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Recombinant human voltage-gated skeletal muscle sodium channels are pharmacologically functional in planar lipid bilayers

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Abstract

Human 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. We report the successful use of ion metal affinity chromatography (IMAC) to purify C-terminal polyhistidine tagged human skeletal muscle voltage-gated sodium (*hSkM1*-HT) channels from *Sf*9 insect cells; *hSkM1* channels were pharmacologically functional when reconstituted into liposomes and incorporated into planar bilayer lipid membranes. *hSkM1*-HT single channel currents activated by veratridine had a conductance of 21 pS and those activated by brevetoxin, 16 pS. Channel activity was inhibited by tetrodotoxin and saxitoxin. This protein is suitable for the development of biosensor and high throughput screening technologies.

Keywords: *hSkM1*; Voltage-gated sodium channel; Ion metal affinity chromatography purification; Patch-clamp; Planar bilayer lipid membrane; Biosensor

1. Introduction

This paper describes the production of purified, recombinant human voltage-gated sodium channels by heterologous expression for use in biosensors as the sensing component for compounds that affect the function of this protein. Voltage-gated sodium channels are crucial components of living cells, being responsible for the rapid depolarisation that occurs during the initial phase of the action potential in excitable cells (Denac et al., 2000; Catterall, 2000). As a major site of action for therapeutic drugs including local anaesthetics, anti-arrythmics, anti-epileptics and analgesics, they are important targets for drug discovery (Clare et al., 2000; Errington et al., 2005). Some marine biotoxins, for example, brevetoxin and saxitoxin, exert their harmful effects via voltage-gated sodium channels. The toxins are produced by algae during toxic algal blooms and contaminate coastal waters worldwide. Saxitoxin is of particular concern as it is the cause of the life-threatening syndrome, paralytic shellfish poisoning in humans (Denac et al., 2000). In addition to contaminating seafood and also drinking water, saxitoxin has been designated as a chemical warfare agent under the Chemical Weapons Convention.

At present the only functional assay to identify potential novel modifiers of sodium channels is the patch-clamp technique which, in its traditional form, is costly, low throughput and requires highly skilled operators. Automated patch-clamp has increased throughput but the other difficulties remain (Shieh, 2004). Saxitoxin and its isomers can be detected chemically using chromatographic analysis, for example, liquid chromatography, LC–MS, or by using antibody-based assays (Lawrence et al., 2004; Jellett et al., 2002). The disadvantage of these methods is that they detect only known isoforms and thus may miss new harmful isoforms that might arise in algal blooms. While chemical detection methods can determine the presence of a compound, they are not indicative of toxicity. The mouse bioassay has been used for many years and is the gold standard in determining the toxicity of algal toxins (AOAC, 1999). However, this method is costly, slow, and it raises questions around ethical justification for the use of live animals. Cell-based detection methods offer an alternative to the mouse bioassay for brevetoxin (Fairey et al., 2001), but they are slow and maintenance of cell cultures is often not feasible in analytical laboratories. These issues have led to an international motivation to replace this assay with a more convenient functional assay that is predictive

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of biological effect (Rossini, 2005; Quilliam, 1999). Moreover, the World Health Organisation has expressed a desire to reduce the regulatory limit for saxitoxin to levels that may be below the limits of detection of approximately 40 μg per 100 g tissue using the mouse bioassay (UNESCO, 2005). A replacement functional assay must therefore be more sensitive. As the human voltage-gated sodium channel is the natural biological target for saxitoxin, it would recognise this dangerous toxin and other analogues that might arise. Thus, it would be ideal as a sensing entity in an ion channel biosensor for the detection of toxins and for drug discovery. The production of purified sodium channels with normal pharmacological function would be an essential component in such a system.

Sodium channels have long been purified from native tissues for reconstitution into bilayer membranes to investigate their functional properties (Miller, 1986). More recently, recombinant sodium channels with added histidine tags have been heterologously expressed, but none have yet been purified then examined as to whether they can still open and close in planar lipid bilayers (Chen et al., 2002; Pincin et al., 2005). To predict the effect of potential drugs or toxins on humans, it is advantageous to use the human form of the sodium channel, thus heterologous expression of this protein offers the only practical source.

The aim of this study was to purify recombinant sodium channels in a manner that preserves their function and is suitable for the development of biosensors and screening technologies. This is the first report to show that purified histidine-tagged human voltage-gated sodium channels are functional when reconstituted into bilayer lipid membranes and that they retain their pharmacological integrity.

