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

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-acetyllactosaminuronic acid (NeuAc) - the most abundant form of sialic acid - induces a specific porin NanC. N-acetyllactosaminuronic acid Channel in the outer membrane of E. coli. Recently, a high resolution structure of NanC (Wirth et al., J.Mol.Biol., 2009 394:781) revealed unique structural features that support Neu5Ac transport. However, patch-clamp experiments showed that NanC conductance is unaffected by sialic acid (Condemine et al., J.Bacteriol., 2005 187:1599). 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-PB B24.) The unitary current increases as the concentration of Neu5Ac in the bilayer increases. As the concentration of Neu5Ac increases, the unitary conductance of NanC increases considerably.

Action of Sialic Acid on Single Channel Function of NanC

A. Titration Experiment

- 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 membrane.
- 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.

B. Symmetric Addition Experiment

- Cis/Trans (ground/voltage side): 250 mM KCl, 20 mM Neu5Ac, pH 7.0
- Control: [G (250 mM KCl, 0 mM sialic acid, pH 7.0) = 114.09 ± 4.16 pS (N = 12)]
- Sialic acid (≥ 7.47 mM in 250 mM KCl, pH 7.0) is completely different effects on OmpF and NanC.
- Sialic acid and HEPES interact and compete giving a net decrease in NanC’s unit ionic conductance.

C. Presence of HEPES

- Cis(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

- 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, 5 mM sialic acid, pH 7.0) = 226.15 ± 9.59 pS (N = 29)
- G (250 mM KCl, 0 mM sialic acid, pH 7.0) = 114.09 ± 4.16 pS (N = 12)
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References


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Sialic Acid

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

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

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].

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

- Sialic acid has completely different effects on OmpF and NanC.
- In OmpF, sialic acid ‘binds’ and ‘closes’ the pores.
- 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.