Fimbriae of uropathogenic *Proteus mirabilis*

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Abstract

*Proteus mirabilis* is a common causative agent of cystitis and pyelonephritis in patients with urinary catheters or structural abnormalities of the urinary tract. Several types of fimbriae, which are potentially involved in adhesion to the uroepithelium, can be expressed simultaneously by *P. mirabilis*: mannose-resistant/*Proteus*-like (MR/P) fimbriae, *P. mirabilis* fimbriae (PMF), uroepithelial cell adhesin (UCA), renamed by some authors nonagglutinating fimbriae (NAF), and ambient-temperature fimbriae (ATF). *Proteus mirabilis* is a common cause of biofilm formation on catheter material and MR/P fimbriae are involved in this process. The considerable serious pathology caused by *P. mirabilis* in the urinary tract warrants the development of a prophylactic vaccine, and several studies have pointed to MR/P fimbriae as a potential target for immunization. This article reviews *P. mirabilis* fimbriae with regard to their participation in uropathogenesis, biofilm formation and as vaccine targets.

Introduction

Among the most common community-acquired and nosocomial infections are those of the urinary tract (Kahlmeter, 2000; Johansen et al., 2006). After *Escherichia coli*, *Proteus mirabilis* is the most frequent etiological agent, especially in patients with urinary catheters or with structural abnormalities of the urinary tract (Warren et al., 1982; Mobley & Belas, 1995). This organism produces several fimbriae and hemagglutinins, including mannose-resistant/*Proteus*-like (MR/P) fimbriae (Adegbola et al., 1983), *P. mirabilis* fimbriae (PMF) (Bahrani et al., 1993), uroepithelial cell adhesin (UCA), renamed by some authors nonagglutinating fimbriae (NAF) (Tolson et al., 1995), and ambient-temperature fimbriae (ATF) (Massad et al., 1994a, b).

Urinary tract infection

The urinary tract is a complicated epithelium-lined tube with an opening to the body surface. It is susceptible to infections by exogenous organisms including bacterial species with specific virulence factors that allow colonization of the urinary tract (Li & Mobley, 2002). Most urinary tract infections (UTIs) are thought to occur via the ascending route. Organisms of fecal origin gain access to the urethra and enter the bladder where they multiply. They can confine themselves there or ascend the ureters to the kidneys. The most virulent and invasive pathogens can break through the single cell-thick barrier afforded by the proximal tubules and enter the bloodstream possibly causing systemic infection (Li & Mobley, 2002).

Individuals at high risk for symptomatic UTIs include neonates, preschool girls, and sexually active women and...
men. In 1991 UTI was the cause of 9.6 million physician visits and 1.5 million hospital case records in the US population. The high frequency places UTIs among the main kidney and urologic diseases in terms of total cost, exceeding even chronic renal failure (Mobley, 2000).

**Bacterial adhesion to uroepithelial tract**

The initial event in the pathogenesis of bacterial infectious diseases is the colonization of the host epithelium, which is usually mediated by fimbrial and nonfimbrial adhesins (Beachey, 1981; Soto & Hultgren, 1999).

Fimbriae are bacterial appendages of varying lengths and diameters, consisting of a polymer of a single polypeptide subunit tipped or interspersed with adhesive proteins. The fimbriae are usually arranged around the bacterial cell in a peritrichous manner, but they can be expressed in a polar orientation. They are thinner, shorter and more numerous than flagella (Edwards & Puente, 1998).

Nonfimbrial adhesins are a growing class of outer-membrane proteins (OMPs) associated with the adhesion of enteric bacteria to the host mucosa (Soto & Hultgren, 1999). Although the major OMP complex of *P. mirabilis* has been characterized, none has been described as having an adhesive function (Moayeri et al., 1991).

As in a wide range of bacterial diseases, adherence plays an important step in the pathogenesis of UTIs (Sareneva et al., 1990). There is evidence suggesting that differences in the ability of host epithelial cells to bind bacteria via specific adhesins influence susceptibility to such infections (Daifuku et al., 1990). It has been shown that fimbriae are indeed responsible for the attachment of *P. mirabilis* to uroepithelial cells (Rozalski et al., 1997; Coker et al., 2000) (Table 1).

