Overcoming the Roadblocks to Structure Determination of the Organic-Ion Channel FocA

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How would Nature design a channel for organic-ions?
Formate nitrite transporter (FNT) family

- ~280 amino acids, 6 transmembrane $\alpha$-helices
- 1,850 family members, from bacteria to yeast
- Many members from pathogens, e.g. *plasmodium* spp.
- Two subfamilies:
  - **Formate Channel** — *FocA*
  - **Nitrite Channel** — *NirC*

Suppmann & Sawers (1994)
Formate is a primary metabolite for bacterial anaerobic growth

E. coli

**Oxygen limited:**

- Pyruvate formate lyase pathway

**Oxygen unlimited:**

- Pyruvate dehydrogenase pathway

E. coli

Formate
Cytoplasmic formate concentration is regulated via FocA

- Channel open, to avoid acidification
- Channel closed, to maintain a reducing environment

E. coli
Mechanistic questions

• What is the structural basis for ion selectivity in FocA?

• How does the cytoplasmic formate concentration gate the channel?
FocA from *Vibrio cholerae* was expressed and purified
FocA from *Vibrio cholerae* forms a pentamer in solution

**EM of purified samples**

**SEC, light scattering, R-index**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>UV absorption (AU)</th>
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<tbody>
<tr>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>0.2</td>
<td>0.15</td>
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<tr>
<td>0.5</td>
<td>0.2</td>
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<tr>
<td>1.0</td>
<td>0.25</td>
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<td>5.0</td>
<td>1.0</td>
</tr>
<tr>
<td>10.0</td>
<td>1.5</td>
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</tbody>
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- Total mass: 303 kDa
- Bound detergent: 142 kDa
- Protein: 161 kDa
- for a pentamer: 155 kDa
Formate transport is difficult to essay in proteoliposomes

pKa of formate: 3.5

At pH 6.5, 0.1% of formate is in the neutral form, formic acid

Membrane is permeable to formic acid

Low conductance by FocA
Concentrative uptake essay for channels and transporters

At equilibrium potential
chemical potential = electrical potential

(Middleton et al., 1994)
FocA transports formate in reconstituted proteoliposomes

- Membrane is permeable to formate
- FocA transport rate >> formic acid diffusion rate
- No ΔpH is required for transport
Crystallization in dodecyl-maltoside

- Initial hits from Mosquito (5 screens approx ~40 hits)
- Optimization → no diffraction
Detergent is the KEY for crystallization
- Screening for protein stability in detergents

Alkyl polyoxyethylene monoethers
Cyclomaltosides
Thiogluicosides
Thiomaltosides
Glucosides
Maltosides
545. Emil Fischer: Einfluss der Configuration auf die Wirkung der Enzyme.
[Aus dem I. Berliner Universitäts-Laboratorium.]
(Vorgetragen in der Sitzung vom Verfasser.)

...
It is known that invertin and emulsin show some similarity to proteic substances and undoubtedly, like them, possess a molecule that is constructed in an asymmetric manner. Their limited action on glucoside may then be explained by the hypothesis that an association of molecule necessary to trigger the chemical process cannot take place without an analogous geometrical construction. To use a picture, I would like to say that enzyme and glucoside have to fit each other like a lock and key in order to exert a chemical effect on each other.

enzyme and glucoside = lock and key

  glucoside = key
Diffraction at synchrotron
FocA crystal structure shows a pentamer

Crystals grown in 20 mM formate – ‘Low Formate Structure’

2.13 Å resolution, $R = 21\%$, $R$-free = 18\%
Topology of FocA is similar to that of aquaporins

AQP1

FocA
Pore in the monomer forms the selectivity filter

Central constriction ring 2.3 Å

Selectivity filter

Periplasmic funnel

Extended narrow pore

Cytoplasmic funnel

Cytoplasmic slit 2.1 Å by 4.1 Å
Ω Loop and TM2b are in two positions in different monomers
Two states of the formate channel

A: closed

E: open
In high-formate FocA structure two formate ions seen at the cytoplasmic slit.

Crystals grown in 120 mM formate

2.5 Å resolution, R = 22%, R-free = 17%
Specific interaction of formate with the filter

Peptide deformylase
Structure basis of formate selectivity
Thr90 moves with $\Omega$ loop and TM2b

A: closed  ↔  E: open