

Energetics of Electron and Proton Transfer in Photosynthetic Reaction Centers

Dissertation zur Erlangung des akademischen Grades des
Doktors der Naturwissenschaften (Dr. rer. nat.)
eingereicht im Fachbereich Biologie, Chemie, Pharmacie
der Freien Universität Berlin

vorgelegt von
Hiroshi Ishikita
石北 央
aus Japan

Oktober, 2005

Die vorliegende Arbeit wurde in der Zeit von Oktober 2000 bis Oktober 2005 unter Anleitung von Prof. Dr. E.-W. Knapp am Institut für Chemie/Kristallographie der Freien Universität Berlin im Fachbereich Biologie, Chemie und Pharmazie durchgeführt.

1. Gutachter: Prof. Dr. E.-W. Knapp, Freie Universität Berlin
2. Gutachter: Prof. Dr. W. Saenger, Freie Universität Berlin

Tag der Disputation: 28/11/2005

Contents

1. Introduction	6
1.1. Photosynthetic reaction center from purple bacteria (bRC)	6
1.2. Photosystem II (PSII)	6
1.3. Photosystem I (PSI)	9
2. Theoretical Background	11
2.1 Overview of the computational procedures	11
2.1.1. Atomic coordinates	11
2.1.2. Generation of hydrogen atoms	11
2.1.3. Variable charges of titratable groups	11
2.1.4. Computation of protonation pattern, pK_a and E_m	11
2.2. Force field	12
2.3. Poisson-Boltzmann equation	13
2.4. Protonation probability	13
2.5. Born energy and background charge	14
2.6. Solution to the protonation probability	15
2.7. Computation of pK_a of a titratable group in protein	15
2.8. Computation of the E_m of a redox-active group in protein	16
2.9. Influence of ionic strength on E_m	17
2.10. Estimation of the ET rate	17
3. Quinones in bRC	18
3.1. Conformational gating in kinetic phase 1	18
3.1.1. Proton uptake of Glu-L212	18
3.1.2. ET-driving force assay	19
3.1.3. 180° propeller twist of Q_B	19
3.1.4. Is the Q_B movement really relevant?	20
3.1.5. H-bond flip of Ser-L223	20
3.2. Varying $E_m(Q_A)$ by flip-flop H bond of Thr-M222	21
3.2.1. H-bond pattern for Q_A	21
3.2.2. H-bond flip of Thr-M222	21
4. Proton transfer in bRC	22
4.1. PT inhibition in D(L213)N mutant and revertant	22
4.1.1. Overview	22
4.1.2. Proton uptake of Glu-H173 in revertant mutants	22
4.1.3. pK_a shift of Glu-H173 upon revertant mutation	23
4.1.4. Main effect of pK_a shifts upon revertant mutation: charge effect or induced conformational change?	24
4.2. PT inhibition by metal binding	24
4.2.1. Overview	24
4.2.2. PT pathways: pK_a decrease upon Cd^{2+} binding	25
4.2.3. Identification of the apparent pK_a of $k_{AB}^{(1)}$	26
4.2.4. Asp-L213, a residue in PT pathways	27
4.2.5. Electrostatic domino: a mechanism of long-range electrostatic influence ...	28
4.2.6. Main electrostatic effects of Cd^{2+} binding	29
5. Quinones in PSII	31
5.1. $E_m(Q_A)$ in PSII	31
5.1. Dependence of $E_m(Q_B)$ on H-bond pattern	32
5.1.1. H-bond flip of D1-Ser264	32
5.1.2. Calculated $E_m(Q_B)$	32

