CHAPTER V

CONCLUSIONS

5.1 Structural Changes of ω-carboxylalkanethiols SAMs

Structural changes of ω-carboxylalkanethiols SAMs at various applied potentials were characterized by SEIRA spectroscopic method. The difference spectra revealed the C=O stretching vibration of protonated carboxyl group at 1690-1700 cm^{-1}, as well as the anti-symmetric and symmetric stretching vibration of deportonated COO^- at 1555-1569 and at 1380-1409 cm^{-1}, respectively. The peak height of the protonated and deprotonated carboxyl-group is more intense for short SAM-lengths compared to those of the longer chains due to stronger proton affinity of the carboxyl-group at short monolayer, which is correlated with the lower pKa value.

Potential-dependent protonation/deprotonation SEIRA difference spectra of the C2-, C5-, C10- and C15-SAMs revealed the presence of reversible spectral changes from monolayers within the potential range between -0.2 and +0.2 V. Whereas the SEIRA difference spectra of C1-SAM revealed the reversible changes in the potential range between -0.1 and +0.2 V. Investigation of Cyt-c bound to these monolayer with an applied potential within this range could be performed without disturbing of the monolayer spectral. A larger potential range for reversible spectral change was found for C10-SAM, which revealed the reversibility of spectral changes in the range of -0.6 to +0.4 V. Irreversible spectral change, which is caused by the reductive-desorption of monolayer from the electrode surface, was critical for the short SAM-lengths. It was already occurred at -0.15, -0.4 and -0.7 V for C1-, C2- and C10-SAMs, respectively.

5.2 Structural and Orientational Changes of Immobilized Cytochrome c on Gold Electrode

SEIRA spectroscopy of immobilised Cyt-c allows detecting redox-linked protein structural changes. The conjugate difference bands at 1693 and 1673 cm^{-1}, tentatively assigned to the β-turn type III segment 67-70, have also been observed in redox-induced difference spectra of Cyt-c in solution whereas the SEIRA signal at 1660-cm^{-1} is a characteristic of the immobilised protein and may reflect subtle redox-dependent structural *and* orientational changes of one or more amide bonds. The redox potentials determined by stationary SEIRA method and CV do not differ significantly from the value in solution and do not display a distance-dependence. Two peptide segments of Cyt-c, i.e., the β-turn III (1693 cm^{-1}) and β-turn II/α-helix (1660 cm^{-1}) of ferric Cyt-c revealed the orientational changes with respect to the surface under the influence of electric field strengths with varying the SAM-lengths and electrode potentials. Within the potential range of -0.1 to +0.1 V, one should detect only the B1 state. Orientational changes of these two peptide segments found to be correlated with the conformational transition from the native B1 Cyt-c to the non-native B2 state, which was not associated with the alteration of secondary structure of Cyt-c as revealed by identical band frequencies of the difference spectra at different potentials and different SAM-lengths.

5.3 Non-Native B2 Conformation of Cytochrome c

Immobilization of Cyt-c on the bare Au-electrode at the equilibrium state could induce the formation of an abundance of the non-native B2 conformation of Cyt-c. Within the potential range from -0.5 to +0.15 V, the B2[6cLS] and B1[6cLS] revealed the redox potentials of -0.4 and +0.05 V, respectively. The latter is consistent with the value in solution. In comparison with the spectrum of native B1 state, the difference spectra of B2 Cyt-c revealed broad, complex bands caused by an alteration of the tertiary structure and an arrangement of the hydrogen-bonding interaction, which involves the movement of peptide backbone of α-helix/β-turn type II (ca. 1659 cm^{-1}) and unordered (ca. 1645 cm^{-1}) structures including the important residues

(His-33 and Met-80, respectively), for the formation of non-native B2 Cyt-c. Contrary to the B1 spectrum, the band at 1660-cm^{-1} is predominant in the B2 state in the reduced form. No reduction changes of the β-turn III (1693 cm^{-1}) was observed, as well as losing in the intensity of the band at 1673 cm^{-1} in the B2 spectra. The reduced forms of the protein segments are more pronounced than the oxidized-forms. A change of the turn structure as visualized by the band at 1680 cm^{-1} was observed.

5.4 Time-Resolved SEIRA Spectro-Electrochemistry

The electron transfer rate constant at C15-SAM, determined by CV on Au, is slightly larger than that on Ag electrodes obtained by SERR spectroscopy, which may be attributed to different SAM structures. For C10-SAM, the electron tunnelling rate constant is the same for both metals. The β-turn type III structural changes (1693/1673 cm^{-1}) occur with the same time-dependence as the electron transfer step whereas the amide bond changes revealed by the 1660-cm^{-1} band proceed with a somewhat slower kinetics, at least for C10- and C15-SAMs. It is very likely that, in analogy to previous findings for SAM-coated Ag electodes [87], rotational diffusion of Cyt-c represents the rate-limiting step of the interfacial redox process at SAM-coated Au-electrodes for SAM lengths shorter than C10. There is no indication that the protein structural (and orientational) changes reflected by the SEIRA difference bands depend on the local electric field strength at the SAM/protein interface. In the distance-independent regime of the interfacial redox process, the rate constant for the limiting step is distinctly larger for Au than for Ag which is attributed to different electric field strengths. Rapid scan and step scan SEIRA spectroscopy provide novel insight into redox-linked protein dynamics and mechanistic details of interfacial processes, thereby complementing the information obtained by electrochemical methods and TR SERR spectroscopy.