Carbohydrate Recognition by the Fimbrial Adhesion Systems of *Escherichia coli*

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Lieven Buts
Vrije Universiteit Brussel
Protein-Carbohydrate Binding Mediates Biological Recognition

- **Carbohydrates** are highly flexible molecules with a complex stereochemistry and a high density of functional groups; this makes them extremely suitable as information carriers.
- The information is encoded by the genome in an indirect way, subject to complex regulation.
- **Lectins** are proteins of non-immune origin that reversibly bind specific carbohydrate structures, without modifying the covalent structure of their ligands.
- Biological recognition processes range from **microbial infection** over intracellular **protein trafficking** to the **differentiation** of cells and tissues in higher organisms.
Carbohydrates as Labels

Blood stream

Site of infection

Rolling (weak adhesion)

Strong adhesion and tissue invasion

Intact, healthy tissue

Carbohydrates

- E-selectin ligands
- L-selectin ligands

Lectins

- E-selectin
- L-selectin
Carbohydrates as Liabilities

Influenza virus particle

Haemagglutinin

Respiratory tract epithelium
Fimbriae or pili bear sugar-binding proteins (adhesins) along their length or at their tips and are involved in the attachment of the bacteria to substrates and host cells; they determine host range and tissue tropism.
Adhesion Systems

Chaperone/usher pathway
Type 1  P-type  F17  
F18  F4/K88  F5/K99

Escherichia coli, Salmonella, ...

Nucleation/precipitation pathway
Thin aggregative pili
Curli

General secretion pathway
Type IV pili
Pseudomonas aeruginosa
Vibrio cholerae

Outer membrane adhesins
Neisseria meningitidis
Moraxella catarrhalis

Invasion systems
CS-1  AIDA

Adhesins of Gram-positive bacteria
F17 Fimbriae

- F17-G: adhesin with GlcNAc specificity
- F17 operon: only 4 genes
- Found in enterotoxigenic Escherichia coli (ETEC) strains infecting livestock

Diagram:
- Fimbria (flexible)
- Usher
- Outer membrane
- Chaperone
The Adhesin has Two Domains

- Lectin domain
- Short linker
- Pilin domain
- Complete Ig fold
The Adhesin has Two Domains

- **Lectin domain**
  - short linker

- **Pilin domain**
  - **Structural pilin subunit**
  - missing strand in incomplete Ig fold

- **N-terminal extension**
The Adhesin has Two Domains

Pilin domain

Lectin domain

short linker

missing strand in incomplete Ig fold

N-terminal extension
The Adhesin has Two Domains

- **Lectin domain**
- **Pilin domain**
- **N-terminal extension**
- **Short linker**

- C
- N
The Adhesin has Two Domains

- Lectin domain
- short linker
F17 Fimbriae

F17-G: adhesin with GlcNAc specificity

F17 operon: only 4 genes

Found in enterotoxigenic Escherichia coli (ETEC) strains infecting livestock
A Specific Chaperone Assists Folding and Assembly

Donor strand complementation

Donor strand exchange
An X-Ray Snapshot

Zavialov et al., Cell, 113; 587-596.
Type 1 Pili

- FimH adhesin
- Adapters
- Pilus rod (rigid)
- Outer membrane
- Usher
- Chaperone

The fim operon is found in human uropathogenic *Escherichia coli* (UPEC) strains. FimH adhesin has a basic specificity for mannose.
Therapeutic Strategies

- General antibiotics
  - Resistance can arise if used inappropriately
  - Persistent and recurrent infections can lead to very long treatment times

- Interfering with pilus assembly
  - “Pilicides” specifically target chaperone function

- Interfering with adhesion
  - Carbohydrate-based binding site blockers target the adhesin-receptor interaction

- Optimal approach depends on circumstances
- Potential for complementarity
F17G Exhibits Significant Natural Variation

F17a-G, F17d-G: bovine enterotoxigenic *E. coli* (ETEC) strains
F17b-G: *E. coli* strains isolated from septicemic calves and lambs
F17c-G: associated with bovine diarrhea or septicemia and with lambs showing nephrosis
F17e-G (*E. coli* strain CK210)
F17f-G (*E. coli* CK377 strain): expressed by non-enterotoxigenic *E. coli* isolated from lambs and goat kids
G fimbriae from human uropathogenic *E. coli* strains were found to be identical in amino acid sequence to F17c fimbriae
Crystal Structure of the F17a-G Lectin Domain

