

**Structure and Dynamics of Neuronal SNARE Complex
Assembly Studied by Electron Paramagnetic Resonance- and
Fluorescence Spectroscopy**

Dissertation
zur Erlangung des akademischen Grades
Doktor der Naturwissenschaften
-Dr. rer. nat.-

im Fachbereich Biologie, Chemie und Pharmazie
der Freien Universität Berlin

vorgelegt von
Diplom-Biochemiker Martin Margittai
Berlin 2001

1. Gutachter: Prof. Dr. Reinhard Jahn
 2. Gutachter: Prof. Dr. Ferdinand Hucho
- Datum der Disputation: 31. Oktober 2001

Contents

1 Introduction	1
1.1 Historical Outline	1
1.2 Towards a Molecular Understanding of SNARE Proteins	4
1.3 A Model for Membrane Fusion	7
1.4 Syntaxin at the Center Stage of Regulation	8
1.5 Electron Paramagnetic Resonance Spectroscopy	10
1.6 Single Molecule Fluorescence Spectroscopy	12
1.7 Aim of this Work	14
2 Materials and Methods	15
2.1 Materials	15
2.2 Plasmid Construction	15
2.3 Protein Expression and Purification	17
2.4 Complex Formation	18
2.5 Spin Labeling and EPR Measurements	18
2.6 Multiangle Laser Light Scattering	19
2.7 Protein Labeling with Fluorescent Dyes	19
2.8 Absorption Spectroscopy	20
2.9 Fluorescence Anisotropy	20
2.10 Single Molecule Measurements	21
2.11 Data Analysis of Multi Parameter Single Molecule FRET	22
2.12 Fluorescence Correlation Spectroscopy	24
2.13 Preparation of Proteoliposomes	25
2.14 Detergent Assisted Insertion	25
2.15 Assembly of SNARE Complexes between Different Membranes	25
2.16 Disassembly of co-reconstituted cis-SNARE Complexes	26
2.17 Other Methods	26
3 Results	27
3.1 Structural Investigation of SNARE Proteins by Electron Paramagnetic Resonance Spectroscopy	27
3.1.1 Individual SNAREs and SNARE Core Complexes in Solution	28
3.1.2 Loop Region of SNAP-25	34
3.1.3 Core Complexes with Truncated SNAREs	35
3.1.4 Binary Complex between Syntaxin and SNAP-25	37
3.2 Conformational Changes of the N-terminal Domain of Syntaxin and Their Influence on SNARE Complex Assembly	41
3.2.1 Preliminary Remarks	41
3.2.2 Kinetics of SNARE Complex Formation Using Syntaxin Variants Containing or Lacking the	

N-terminal Regulatory Domain.....	42
3.2.3. Analysis of Syntaxin Conformations by Single Molecule Fluorescence Spectroscopy	46
3.3 Interactions between Syntaxin and Synaptobrevin Involving the Transmembrane Domains.....	57
3.3.1 General Remarks	57
3.3.2 Interactions between the Transmembrane Domains of Syntaxin and Synaptobrevin	58
3.4 Formation of trans-SNARE Complexes.....	62
4 Discussion.....	65
4.1 Structural Aspects of the SNARE Motif.....	66
4.2 The N-terminus of Syntaxin	67
4.2.1 A Weak Interaction between Syntaxin's SNARE Motif and its N-terminus.....	67
4.2.2 Kinetic Analysis of the Back-Folding.....	70
4.2.3 Implications for Munc-18-Binding	70
4.3 Syntaxin in the Membrane	72
4.4 Syntaxin in Complex with SNAP-25.....	74
4.5 Multiple Conformations of Syntaxin's H3-domain	75
4.6 Syntaxin at the Final Step of Membrane Fusion	76
4.7 Syntaxin in Complex with Synaptobrevin	76
4.8 Outlook.....	77
5 Summary.....	79
6 Zusammenfassung	81
7 References	83
8 Appendix	96
8.1 Abbreviations	96
8.2 Acknowledgements	97
8.3 Curriculum Vitae.....	98
8.4 Publications	99