2. Materials and methods

2.1. Materials

CD4 antibody-labelled beads were purchased from Dynal Biotech Pty Ltd. (Victoria, Australia). Phosphoethanolamine (PE), phospho-L-serine (PS) and cholesterol (CH) were from Avanti Polar Lipids. Phosphatidylcholine (PC) was extracted from egg yolk (Singleton et al., 1965). Saxitoxin (STX) dihydrochloride was obtained from the National Research Council of Canada. Brevetoxin (PbTx-1) was from Calbiochem (San Diego, United States). Veratridine (VTD) was purchased from Sigma. Tetrodotoxin (TTX) and anti-Pan Na_v were purchased from Alomone Labs (Jerusalem, Israel).

2.2. Molecular biology

hSkM1 cDNA was provided in the mammalian expression vector, pRc/CMV (George et al., 1992). A 6× histidine fusion tag was introduced to the C-terminus of *hSkM1* giving the amino acid sequence, +KESLVHHHHHH. PCR was used to amplify a fragment from a *Cla*I site (forward primer 5′-AC GTC AAG AAG GAG TCG GG-3′) to a new *Xba*I site at the C-terminus (reverse primer 5′-TT TCT AGA CTA ATG GTG ATG GTG ATG GTG GAC AAG AGA CTC CTT GAC ACC TG-3′). The resulting ∼1 kb PCR product was amplified in a TOPO-TA vector, transferred back into pRC/CMV at *Cla*I and *Xba*I restriction endonuclease sites, then sub-cloned into pFastBac at *Not*I and *Xba*I sites. Base changes were confirmed by DNA sequencing.

2.3. hSkM1-HT expression in Sf9 cells

*Sf*9 cells were grown in Grace's Insect medium (Sigma) supplemented with 10% foetal bovine serum, 2% yeastolate, 3.3 mg/ml lactalbumin, pH is 6.2. Cells were routinely seeded at a density of 5×10^5 cells/ml and grown to 2×10^6 cells/ml in spinner flasks at 25 °C. Recombinant baculovirus was produced in *E. coli* by site-specific transposition using the Bac-to-Bac™ baculovirus expression system (Invitrogen, Carlsbad, CA). Briefly, pFastBac/*hSkM1*-HT was transformed into DH10-Bac and colonies were selected on the basis of their resistance to the antibiotics, kanamycin/gentamycin/tetracycline and by blue/white colony screening using IPTG and X-gal. Generation of recombinant bacmid was confirmed by PCR analysis. Purified bacmid DNA was used to infect *Sf*9 insect cells and the resultant P1 viral supernatant propagated to obtain stocks of recombinant baculovirus. Virus titre was determined by plaque assay. For protein production, cells were grown to a density of 2×10^6 cells/ml, then infected with baculovirus (1×10^7 PFU/ml). Protein production in *Sf*9 cells infected with *hSkM1*-HT recombinant baculovirus was verified by electrophoresis and Western blot analysis using an anti-Pan Na_v channel antibody (1/400), a secondary peroxidase conjugated goat anti-rabbit antibody (1/2000), and visualised using chemiluminesence (ECL™, Amersham, England).

2.4. Purification of hSkM1-HT protein and reconstitution into liposomes

Virus-infected *Sf*9 cells were harvested 72 h post-infection and washed with phosphate-buffered saline buffer. In some cases cells were stored at −80 °C until use. The cells were pelleted and resuspended in lysis buffer (100 mM NaCl, 50 mM Tris–HCl, 10% glycerol, 2% nonidet P-40, protease inhibitor and 0.5% PE + PS + PC + CH in the ratio of 5:3:1:1, pH 7.9). Cells were gently agitated in lysis buffer for 20 min at 4 °C, then centrifuged at 100,000 × *g* for 40 min at 4 °C. The supernatant was passed through a column that was preloaded with Ni-NTA resin (Invitrogen) by gravity flow and washed with 10 volumes of binding buffer (500 mM NaCl, 20 mM Tris–HCl, 5 mM imidazole, 10% glycerol, 0.5% PC, pH 7.9) followed by 10 volumes of wash buffer (binding buffer containing 15 mM imidazole). The protein was eluted with 5 volumes of elution buffer (500 mM NaCl, 20 mM Tris–HCl, 250 mM imidazole, 10% glycerol, protease inhibitor, pH 7.9). Phospholipids were added to the eluate in a ratio of 5:3:1:1, PE + PS + PC + CH (0.5%) prior to passing it through a Gel D column (Pierce) to remove the detergent. The protein was eluted with 4 volumes of 10 mM phosphate buffer containing 0.5% PE + PS + PC + CH in ratio 5:3:1:1, pH 7.2, to form proteoliposomes. Liposomes were stored in a reconstitution buffer containing: 300 mM NaCl, 15 mM HEPES (*N*-[2-hydroxyethyl]-piperazine-*N*′-[2-ethanesulfonic acid]), 200 mM sucrose, adjusted to pH 7.4 with KOH, and were either used

immediately for planar lipid bilayer experiments, or stored at −80 °C for later use.