### Fimbriae and adhesins of *P. mirabilis*

#### UCA/NAF

Wray et al. (1986) isolated and identified UCA from an isolate of uropathogenic *P. mirabilis*. This fimbria was found to be organized as long and flexible rods, with a thin filament of 4 nm predominating. UCA was found to be responsible for the attachment of bacteria to the uroepithelial cells. Its subunit responsible for adhesion has a molecular mass of 17.5 kDa. The N-terminal amino acid sequence of this protein revealed much less homology between UCA and the urinary tract adhesins of *E. coli* (20% and 25% homology with the P fimbriae and type 1 fimbriae, respectively). These findings suggest that UCA was formerly an adhesin for the intestinal epithelium. The genetic organization of UCA biogenesis is still unknown and the only cloned gene thus far is *ucaA*, which encodes the subunit responsible for adhesion (Cook et al., 1995).

The term NAF has been proposed as an alternative name for UCA, in order to distinguish it from the other *P. mirabilis* fimbriae (Tolson et al., 1995). Although UCA and NAF fimbrial amino acid sequences have been shown to be the same (Latta et al., 1998), some authors still employ the UCA denomination, reflecting a lack of consensus on the nomenclature for this adhesin.

The conditions of NAF expression were studied by Tolson et al. (1995). They found that seven out of eight strains of *P. mirabilis* clearly produced this fimbrial adhesin in relatively comparable quantities when grown on Luria agar. However, the molecular masses of NAF subunits for the different strains ranged from 23 to 29 kDa. When harvested from Luria agar, none of the eight *P. mirabilis* strains was able to agglutinate erythrocytes of different species, either in the presence or the absence of D-mannose. The authors found that *P. mirabilis* grown on Luria agar was able to adhere to HEp-2 cells and that the binding was significantly eliminated by preincubation with monoclonal antibodies specific for NAF (Tolson et al., 1997).

Lee et al. (2000) have shown that NAF of *P. mirabilis* recognized the common carbohydrate sequence GalNacβ1-4Gal present in asialo-GM1 and asialo-GM2 glycosphingolipids. Glycolipids containing the GalNacβ1-4Gal isoreceptors are highly targeted by many pathogenic bacteria (Lingwood, 1992).

#### Ambient-temperature fimbriae

Massad et al. (1994a) described ATF employing one isolate of *P. mirabilis*. The purified fimbria revealed a subunit with an apparent molecular size of 24 kDa, where the N-terminal amino acid sequence does not demonstrate similarity to that of any other fimbriae or other protein in the SWISS PROT database. The optimal expression of this fimbria was observed in static culture in Luria broth at 23 °C for 48 h. There was no detectable expression in Luria broth at 42 °C, in liquid minimal medium or during growth on solid media.

### Table 1. Fimbriae of Proteus mirabilis and their involvement in pathogenicity and biofilm formation and as antigen candidates for vaccination

<table>
<thead>
<tr>
<th>Type of fimbria</th>
<th>Pathogenicity in UTI</th>
<th>Biofilm formation</th>
<th>Antigen for vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCA/NAF</td>
<td>Adhesion to uroepithelial cells <em>in vitro</em></td>
<td>Not studied</td>
<td>Yes</td>
</tr>
<tr>
<td>ATF</td>
<td>Not associated</td>
<td>Not studied</td>
<td>Not studied</td>
</tr>
<tr>
<td>PMF</td>
<td>Colonization of bladder and kidneys</td>
<td>Not studied</td>
<td>Yes</td>
</tr>
<tr>
<td>MR/P</td>
<td>Colonization of bladder and kidneys</td>
<td>Biofilm formation in an <em>in vitro</em> assay</td>
<td>Yes</td>
</tr>
</tbody>
</table>
The \textit{P. mirabilis} strains HI4320 and HU1069 were grown under various conditions and the level of hemagglutination and expression of ATF were measured. Expression of ATF did not correlate with either MR/P or mannose-resistant \textit{Klebsiella}-like fimbriae hemagglutination and hemagglutination was not apparent in either strain when cells were maximally expressing ATF (Massad 	extit{et al}., 1994a, b).