2-acetamido-2-deoxy-D-glucopyranose

methyl 2-acetamido-2-deoxy-1-seleno-β-D-glucopyranoside

(Oscarson lab)
Crystal Packing Variation
## Crystal Quality Variation

<table>
<thead>
<tr>
<th></th>
<th>Time to obtain crystals</th>
<th>Maximum resolution</th>
<th>Suitability for soaking with new ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td>F17aG</td>
<td>Minutes</td>
<td>1.1 Å</td>
<td>+</td>
</tr>
<tr>
<td>F17bG</td>
<td>Days</td>
<td>2.1 Å</td>
<td>++</td>
</tr>
<tr>
<td>F17cG</td>
<td>Days</td>
<td>1.8 Å</td>
<td>-</td>
</tr>
<tr>
<td>F17dG</td>
<td><strong>No crystals obtained</strong></td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>F17eG</td>
<td>Days</td>
<td>2.4 Å</td>
<td>--</td>
</tr>
<tr>
<td>F17fG</td>
<td>Days</td>
<td>1.5 Å</td>
<td>-</td>
</tr>
</tbody>
</table>

Prokaryotic genetic diversity is a source of complications and opportunities.
Carbohydrate Complexes

GlcNAc(β1-3)Gal

β-methyl-paranitrophényl-GlcNAC
Surface Plasmon Resonance Binding Experiments
Surface Plasmon Resonance Binding Experiments

Glc\(\beta\)-1-OMe

Glc\(\alpha\)-1-OMe
## Surface Plasmon Resonance Binding Experiments

<table>
<thead>
<tr>
<th>K_a (1/mM)</th>
<th>F17a-G</th>
<th>F17d-G</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAG</td>
<td>0.85 ± 0.09</td>
<td>1.00 ± 0.21</td>
</tr>
<tr>
<td>NAG_2</td>
<td>1.09 ± 0.04</td>
<td>0.97 ± 0.08</td>
</tr>
<tr>
<td>NAG_3</td>
<td>1.02 ± 0.08</td>
<td>1.33 ± 0.11</td>
</tr>
<tr>
<td>NAG_4</td>
<td>1.62 ± 0.08</td>
<td>1.92 ± 0.11</td>
</tr>
<tr>
<td>β-Me-SeNAG</td>
<td>4.58 ± 0.17</td>
<td>4.59 ± 0.56</td>
</tr>
<tr>
<td>NAGβ1-2Man</td>
<td>2.78 ± 0.10</td>
<td>2.99 ± 0.14</td>
</tr>
<tr>
<td>tri</td>
<td>0.64 ± 0.05</td>
<td>0.55 ± 0.08</td>
</tr>
<tr>
<td>penta</td>
<td>1.19 ± 0.04</td>
<td>1.24 ± 0.05</td>
</tr>
<tr>
<td>GlcN</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>α-Me-Glc</td>
<td>Not detectable</td>
<td>Not detectable</td>
</tr>
<tr>
<td>β-Me-Glc</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Gal</td>
<td>Not detectable</td>
<td>Not detectable</td>
</tr>
<tr>
<td>GalNAc</td>
<td>Not detectable</td>
<td>Not detectable</td>
</tr>
</tbody>
</table>
Type 1 Pili

- **Tip fibrillum** (flexible)
- **Adapter**
- **Pilus rod** (rigid)
- **Outer membrane**
- **Usher**
- **Chaperone**

**fim** operon

Found in human uropathogenic *Escherichia coli* (UPEC) strains

FimH adhesin has a basic specificity for mannose
FimH Recognizes Terminal Mannose Residues
A Serendipitous Ligand Reveals Remarkable Affinities
A Competition Assay for Affinity Measurements

