Application Note

Transporter:	**Ec-ClC**
Sample:	**Proteoliposomes**
Origin:	*E. coli*
Tools:	**SURFE²R N1**

Electrophysiological recordings of the H^+/ Cl^- exchanger Ec-ClC on the SURFE²R N1

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Samples kindly provided by Dr. Merritt Maduke, Stanford University, USA

Summary

ClCs are a family of chloride ion channels and transporters with important physiological roles including regulation of the membrane potential, transepithelial salt transport and ion homeostasis[1,2]. To date, 9 members of the ClC family have been identified in mammals[1,2], the first 4 (ClC-1, ClC-2, ClC-Ka, and ClC-Kb) are located on the plasma membrane where they act as chloride ion channels whereas the remaining 5 are located in intracellular organelles (ClC-3-7) and are chloride-proton exchangers[1-3]. These transporters are important for endosome, lysosome and synaptic vesicle acidification[1,2], and mutations in, e.g. ClC-5 underlie the rare chronic kidney disorder, Dent's Disease, and mutations in ClC-7 underlie osteopetrosis[2,4], a rare inherited bone hardening disorder. Given their ubiquitous expression and importance in physiological processes, they are important potential drug targets.

ClC from *Escherichia coli* (Ec-ClC or ClC-ec1) is closely related to its mammalian counterparts and is a Cl^-/H^+ exchanger[3,5]. It transports 2 Cl^- into the cell, coupled to the efflux of 1 H^+[3,5]. Therefore Ec-ClC is an electrogenic transporter, generating a net charge flow. In *E. coli,* the Ec-ClC mediates acid resistance of enteric bacteria by promoting H^+ extrusion[1,5,6].

Here we present Ec-ClC activity measurements on the SURFE²R N1 instrument using proteoliposomes reconstituted with purified Ec-ClC at different lipid-to-protein ratios.

Results

To activate Ec-ClC on the SURFE²R N1, a sensor with attached Ec-ClC proteoliposomes pre-incubated in chloride-free buffer solution was inserted into the device and perfused with a buffer containing chloride. Application of chloride leads to transient currents with negative amplitude, reflecting the net transport of one negative charge into the proteoliposomes. The sensor was flushed with chloride-free buffer solution before and after Ec-ClC activation (Figure 1A). The half saturation of Ec-ClC mediated chloride translocation was investigated by applying different chloride concentrations sequentially on the same sensor. An EC_{50} value of 12.3 ± 0.6 mM (n = 4 sensors) was determined (Figure 1B).

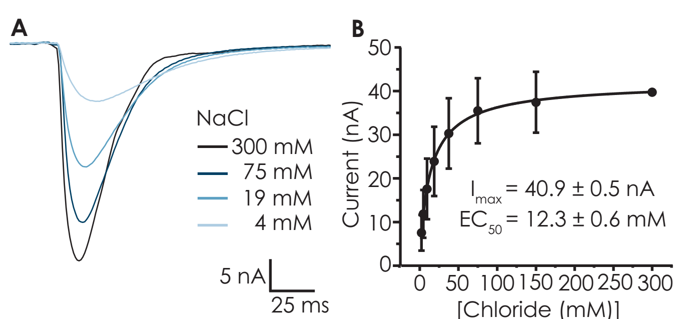

Figure 1: Typical Ec-ClC current response on the SURFE²R N1. **A** When applying chloride to the sensor a transient negative current occurs. **B** Concentration response curves for chloride was tested cumulatively on the same population of proteoliposomes. Data are average ± S.E.M of 4 sensors.

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Since the direction of transport is dictated by the electrochemical gradients, we were able to detect the opposite exchange activity by pre-loading the proteoliposomes with chloride and activating Ec-ClC by removing chloride from the external buffer solution. In this case we measured transient positive currents, reflecting the net charge translocation of one negative charge out of the proteoliposomes (Figure 2).

