SINGLE CHANNEL MEASUREMENTS OF N-ACETYLNEURAMINIC ACID-INDUCIBLE CHANNEL (NANC) IN E. COLI

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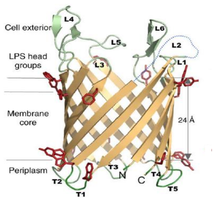
Abstract

Escherichia coli can use N-acetylneuraminic acid (Neu5Ac) as its sole carbon source even if the general outer membrane proteins OmpF and OmpC are not expressed: NanC - a monomeric outer membrane channel - allows Neu5Ac to move into the bacterial periplasm. Recently, a high resolution structure of NanC in two different crystal forms was reported by Wirth et al., J.Mol.Biol., (2009) 394:718 (PDB codes: 2WJQ and 2WJR). Our goal is to determine appropriate 'baseline' ionic conditions to study the transport of Neu5Ac through NanC using single channels in lipid bilayers. Measurements of single channel currents showed that NanC has two modes of time dependent behavior ('gating'). In the many situations we have tested, the modes are not induced or changed by surrounding ionic conditions or voltage. Single channels of NanC at pH 7.0 have: (1) a large conductance (around 100 pS to 800 pS in 100 mM KCl to 3M KCl) that varies with the polarity of the applied voltage; (2) anion over cation selectivity ($V_{reversal}$ around +16 mV in 250 mM KCl || 1 M KCl); (3) voltage-dependent gating (channel closures above ±200 mV). Single channel conductance of NanC decreases about 50% when HEPES concentration is increased from 100 µM to 100 mM in 250 mM KCl at pH 7.4, consistent with the two HEPES binding sites observed in the crystal structure (PDB code: 2WJR). Studying alternative buffers, we found that phosphate interferes with the channel conductance, whereas TRIS could not be used because it reacts with Ag/AgCl electrodes producing artifacts even in the presence of Agar-KCl bridges. Our further studies of NanC will use no pH buffers, but low concentration (250 mM) salt solutions adjusted to neutral pH 7.0.

NanC Structure



PDB code: 2WJQ

Pore Architecture

track 1 track 2

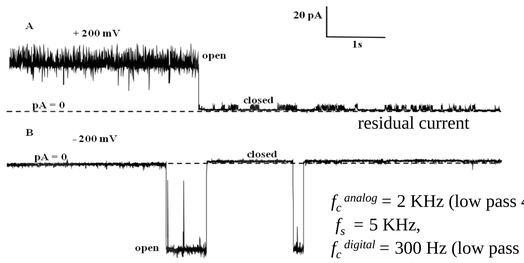
Wirth et al., J.Mol.Biol. (2009), 394, 718-731

Two separate **electropositive tracks**

- High resolution structure (at 1.8Å), 28 Å high, **12-stranded** β-barrel, **monomer**, average pore diameter 6.6Å. **No loop occluding the pore**.
- Contains 215 residues, family of small monomeric KdgM-related porins (acidic sugar/oligosaccharide transporters, poorly characterized).
- Bulky side chains extending, **constriction** occurs close to its center, with a **minimum diameter of 5.8 Å**.

Single Channel Measurements of NanC in Planar Lipid Bilayer

Cis (ground side): 500 mM KCl, 20 mM HEPES, pH 7.4
Trans (voltage side): 500 mM KCl, 20 mM HEPES, pH 7.4



f_c^{analog} = 2 KHz (low pass 4-pole Bessel filter)
f_s = 5 KHz,
$f_c^{digital}$ = 300 Hz (low pass 8-pole Bessel filter)
Corrected for leakage and offset current.

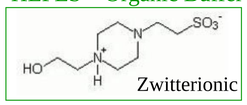
- **Voltage-gated. Closures at V ≥ ±150 mV.**
- **Large unit conductance, G = 215.80 ± 0.96 pS, (N = 15).**

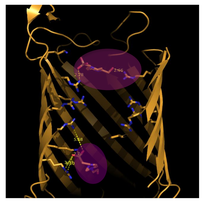
References

1. G. Condemine et al. (2005), Function and Expression of an N-Acetylneuraminic Acid –Inducible Outer Membrane Channel in *Escherichia Coli*, J.Bacteriol., 187(6), 1959-1965.
2. C. Wirth et al. (2009), NanC Crystal Structure, a Model for Outer-Membrane Channels of the Acidic Sugar-Specific KdgM Porin Family, J.Mol.Biol., 394, 718-731.