The ATF biogenesis genes were identified and sequenced, and the putative promoter (−10 and −35) sequences and ribosomal binding site (AGGA) were identified. Downstream of this region, one 558-bp ORF was located and designated \textit{atfA}. This ORF predicts a 186-amino acid polypeptide of 19.1 kDa, which was found to be similar to the structural subunit polypeptide of type 1 fimbriae from \textit{Salmonella typhi} (41%), \textit{Salmonella typhimurium} (40%) and \textit{E. coli} (38%). A second ORF located 102 bp downstream from \textit{atfA} was identified and designated \textit{atfB}. The 675-bp sequence predicts a 225-amino acid polypeptide of 25.3 kDa. This sequence shared 44.8% identity with the FimC chaperone protein for type 1 fimbria biogenesis of \textit{E. coli}. A third ORF, 40 bp downstream from \textit{atfB} was identified and designated \textit{atfC}. The \textit{atfC} 2529-bp sequence predicts a 843-amino acid polypeptide of 94.3 kDa. This sequence shared 44.4% identity with the FimD outer membrane usher protein of \textit{E. coli}. No putative regulatory or minor pilin genes were evident (Massad 	extit{et al}., 1996).

Zunino 	extit{et al} (2000) isolated a mutant of \textit{P. mirabilis} that was unable to synthesize ATF. They confirmed that ATF are not required for \textit{P. mirabilis} hemagglutination. This ATF mutant was also used in a mouse ascending UTI model and no significant differences were noted between numbers of infected animals or organs, or between the levels of colonization of kidney or bladders between the wild-type or mutant infections. This indicates that ATF mutagenesis did not affect the infective ability of \textit{P. mirabilis}. Therefore, ATF are more likely to play a role in the survival of \textit{P. mirabilis} in the external environments of a mammalian host due to its optimal expression temperature.

\section*{\textit{P. mirabilis} fimbriae}

The PMF were isolated and their operon nucleotide sequence was determined by Massad & Mobley (1994). The PMF were isolated and their operon nucleotide sequence was determined by Massad & Mobley (1994). The \textit{pmf} operon revealed six ORFs with five predicted polypeptides: \textit{PmfA} (18.9 kDa), the major fimbrial subunit; \textit{PmfC} (93.1 kDa), the usher; \textit{PmfD} (28.2 kDa), the chaperone; \textit{PmfE} (38.9 kDa), the minor fimbrial subunit; and \textit{PmfF} (19.6 kDa), which is the adhesin. Such genetic organization resembles that of uropathogenic \textit{E. coli} fimbriae (Soto & Hultgren, 1999).

The role of PMF in the virulence of \textit{P. mirabilis} was determined by two studies. In the first, an isogenic \textit{pmfA} fimbrial mutant colonized the bladders of transurethrally challenged CBA mice in numbers 83-fold lower than those of the wild-type strain (Massad \textit{et al}., 1994b). However, the mutant colonized the kidneys in numbers similar to those of the wild-type strain. The authors suggested that the role of PMF was just in colonization of the bladder but not in the kidney tissue. The second study also evaluated a \textit{pmfA} mutant and the role of PMF in colonization, and virulence in UTI was assessed using a cochallenge ascending UTI model in CD-1 mice (Zunino \textit{et al}., 2003). After 7 days of infection, the mutant and the wild-type strains were enumerated on nonswarming Luria–Bertani (LB) agar. The number of viable bacteria counted from bladder and kidneys showed that the mutant strain was significantly out-competed by the wild-type strain in colonizing the bladders and kidneys. This suggests that PMF play a role in localization of uropathogenic \textit{P. mirabilis} to the bladder and kidney.

