SIALIC ACID TRANSPORT IN E. COLI: ROLE OF OUTER MEMBRANE PORIN NANC

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Abstract

Sialic acid is a nutrient of bacteria important in host-pathogen interactions. The mechanism of transport of sialic acid from outer membrane to periplasmic space of *Escherichia coli* is not known. N-acetylneuraminic acid (Neu5Ac) - the most abundant form of sialic acid - induces a specific porin NanC (N-acetylneuraminic acid Channel) in the outer membrane of *E. coli.* Recently, a high resolution structure of NanC (Wirth et al., J.Mol.Biol., (2009) 394:718) revealed unique structural features that support Neu5Ac transport. However, patch-clamp experiments seemed to show that NanC conductance is unaffected by sialic acid (Condemine et al., J.Bacteriol., (2005) 187:1959). We report single channel current measurements of NanC in bilayers in the presence of Neu5Ac. Neu5Ac changes gating and considerably increases the ionic conductance of NanC in 250 mM KCl, pH 7.0. (See our other NanC poster 3136-Pos B241.) The unitary current through NanC increases when 7-12 mM of Neu5Ac is added to the grounded side of the bilayer. A distinct steady voltage dependent current (sub-level) is observed that seems to add to the unitary current. The single channel slope conductance of NanC increases by 51% in the presence of 7 mM Neu5Ac and by 74% in 55 mM. The effect of Neu5Ac on the unitary current through NanC seems to saturate at higher Neu5Ac concentrations. The unit conductance of NanC also increases when 20 mM Neu5Ac is added to both sides of the bilayer. It is likely that some of the current is carried by Neu5Ac. Interestingly, Neu5Ac reduces the ionic conductance of trimeric OmpF (Outer membrane porin F) under the same conditions: frequent, long closures are seen. Thus, we provide evidence that sialic acid translocation is specifically facilitated by NanC, and not by the general porin OmpF.

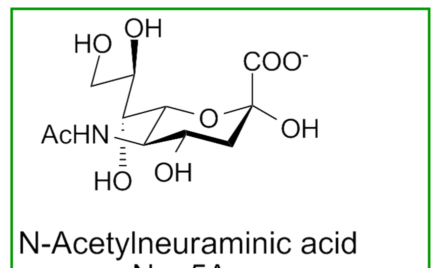
Sialic Acid

Chemical structure

- Family of negatively charged 9 carbon sugar acids.
- N-acetylneuraminic acid (Neu5Ac), most common form of sialic acid.

Function

- Carbon rich nutrient.
- Assists bacterial colonization.
- Survival in host environment.
- Bacterial virulence.



N-Acetylneuraminic acid
Neu5Ac

Sialic acid uptake in *E. coli*

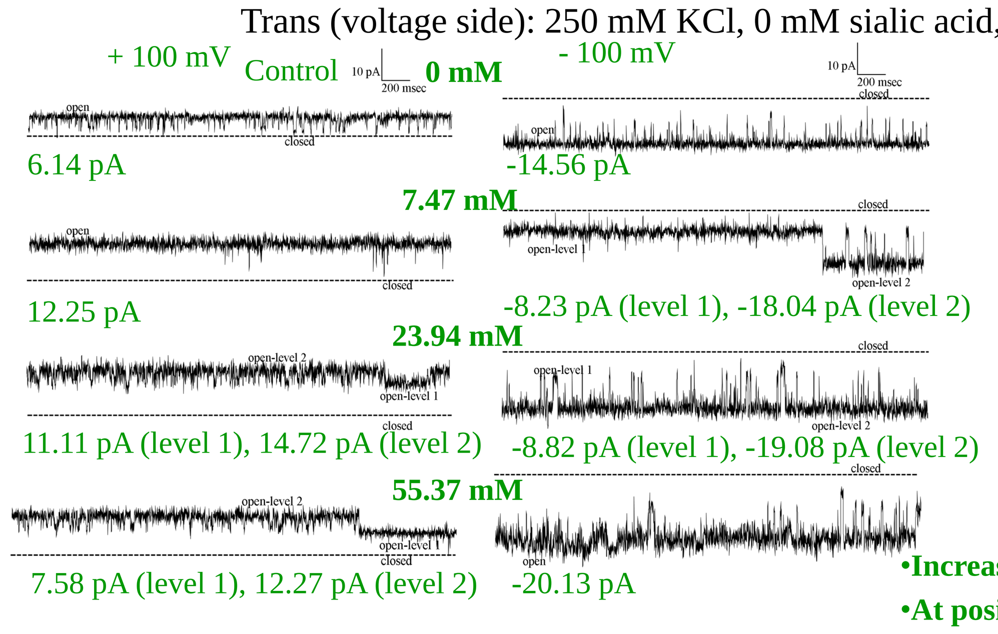
- A specific porin NanC is induced in the outer membrane if OmpF & OmpC are absent [1, 3].
- Previous single channel measurements could not demonstrate Neu5Ac specificity of NanC [1].
- Characterized at the inner membrane level but ambiguous at the outer membrane [2].

