Protein-Vesicle Interaction Analysis

Application Note NT-MO-002

Interactions of Liposome embedded SNARE Proteins

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Abstract

SNARE protein complexes play a key role in membrane fusion processes, e.g. the fusion of synaptic vesicles with the pre-synaptic plasma membrane in response to elevated Ca^{2+} levels. This study aimed to demonstrate the use of MicroScale Thermophoresis for the detection of protein-protein interaction in more complex assay systems like in this case the interaction of membrane proteins embedded into liposomes.

Introduction

Synaptical signal transduction to neighboring neuronal cells occurs mainly by chemical signal transduction.

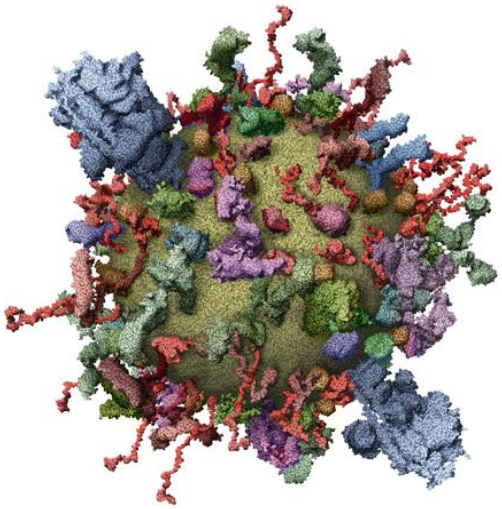

Fig. 1 Molecular modell of a synaptic vesicle *(Reinhard Jahn, Max-Planck-Institut für biophysikalische Chemie, Göttingen, Germany)*

Neurotransmitters are released from the pre-synaptic cell inducing activation of ion-channels which in turn induces an electrical signal in the post-synaptical cell. The neurotransmitters are stored in multiple pre-synaptic vesicles closely attached to the plasma membrane to ensure a fast release upon incoming signals. Voltage-gated Ion channels lead to elevated Ca^{2+} levels in the pre-synaptic cell and induce fusion of the synaptic vesicles with the plasma membrane with the subsequent release of the neurotransmitters into the synaptic cleft.

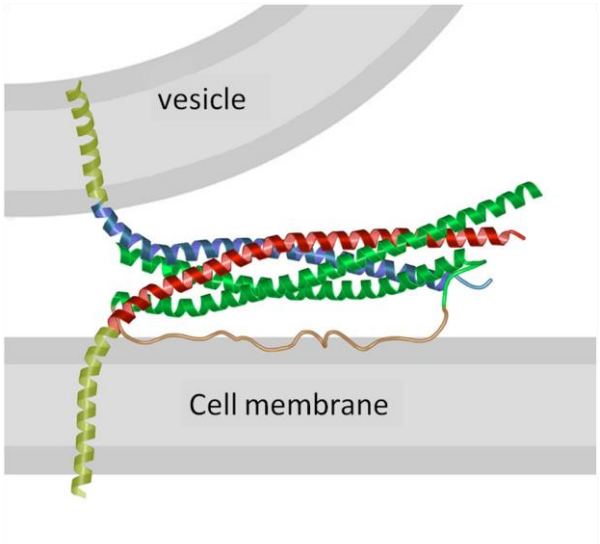

Fig. 2 The SNARE-complex attaches vesicles to the cell membrane. Three proteins – syntaxin (red), synaptobrevin-2 (syp-2) (blue) and SNAP25 (green) – are forming a helical complex. Syntaxin and synaptobrevin-2 are integral membrane proteins bringing vesicle and cell membrane in close contact. *(Reinhard Jahn, Max-Planck-Institut für biophysikalische Chemie, Göttingen, Germany)*

In eukaryotes, most intracellular membrane fusion reactions are mediated by the interaction of complementary SNARE proteins that are present in both fusing membranes. SNARE complexes consist of SNAP25 and the membrane protein syntaxin which is embedded in the plasma

membrane as well as synaptobrevin which is integrated into the vesicle membrane. By forming the SNARE complex the vesicles are closely attached to the pre-synaptic plasma membrane and form a pool of neurotransmitter containing releasable vesicles (Fig.2).

Results

For the experiment, two different liposome populations were combined. One liposome population contains the neuronal SNARE protein synaptobrevin-2 (syb-2), while the other contains a receptor complex consisting of SNAP-25 and syntaxin-1A (ΔN-complex) associated with a syb49-96 peptide labeled with Alexa Fluor 488 (Fig.3). Full-length syb-2 binds to the ΔN-complex (acceptor SNARE complex) and a *cis*-SNARE complex is formed. This results in the replacement of the fluorescently labeled syb49-96 fragment and is directly followed by membrane fusion. For this particular experiment a competitive approach (labeled peptide competition instead of incorporating a label in a liposome) has been used to focus on the interaction between the membrane receptors and not the following process of liposome fusion (Fig.3).