2.5. Patch-clamping

Human embryonic kidney (HEK) cells were grown in a mix of Dulbecco's Modified Eagle Medium containing 2.5 mM HEPES, supplemented with minimal essential amino acids and 10% foetal bovine serum. They were plated onto 24-well plates, grown to 95% confluency and transfected 24 h later with 10 μg of pRcCMV/*hSkM1*-HT and 2 μg CD4 (pcDNA), using 2 μl Lipofectamine 2000™, then replated onto cover slips 24 h later. CD4 antibody-labelled beads were used to identify transfected cells for patch-clamp experiments.

Macroscopic currents were recorded from whole-cell membrane patches at 3 days post-transfection of HEK cells. The pipette solution contained: 10 mM EGTA, 35 mM NaCl, 10 mM HEPES, 105 mM CsCl, pH 7.2. The bath solution contained: 150 mM NaCl, 2 mM KCl, 1.5 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM 2-(*N*-morpholino) ethane sulfonic acid (MES), pH 6.2. Pipettes were made from borosilicate glass using a 2-stage microelectrode puller (List-Medical, Germany) and had resistances of 3–5 MΩ when filled with pipette solution. Macroscopic currents were recorded from voltage-clamped cells at a holding potential of −100 mV. Whole-cell experiments were performed with leak and capacitance compensation by the P/5 method. Currents were recorded with an EPC-9 amplifier and Pulse Version 8.53 data collection software (HEKA, Germany). Data were filtered at 5 kHz and sampled at 10 μs intervals. Fast capacitance compensation was used to cancel the fast transient and leak subtraction was used. Data were analysed using Pulse Tools 8.67 and Sigma Plot 7.0 software.

2.6. Planar lipid bilayers

Planar bilayer lipid membranes (p-BLM) were formed from a mixture of 5% PC and 2% CH in *n*-octane across a 200 μm diameter hole in a polystyrene cup separating two chambers. The solution in each chamber contained 1 ml of sterile filtered: 10 mM HEPES, 300 mM NaCl, pH 7.4. The two chambers were kept at the same volume to eliminate hydrostatic pressure differences. Single channel recordings from planar bilayer membrane experiments were filtered at 3 kHz, sampled at 100 μs intervals and analysed using TAC X4.1.5 software.

3. Results

3.1. Effect of the histidine tag on hSkM1-HT function

We modified the *hSkM1* gene by adding a 6× histidine tag at the C-terminus (*hSkM1*-HT) in order to purify the protein using ion metal-affinity chromatography (IMAC) The effect of a C-terminal poly-histidine tag on the function of *hSkM1* has been reported previously and shown not to alter channel function when expressed in human embryonic kidney cells (Chen et al., 2002). In the current study, we also verified the integrity of our recombinant *hSkM1*-HT protein when expressed in human embryonic kidney cells using the patch-clamp technique.

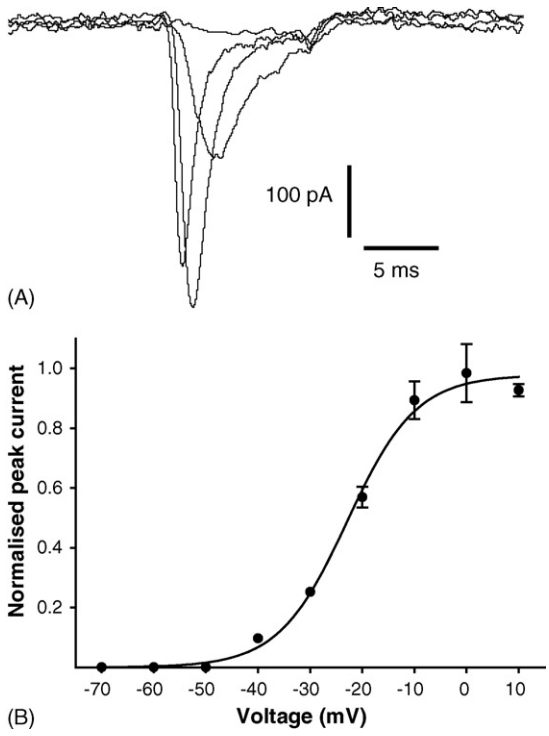

Fig. 1. Voltage-activation of *hSkM1*-HT channels expressed in HEK293 cells. (A) Whole-cell current recordings at applied potentials from −40 to +20 mV in 20 mV increments. (B) Normalised peak current from three cells plotted as a function of membrane potential. The curve is a fit of a Boltzmann equation to the data.