5.1.3. Driving force for ET from Q_A^- to Q_B	32
5.1.4. Similarity of Ser H-bond flip with bRC	33
5.2. Proton uptake at D1-His252 induced by the formation of Q_B^-	33
5.2.1. Absence of long PT pathways for Q_B in PSII	33
5.2.2. Proton uptake at D1-His252	34
5.2.3. pK_a of D1-His252	34
5.2.4. Possible Cd^{2+} binding site at the acceptor side of PSII	34
5.2.5. D1-Ser264 mutants	34
6. Quinones in PSI	36
6.1. Native PSI protein-pigment complex	36
6.1.1. Calculated $E_m(A_1)$ in native PSI	36
6.1.2. Kinetics of ET from A_1^- to F_X in native PSI: a single or double branch ET activity?	37
6.1.3. Difference between $E_m(A_{1A})$ and $E_m(A_{1B})$	37
6.2. P700- F_X core	38
6.2.1. P700- F_X core treatment	39
6.2.3. Shift of $E_m(F_X)$ upon F_X -P700 core treatment	39
6.3. Difference between $E_m(A_1)$ of PSI and $E_m(Q)$ of PSII and bRC	41
6.3.1. H-bond pattern for quinones	41
6.3.2. Fe_4S_4 center F_X in PSI and the Fe-complex in bRC/PSII	41
7. The non-heme iron complex near quinones	43
7.1. Fe-complex in bRC	43
7.1.1. Fe-complex with Glu	43
7.1.2. $E_m(Q_A)$ in Fe-depleted bRC	43
7.1.3. $E_m(Q_B)$ in Fe-depleted bRC	44
7.2. Fe-complex in PSII	45
7.2.1. Fe-complex with bicarbonate	45
7.2.2. D- <i>de</i> loop near the Fe-complex in PSII	46
7.2.3. pH-dependence of $E_m(Fe)$ observed experimentally	46
7.2.4. Deprotonation of D1-Glu244 upon oxidation of Fe-complex	47
7.2.5. Residues responsible for pH dependence of $E_m(Fe)$	47
7.2.6. Proton network for the Fe-complex	48
7.2.7. Influence of the Q_A/Q_B redox state on $E_m(Fe)$	48
7.2.8. Light-induced oxidation of the Fe-complex by exogenous Q_B	49
7.2.9. Proton release from D1-His252	49
7.2.10. EPR signals at $g = 1.82$ and $g = 1.9$ relate to ability of D1-His252 to deprotonate or not	50
8. Oxidation power of chlorophylls	53
8.1. $E_m(P870)$ in bRC	53
8.1.1. Monomer $E_m(P_{L/M})$ in bRC	53
8.1.2. Dimer $E_m(P870)$ in bRC	54
8.2. $E_m(P700)$ in PSI	55
8.2.1. Monomer $E_m(P_{A/B})$ in PSI	55
8.3. $E_m(P680)$ in PSII	55
8.3.1. $E_m(P_{D1/D2})$ and $E_m(Chl_{D1/D2})$ in PSII	55
8.3.2. Comparison of $E_m(Chl_{D1/D2})$ with $E_m(B_{L/M})$ and $E_m(A_{-1A/B})$	56
8.4. What shifts $E_m(P680)$ in PSII to such high values?	56
8.4.1. Overview of the 600 mV E_m difference between $P_{D1/D2}$ and $P_{A/B}$	56
8.4.2. Side-chain charges in PSII	57
8.4.3. Protonation patterns of titratable residues in PSII	57

8.4.4. Influences of side-chain and the Mn-cluster on $E_m(P_{D1/D2})$	58
8.4.5. Backbone dipole of His-providing α -helix stabilizes $Chl a^+$ by 130 mV in PSI relative to PSII.....	58
8.4.6. Protein backbone near luminal helix up-shifts $E_m(P_{D1/D2})$ by 90-110 mV in PSII relative to $E_m(P_{L/M})$ in bRC	59
9. Proton transfer in water oxidation	63
9.1. Proton exit pathway	63
9.1.1. Hydrophilic channels in the luminal side of PSII	63
9.1.2. Calculated pK_a values for residues along proton exit pathway	63
9.1.3. D1-Asp59, D1-Arg64 and PsbO-Arg152 in the proton exit pathway	64
9.1.4. Residues near the proton exit pathway	65
9.1.5. An alternative channel and the Cd^{2+} binding site	65
9.1.6. Influence of the extrinsic proteins on the energetics of the proton exit pathway.....	67
10. Photoprotective devices in PSII.....	69
10.1. Photoprotection by high-potential Q_A form.....	69
10.1.1. Photoprotection by shifting $E_m(Q_A)$	69
10.1.2. Varying $E_m(Q_A)$ by flip-flop H bonds.....	70
10.1.3. Mn-depleted PSII.....	70
10.1.4. Dynamics of the H-bond flip of D2-Thr217.....	71
10.1.5. Are reorientations of residues relevant for the Q_A^- stabilization?.....	72
10.2. Photoprotection by β -carotene near D2 protein (Car_{D2}), Chl_Z and cytochrome b_{559}	72
10.2.1. Electron hole transfer from $P680^+$ to cyt b_{559}/Chl_Z	72
10.2.2. Involvement of Car_{D2} in the pathway	72
10.3. Photoprotection by β -carotene near D1 protein (Car_{D1}).....	74
10.3.1. Car_{D1} and Car_{D2}	74
10.3.2. Car_{D1} is Car_{489}	75
10.3.3. Involvement of Car_{D1} in photoprotection	75
10.3.4. Cluster of Car near Car_{D1}	77
Zusammenfassung	78
Acknowledgement	82
References	83
Curriculum Vitae	97