Summary

The pleckstrin protein is the major protein kinase C (PKC) substrate in blood platelets. Its phosphorylation triggers processes that ultimately lead to platelet activation which initiates blood clotting and wound closure. Pleckstrin consists of three domains: the prototypic pleckstrin homology (PH) domains at each terminus and a central DEP domain. The three PKC phosphorylation sites all lie in the linker sequence between the N-terminal PH domain (N-PH) and DEP. The structures of both N-PH and DEP have been previously solved.

In the first part of this thesis, the novel high-resolution nuclear magnetic resonance (NMR) structure of pleckstrin's C-terminal PH (C-PH) domain is presented. By biochemical and NMR analysis, C-PH is found to bind specifically to phosphatidylinositol-3,4-bisphosphate (PtdIns(3,4)P_2), an important lipid second messenger in platelets. The interaction between C-PH and PtdIns(3,4)P_2 involves conserved motifs on the β1 and β2 strands as well as determinants in the β1–β2 loop which form a basic patch at the "open" side of the PH domain. Thus, C-PH may play an important role in a control mechanism in addition to PKC phosphorylation that directly regulates pleckstrin's activity and/or localisation in response to PtdIns(3,4)P_2.

In the second part of this work, the DEP_C-PH double-domain construct is studied by NMR and the phosphoinositide binding properties of all pleckstrin domains and constructs are analysed biochemically. Based on these data, C-PH appears to be a sensor for PtdIns(3,4)P_2 that is independent of the remainder of the molecule.

In the last part of this thesis a new strategy to obtain medium- to high-resolution structures of multi-domain proteins by NMR is developed and applied to pleckstrin double-domain constructs, involving a systematic mutagenesis scheme and site-directed spin-labelling. Methods for calibrating restraints derived from paramagnetic relaxation enhancement (PRE) caused by the spin-labels are implemented in a structure calculation of the pleckstrin N-PH_DEP double-domain construct. The preliminary structure of N-PH_DEP shows that the two domains are closely associated, which may obstruct the functional regions of N-PH. Thus, an "auto-inhibition" model of unphosphorylated pleckstrin is proposed.