Immobilized antibody targeting the FimH binding site

Carbohydrate ligands interfere with antibody binding, preventing the binding of FimH to the chip
<table>
<thead>
<tr>
<th>Ligand</th>
<th>$K_d$</th>
<th>$\Delta G^\circ$ (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mannose</td>
<td>2.3 µM</td>
<td>-7.6</td>
</tr>
<tr>
<td>methyl 2-deoxy-(\alpha)-D-mannopyranoside</td>
<td>300 µM</td>
<td>-4.8</td>
</tr>
<tr>
<td>glucose</td>
<td>9.24 mM</td>
<td>-2.8</td>
</tr>
<tr>
<td>galactose</td>
<td>100 mM</td>
<td>-1.4</td>
</tr>
<tr>
<td>fructose</td>
<td>31 µM</td>
<td>-6.1</td>
</tr>
<tr>
<td>sucrose</td>
<td>12.8 mM</td>
<td>-2.6</td>
</tr>
<tr>
<td>turanose</td>
<td>7.6 mM</td>
<td>-2.9</td>
</tr>
</tbody>
</table>
Fructose, present for about 5% in fruit juices, inhibits type 1 fimbrial adherence.
Inhibitor Design

**Alkyl mannose derivatives**

- Methylmannose (Man-C$_1$)
- Butylmannose (Man-C$_4$)
- Octylmannose (Man-C$_8$)

**Aromatic substituents**

- Methylumbelliferylmannose (MU-Man)
- Paranitrophenylmannose (PNP-Man)
<table>
<thead>
<tr>
<th>Ligand</th>
<th>$K_d$ SPR (nM)</th>
<th>$\Delta G^\circ$ SPR (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mannose</td>
<td>2.3 $10^3$</td>
<td>-7.6</td>
</tr>
<tr>
<td>methyl$\alpha$man</td>
<td>2.2 $10^3$</td>
<td>-7.7</td>
</tr>
<tr>
<td>ethyl$\alpha$man</td>
<td>1.2 $10^3$</td>
<td>-8.1</td>
</tr>
<tr>
<td>propyl$\alpha$man</td>
<td>300</td>
<td>-8.9</td>
</tr>
<tr>
<td>butyl$\alpha$man</td>
<td>151</td>
<td>-9.3</td>
</tr>
<tr>
<td>pentyl$\alpha$man</td>
<td>25</td>
<td>-10.4</td>
</tr>
<tr>
<td>hexyl$\alpha$man</td>
<td>10</td>
<td>-10.9</td>
</tr>
<tr>
<td>heptyl$\alpha$man</td>
<td>5</td>
<td>-11.3</td>
</tr>
<tr>
<td>octyl$\alpha$man</td>
<td>22</td>
<td>-10.4</td>
</tr>
<tr>
<td>pNP$\alpha$Man</td>
<td>44</td>
<td>-10.0</td>
</tr>
<tr>
<td>MeUmb$\alpha$Man</td>
<td>20</td>
<td>-10.5</td>
</tr>
</tbody>
</table>
A Linear Correlation Between Alkyl Chain Length and Energy

\[ \Delta G \text{ (kcal/mol)} \]

\[ \text{number of carbon atoms} \]

Slope: -0.64 ± 0.03
Correlation: -0.99 ± 0.18
Docking Energy Histograms

![Docking Energy Histograms](image)
Future Directions

Target tissue
- Recovery and digestion of glycoconjugates

Glycoprotein mix
- Treatment with proteases and glycosidases

Mixture of fragments
- Selection with lectin/adhesin

Binding fragments
- Mass spectrometry and database search

Identification
Integration

Structure

Thermodynamics

A + B \xrightleftharpoons{K_a} AB

K_a = \frac{[AB]_{eq}}{[A]_{eq} \cdot [B]_{eq}}

\Delta G = -RT \ln(K_a)

\Delta G = \Delta H - T \Delta S

Experiments

BiaCore

ITC, DSC

Spectroscopic titrations

Docking

Structure determination

Crystallography

NMR
K88 Fimbriae

D

G

? 

outer membrane

Usher

E

Chaperone
Conclusions
Conclusions

- **X-ray crystallography** reveals the structures of adhesins and their interactions with carbohydrate receptors in atomic detail
- **Surface plasmon resonance** measurements now enables the detection of the binding of mono- and oligosaccharides, using comparatively small amounts of protein and ligand
- Analysis of the combined data from these techniques provides insights in the **specificity profiles** of the adhesins, forming the basis for the design of novel **adhesion inhibitors**
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