Whole-cell currents were recorded from the HEK cells expressing *hSkM1*-HT (Fig. 1A). Cells were held at a negative membrane potential (−100 mV), and voltage pulses were applied stepwise from −100 to +100 mV, in 10 mV increments. Rapidly activating and inactivating sodium currents were recorded at potentials more depolarised than −40 mV and reversed close to the Nernst potential for sodium. HEK cells are known to have endogenous inward sodium currents that are typically 50 pA in amplitude, therefore currents greater than 300 pA are accepted as being those expressed (Cummins et al., 1993). The peak amplitude of the current response for *hSkM1*-HT shown in Fig. 1A was 400 pA. Current–voltage data for the peak inward currents were fitted with a Boltzmann function, $I = I_{max}(1/(1 + e^{-(V-V_{1/2})zF/RT}))$, and normalised to the estimated maximum (Fig. 1B). The half-maximal voltage for activation ($V_{1/2}$) was estimated at −23 mV, and the slope (z) at 5.9. These results showed that the C-terminal histidine tag did not affect voltage-activation as the function of *hSkM1*-HT was normal.

3.2. Channel protein expression and purification

Following the confirmation of function of *hSkM1*-HT, its expression in *Sf*9 insect cells was confirmed by gel electrophoresis and immunodetection (Fig. 2A). Protein expression was maximised by establishing the optimal multiplicity of infection

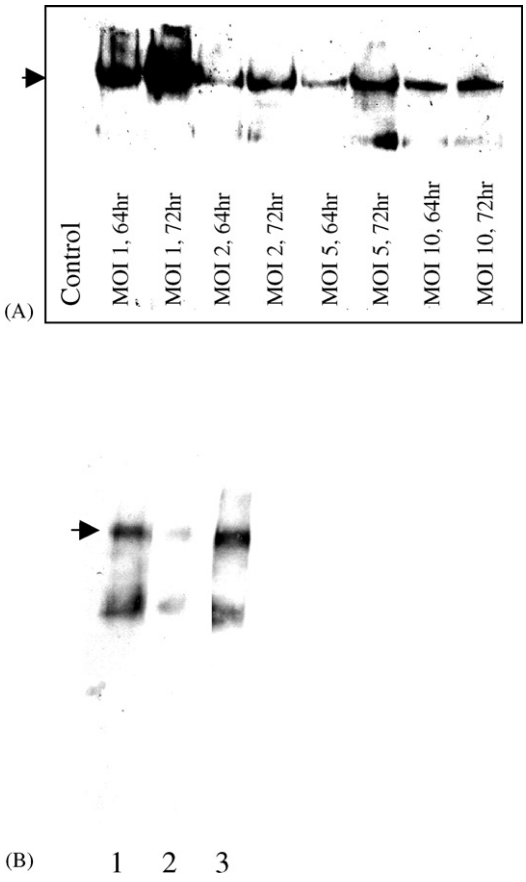

Fig. 2. Expression and purification of *hSkM1*-HT from *Sf*9 insect cells. (A) Western blot of insect cells infected at different MOIs and harvested at different times. Cells were infected with *hSkM1*-HT recombinant baculovirus and protein expression detected using an anti-Pan Na_v antibody. The control was uninfected *Sf*9 cells. (B) Western blot of purified protein and proteoliposomes. Lane 1: freshly purified protein; lane 2: purified protein stored at 4 °C overnight; lane 3: purified protein in liposomes stored at 4 °C overnight.

(MOI) and post-infection time for harvest of infected *Sf*9 insect cells. An MOI of 1 after 72 h of infection gave the highest level of protein expression and was routinely used. The purification procedure was optimised by using a rapid purification and reconstitution protocol that involved adding phospholipids during the purification steps. To purify the protein in a form that would remain functional it was solubilised under non-denaturing conditions using a non-ionic detergent (2% nonidet P-40). Lipid was added to binding and washing buffers to help maintain the conformational integrity of the protein (Agnew et al., 1986). The protein migrated on an SDS/Tris-glycine gel, at a molecular mass of 260 kDa, as determined by Western blot analysis using anti-Pan Na_v antibody (Fig. 2B). It degraded in the elution buffer after only 12 h at 4 °C, even in the presence of protease inhibitor. However, if the freshly purified *hSkM1*-HT protein was reconstituted immediately into liposomes, it remained stable for at least 4 months at −80 °C.