\section*{Mannose-resistant \textit{Proteus}-like fimbriae}

The MR/P fimbriae are the most well-studied fimbriae of \textit{P. mirabilis}. MR/P fimbriae were isolated and purified for the first time by Sareneva \textit{et al} (1990) from \textit{P. mirabilis} strain 3087. These authors demonstrated that the tubular elements of the kidney, but not the glomerular elements, were the tissue-binding site of these fimbriae in humans. \textit{Proteus mirabilis} strain 3087 agglutinated human erythrocytes in a mannose-resistant manner, indicating the presence of the MR/P type of fimbria. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis of the fimbrial preparation demonstrated that the major MR/P subunit appeared to have a molecular mass of 21 kDa. Ultrastructural analysis showed MR/P fimbriae as filament of c. 7 nm in diameter (Sareneva \textit{et al}., 1990).

The MR/P fimbriae were also characterized by Bahrami \textit{et al} (1991, 1993, 1994) using \textit{P. mirabilis} strain HI4320. The MrpA protein, the major subunit of this fimbria type, has a molecular mass of 18.5 kDa and is encoded by the \textit{mrpA} gene.

The discrepancy in the results regarding the molecular mass of MrpA (21 and 18.5 kDa) indicates that although Sareneva \textit{et al} (1990) and Bahrami \textit{et al} (1991) have described functionally and morphologically similar fimbriae, they may be heterogeneous. The fact that the MR/P receptor has not been identified points to the possibility that the mannose-resistant hemagglutination observed may actually include several distinct fimbrial types (Bahrami \textit{et al}., 1991).

This fimbria type belongs to a family of bacterial fimbriae modelled after P fimbriae of uropathogenic \textit{E. coli}, which is assembled through the chaperone–usher pathway (Soto & Hultgren, 1999). The MR/P gene cluster contains two divergent transcripts, \textit{mrpABCDEFGHJ} (\textit{mrp} operon) and \textit{mrpL}.
mrpA encodes the major structural fimbrial subunit, mrpBEFG encodes the smaller subunits, mrpD encodes the chaperone, mrpC encodes the usher, mrpF encodes the pilin of fimbriae and mrpJ encodes a protein that represses transcription of the flagellar regulon (Bahrami & Mobley, 1994; Li et al., 1999, 2001). mrpJ encodes a recombinase that switches the invertible element from ON to OFF (transcription of the mrp operon) or from OFF to ON, which prevents the transcription of the mrp operon (Zhao et al., 1997). Gene products of the mrp operon (except for mrpJ) have an apparent functional homology in P fimbriae of uropathogenic E. coli (Bahrami & Mobley, 1994; Li et al., 1999). Li et al. (2001) demonstrated that MrpJ represses the transcription of flhDC flagellar master operon, the function homologue to PapX of E. coli P fimbriae.

Different groups have demonstrated the participation of MR/P fimbriae in the colonization of the urinary tract by P. mirabilis. Bahrami et al. (1994) constructed a MR/P fimbrial mutant, and to assess its virulence, 40 CBA mice were challenged transurethrally with either the wild-type or the mutant strains. One week later, the mice challenged with the wild-type strain showed significantly more severe renal damage and bladder colonization than did the mice challenged with the mutant. Zunino et al. (2001) studied a clinical isolate of P. mirabilis and an isogenic mutant unable to express MR/P fimbriae, using different experimental approaches. They were tested for their ability to cause infection in an ascending coinfection model of UTI and in a hematogenous model in mouse. In both models the mutant was less able than the wild-type strain to colonize the lower and upper urinary tracts, although infectivity was not abolished. When these strains were used in an in vitro assay to evaluate bacterial adherence to cultured epithelial cells, aggregative adherence of MR/P fimbriae was significantly decreased in the number of adherent MR/P fimbriae per cell could be observed after 1 h of incubation.

Biofilm formation of P. mirabilis on catheter material is a well-documented phenomenon (Morris et al., 1997; Sabbubba et al., 2002; Jansen et al., 2004). Biofilm is a microbiially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other, embedded in a matrix of extracellular polymeric substances that they have produced (Donlan & Costerton, 2002).

