

Structural and functional studies of the yeast TRAPP associated protein Tca17 and organization of human TRAPP tethering complexes

Dissertation zur Erlangung des akademischen Grades des
Doktors der Naturwissenschaften (Dr. rer. nat.)

eingereicht im Fachbereich Biologie, Chemie, Pharmazie
der Freien Universität Berlin

vorgelegt von
M.Sc.-Chem. Chengcheng Wang
aus Hunan, China

Berlin, 2010

1. Gutachter: Prof. Dr. Udo Heinemann

2. Gutachter: PD. Dr. Michael Veit

Disputation am: 25.02.2011

Selbstständigkeitserklärung

Diese Arbeit wurde im Zeitraum von März 2006 bis November 2010 in der Forschungsgruppe Makromolekulare Strukturen und Interaktionen, unter der Leitung von Prof. Dr. Udo Heinemann, am Max-Delbrück-Centrum für Molekulare Medizin in Berlin-Buch angefertigt.

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbstständig und auf Grundlage der angegebenen Hilfsmittel angefertigt habe.

Table of contents

1 Introduction	1
1.1 Vesicular transport	1
1.1.1 Modular organization of vesicular transport.....	2
1.1.2 Vesicle transport from ER to Golgi	3
1.2 SNARE proteins	5
1.2.1 Longin domain of SNARE proteins.....	6
1.3 Tethering factors	8
1.3.1 Coiled-coil tethers.....	8
1.3.2 Multi-subunit tethering complexes	9
1.3.3 TRAPP complex	11
1.3.4 Regulation of tethering factors.....	17
1.4 Objectives	18
2 Materials	19
2.1 Instruments	19
2.2 Chemicals, enzymes, kits.....	21
2.3 Strains and plasmids.....	23
2.4 Media and buffers	24
2.5 Synthetic oligonucleotides.....	31
2.5.1 Gene-specific primers.....	31
2.5.2 Vector-specific primers.....	31
3 Methods	32
3.1 Molecular biology methods	32
3.1.1 Polymerase chain reaction (PCR)	32
3.1.2 DNA gel electrophoresis.....	34

3.1.3 DNA purification.....	34
3.1.4 Restriction endonuclease-based cloning.....	34
3.1.5 Transformation of chemically competent <i>E. coli</i> cells.....	35
3.2 Protein purification and characterization	36
3.2.1 Test expression	36
3.2.2 Expression in <i>E. coli</i>	36
3.2.3 Production of seleno-methionine labeled protein	37
3.2.4 Cell lysis	37
3.2.5 Affinity chromatography.....	38
3.2.6 Determination of protein concentration.....	39
3.2.7 Cation exchange chromatography.....	40
3.2.8 Tag removal.....	40
3.2.9 Gel filtration chromatography	41
3.2.10 Protein SDS-PAGE	41
3.2.11 Staining of SDS-PAGE	42
3.2.12 Concentration of protein	43
3.2.13 Circular dichroism (CD) spectrum	43
3.2.14 Protein buffer screen	43
3.3 Protein crystallography.....	45
3.3.1 Crystallization	45
3.3.2 Crystal mounting and cryo-protection.....	47
3.3.3 Diffraction data collection	48
3.3.4 Data processing.....	50
3.3.5 Calculation of the electron density.....	51
3.3.6 Model building	57
3.3.7 Model refinement.....	58

3.4 Biophysical and biochemical methods	59
3.4.1 Analytical ultracentrifugation (AUC).....	59
3.4.2 Static light scattering (SLS)	60
3.4.3 Pull-down assays.....	60
3.4.4 Co-immunoprecipitations (CoIPs).....	61
3.4.5 Western blot	61
3.4.6 Immunodetection	62
4 Result.....	63
4.1 Cloning and purification of NIBP and Ehoc-1.....	63
4.1.1 Fragments designing	63
4.1.2 Purification of Ehoc-1 fragment	64
4.2 Structural and biophysical study of NIBP and Ehoc-1.....	65
4.2.1 CD spectrum of E211	65
4.2.2 Crystallization of E211	65
4.2.3 Interactions of NIBP and Ehoc-1	66
4.3 Structure determination of Tca17.....	67
4.3.1 Expression of Tca17 in <i>E. coli</i>	67
4.3.2 Purification of Tca17	67
4.3.3 Protein buffer screen	68
4.3.4 Crystallization of Tca17	71
4.3.5 Data collection and processing.....	72
4.3.6 Structure determination	73
4.4 The structure of Tca17	76
4.4.1 Longin fold of Tca17 structure	76
4.4.2 Comparison with Bet5 subfamily proteins	79
4.4.3 Oligomerization state of Tca17	80
4.5 Interaction of Tca17 with TRAPP subunits.....	86

5 Discussion	88
5.1 Organization of the mammalian TRAPP complex.....	88
5.2 Characterization of Tca17	89
5.2.1 Oligomerization state of Tca17.....	89
5.2.2 Sequence conservation of Tca17/TRAPPC2L	89
5.3 Electrostatic potential distribution.....	91
5.3.1 Comparison among Bet5 subfamily members.....	91
5.3.2 Electrostatic potential on Tca17 surface.....	92
5.4 Possible interaction of Tca17 and TRAPP I subunits.....	96
5.4.1 Possible interaction with Bet3.....	96
5.4.2 Possible interaction with Trs31.....	97
5.4.3 Possible interaction with Trs33p.....	99
5.5 Conclusion	100
6 Summary / Zusammenfassung.....	101
 Appendix A: References.....	105
Appendix B: Crystallization screen formulations.....	111
Appendix C: Multiple sequence alignment.....	114
Appendix D: Abbreviations	116