7. SUMMARY

MOLECULAR DETERMINANTS FOR THE PALMITOYLATION OF VIRAL AND CELLULAR MEMBRANE PROTEINS

The covalent attachment of fatty acids - commonly palmitic acid - in a thioester-type linkage is a widespread modification of viral and cellular polypeptides. While constantly further palmitoylated proteins are being identified, many questions remain open regarding the molecular mechanisms for palmitoylation. So far specific sequence motives for palmitoylation could be discovered only for cytoplasmic proteins. With integral membrane proteins the hydrocarbon chain is usually bound to cysteine residues located close to the boundary between the transmembrane region and the cytoplasmic tail. Inspection of the amino acids in the vicinity of the acylated cysteine residues reveals no obvious ‘consensus-signal’ for palmitoylation. This implies that acylated membrane proteins contain complex conformational signals for palmitoylation that are mainly located within the cytoplasmic half of the transmembrane domain, but also involve the cytoplasmic region. Therefore the aim of this study was to resolve the influence of the transmembrane region for palmitoylation as well as the particular contribution of amino acid residues within the transmembrane domain using a series of new chimeric and mutant proteins derived from the acylated proteins Influenza virus haemagglutinin, CD4 and CD8 receptor protein as well as from the non-acylated Sendai virus fusion protein.

While Sendai virus fusion protein is not palmitoylated even after introducing of a cysteine residue into the potential acylation region, substitution of the transmembrane domain or both transmembrane and cytoplasmic region for the corresponding regions of Influenza virus haemagglutinin or CD4- or CD8 receptor protein is sufficient for palmitoylation. Non-hydrophobic amino acid residues on the hydrophilic face of the TMD-helix may support palmitoylation of membrane proteins. Sequence alignment of some palmitoylated proteins shows frequent occurrence of glycine in the transmembrane region close to the cytoplasmic tail. Replacement of cytoplasma-near glycine and phenylalanine residues in the transmembrane region leads to a lower acylation rate of the chimerae, while distant glycine residues have no significant influence on palmitoylation rate. This may be due to changes in the protein adjustment in the membrane and therefore disturbed interaction with the membrane-based enzyme palmitoyltransferase. Non-hydrophobic motives in the otherwise strictly hydrophobic transmembrane helix therefore represent molecular signals for palmitoylation.