5 Summary

Here I present the cloning, expression, purification and structural characterization of the intracellular domain of the δ-subunit of the nicotinic acetylcholine receptor from *Torpedo californica* and *Rattus norvegicus*.

Escherichia coli was chosen as an appropriate expression system. The expression in eukaryotic cells was too low for further investigations due to the toxicity of the investigated protein.

First the expression and purification of the intracellular domain of the nAChR was optimized using different fusion proteins. The expression of Strep-fusion proteins resulted in low amounts insoluble protein. In contrast, the hexahistidin-fusion protein of the intracellular domain of the nAChR was expressed in high amounts. Although the hexahistidin-fusion proteins were insoluble performing purification under denaturating conditions, pure protein (indeed from Coomassie stained SDS-PAGE) could be obtained in one step.

While no biochemical test for the activity of the investigated domain is available right now, the purification protocol should be established under non-denaturating conditions. Therefore different fusion proteins were generated to enhance the solubility of the investigated protein. The expression as a soluble protein and purification under native conditions could be obtained using GST- and MBP-fusions. Unfortunately, the resulting fusion proteins turned out to be instable and multiple degradation products could be observed. Cleavage of the fusion partner with different aminoacid-sequence-specific proteases (factor Xa, enterokinase, genenase I) resulted in enhanced degradation of the intracellular domain of the nAChR.

As an alternative approached, Intein- and SUMO-fusion proteins were expressed. Both fusions resulted in soluble protein. However, isolation of the intracellular domain of the nAChR by an Intein-mediated internal protein splicing mechanism could not be reached.

The SUMO-fusion protein could be purified under native conditions. The removal of the fusion partner with the tertiary-structure recognizing SUMO-protease was successful. However, isolation of the intracellular domain of the nAChR from the uncleaved construct could not be reached due to the instability of the domain in the absence of detergents.

As enrichment of the intracellular domain of the nAChR was not successful under native conditions, the hexahistidin-fusion proteins had to be purified from inclusion bodies and refolded. The fusion protein from *Torpedo* could be renaturated by gelfiltration or dialysis. A sample of 0.3 mg/ml fusion protein was available for structural characterization. The renaturation of the intracellular domain of the nAChR from rat was only successful in the presence of the detergents dodecylmaltoside and dodecylphosphocholine.

A characterization with CD spectroscopy revealed a high portion of disordered conformation of the investigated protein. This conformation was further proven by experiments with limited proteolysis, since no fragments with compact structures could be formed.

In the presence of dodecylphosphocholine the hexahistidin-fusion proteins could be concentrated to 6 mg/ml enabling NMR-spectroscopic investigations. The record of a ^{15}N-HSQC-Spektrum confirmed the unstructured confirmation of the investigated proteins. Analytical ultracentrifugation showed that the intracellular domain of the nAChR is a monomer in association with dodecylphosphocholine.

An influence on the secondary structure of the intracellular domain of the nAChR was investigated with variations of the construct. The introduction of a phosphorylation or mimicking the serine-phosphorylation by a serine-aspartate-mutation did not show an effect on the secondary structure. A change in the length of the N- or C-Terminus of the intracellular domain of the nAChR had also no influence on the structure of the investigated protein.

In agreement with the experimental findings, also secondary-structure predictions anticipate a high content of disordered conformation for the intracellular domain of the nAChR.

I suggest that a binding of an interaction partner induced an ordered secondary structure. This hypothesis could not be investigated in more detail in the presented work.