain was more stable than all matrilin VWA domains, showed a 10-15°C higher denaturation temperature, and produced a good quality NMR-spectrum. The structure of this VWA domain was solved by crystallography. The crystal structure of the muscular dystrophy causing R1064Q mutant of the collagen VI α3N5 domain was also determined. The mutant structure showed the same fold as the wild type N5 domain, but with the mutated residue taking a different orientation. This structure may help in the

analysis of the disease causing mechanisms. The secretion of the domain was not affected by the mutation and in initial binding studies no specific binding partners could be identified for the N5 domain. The new structure will be used to improve models of the N-terminal region of the collagen VI α3-chain and may serve as an improved template for modelling of matrilin VWA domains.