

Institute of Complex Systems
Strukturbiochemie (ICS-6)

Molecular Mechanisms of Signal Transduction in Two-Component Signaling Systems

Andrii Ishchenko

Contents

Contents.....	2
Introduction	6
Acknowledgements	10
List of Abbreviations	11
1. Literature review	12
1.1. Two component signal transduction systems	12
1.2. The complex of Sensory Rhodopsin II (SRII) with its cognate transducer	14
1.2.1. General characteristics and function in vivo	14
1.2.2. Photocycle of Sensory Rhodopsin II. Intermediate states	18
1.2.3. The role of HAMP domains in cell signaling.....	19
1.2.4. Known structures of HAMP domains and molecular modeling	20
1.2.5. The molecular mechanism of signal transduction	23
1.2.6. O-like D75N mutant of SRII	26
1.2.7. Signal abolishment in the G83F mutant of HtrII.....	28
1.3. HAMP domain containing histidine kinases NarX and NarQ from <i>E. coli</i>	28
1.4. Crystallization of membrane proteins.....	30
2. Materials and Methods	33
2.1. Materials and Equipment.....	33
2.2. Molecular biology methods	41
2.2.1. Plasmid DNA isolation.....	41
2.2.2. Amplification of DNA.....	41
2.2.3. Analysis and extraction of DNA from agarose gels	41

2.2.4. DNA restriction	42
2.2.5. Ligation of DNA	42
2.2.6. Transformation of plasmids into <i>E. coli</i>	42
2.3. Heterologous expression and purification of SRII in <i>E. coli</i>	43
2.4. Heterologous expression and purification of truncated HtrII in <i>E. coli</i>	45
2.5. Biophysical methods for protein properties determination.	47
2.5.1. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and western- blotting (WB)	47
2.5.2. Size-exclusion chromatography (SEC)	47
2.5.3. Mass-spectrometry (MALDI-TOF).....	50
2.6. Crystallization of the SRII/HtrII complex	50
2.7. Treatment of the X-Ray diffraction data	52
2.7.1. Determination of twinning fraction.....	52
2.7.2. Determination of phases by molecular replacement	52
2.7.3. Model building and refinement	54
2.7.4. Obtaining the structures of intermediate states	54
3. Results and Discussion.....	55
3.1. Overall characteristic of the ground state structure of the SRII/HtrII complex	55
3.2. The structure of the D75N mutant. Implications from the structure	60
3.3. The structure of the M state intermediate. Signal transduction.....	65
3.4. “U” and “V” shapes of the complex. Biological relevance	69
3.5. Structure of the signal transferring triple mutant of bacteriorhodopsin in complex with HtrII.....	73
3.6. Expression, purification and crystallization of truncated parts of HtrII	75
3.7. The structure of SRII in complex with HtrII-135 and G83F mutation of HtrII-135 .	80

3.8. Expression, purification, characterization and crystallization of chemoreceptors NarQ and NarX.....	86
Main results and perspectives overview	91
Concluding remarks.....	92
References	95
Appendix I.....	103
Appendix II.....	107

Jül-4362
Juni 2013
ISSN 0944-2952