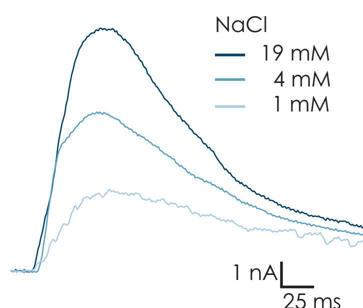

Figure 2: Typical Ec-ClC current response on the SURFE²R N1. When pre-loading proteoliposomes with chloride and removing chloride in the external buffer solution a positive current occurs.

To confirm that the currents were indeed mediated by Ec-ClC, we applied the specific Ec-ClC inhibitor, OADS[7], at 100 µM in all buffer solutions. After just 1 s after adding OADS, we observed 50% inhibition, and after 5 minutes incubation we obtained up to 80% inhibition. After rinsing with OADS-free buffer solution we could partially recover the signal (approx 50%; Figure 3A).

In addition to inhibition of the current, we also investigated the impact of lipid-to-protein ratio on the Ec-ClC response. The signal-to-noise was clearly dependent on the Ec-ClC density where increasing the lipid-to-protein ratio resulted in smaller current responses. Liposomes not containing Ec-ClC on the other hand do not generate any current response in the SURFE²R assay (Figure 3B).

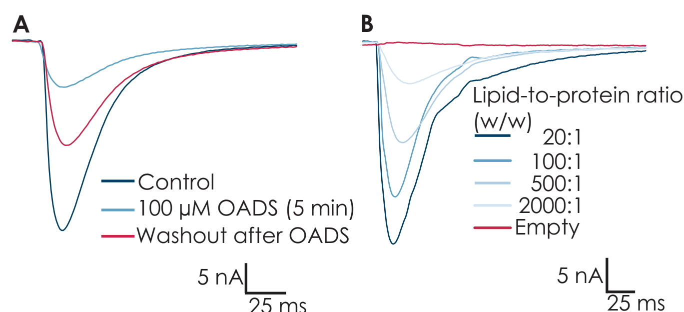

Figure 3: A Transient currents mediated by Ec-ClC were blocked by OADS. Current traces are shown after activation by chloride before (dark blue), after 5 min incubation in 100 µM OADS (light blue), and after washout (red). **B** Current response on the SURFE²R N1 for proteoliposomes with different protein densities attached to different sensors. Lipid-to-protein ratio (w/w) is shown and the lower the protein density (i.e. higher ratio) the smaller the current response. Empty liposomes generate no current response (red).

In conclusion, the SURFE²R N1 can be used to reliably measure Ec-ClC activity. This assay can easily be adopted for other transporters of the ClC family and provides a useful tool in future drug discovery.

References

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Methods

Protein purification and reconstitution

Protein was purified according to ref. 3. For reconstitution, *E.coli* polar lipid extract (Avanti) was used. Samples were kindly provided by Dr. Merritt Maduke, Stanford University, USA.

Buffers

Ec-ClC experiments were performed by the exchange of a chloride free ("control") buffer for a chloride containing ("activating") buffer. When the reverse transport mode was investigated, the sensor was first flushed with activating solution, followed by activation using the control solution. Control buffer contained: 10 mM MES, 5 mM $MgSO_4$, (100 + x) mM K_2SO_4, pH 5.2. Activating buffer contained: 10 mM MES, 5 mM $MgSO_4$, 100 mM K_2SO_4, 2*x mM KCl, pH 5.2.

SURFE²R N1 sensor preparation

According to the Nanion standard procedure "SURFE²R N1 Protocols: Sensor Preparation Guide". Sensors are prepared in control buffer. For the reverse transport mode, sensors are prepared in activating buffer. Proteoliposomes were diluted 1:10 before sonication in a water bath sonicator.

SURFE²R N1 measurement workflow

ClC can be activated by providing chloride. There is no additional driving force required. Therefore, any 2-buffer Nanion standard protocol is suitable. ClC activity is higher in the acidic pH range.

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