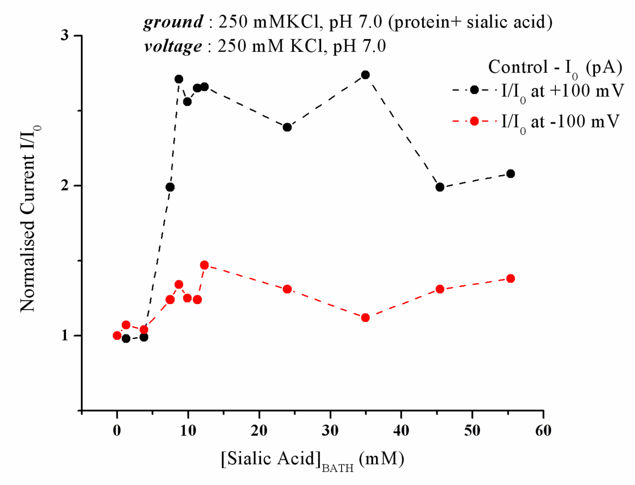
Action of Sialic Acid on Single Channel Function of NanC

A. Titration Experiment

Cis (ground side): 250 mM KCl, pH 7.0, Neu5Ac (1.26 mM to 55.37 mM)

Trans (voltage side): 250 mM KCl, 0 mM sialic acid, pH 7.0





- **Increase in current with increase in amount of Neu5Ac.**
- **At positive voltage the current increase is larger.**

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)

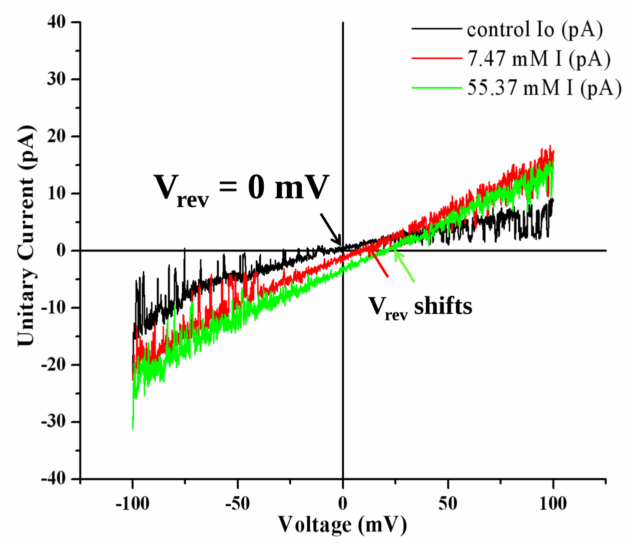
References

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. E. Severi et al. (2007), Sialic Acid Utilization by Bacterial Pathogens, Microbiol., 153, 2817-2822.
3. 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.