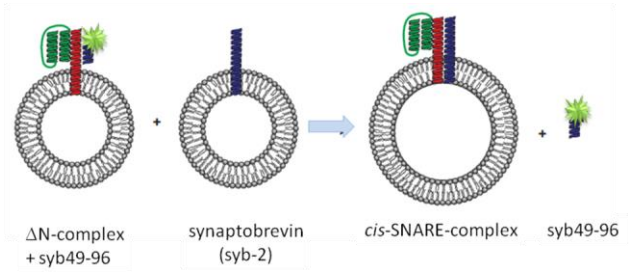

Fig. 3 Liposome embedded membrane receptor interactions. The interaction of two liposome embedded membrane receptors – synaptobrevin-2 (blue) and the ΔN-complex consisting of syntaxin (red) and SNAP-25 (green) (acceptor SNARE complex) associated with a labeled syb49-96 peptide leads to the release of the syb49-96 peptide due to higher affinity of the full-length syb-2 receptor. The process of liposome docking is followed by liposome fusion.

The result of a thermophoresis experiment as a function of the concentration of unlabeled syb-2 liposomes is shown in Fig.4. The concentration of ΔN-complex/labeled syb49-96 liposomes has been kept constant. The 50 % point of the binding curve is found at about 450 nM. The change in thermophoretic amplitude shows the dissociation of the syb49-96 fragment due to competition with the liposome embedded full-length synaptobrevin, which has a higher affinity to the ΔN-complex. Since this dissociation is irreversible, the result reflects the point at which 50 % of active acceptor SNAREs are bound. The binding curve that is obtained (in equilibrium) shows a relatively strong change from the region of very high concentrations of (unlabeled) syb-2 liposomes towards low concentrations where the MST signal change is only small because little of the syb49-96 is dissociated.

As a control, plain liposomes containing no synaptobrevin have been titrated to the ΔN-complex/syb49-96 liposomes. As expected no change of the thermophoretic signal is observed (Fig.4).

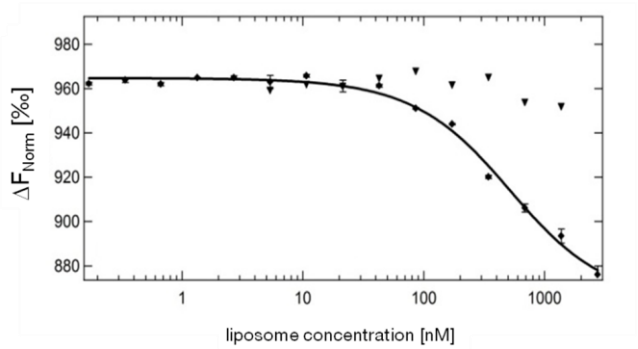

Fig. 4 Liposome embedded membrane receptor interactions measured with MicroScale Thermophoresis. The interaction of two liposome embedded membrane receptors – synaptobrevin-2 (blue) and the ΔN-complex associated with labeled syb49-96 peptide is measured with MicroScale Thermophoresis. Upon receptor-receptor interaction of the ΔN-complex and full-length synaptobrevin, the fluorescently labeled syb49-96 peptide is released (black diamonds). As a control plain liposomes containing no syb-2 receptor have been used (black triangles). Error bars of synaptobrevin liposomes represent standard error of n = 3 measurements.

Conclusion

This experiment demonstrates that even complexes with a size of several 100nm can be analyzed with MST. The use of liposomes allows to measure membrane associated proteins and trans-membrane proteins at conditions that are, in comparison to other approaches, close to the native conditions.

Material and Methods

Assay conditions
SNARE proteins were expressed and purified as described (Schuette *et al*. 2004). Proteoliposomes used in this experiment were prepared as described in 20 mM HEPES, pH 7.4, with 150 mM KCl (Pobbati *et al*. 2006). Lipid composition was in 5:2:2:1 ratio of brain L-α-phosphatidylcholine, L-α-phosphatidylethanolamine (PE), L-α-phosphatidylserine and cholesterol (Avanti). Protein:lipid ratio was 1:4000.

For the experiment described here, a 20 mM HEPES, 150 mM KCl at pH 7.4 buffer has been used. The measurement was performed in standard treated capillaries (Cat#K002).

Instrumentation
The measurements were done on NanoTemper Monolith NT.015 and NT.115 instruments.
The measurement was performed at 40 % LED and 40 % MST power, Laser-On time was 30 sec, Laser-Off time 5 sec.

References

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