Protein-Ion Interaction Analysis

Application Note NT006

Binding of Calcium Ions to Synaptotagmin

measured with fluorescence label and label-free

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Abstract

The synaptic vesicle protein synaptotagmin 1 is the main calcium sensor of neuronal exocytosis. Calcium binds to its cytosolic portion that consists of tandem C2-type domains. In this work we show that MicroScale Thermophoresis is a valuable tool to measure binding of ions to proteins, with and without the use of a fluorescent label.

Introduction

Neuronal excocytosis is the process were small synaptic vesicle fuse with the plasma membrane, thereby releasing neurotransmitter in the synaptic cleft. The process is triggered by a rapid increase of the cytoplasmic calcium concentration.

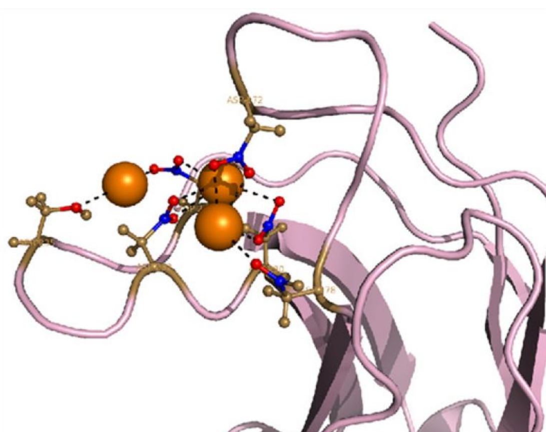

Fig. 1 Calcium binding site of synaptotagmin (Radhakrishnan *et al* 2009)

Calcium binds to synaptotagmin 1, the calcium sensor of neuronal exocytosis, which than actively promotes exocytotic fusion (for review, see Chapman *et al*. 2008). Synaptotagmin 1 consists of a single transmembrane region followed by a large unstructured linker and two C2-type domains, called C2A and C2B.
We employed MicroScale Thermophoresis to measure the intrinsic calcium binding affinities of the calcium binding synaptotagmin 1 C2AB domain and found a very good agreement with Isothermal Titration Calorimetry (ITC) experiments.

Results

As shown in Fig. 2 and 3 the binding of calcium ions to synaptotagmin was observed as a clear and strong response in MST signal, while no signal was observed at increasing magnesium ion concentrations.

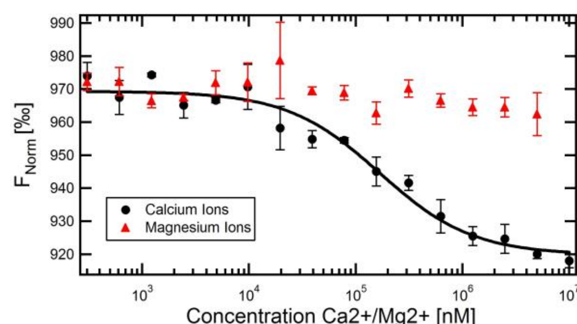

Fig. 2 Binding of Calcium ions to NT-647 labeled synaptotagmin 1 C2AB. The difference in thermophoretic mobility is measured by attaching a fluorescence label to the synaptotagmin 1 (n = 2 measurements).

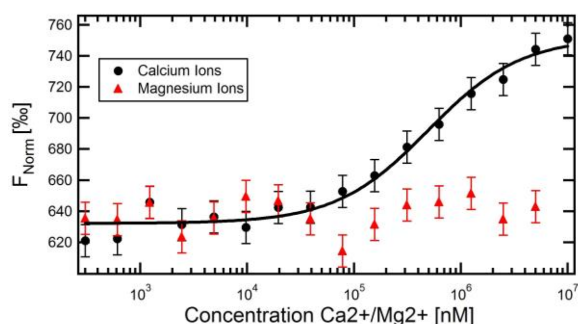

Fig. 3 Label-free binding of calcium ions synaptotagmin 1 C2AB. Themophoresis is measured by measuring the fluorescence of the intrinsic tryptophanes (n = 1 measurement, error bars by average noise of negative control).

Both measurements were performed with NT-647-labeled synaptotagmin in the Monolith NT.115 instrument (Fig.2) as well as in the NT.LabelFree instrument (Fig.3).
Since synaptotagmin 1 binds a total of 5 calcium ions, the MST-Signal comprises of different binding events. The data sets are fitted with a line to guide the eye. The dissociation constants from double digit µM to mM (Radhakrishnan *et al.* 2009) are in good agreement with the literature values of 50 µM to 3 mM. Higher resolution to obtain information on the individual binding sites can be obtained by independent repeats of the experiment.

Conclusion

The study provides an example that MicroScale Thermophoresis allows to measure binding of ions to proteins by following the change in thermophoresis of the comparably much larger protein. It also illustrates that these interactions can be measured even without labeling of the protein; we showed that results of labeled protein are comparable to unlabeled proteins, measured by intrinsic tryptophan fluorescence.

Material and Methods

Assay Conditions

All protein constructs used were from *Rattus norvegicus* and cloned into the expression vector pET28a. Expression constructs of the full-length protein (aa1–21) has been described before (Stein *et al.* 2007).
For the NT.115 experiment synaptotagmin-1 C2AB fragment was labeled with the Monolith NT™ Protein Labeling Kit RED (Cat#L001) according to the supplied labeling protocol.
Labeled synaptotagmin 1 C2AB was used at a concentration of ~ 40 nM. For the label-free synaptotagmin 1 C2AB was used at 1 µM. Calcium and magnesium chloride were titrated in 1:1 dilutions beginning at a final concentration of 10 mM.
Labeled protein approach: A dilution of calcium chloride starting at 20 mM in 20 mM HEPES, 150 mM KCl at pH 7.4 was prepared. 10 µl of the ion containing solution was mixed with 10 µl of 80nM protein diluted in 20 mM HEPES, 150 mM KCl at pH 7.4 containing 0.5 mg/ml BSA. After mixing, the samples were incubated for 10 minutes and filled into hydrophobic capillaries (Cat# K003). A similar experiment was recently published (van den Bogaart *et al.* 2011) using a cysteine labeled synaptotagmin protein.
Label-free approach: 10 µl of a 2 µM synaptotagmin C2AB solution was mixed with same serial dilution of calcium ions prepared before.
As a negative control, for the labeled and label-free approach a 1:1 serial dilution of magnesium chloride beginning at 10 mM final was prepared and mixed with the respective protein preparation. The protein dilution buffer (20 mM HEPES, 150 mM KCl at pH 7.4) did not contain any BSA.

Instrumentation

The measurements were done on a NanoTemper Monolith NT.115 and the NT.LabelFree instrument.
The measurement was performed at 40 % LED (NT.115) and 80 % UV-LED (NT.LabelFree) both at 40 % MST power, Laser-On time was 30 sec, Laser-Off time 5 sec.

References

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