ABSTRACT

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Voltage-gated sodium channels (Na_vs) are membrane proteins from the superfamily of voltage-gated ion channels (VGIC), and are present in every excitable cell where they participate in the propagation of action potentials by changing the Na^+ permeability of the cell membrane. Eukaryotic Na_vs are pseudo homotetrameric polypeptides, comprising four repeats of six transmembrane segments (S1-S6), where S1 to S4 form the voltage-sensing domain and S5 and S6 create the pore domain with the selectivity filter on the extracellular site. Whereas the ion selectivity of the voltage-gated potassium channels has been elucidated on the molecular level in great detail, little is known about this for the voltage-gated sodium channels.

To allow future studies of the selectivity filter of Na_v by the means of 2D IR spectroscopy, a technique able to provide bond-specific structural information on the picosecond to millisecond time scales, large quantities of the purified channel are needed. Eukaryotic Na_vs are difficult to obtain in these amounts. Therefore we have chosen a prokaryotic pore-only Na_v from *Silicibacter pomeroy* (Na_vSp1p) as a model system. Furthermore, 2D IR studies require site-specifically isotope labeled samples which can only be produced in cell-free expression systems.

In this work, we report the first cell-free expression of a voltage-gated sodium channel and its subsequent purification. To prepare the isotope labeling, three amino acids, which are most likely involved in the ion selectivity of Na_vSp1p, were identified and mutated to amber codons. This will allow 2D IR spectroscopy studies and will help to reveal the principle of sodium ion selectivity in Na_vs.