Chapter 15

Structural Studies of Yersinia Adhesin YadA
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1. INTRODUCTION

Adhesion of human pathogenic Yersiniae to host cells is mediated by several mechanisms, which include the chromosomally encoded proteins invasin and Ail (Isberg & Falkow 1985; Miller & Falkow, 1988) and the Yersinia virulence plasmid (pYV) encoded Yersinia adhesin, YadA (Heesemann & Grüter, 1987). The expression of the yadA gene is temperature regulated in Y. enterocolitica and Y. pseudotuberculosis (Skurnik & Toivanen, 1992). YadA is not expressed in Y. pestis, because of a single-base-pair deletion in the yadA gene (Rosqvist et al., 1988). YadA is involved in several virulence-related functions like binding to the extracellular matrix proteins (for a review see El Tahir & Skurnik, 2001). The collagen-biding ability of YadA in Y. enterocolitica is directly related to its virulence: loss of the collagen-binding ability of YadA leads to avirulence in mice (Tamm et al., 1993; Roggenkamp et al., 1995).

Wild type YadA of Y. enterocolitica serotype O:3 is a 430-amino-acid outer membrane protein, translated with a 25-amino-acid signal peptide that is processed during transportation. On SDS-PAGE YadA forms stable aggregates of molecular weight 160 to 240 kDa suggesting oligomerization of three monomers (Gripenberg-Lerche et al., 1995). Recent electron micrographs show that YadA adopts a lollipop shaped form which consists of a C-terminal membrane anchor domain, a coiled-coil stalk domain and a globular N-terminal head domain (Hoiczyk et al., 2000). The head domain
consists of amino acids 26-224 and it includes seven out of eight NSVAIGXXS motifs, which are required for YadA mediated collagen binding (El Tahir et al., 2000).

2. MATERIALS AND METHODS

For the crystallographic studies \textit{yadA}_{26-241} of \textit{Y. enterocolitica} O:3 was cloned to a vector with an N-terminal His\textsubscript{6}-tag and transformed to M15(pREP4). For the production of selenomethionyl labelled protein, mutations I130M and I157M were made to NSVAIG--S motifs, which were anticipated to be on the surface of the protein (Nummelin et al., 2002). YadA was purified from cytoplasmic fraction of broken bacterial cells by a metal chelating affinity column followed by size exclusion chromatography to separate the soluble protein from the aggregated form. For the YadA\textsubscript{26-241}-SeMet, the bacterial cells were grown in minimal medium with selenomethionine.

Crystal screening was done using sparse matrix screens and a sitting-drop vapor-diffusion method. The crystals were flash frozen and native data were collected at beam line X11 in the DESY, EMBL Hamburg. The MAD data were collected at BM-17, ESRF, Grenoble. Space group assignment and unit cell parameters were defined using DENZO (Otwinowski & Minor 1995).

3. RESULTS

Crystals grew within one week using 12\% PEG 8000, 0.1 M Na-cacodylate pH 6.5 and 0.1 M Na-acetate as precipitant and a protein concentration of 8 mg ml\textsuperscript{-1}. The crystals belonged to space group R3 and diffracted to 1.55 Å resolution (Table 1). Expression of a selenomethionyl-incorporated protein led to crystals under similar conditions to native. However, the anomalous signal of arsenic interfered the selenium signal, so the buffer was changed to Tris-HCl. The selenomethionyl-protein was also less soluble and so a lower protein concentration of 2.5 mg ml\textsuperscript{-1} was used (Nummelin et al., 2002). The hexagonal crystals belonged to space group R32 and diffracted to 2.0 Å (Table 1).
Table 1. Data collection statistics for native YadA_{26-241} and selenomethionyl-labelled YadA_{26-241}-SeMet data. The values for highest resolution shell 1.61-1.55 Å for native data are on parenthesis.

<table>
<thead>
<tr>
<th></th>
<th>YadA_{26-241}</th>
<th>YadA_{26-241}-SeMet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unit cell a, b, c (Å)</td>
<td>67, 67, 222</td>
<td>65, 65, 230</td>
</tr>
<tr>
<td>Completeness (%)</td>
<td>99.7 (98.6)</td>
<td>100.0</td>
</tr>
<tr>
<td>1/σ(I)</td>
<td>20.2 (3.1)</td>
<td>21.2</td>
</tr>
<tr>
<td>R_{merge} (%)</td>
<td>5.2 (39.2)</td>
<td>8.0</td>
</tr>
<tr>
<td>Phasing power</td>
<td>0.80</td>
<td>8.5</td>
</tr>
<tr>
<td>FOM</td>
<td>0.28</td>
<td>9.6</td>
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</table>

Two selenium atoms per asymmetric unit were found using MAD data, and initial phases and maps were calculated with SOLVE (Terwilliger & Berendzen 1999). The quality of the maps did not correspond to the resolution of the data and the tracing of the polypeptide was difficult. The bad map quality was a result of the location of the selenium atoms very close to one another and in close proximity to crystallographic threefold axis (Figure 1). This resulted in low phasing power and only the central part of the molecule could be traced. The model has been partly refined, and additional phasing power is needed to solve the structure completely.

Even with the partial model it can be seen that the central part of the molecule forms a beta solenoid, which has the collagen binding NSVAIG—S motifs inside the trimer. The motifs are thus actually structural motifs that are needed to form a hydrophobic core inside the trimer and most likely they do not interact directly with collagen.
REFERENCES


Miller, V.L., and Falkow, S., 1988, Evidence for two genetic loci in _Yersinia enterocolitica_ that can promote invasion of epithelial cells, _Infect. Immun._ 56, 1242-1248.


Otwinowski, A., and Minor, W., 1995, _DENZO_. Yale University, New Haven, Connecticut, USA.