Action of Sialic Acid on Single Channel Function of NanC

A. Titration Experiment

Unitary I-V Traces vs. $[Neu5Ac]_{BATH}$



Control: $[Neu5Ac]_{BATH}$ = 0 mM, G = 96.23 pS

$[Neu5Ac]_{BATH}$ = 7.47 mM, G = 144.89 pS

$[Neu5Ac]_{BATH}$ = 55.37 mM, G = 166.54 pS

Summary

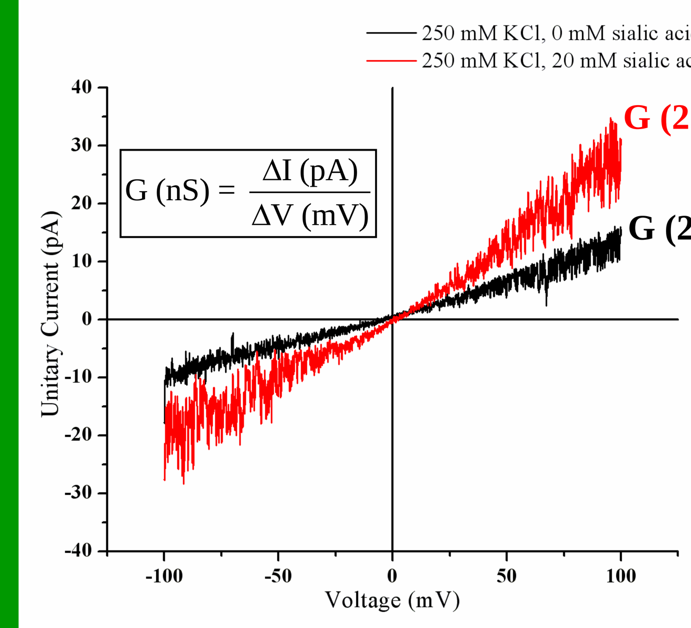
- **Sialic acid (≥ 7.47 mM in 250 mM KCl, pH 7.0) is required for a noticeable change in the action of NanC on the bilayer.**
- **Addition of sialic acid (ground side) changes the channel amplitude (increases) and channel activity (gates more often and goes into sub-levels).**
- **Unit slope conductance of NanC increased from control by 51 % at 7.47 mM and by 74 % at 55.37 mM sialic acid in 250 mM KCl, pH 7.0.**
- **Sialic acid shifts the reversal potential from 'zero' if sialic acid is added only to one side, to the 'ground' side of the bilayer.**
- **Action of sialic acid depends on the sign of the voltage.**

Action of Sialic Acid on Single Channel Function of NanC

B. Symmetric Addition Experiment

Cis/Trans (ground/voltage side): 250 mM KCl, 0 mM Neu5Ac, pH 7.0

Cis/Trans (ground/voltage side): 250 mM KCl, 20 mM Neu5Ac, pH 7.0



G (250 mM KCl, 20 mM sialic acid, pH 7.0) = 226.15 ± 9.95 pS (N = 29)

G (250 mM KCl, 0 mM sialic acid, pH 7.0) = 114.09 ± 4.16 pS (N = 12)

Summary

- **Sialic acid contributes a steady amount of current (seen as a distinct sub-level) that adds to the ionic current carried by single NanC.**
- **Unit conductance of NanC in presence of sialic acid increased significantly.**

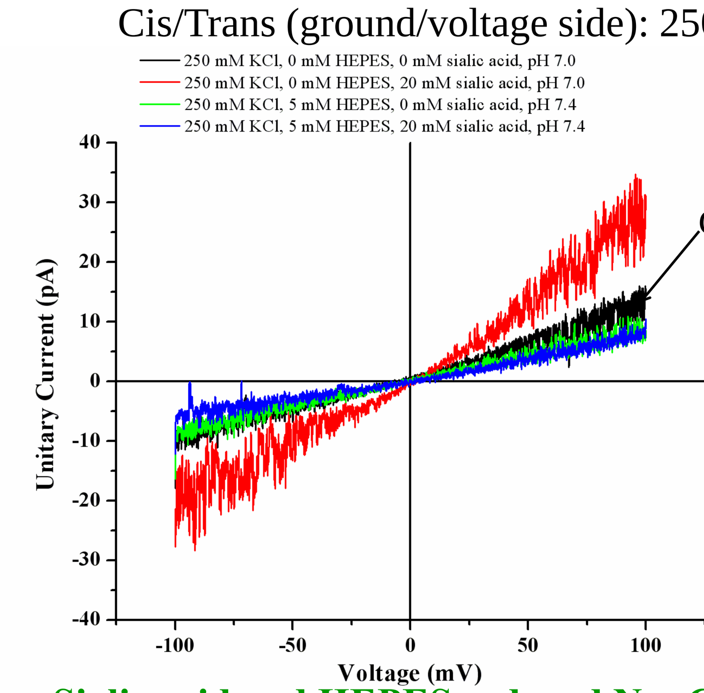
Filtered I-V traces corrected for leakage and offset

Unit slope conductance G determined between -30 mV to +30 mV

Action of Sialic Acid on Single Channel Function of NanC

C. Presence of HEPES

Cis/Trans (ground/voltage side): 250 mM KCl, 5 mM HEPES, 20 mM Neu5Ac, pH 7.0



Control vs. Sialic Acid With/Without HEPES

Slope conductance in symmetric ionic conditions

Control

G (250 mM KCl, 0 mM HEPES, 0 mM sialic acid, pH 7.0) = 114.09 ± 4.16 pS (N = 12)

