Abstract

The small GTPase Ras is a proto-oncogenic protein that can be found in 20% of all tumours. In vivo Ras is bound to the plasma membrane by a lipid anchor and plays a central role as a molecular switch in numerous signal transduction pathways. There are until now only few biophysical analyses of Ras in its native membrane environment. Therefore, the objective of this thesis was to establish the ATR-FTIR-spectroscopic analysis of membrane-bound Ras. In a first step solid supported lipid bilayers were assembled on the internal reflection element, allowing the subsequent immobilisation of lipidated Ras in its native conformation. The protein was bound to the membrane such that its α-helices were oriented in a perpendicular angle with respect to the membrane. The analysis of the secondary structure indicated that membrane binding of Ras leads only to minor effects on the protein structure. Moreover, the secondary structure analysis suggested that the lipid anchor exists predominantly in a β-sheet conformation. By using the γ-phosphate analogue BeF$_3^-$ difference spectra of membrane-bound Ras between ON and OFF state could be obtained for the first time. Since these spectra were very similar to difference spectra of Ras in solution one can assume that the conformation of the nucleotide binding pocket of membrane-bound Ras is identical to that of Ras in solution. Establishment of the nucleotide exchange of membrane-bound Ras enabled the characterisation of its intrinsic GTP hydrolysis. The kinetics and the hydrolysis difference spectra obtained with membrane-bound Ras were nearly identical to those measured in solution. This shows that neither the membrane immobilisation nor the orientated binding influence the nucleotide binding or the hydrolysis activity. In a first set of protein-protein interaction experiments with membrane-bound Ras and its effector NoreRBD membrane-induced changes in the affinity could not be detected. The successful establishment of ATR-FTIR-spectroscopic analyses of membrane-bound Ras now enables the more profound investigation of many interesting questions, e. g., the difference spectroscopic characterisation of oncogenic Ras mutants and the influence of putative pharmacologically relevant small molecules on the Ras active state. Moreover, the newly established technique now allows to gain deeper insight into the interaction of Ras with effector molecules, GAPs and GEFs in the vicinity of the membrane. By varying the lipid composition and mutation of potentially membrane-interacting residues the molecular basis and biological consequence of the specific membrane orientation of Ras could be elucidated.