3.3. Channel activity in planar-BLMs

To determine whether *hSkM1*-HT channels were functionally intact following the purification procedure, proteoliposomes were used in planar lipid bilayer experiments. Proteoliposomes (20–40 μl) containing *hSkM1*-HT were added to both cis and trans chambers of the p-BLM, therefore we expect that they were inserted into the BLM in both orientations. The pharmacological integrity of the channel was investigated by examining the ability of known activators and inhibitors that bind to different sites on the channel, to modulate its activity. As sodium channel currents rapidly inactivate, we used the channel activator veratridine which slows their inactivation and allows the channels to be observed under steady state conditions where the membrane potential is held constant. Control experiments were carried out using liposomes prepared from uninfected cells that underwent the same purification procedure as for infected cells (Fig. 3A). No channel activity was observed in five experiments. To verify that the recorded trace was not obtained from channels that were continually in the open state in the presence of veratridine, we added TTX and no change in the current amplitude was recorded (Fig. 3B). This indicated that TTX-sensitive endogenous sodium channels and other proteins in *Sf*9 cells, detergent and chemicals used in the purification did not contribute to the channel activity recorded in these bilayers. Protein purified from cells infected with *hSkM1*-HT recombinant baculovirus showed channel activity in the presence of 100 μM veratridine (VTD) (Fig. 3C). The potent and selective sodium channel blocker, tetrodotoxin (TTX), at a concentration of 100 nM, completely inhibited channel activity after 9 min (Fig. 3D). This confirmed the identity of the activity as that of sodium channels. Veratridine-activated channels were also inhibited by 100 nM saxitoxin (Fig. 3E and F). In the example shown in Fig. 3E, there were at least two active channels that were partially inhibited after 7 min of exposure to saxitoxin and were completely inhibited after a further 20 min of exposure (Fig. 3F and G). Multi-channel openings activated by veratridine were common and had a slope conductance of 21.2 ± 0.7 pS (mean ± 1S.E.M.), determined from five experiments (Fig. 3H). Because the proteoliposomes remained in the chambers throughout each experiment, channel incorporation could increase with time. However, we found that the activity seen after 15–30 min of stirring was usually unchanged after 60 min of recording.

For use of the purified sodium channel in biosensors to detect the presence of environmental toxins, the latter must alter its activity to a significant degree. We examined effects of two key algal toxins that act via sodium channels; brevetoxin (PbTx-1) which increases activation, and saxitoxin (STX), which is an inhibitor. Subconductance levels were prevalent in the single channels recorded in the presence of 60 nM PbTx-1 (Fig. 4A). STX (50 nM) largely inhibited channel activity after 6 min (Fig. 4B). When the channel current activated by PbTx-1 at different voltages was plotted (Fig. 4C), the slope indicated a conductance of 16.1 ± 0.5 pS, that was significantly different from that for veratridine-activated channels (*t*-test, $p = 0.0026$).

4. Discussion

After addition of six histidine residues to the C-terminus, *hSkM1* channels remain functional and their activation properties are similar to those reported previously (Chen et al., 2002).

