Pharmacological function of recombinant human skeletal muscle sodium channels are preserved following ion metal affinity chromatography purification and reconstitution into bilayer lipid membranes

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Voltage-gated sodium ion channels are major sites of action for drugs and toxins that modulate cellular excitability, and are therefore key molecular targets for ion channel research, high throughput screening for new drugs, and toxin detection. Protein suitable for these applications must be produced in a functionally active form. The aim of this study was to purify recombinant sodium channels in a manner that preserves their function and may therefore be suitable for use in future biosensors and screening technologies. Human voltage-gated skeletal muscle sodium channels modified by addition of a 6x-histidine tag at the C-terminus of the gene (hSkM1-HT) were expressed in Sf9 insect cells and purified using ion metal affinity chromatography. Following this purification method, hSkM1-HT protein was pharmacologically functional when reconstituted into liposomes and incorporated into planar bilayer lipid membranes (BLMs) (Zhang *et al.*, 2006). Channel activities were characterised using the neurotoxins veratridine and brevetoxin (PbTx-1) to active the channels, and tetrodotoxin (TTX) and saxitoxin (STX) to inhibit them. As these inhibitors act only from the extracellular side of the channel and are not able to cross the BLM, they are ideal molecular tools to investigate channel orientation. We found that the extracellular side of the channel molecules could face either side of a planar-BLMs, but they were more likely to face the side to which liposomes were added. Our results show that metal affinity chromatography is a suitable method to obtain functional recombinant sodium channels.

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