Chapter 1
Introduction

The metabolism of living cells crucially depends on electron transfer reactions, which are involved in a variety of anabolic and catabolic processes. The physiological role of redox proteins and electron transfer reactions is a topic of growing interest and research activity in enzymology. Redox proteins represent a very large group of enzymes. Their cofactors or redox centers are embedded in structural domains with a wide variety of functions. Especially the heme-proteins represent a very spread group of proteins, involved in electron transfer (cytochromes), catalysis (catalases, peroxidases), detoxification (cytochrome 450) and transport and storage of small molecules (hemoglobin, myoglobin). In order to understand the influence of the protein environment on properties of the heme, synthetic polypeptides and proteins were synthesized, recently (Choma et al., 1994; Bryson et al., 1995; Huffman et al., 1998; Rau & Haehnel, 1998; Rau et al., 1998; Sharp et al., 1998). The *de novo* design of redox proteins is an attractive approach to investigate the factors involved in electron transfer and to create proteins with novel functions. Recent developments in peptide synthesis and in chemoselective ligation to link unprotected peptides, as well as the increasing knowledge from the crystal structures of native proteins has inspired the design and chemical synthesis of such artificial proteins.

Nature has selected some of the redox-active molecules and assembled them within a protein matrix to facilitate essential functions such as substrate binding, electron transfer, light-induced charge separation, energy conversion, and chemical catalysis. The physical and chemical properties of the cofactors are adjusted by the protein environment to perform the desired function satisfactorily and with high efficiency. Thus, the function of native proteins is achieved through complexity of their structures. The aim of protein design is to uncover the construction principle of native proteins in order to simplify the problem and to synthesize minimalistic proteins that assemble working arrays of cofactors and reproduce native-like function. They have been named as "molecular maquettes". These synthetic proteins exhibit key properties of their native counterparts with respect to the physico-chemical characteristics of the cofactors and the influence that the peptide environment exerts on their properties.

Electrostatic interactions play a central role in a variety of biochemical processes. The importance of the electrostatic effects in understanding the behavior of proteins has been recognized in many contexts, including enzymatic catalysis, solvation, ligand binding, docking, protein-protein association, determining the pK_a values of titratable amino acid side chains and redox potentials of cofactors (Perutz, 1978; Warshel, 1981; Warshel et al., 1984; Klapper et al., 1986; Warshel & Aqvist, 1991; Gilson, 1995; Nakamura, 1996). The combination of experimental and theoretical research reveals new insight in protein structure and function. Especially site-directed mutation experiments and structural investigations by NMR and X-ray crystallography has increased the understanding of protein function. With the advent of site-specific NMR assignment procedures, it has become possible to identify the titration behavior of individual residues. Other methods such as IR spectroscopy, potentiometry, the assessment of proton uptake or release, or the measurement of the pH dependence of free energy changes can describe the titration of biological macromolecules as a whole. All these methods provide very important informations about proteins. Classical electrostatics has also proved to be a successful quantitative tool yielding accurate descriptions of electrostatic potentials, pH-dependent properties of proteins, ionic strength-dependent phenomena, and the solvation free energies of organic molecules. In order to correlate the protein structure with its function quantitatively, the calculation of intra- and intermolecular electrostatic interactions and energies is required.

A detailed characterization of the strength and nature of these interactions requires an understanding of the physico-chemical properties of molecules in aqueous solution. Although

the solvent effects on solute properties have been understood for many years, methods that translate these principles into accurate prediction of experimental observables have been developed in the last two decades. The use of classical electrostatics as an approach to study charged and polar macromolecules in aqueous solution became possible through the development of powerful computers and fast numerical and computational methods to solve the Poisson-Boltzmann equation for solute molecules that have complex shapes and charge distributions. Understanding the properties of proteins in aqueous solution requires models of the solute, the solvent and the interactions between them. In the molecular mechanics approach, solute and solvent molecules are described in terms of a "force field" where nonbonded van der Waals and electrostatic terms account for most of the details of intermolecular interactions. A proper description of solvent effects at the molecular level requires the calculation of the mutual interactions of a large number of molecules and the averaging of these over many solvent configurations and different protonation states of the protein. Therefore, for many applications, the explicit treatment of solvent molecules and mobile ions is not feasible. An alternative approach involves continuum electrostatics of macroscopic solvent models, where solvent properties are described in terms of average values. These methods enable to describe solute molecules in atomic detail while treating the solvent in terms of average properties.

As one can already notice from this short introduction, the major interest of my investigations in this doctoral work was focused on the electrostatic interactions in proteins and on the electron transfer processes occurring in redox-active proteins. Among them, the heme-proteins that have axially coordinated histidines to the heme iron, as well as the native and artificial cytochrome *b* (Cb), are especially in the focus of my study. Attention was concentrated on the protein energetics, the calculations of the protonation and oxidation patterns in redox proteins and evaluation of the redox potentials of their cofactors. In chapter 2, the basic electrostatic theory, that I used to investigate the titration behavior of proteins, is explained. Chapter 3 provides some necessary informations about the structure, function and working environment of the native cytochrome bc_1 complex in the mitochondrial electron transport chain, which are important to understand the biochemical role and redox heterogeneity of the two Cb hemes.

The results of the theoretical work presented here, can be divided into three parts, which are mutually connected. In the first part (chapter 4), I analyzed the factors determining the orientation of the histidine axially coordinated to heme by searching in the Protein Data Bank (PDB) of 432 heme-protein structures and evaluating the corresponding relevant interactions using the CHARMM22 force field. This study was particularly useful for our further investigations, since it is known that the redox potential of heme depends on the type, conformation and orientation of the axial ligands.

In the second part (chapter 5), I generated the atomic coordinates of the model structure of an artificial protein from scratch, which was recently synthesized to mimic the central part of the native Cb. The stability of the computer generated structure was tested by monitoring the conformational changes and fluctuations during a long-term molecular dynamics simulation and comparing the results with values obtained from the crystal structure of native Cb.

The third part consists of the continuum electrostatic calculations by solving the linearized Poisson-Boltzmann equation (LPBE) with a subsequent Monte Carlo titration of the protein. This approach, I applied on the artificial and native Cb (chapter 6) to examine the titration behavior of ionizable residues and to evaluate the redox potential of heme groups, as well as to investigate the energetics of the photoactivation process in the DNA photolyase from *E. coli* (in chapter 7). The factors that determine the redox potential of the two hemes in the artificial Cb were analyzed in terms of the influence of different structural elements.