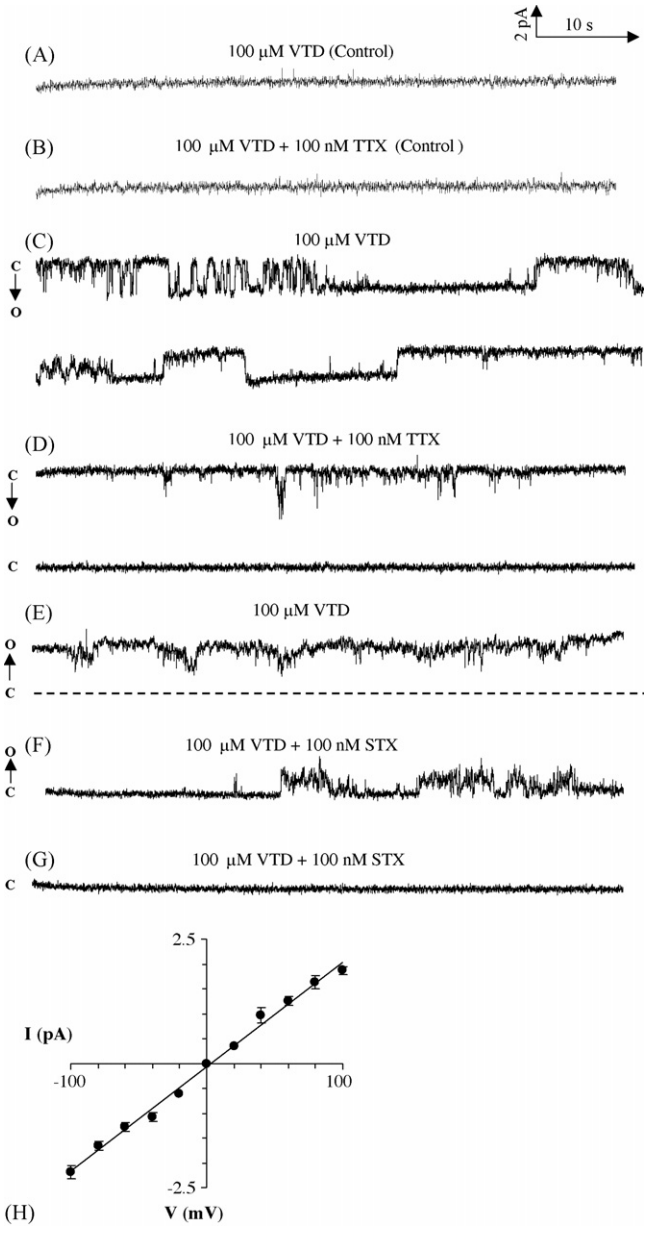

Fig. 3. Reconstituted *hSkM1*-HT channels activated by veratridine. Representative channel recordings are shown for protein reconstituted into liposomes. Purified, uninfected *Sf*9 cells held at −80 mV in the presence of: (A) 100 μM veratridine and (B) after addition of 100 nM tetrodotoxin. Protein purified from cells infected with *hSkM1*-HT recombinant baculovirus, recorded at −80 mV in the presence of: (C) 100 μM veratridine, and (D) 8 min after 100 nM tetrodotoxin was added. Channel activity for *hSkM1*-HT at +80 mV in the presence of: (E) 100 μM veratridine, and (F) 7 min and (G) 27 min after 100 nM tetrodotoxin was added. The baseline (0 pA) is indicated by the broken line. The records shown were filtered at 300 Hz. The arrow indicates the direction of channel opening; C is closed and O is open. (H) Current–voltage relationship for single *hSkM1*-HT channels activated by veratridine in symmetrical 300 mM NaCl solutions ($n = 5$).

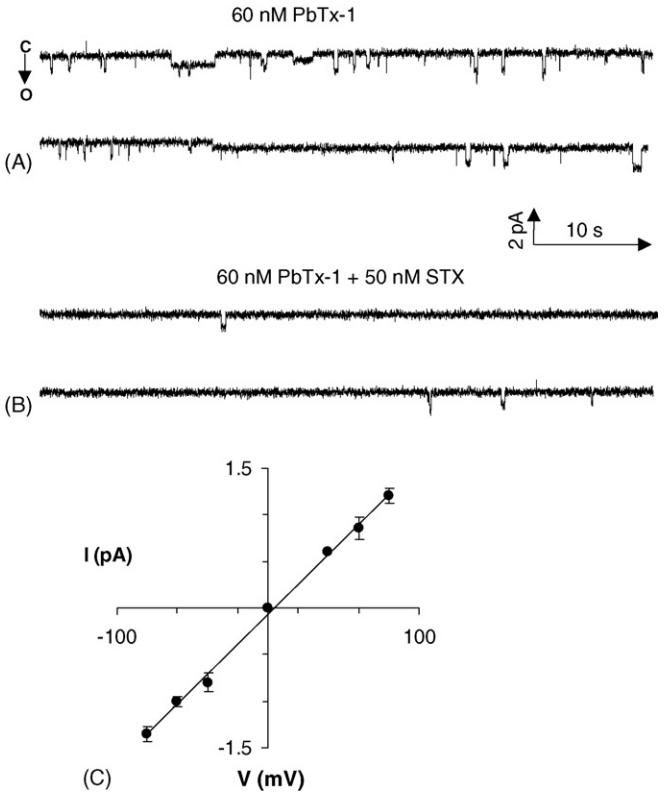

Fig. 4. Reconstituted *hSkM1*-HT channels activated by brevetoxin. Channel activity obtained at −80 mV in the presence of: (A) 50 nM PbTx-1 and (B) 6 min after 50 nM STX was added. The records shown were filtered at 300 Hz. (C) Current–voltage relationship for single *hSkM1*-HT channels activated by PbTx-1 in symmetrical 300 mM NaCl solutions ($n = 3$).

