Proteins in Action: Monitored by tr(time-resolved) FTIR spectroscopy

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In the Postgenom era one of the remaining major challenges is the detailed understanding of protein networks within the living cell. Currently, there is a large gap between the detailed understanding of proteins in vitro and their description in interaction pathway maps in systems biology. In order to contribute to a more detailed understanding of protein interactions a combined approach of x-ray structure analysis, time-resolved FTIR spectroscopy, Molecular Dynamic and QM/MM biomolecular simulations is used.

Time-resolved FTIR difference spectroscopy can be used to monitor the reactions within proteins at atomic resolution with ns time-resolution up to days [1]. This provides in combination with structural models in addition spatial resolution. Complementary, by QM/MM simulations theoretical IR spectra can be obtained. More quantitative information is thereby deduced from the IR spectra.

Based on fast scan studies on bacteriorhodopsin the key catalytic residues, asp 85 and asp 96 and their protonation kinetics are identified and summarized in a first detailed proton pump model [2]. Based on succeeding step scan FTIR measurements the interplay between protein bound water molecules, a strongly hydrogen bonded water, a dangling water and a protonated water complex is elucidated in detail. It results in a controlled Grotthus proton transfer from the central proton binding site to the protein surface [3]. A similar mechanism might apply in the photosynthetic reaction center [4].

Using caged GTP the GTPase mechanism of the protooncogen Ras is investigated [5]. The ras protein switches external signals to the nucleus. It is down regulated by a protein-protein interaction with the GAP protein which catalyses the GTP hydrolysis by five orders of magnitude. Oncogenic mutations in Ras prevent this catalysis, which results in uncontrolled cell growth. The Ras-GAP protein interaction is be studied time-resolved [6,7]. This provides a detailed thermodynamic characterisation of the catalytic mechanism. It is shown that the movement of a catalytic GAP-“arg-finger” into the GTP binding site, pushes water molecules out of the binding pocket. Thereby the activation entropy is increased and the hydrolysis is catalysed (8,9). The studies proves that the trFTIR approach can be applied to protein-protein interactions. Beside reaction within the active site of a protein, also the surface change of a protein, which controls the protein-protein interactions is monitored (10).

In order to investigate the protein interactions closer to physiological conditions the ATR (attenuated total reflection) technique is applied. Using this approach for the first time the folding of the prion protein bound to a rafts lipid bylayer is studied (11).

References

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