Summary of the PhD thesis (2014)

Investigation of electroneutral M. jannaschii NhaP1 and electrogenic H. pylori NhaA: A common transport mechanism for CPA1 and CPA2 Na^+/H^+ antiporters

Transmembrane exchange of Na^+ for H^+ exists in virtually all organisms and is essential for survival. The mediators of this process, Na^+/H^+ antiporters, belong to the monovalent cation:proton antiporter (CPA) superfamily that has as main subgroups the electroneutral CPA1 and the electrogenic CPA2 families. The most studied CPA transporter is NhaA from *Escherichia coli* (EcNhaA), the prototype of the CPA2 family. The pH-regulated activity exhibited by EcNhaA was recently explained by a model that proposes the existence of competition between the two substrates (Na^+ and H^+) for a common binding site [1]. This model provides an inherent self-regulatory mechanism at extreme pH values and it was tempting to speculate that it might apply to all CPA antiporters.

AIM OF THE WORK. The aim of the study was thus further investigation into the transport mechanism and pH regulation of Na^+/H^+ antiporters. Two antiporters were chosen as experimental models: the NhaA transporter from *Helicobacter pylori* (HpNhaA) and the NhaP1 transporter from *Methanocaldococcus jannaschii* (MjNhaP1).

RESULTS. HpNhaA shows a high sequence homology to EcNhaA, but has been reported [2] to possess a pH independent activity within the pH range 6.5 to 8.5 by the use of fluorimetric methods. Solid supported membrane (SSM)-based electrophysiological investigation revealed that, in contradiction to previously published findings, the activity profile of HpNhaA is remarkably similar to that of EcNhaA and shows a clear pH dependence. Based on the experimental data, the kinetic parameters of HpNhaA were determined and it could be shown that HpNhaA was excellently described by the competition model. In order to clarify why previously published results [2] contradict the findings of this work, the previously used assay was also employed. It was shown that, under different conditions of protein expression and in response to varying Na^+ concentrations, the pH-dependent activity profile of HpNhaA is changed. Therefore, the previously reported pH-independent behavior of HpNhaA was a result of the limitations of the fluorescent assay and the experimental conditions that were previously used. The kinetic properties of HpNhaA were compared to those of two other enterobacterial Na^+/H^+ antiporters and all three antiporters were shown to be highly similar.

MjNhaP1 was, at the time, the only CPA1 exchanger about which structural information was available. Functionally important regions in MjNhaP1 share a high sequence homology with the corresponding regions of eukaryotic CPA1 transporters. Therefore, a better understanding of the transport mechanism and pH regulation of MjNhaP1 could also provide insights into the function of human NHE Na^+/H^+ exchangers. Investigation of MjNhaP1 by SSM-based electrophysiology revealed that the substrate translocation reactions are electrogenic in this overall electroneutral exchanger. This allowed the first detailed kinetic characterization of an electroneutral Na^+/H^+ antiporter using an electrophysiological technique. It was shown that the transport properties and pH regulation of MjNhaP1 are also excellently described by the competition model. This finding is makes it very likely that the competition model can be generally applied to all CPA Na^+/H^+ exchangers. Electrophysiological investigation of MjNhaP1 coupled with measurements employing atomic absorption spectrometry permitted the determination of kinetic parameters for MjNhaP1. A number of mutants of MjNhaP1 were also investigated, and conclusions could be drawn regarding the relevance of these mutations on the function of MjNhaP1.

CONCLUSION. In conclusion, the competition model of Na^+/H^+ exchange was shown to be equally applicable to electrogenic CPA2 and electroneutral CPA1 exchangers. This model allows the adaptation of Na^+/H^+ exchangers to a wide variety of physiological and environmental conditions of the host organisms. Moreover, it seems very likely that eukaryotic Na^+/H^+ exchangers follow the same general mechanism of transport as the prokaryotic exchangers investigated so far.

REFERENCES

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Dr. Octavian Călinescu