The voltage that gave half-maximal activation of the channels (−23 mV) was as expected for *hSkM1* channels expressed in mammalian cells (Chahine et al., 1994; Wang et al., 1996; O'Leary, 1998; Bennett, 2002). Our results show for the first time that after undergoing purification using immobilised metal affinity chromatography and reconstitution into liposomes, *hSkM1*-HT functions normally as an ion channel in planar lipid bilayers. That voltage-activation and pharmacological modulation by four different compounds were unchanged indicates that the binding sites for these compounds were intact and that the protein had retained its overall structural conformation during solubilisation, purification and reconstitution.

In optimising the metal affinity chromatography method for the sodium channel we identified key factors that were important for improving protein yield. Because the protein was prone to degradation once removed from the cells, even in the presence of protease inhibitor, we shortened the length of the procedure to take 8 h from the harvest of infected cells until the formation of proteoliposomes. Since the protein was more stable when in liposomes, *hSkM1*-HT protein was reconstituted immediately following purification. Under these conditions we obtained channel activity after liposomes had been stored at −80 °C for at least 4 months.

The compounds examined for their ability to modulate the modified sodium channel bind to different sites and affect its function in different ways. Veratridine and brevetoxin promote sodium channel opening by binding to receptor sites 2 and 5, respectively, whereas TTX and STX bind to receptor site 1 at the external mouth of the pore (Denac et al., 2000). Since veratridine can activate a minimal pore only version of the sodium channel but TTX requires the full length channel for its effect, we used TTX to probe the structural integrity of the channel (Chen et al.,

2002; Pincin et al., 2005). The inhibitory effects of TTX and STX that we observed confirmed the identity of the activity as that of sodium channels that were structurally intact. Inhibition of sodium channels by 50 nM STX corresponds to a concentration of 2 μg STX equivalent per 100 g tissue, which is well below the regulatory limit of 80 μg per 100 g tissue.

The *hSkM1*-HT channel activity that we recorded had a single channel conductance of 21.2 pS when activated by 100 μM veratridine. This is slightly lower than that reported for *hSkM1* channels activated by voltage alone, which have a slope conductance of 24.9 pS (Chahine et al., 1994). This difference might be due to the mode of action of veratridine as it is known not only to increase channel opening but also to reduce the single channel conductance of sodium channels (Barnes and Hille, 1988; Sigel, 1987; Corbett and Vander Klok, 1994). It has been suggested that veratridine stabilises the open conformation by blocking the open channel to reduce its unitary conductance (Wang and Wang, 2003a). Of the multiple current levels present in our recordings, some were due to the presence of more than one channel in the bilayer, while others were apparent subconductances. The fact that we regularly saw transitions in 20 pS steps suggests that the smaller conductance levels were indeed substates. The presence of different conductance levels is reminiscent of the situation in chick skeletal muscle, where sodium channel openings are modified by veratridine, such that in addition to the normal conductance, a lower conducting state also appears (Sigel, 1987). Similar effects have also been reported in neuronal sodium channels (Barnes and Hille, 1988).

When activated by 60 nM PbTx-1, *hSkM1*-HT channels had a single channel slope conductance of only 16.1 pS. In addition, subconductance levels were clearly visible. Reduced conductance by PbTx-3 in neuronal sodium channels and the occurrence of subconductance states have been reported (Gawley et al., 1995; Jeglitsch et al., 1998; Purkerson et al., 1999). The reason for the different conductance levels among brevetoxin-, veratridine- or voltage-activated *hSkM1* channels is not known. Residues important for persistent opening by veratridine are located in the S6 region of domains 1, 3 and 4 (Wang and Wang, 2003a) and those important for brevetoxin binding are in S6 of domain 1 and S5 of domain IV (Trainer et al., 1994). These compounds may therefore interfere with the movement of the S6 region and alter gating to stabilise different open conformations with different conductances (Wang and Wang, 2003b). Alternatively, as S6 is thought to line the pore, these compounds might partially occlude the ion permeation pathway to different extents and thus reduce the single channel conductance to different levels.

5. Conclusion

This study has taken the natural biological target for some harmful marine toxins, and detected their presence using a real-time assay that is a direct measure of physiological function. Further development of the planar lipid bilayer technique to make it robust, reusable and compact would aid in ion channel research and drug discovery, and would enable its use in biosensors for toxin detection. We have shown that metal affinity chromatography is a suitable method to obtain functional recombinant sodium channels. Their future use as recognition entities in biosensors for algal toxins could provide an alternative to the mouse bioassay as the international standard for determining safe toxin levels in shellfish. Furthermore, it would complement existing methods in the detection of novel harmful compounds that might arise in algal blooms.

