

Preface

This thesis was prepared from October 1998 to October 2001 in the group of Professor Ernst-Walter Knapp at the Freie Universität in Berlin. Here, I would like to address my special thanks to all people who helped and supported me during my PhD work. Especially, I wish to thank Professor Ernst-Walter Knapp, my supervisor, for giving me opportunity to work as a PhD student in his group. He suggested the interesting topics of this thesis to me, and he always had time to give me useful advises.

I wish to acknowledge Dr. Matthias Ullmann for his help which was essential in the beginning of my PhD work. I am thankful to Dr. Peter Vagedes, who was open for many helpful discussions. I am grateful to Dr. Björn Rabenstein for explaining me the background of the titration calculations.

Specially, I am thankful to Dr. Snezana Zarić for her collaboration on a project about new type of cation- π interactions in metalloproteins, Aleksandra Zmirić and Daniel Winkelmann for collaboration in the calculations of energetics of radical transfer in DNA photolyase from *E. Coli* and *A. Nidulans*, and Philip Voigt for collaboration in evaluation of the redox potentials of the four hemes in a bacterial photosynthetic reaction center.

Programs with which parts of the data in this thesis have been calculated, were kindly provided by Prof. Peter Kollman (RESP), Prof. Donald Bashford (MEAD) and Dr. Björn Rabenstein (KARLSBERG).

During my work here, Bernd Melchers, Björn Rabenstein, Timm Essigke and Daniel Winkelmann took care for keeping computer network running and for installing many useful programs.

The typesetting of this thesis was done by using MICROSOFT WORD program. Molecular graphics were drawn with programs – MOLSCRIPT of Per Kraulis and ISISDRAW provided by MDL, while most of the figures were prepared with SHOWCASE. The plots were done by using XMGR and postprocessed with XFIG.

I am particularly grateful to the Graduiertenkolleg "*Dynamik und Evolution zellulärer und makromolekularer Prozesse*" (GRK 268) for financial support of this work.

Finally, I want to thank my family for permanent support during all these years.

Writing the last parts of this PhD thesis, on September 11th, a tragic event happened, when several thousands of innocent people died in terror attacks on New York City and Washington. This PhD work, I dedicate to all victims from the World Trade Center in New York and Pentagon in Washington.

Dragan Popović

Freie Universität Berlin
October 2001

Contents

1. Introduction	1
2. Titration curves of proteins	3
2.1. Introduction	3
2.1.1. Acid-base equilibrium of a single titratable group	3
2.1.2. Redox equilibrium of a single redox-active group	4
2.1.3. The model of a protein in solution	5
2.2. Continuum electrostatic calculations	7
2.2.1. The Poisson-Boltzmann equation	7
2.2.2. A single titratable group in a protein	11
2.2.3. Protonation state energy	13
2.2.4. Redox reactions	16
2.2.5. Protonation and oxidation probability	17
2.2.6. The coupling of protonation and redox reactions	18
2.2.7. pH dependent pK_a values and solution redox potential dependent E^0 values	19
2.3. Monte Carlo titration	20
2.3.1. The sampling method	20
2.3.2. Additional features	21
3. The respiratory electron transport chain	26
3.1. Coupling of oxidative phosphorylation to electron transport	26
3.2. Electron transport	27
3.2.1. The sequence of electron transport	28
3.2.2. Complex III	29
4. Orientation of axially ligated imidazoles in heme-proteins	33
4.1. Introduction	33
4.2. Methods	34
4.2.1. Data mining in the PDB	34
4.2.2. Molecular force field computations	36
4.3. Results and Discussions	36
4.3.1. General overview of PDB data	36
4.3.2. Hydrogen bonding scheme of imidazole ligated to heme	38
4.3.3. Role of propionic acids	43
4.3.4. Influence of histidine backbone	47
4.3.5. Imidazole-heme conformations for different groups of heme-proteins	47
4.3.6. Mutual orientation of two axially coordinated histidines	52
4.4. Conclusions	53
5. Modeling and structure validation of the artificial cytochrome b	55
5.1. Introduction	55
5.1.1. Protein design	55
5.2. Methods	58
5.2.1. Generation of atomic coordinates	58
5.2.2. Preparation of the native Cb chain for MD simulation	60
5.2.3. MD simulation	60
5.3. Results and Discussions	60
5.3.1. Structural relaxation of the artificial cytochrome b	60
5.3.2. Salt bridges in the artificial Cb	61
5.3.3. Comparison of heme conformations in artificial and native Cb	63
5.3.4. Stability of the artificial and native Cb	66