NanC and HEPES Binding

HEPES – Organic Buffer

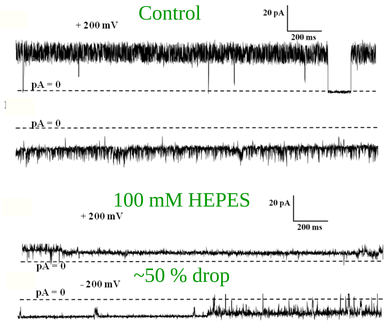




PDB Code: 2WJR
Wirth et al., J.Mol.Biol. (2009), 394: 718-731

Cis (ground side): 250 mM KCl, X mM HEPES, pH 7.4
Trans (voltage side): 250 mM KCl, X mM HEPES, pH 7.4

Control

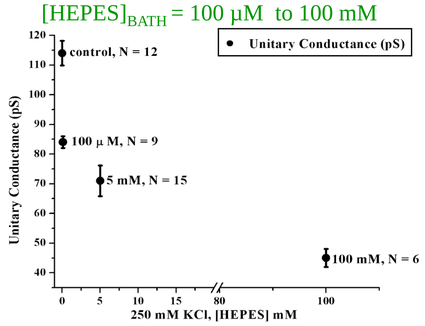


- **Binding site for HEPES revealed at 100 mM.**
- **Single channel experiments show that HEPES binds to NanC.**

Effect of Buffers on NanC

Cis (ground side): 250 mM KCl, X mM HEPES, pH 7.4
Trans (voltage side): 250 mM KCl, X mM HEPES, pH 7.4

$[HEPES]_{BATH}$ = 100 µM to 100 mM



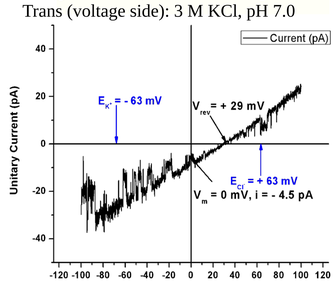
- **Unit conductance decreases considerably in presence of HEPES.**
- **TRIS introduced a drift, affected Ag/AgCl electrodes (with salt bridges).**
- **K-phosphate changed unit conductance.**

- **NanC ionic conductance is changed by HEPES and other buffers.**
- **Suggests experiments should be done without buffers.**

Single Channel Function of NanC: Selectivity

Measurement of Reversal Potential

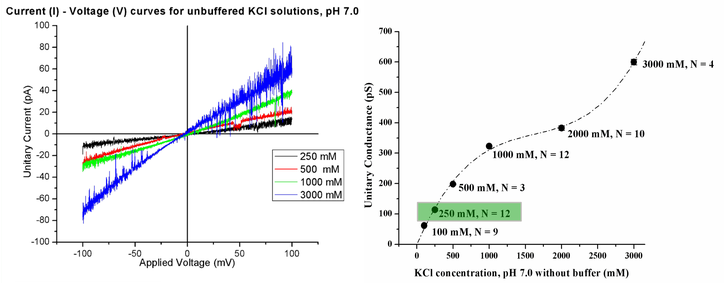
Cis (ground side): 250 mM KCl, pH 7.0
Trans (voltage side): 3 M KCl, pH 7.0



Expt.	Cis (ground) KCl (mM)	Trans (voltage) KCl (mM)	LJP (mV)	E_{Cl^-} (mV)	E_{K^+} (mV)	V_{rev}* (mV)
1	250	1000	-0.7	+ 35	-35	+15.89±1.01 (N = 2)
2	250	3000	-1.2	+ 63	-63	+28.31±0.37 (N = 9)

- **NanC exhibits anion selectivity.**

Single Channel Function of NanC: Conductance



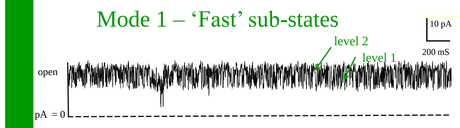
$$G\ (nS) = \frac{\Delta I\ (pA)}{\Delta V\ (mV)}$$ Slope conductance determined between -30 mV to +30 mV.

- **Unit conductance increases with increasing salt concentration.**
- **Working concentration: 250 mM KCl, pH 7.0 (without buffer).**

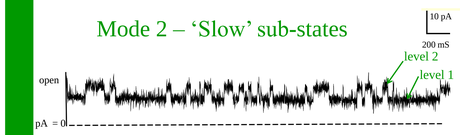
Single Channel Function of NanC: Gating

Cis (ground side): 250mM KCl, 0 mM HEPES, pH 7.0
Trans (voltage side): 250mM KCl, 0 mM HEPES, pH 7.0

Mode 1 – 'Fast' sub-states



Mode 2 – 'Slow' sub-states



Sub-conductances observed:

- **Mode 1 observed ~ 95%.**
- **At large salt concentration.**
- **In presence of HEPES.**
- **In unbuffered salt solutions.**
- **Native spontaneous property, not induced.**

Single Channel Function of NanC in Bilayer Summary

- NanC functions as a monomer.
- Large unit slope conductance, 62 pS-600 pS (100 mM KCl-3 M KCl, pH 7.0).
- NanC is voltage-gated, closes at $V \geq \pm 150$ mV.
- Two distinct modes of function (fast or slow sub-conductance states).
- Anion selectivity, V_{rev} close to E_{Cl^-}.
- NanC interacts with the buffers HEPES and Phosphate.
- TRIS makes measurements difficult.
- **Native function of NanC identified in bilayer.**
- **Condemine et al. could not show Neu5Ac specificity (up to 50 mM) in high salt concentration with HEPES (800 mM KCl, 10 mM HEPES-KOH, pH 7.4). In 250 mM KCl, pH 7.0 (without buffer) with Neu5Ac, we find an increase in current flow through NanC (3123-Pos B228).**