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References

Agnew, W.S., Rosenberg, R.L., Tomiko, S.A., 1986. Reconstitution of the sodium channel from electrophorus electricus. In: Miller, C. (Ed.), Ion Channel Reconstitution. Plenum Press, New York.
AOAC, 1999. Association of Official Analytical Chemists, Arlington, VA.
Barnes, S., Hille, B., 1988. J. Gen. Physiol. 91, 421–443.
Bennett, E.S., 2002. J. Physiol. 538, 675–690.
Catterall, W.A., 2000. Neuron 26, 13–25.
Chahine, M., Bennett, P.B., George Jr., A.L., Horn, R., 1994. Pflugers Arch. 427, 136–142.
Chen, Z., Alcayaga, C., Suarez-Isla, B.A., O'Rourke, B., Tomaselli, G., Marban, E., 2002. J. Biol. Chem. 277, 24653–24658.
Clare, J.J., Tate, S.N., Nobbs, M., Romanos, M.A., 2000. Drug Discov. Today 5, 506–520.
Corbett, A.M., Vander Klok, M.A., 1994. Biochem. Biophys. Res. Commun. 199, 1305–1312.
Cummins, T.R., Zhou, J., Sigworth, F.J., Ukomadu, C., Stephan, M., Ptacek, L.J., Agnew, W.S., 1993. Neuron 10, 667–678.
Denac, H., Mevissen, M., Scholtysik, G., 2000. Naunyn Schmiedebergs Arch. Pharmacol. 362, 453–479.
Errington, A.C., Madison, K., Kumar, A., Lees, G., 2005. Curr. Anaesth. Crit. Care 16, 81–87.
Fairey, E.R., Bottein Dechraoui, M.Y., Sheets, M.F., Ramsdell, J.S., 2001. Biosens. Bioelectron. 16, 579–586.
Gawley, R.E., Rein, K.S., Jeglitsch, G., Adams, D.J., Theodorakis, E.A., Tiebes, J., Nicolaou, K.C., Baden, D.G., 1995. Chem. Biol. 2, 533–541.
George Jr., A.L., Komisarof, J., Kallen, R.G., Barchi, R.L., 1992. Ann. Neurol. 31, 131–137.
Jeglitsch, G., Rein, K., Baden, D.G., Adams, D.J., 1998. J. Pharmacol. Exp. Ther. 284, 516–525.
Jellett, J.F., Roberts, R.L., Laycock, M.V., Quilliam, M.A., Barrett, R.E., 2002. Toxicon 40, 1407.
Lawrence, J.F., Niedzwiadek, B., Menard, C., Biré, R., Burdaspal, P.A., Ceredi, A., Davis, B., Dias, E., Eaglesham, G., Franca, S., Gallacher, S., Graham, D., Hald, B., Heinze, L., Hellwig, E., Jonker, K.M., Kapp, K., Krys, S., Kurz, K., Lacaze, J.P., Gago Martinez, A., McNabb, P., Ménard, C., Milandri, A., Nsengiyumva, C., Pereira, P., Pineiro, N., Poletti, R., Riddell, G., De Astudillo, L.R., Selwood, A., Stern, A., Tiebach, R., Den Top, H.V., Van Egmond, H.P., Wezenbeek, P., Yen, I.C., 2004. J. AOAC Int. 87, 83.
Miller, C., 1986. Ion Channel Reconstitution. Plenum Press, New York and London.
O'Leary, M.E., 1998. Can. J. Physiol. Pharmacol. 76, 1041–1050.
Pincin, C., Ferrera, L., Moran, O., 2005. Biochem. Biophys. Res. Commun. 334, 140–144.
Purkerson, S.L., Baden, D.G., Fieber, L.A., 1999. Neurotoxicology 20, 909–920.
Quilliam, M.A., 1999. J. AOAC Int. 82, 773–781.
Rossini, G.P., 2005. Toxicology 207, 451–462.

Shieh, C.C., 2004. Drug Discov. Today 9, 551.
Sigel, E., 1987. J. Physiol. 386, 73–90.
Singleton, W.S., Gray, M.S., Brown, M.L., White, J.L., 1965. J. Am. Oil Chem. Soc. 42, 53–56.
Trainer, V.L., Baden, D.G., Catterall, W.A., 1994. J. Biol. Chem. 269, 19904–19909.
UNESCO, 2005. Report of the Joint FAO/IOC/WHO Ad Hoc Expert Consultation on Biotoxins in Bivalve Molluscs (Oslo, Norway).
Wang, D.W., George Jr., A.L., Bennett, P.B., 1996. Biophys. J. 70, 238–245.
Wang, G.K., Wang, S.Y., 2003a. J. Physiol. 548, 667–675.
Wang, S.Y., Wang, G.K., 2003b. Cell Signal 15, 151–159.