6. Redox potential and protonation pattern of native and artificial cytochrome b	67
6.1. Methods.....	67
6.1.1. Computation of heme redox potentials and protonation and redox patterns in proteins...	67
6.1.2. Titratable groups.....	69
6.1.3. Atomic partial charges.....	69
6.1.4. Preparation of structures for electrostatic computations.....	70
6.2. Results and Discussions.....	71
6.2.1. Calculations on the artificial cytochrome b.....	71
6.2.1.1. Protonation pattern of titratable groups.....	71
6.2.1.2. Experimental values of redox potentials.....	72
6.2.1.3. Calculated heme redox potentials.....	72
6.2.1.4. Role of the Phe and Trp and the electrostatic coupling of the hemes.....	73
6.2.1.5. Influence of dielectric medium and specific charge distribution.....	75
6.2.1.6. Influence of different residues on the redox potentials.....	77
6.2.2. Calculations on the native cytochrome b.....	78
6.2.2.1. Protonation pattern of titratable groups.....	78
6.2.2.2. Experimental values of hemes redox potentials.....	79
6.2.2.3. Calculated redox potentials in the whole Cbc ₁ complex.....	79
6.2.2.4. Calculated redox potentials of the hemes in the Cb subunit.....	80
6.2.3. Redox titration.....	82
6.3. Conclusions.....	84
7. Radical transfer in DNA photolyase	86
7.1. Introduction.....	86
7.1.1. Splitting the pyrimidine dimer by photolyase.....	86
7.1.2. Mechanism of photoactivation.....	88
7.2. Methods	91
7.2.1. Calculations of protonation and redox patterns.....	91
7.2.2. Titratable groups.....	93
7.2.3. Atomic partial charges.....	94
7.3. Results and Discussions	95
7.3.1. Protonation states in photolyase.....	95
7.3.2. Redox potentials of tryptophan triad.....	98
7.3.3. Energetics and reaction rates of the photoactivation process.....	101
7.4. Conclusion.....	104
A. Abstract	105
B. Zusammenfassung (German abstract)	108
C. Abbreviations, Constants and Symbols	111
D. The pK_a and E⁰ values of the model compounds	114
E. Atomic partial charges of titratable groups	115
F. List of the PDB codes for different groups of the heme-proteins	121
G. Comparison the amino acid sequences of four-helix bundles	123
H. Molecular mechanics force field	124
I. Modeling steps to generate atomic coordinates of the artificial cytochrome b	126
<i>Curriculum vitae</i> of the author	129
Bibliography	131

List of Figures

2.1.	A protein molecule in heterogeneous dielectric medium.....	6
2.2.	Thermodynamic cycle for protonation reactions in different environments.....	11
2.3.	A model compound for a titratable group	14
2.4.	Treatment of two strongly coupled sites in the MC titration	22
2.5.	Treatment for the residues with more than two possible protonation states.....	24
3.1.	The X-ray structure of the complex III from bovine heart	30
3.2.	Electron transfer pathway in the complex III.....	32
4.1.	Definition of imidazole-heme conformation by three torsion angles α , β and γ	37
4.2.	Distributions of the torsion angle α of different heme-proteins from the PDB	37
4.3.	Possible H-bonds of imidazole coordinated to heme.....	41
4.4.	Distribution of the torsion angle α for all bis-Cc and Cb heme-proteins	44
4.5.	Calculated interaction energies of imidazole with heme as a function of torsion angle α (both PR up).....	46
4.6.	Calculated interaction of imidazole with heme as a function of torsion angle α (PR on opposite sides).....	46
4.7.	Survey of imidazole-histidine backbone angles β derived from the PDB.....	48
4.8.	Calculated interaction energies between imidazole and its histidine backbone	48
4.9.	Distribution of the torsion angles α and γ for different heme-proteins.....	50
4.10.	Distribution of the torsion angle β for different groups of heme-proteins.....	51
4.11.	Histidine-heme conformation in Hb and CcPo group of proteins.....	51
4.12.	Distribution of the relative orientations of bis-histidine planes.....	53
5.1.	Crystal structure of the native Cb chain from bovine heart	57
5.2.	Side (a) and top (b) view of the artificial Cb	57
5.3.	Helical wheel projection of the artificial four-helix bundle	59
5.4.	Time evolution of the RMS deviations for the artificial and native Cb.....	65
6.1.	Titration curves of four arginines and propionates of the artificial Cb	71
6.2.	pH dependence of the heme redox potentials in artificial and native Cb.....	74
6.3.	Redox titration of the artificial and native Cb.....	83
7.1.	Photoactivation and photorepair processes in DNA photolyase	87
7.2.	X-ray structure of DNA photolyase from <i>E. coli</i>	88
7.3.	Catalytical core of the DNA photolyase from <i>E. coli</i>	90
7.4.	pH dependence of deprotonation free energies of tryptophan cation radicals	97
7.5.	pH dependence of redox potentials of three tryptophans and tyrosine in photolyase and in water.....	99
7.6.	The energy scheme of photoactivation process in DNA photolyase of <i>E. coli</i>	100

List of Tables

3.1.	Components of the mitochondrial electron transport chain.....	29
4.1.	Possible H-bonds of imidazole axially ligated to heme.....	42
5.1.	RMS deviation between structures of the different modeling steps and the final structure of the artificial Cb.....	62
5.2.	RMS deviation between the two hemes in artificial Cb for the different steps of modeling.....	62
5.3.	The heme conformations in the native and artificial cytochrome b.....	64
5.4.	Relative orientation of the hemes in native and artificial Cb.....	64
6.1.	Redox potentials of hemes in artificial Cb at pH= 7 and $E_{\text{sol}}= 0$ meV.....	76
6.2.	Redox potentials of the hemes in native Cb at different pH values	81
7.1.	Residues with non-standard protonation state in photolyase from <i>E. coli</i>	96

