CHAPTER I

INTRODUCTION

Immobilisation of redox enzymes on electronic conducting materials is a key issue in the design and construction of bioelectronic devices that are of actual or potential importance for biotechnological applications [1]. These hybrid systems aim to utilise the evolutionarily optimised electron transfer properties as well as substrate and product specificity of biocatalysts. The central challenge, however, is to achieve an efficient electronic coupling of the protein to inorganic materials, which is essential for the performance of bioelectronic devices. The enormous research efforts in this field follow empirical "trial-and-error" approaches or rational design principles to optimiSe the desired functional properties of the bioelectronic hybrid system. The latter strategy requires deeper insight into the molecular processes of immobilised proteins and the factors that control the electronic coupling with the conducting support. Progress in this respect strongly depends on appropriate techniques that may provide information about the structure and reaction dynamics of immobilised proteins.

Surface enhanced vibrational spectroscopies fulfil these requirements [2]. Among them surface enhanced resonance Raman (SERR) spectroscopy can be considered as an established technique for probing the molecular structure of the redox site and its dynamics and reactivity in interfacial processes [3,4]. For many systems, specifically for heme proteins, a drawback of this technique is the restriction to Ag surfaces since this metal limits the accessible electrode potential range. Moreover, Ag cations are known to attack proteins and thus may cause their denaturation [5]. Finally, SERR spectroscopy only probes the redox site but does not provide direct information about the protein structure and dynamics. In this respect, surface enhanced infrared absorption (SEIRA) spectroscopy represents a promising complementary method as demonstrated by the pioneering work of Ataka and Heberle [6-8]. The authors have successfully employed SEIRA spectroscopy to probe soluble and membrane bound proteins immobilised on coated Au electrodes. In general, the technique is used in the

difference mode and thus reveals potential-dependent changes of the protein structure and its orientation. Specifically, it allows analysing redox transitions of heme proteins and enzymes.

In this work, structural/conformational changes of Cyt-c has been investigated by stationary SEIRA spectroscopy and the combination of SEIRA spectroscopy with time-resolved methods to monitor the electron transfer kinetics and protein dynamics of immobilised cytochrome c (Cyt-c) is reported for the first time. The approach is based on the rapid-scan and step-scan methods that are well established in conventional Fourier-transform IR spectroscopy [2]. Similar to time-resolved SERR spectroscopy [2,3], the processes to be studied are initiated by a rapid potential jump at the working electrode such that the subsequent relaxation processes can be probed by SEIRA spectroscopy, operated in the rapid scan and step scan mode for processes slower and faster than 100 ms, respectively. The present results demonstrate that SEIRA spectroscopy in conjunction with SERR spectroscopy and electrochemical methods may provide novel insights into the mechanism and dynamics of interfacial processes of proteins.

Scope of the Research

The experiments in this research work were carried out on an Au/SAM/Cyt-c system. Self-assembled monolayers (SAMs) of ω-carboxylalkanethiols were used to form biocompatible films, in which the local electric field strength could be controlled by a variation of the SAM chain lengths and an applied electrode potential. The work was divided into five parts: i) studies of the electric field dependent stability and related spectral changes of the monolayers, ii) investigations of structural/orientational changes, as well as conformational transitions of Cyt-c under the influence of EF strength, iii) studies of the non-native B2 state of Cyt-c, iv) elucidation of the protein dynamics from Cyt-c, and v) determination of the ET rate constant by cyclic voltammetry. Parts i-iii were carried out with SEIRA spectro-electrochemistry in the stationary mode, while part iv was performed in the respective time-resolved mode.

Objective of the Research

The research aim is a deeper understanding of the influence of the electric field strength on the mechanism and dynamics of redox proteins, as well as the protein structural and orientational changes by means of stationary and time-resolved SEIRA spectro-electrochemistry. Addionally, the SEIRA technique has been used to study the non-native B2 state of Cyt-c.