G (250 mM KCl, 0 mM HEPES, 20 mM sialic acid, pH 7.0) = 226.15 ± 9.95 pS (N = 29)

G (250 mM KCl, 5 mM HEPES, 0 mM sialic acid, pH 7.4) = 70.70 ± 5.17 pS (N = 15)

G (250 mM KCl, 5 mM HEPES, 20 mM sialic acid, pH 7.4) = 66.21 ± 0.74 pS (N = 26)

Sialic acid and HEPES reduced NanC's unit current significantly vs. the control (250 mM KCl, 0 mM HEPES, 0 mM Neu5Ac, pH 7.0).

Single Channel Function of NanC (Sialic Acid/HEPES) Summary

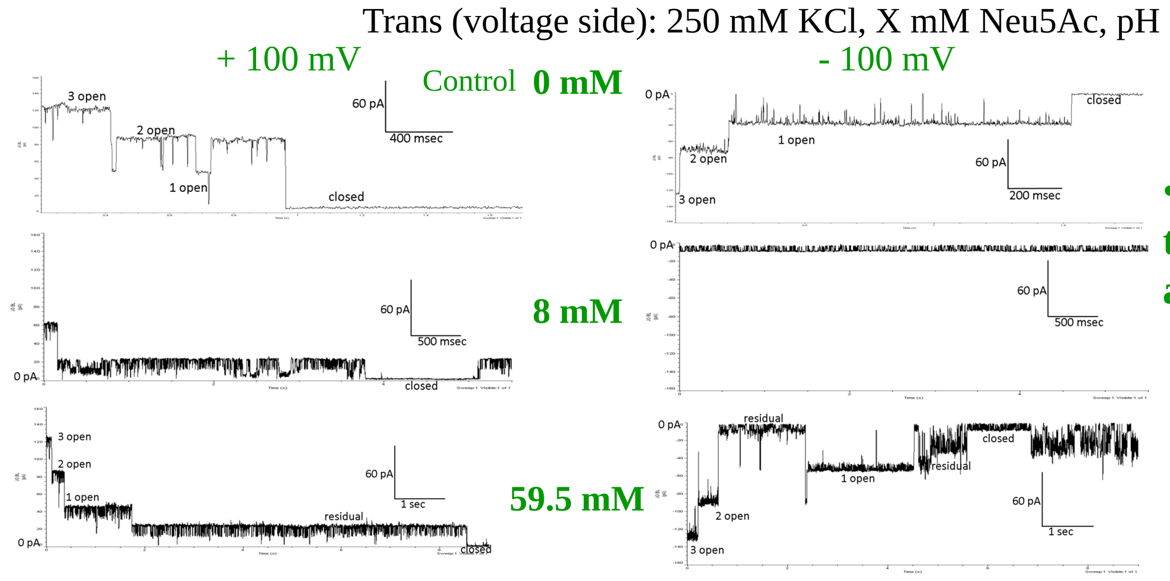
- **NanC has affinity for HEPES and conducts sialic acid.**
- **HEPES decreases the unit ionic conductance of NanC.**
- **Sialic acid increases NanC's unit ionic conductance in the absence of HEPES.**
- **Gating changed. Distinct sub-current levels observed.**
- **Sialic acid decreased the unit ionic conductance of NanC in the presence of HEPES.**
- **Sialic acid and HEPES interact and compete giving a net decrease in NanC's unit ionic conductance.**

Action of Sialic Acid on Trimeric OmpF

Symmetric Addition Experiment

Cis (ground side): 250 mM KCl, X mM Neu5Ac, pH 7.0

Trans (voltage side): 250 mM KCl, X mM Neu5Ac, pH 7.0



- **Gating changes, trimer disappears and appears.**

Sialic Acid Transport in *E. coli*: Role of NanC vs. OmpF Summary

- **Sialic acid has completely different effects on OmpF and NanC.**
- **In OmpF, sialic acid 'binds' and 'closes' the pore(s).**
- **In NanC, sialic acid adds to the ionic current and increase single channel conductance.**
- **In OmpF, sialic acid 'binds' and 'unbinds' the 3 pores.**
- **OmpF has high affinity for sialic acid compared to NanC.**

These results suggest that OmpF is not as efficient as NanC in allowing the passage of sialic acid into the periplasmic space of *E. coli.*