Application Note

Channels: endogenous
Recorded from: Spheroplasts
Tools: Port-a-Patch®

Preparation and recordings on Nanion´s Port-a-Patch® of native E. coli Spheroplasts

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Summary

Bacterial membranes are not easy to patch clamp. Since bacterial ion channels are of increasing interest, we started to optimize the protocols for patch clamping bacterial spheroplasts with the Port-a-Patch.
Bacterial spheroaplasts can be prepared up to a size of 5 µm. They consist of the inner bacterial membrane. This technique was first used for patch clamp experiments by Boris Matrinac in 1987 and let to the discovery of mechanosensitive channels in E. coli. Here we describe the preparation of Spheroplasts out of E. coli and show ion channel currents recorded with the Port-a-Patch.

Results

The E. coli strain C41 was used for the preparation of bacterial spheroplasts. To be able to patch clamp the small membranes, NPC-1 chips with a high resistance (10-15 MΩ) were used. When patch clamping, success rates of 70 % were reached (90% catching, 70 % reaching a Giga-Ohm seal).

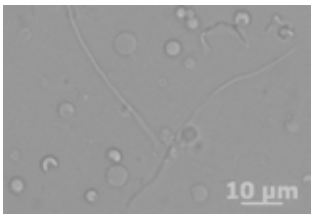

Figure 1: Spheroplast preparation out of C41 E. coli strain.

E. coli spheroplasts showed an endogenous current with slow kinetics and rectifying properties (fig. 2). In Nanion's standard solutions with internal potassium, the averaged maximum current response to a ramp protocol from -100 mV to 100 mV was 880 ± 560 pA at +100 mV, with a slightly positive reversal potential.

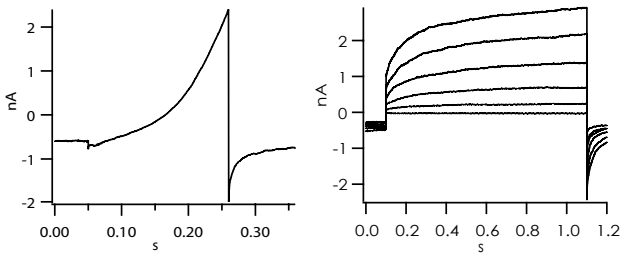

Figure 2:
Endogenous spheroplast currents. Left: Ramp from -100 mV to +100 mV in 200 ms. Right: Voltage steps from 0 mV to 100 mV. Holding potential in both cases was -80 mV. Nanions standard solutions, with additional 400 mM sucrose in the external solution, were used.

Also single channels with a conductance of about 1 nS could be observed, possibly native MscL (fig. 3). Spheroplasts reacted also sensitive to external pH (data not shown).

Figure 3: Singe channel activity in spheroplasts with a conductance of about 1 nS.

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Recordings with solutions containing a reduced chloride concentraton indicated that the observed current is mainly caused by chloride. The reversal potential in Nanion's standard solutions was +15.5 mV (fig. 4), meaning that chloride can't be the only permeable ion. Calculated reversal potentials in these solutions are: Potassium -83 mV, sodium +66 mV, chloride -23 mV and calcium >> +100 mV. Based on this insight, solutions were designed which repress the endogenous current. These conditions could then be used to study ion channels which were overexpressed in E. coli (fig. 5).

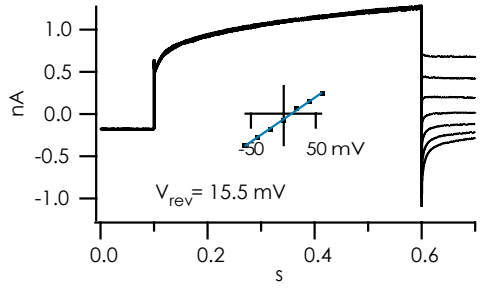
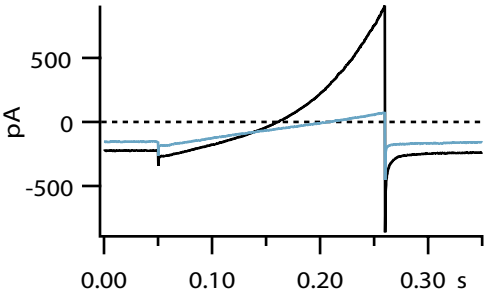


Figure 4: Calculation of the reversal potential by tail current analysis in Nanion's standard solutions.



Internal Solution:
120 mM KF
20 mM KCl
10 mM HEPES (pH 7)

External Solution 1:
3 mM KCl
80 mM NaCl
10 mM MgCl2
35 mM CaCl2
10 mM HEPES
+ agar bridge

External Solution 2:
50 mM K-MSA
35 mM Ca-MSA
80 mM Na-MSA
10 mM HEPES
+ agar bridge

Figure 5: Solutions designed to repress the endogenous current. They can be used to study other channels expressed in E. coli. The current shown in response to a voltage ramp (200 ms ramp from -100 mV to +100 mV) is based on potassium.

References

1. Boris Martinac, Matthew Buenchner, Anne Delcour, Julius Adler and Ching Kung, 1987. Pressure-sensitive ion channels in Escherichia coli. *Proc. Natl. Acad. Sci. USA*, Vol. 84, pp. 2297-2301.

Methods

Preparation of Spheroplasts: Inoculate a single colony from a prepared agar plate in 5 ml modified LB medium and incubate aerobically at 250 rpm and 37 °C until A600 reached ~0.5. Add 60 µg/ml cephalexine for about 1 hour, control length of cells by microscope. Harvest 2 ml of the culture at 3*10^{3}xg for 1 min in 2 ml tube. Resuspend pellet in 500 µl of 1 M glucose by inverting. Add successively 30 µl 1 M Tris-HCl, pH 8, 24 µl of 5 mg/ml lysozyme, 6 µl of 5 mg/ml DNase and 6 µl of 125 mM EDTA-NaOH, pH 8 and mix in between by inverting. Incubate 15 min at room temperature. Add 100 µl stop solution (10 mM Tris-HCl, pH 8, 0.7 sucrose, 20 mM $MgCl_2$). Spheroplasts can be stored at -80 °C.

Electrophysiology: Experiments were performed with the Port-a-Patch, using NPC-1 borosilicate glass chips with a resistance of 10-15 MΩ. If nothing else is indicated Nanion's standard solutions with additional 400 mM sucrose in the external solution were used: Internal: 50 mM KCl, 10 mM NaCl, 60 mM KF, 20 mM EGTA, 10 mM Hepes, KOH pH 7.2 External: 140 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 5 mM Glucose, 10 mM Hepes, 400 mM sucrose, NaOH pH 7.4.

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