Jansen et al. (2004) tested the role of MR/P fimbriae in biofilm formation, employing the wild-type strain HI4320 and mutants phase-locked for either constitutive expression (MR/P ON) or inability to express MR/P fimbriae (MR/P OFF). The MR/P ON formed significantly more biofilm than did either P. mirabilis HI4320 or MR/P OFF. Similar biofilm was observed for P. mirabilis HI4320 and MR/P OFF. MR/P ON formed a three-dimensional biofilm structure as early as 18 h after the initiation of the biofilm, while MR/P OFF and HI4320 did not. However, after 7 days MR/P OFF and MR/P ON formed 12-μm-thick biofilms, while P. mirabilis HI4320 formed a 65-μm-thick biofilm.

In the characterization of the adherence of uropathogenic P. mirabilis to cultured epithelial cells, aggregative adherence to HEP-2 cells, which is characteristic of enteraggregative E. coli (Nataro et al., 1987), was observed among 35 isolates. A mutation of mrpA in one of these isolates demonstrated that MR/P is involved but is not sufficient in the establishment of this characteristic adherence pattern, as well as in biofilm formation on polystyrene (S.P.D. Rocha, unpublished data).

Other fimbriae

Two other fimbriae were detected in P. mirabilis: mannose-resistant Klebsiella-like (MR/K) fimbriae and P. mirabilis P-like fimbriae.

MR/K fimbriae, which is a type III fimbria associated with the ability to agglutinate tannic acid-treated erythrocytes from different species in the presence of mannose (Old Adegbola, 1983), is more characteristic of Proteus penneri than P. mirabilis strains (Yakubu et al., 1989). The detection of MR/K in P. mirabilis was deduced by its hemagglutination pattern displayed in comparison with the MR/K hemagglutination pattern of Klebsiella pneumoniae (Gerlach et al., 1989). Sareneva et al. (1990) demonstrated with P. mirabilis 2456 that MR/K fimbriae bound strongly to the Bowman's capsule of the glomeruli and to the tubular basement membranes and did not adhere to epithelial cells of urinary sediment. Interestingly, these authors showed that MR/K fimbrial extraction showed multiple peptides, but no conclusions were drawn as to whether they correspond to different variants or different types of MR/K fimbriae. Cloning of MR/K genetic determinants of P. mirabilis could indicate which polypeptide corresponds to the correct adhesin subunit. However, attempts to clone the MR/K fimbria biogenesis genes of P. mirabilis have been unsuccessful (Bahrami et al., 1993). The lack of further studies investigating this fimbria type in P. mirabilis is probably because of the fact that it is not a common type in P. mirabilis.

Proteus mirabilis P-like fimbriae were isolated from a canine isolate of P. mirabilis by Bijlsma et al. (1995) and were not found to be among the P. mirabilis isolated from humans.

Vaccine development

As P. mirabilis are opportunistic uropathogens that cause UTIs in hosts with anatomically and functionally abnormal urinary tracts, preferentially affecting some individuals, such as women with recurrent UTIs and those early in the course of long-term catheterization, prevention for these
patients is necessary (Warren et al., 1982; Mobley & Belas, 1995; Kahlmeter, 2000; Mobley, 2000; Johansen et al., 2006). Li et al. (2004) described the use of a fimbrial component, MrpH, as a vaccine to prevent infection by P. mirabilis employing a CBA mouse model of ascending UTI to test the efficacy of vaccination. Four routes of vaccination were examined: subcutaneous, intranasal, oral and transurethral. Protection was assessed using live bacteria and vaccination with formalin-killed bacteria, purified MR/P fimbria, the MrpH fimbrial adhesin, and the truncate MrpH fimbrial adhesin. The truncate MrpH vaccine for the intranasal route protected 75% of mice from P. mirabilis UTIs.

Pellegrino et al. (2003) expressed and purified recombinant structural fimbrial antigens MrpA, PmfA and UcaA and employed them for vaccination and protection evaluated in the ascending and hematogenous model of UTI in CD-1 mice. Subcutaneous vaccination with MrpA protected mice in both models, demonstrating that, indeed, the MR/P fimbria is an important vaccine target against P. mirabilis UTI. Vaccination with UcaA only protected mice that were challenged intravenously; PmfA elicited a significant urinary antibody response, but this protein was unable to confer protection against P. mirabilis experimental challenges.

Scavone et al. (2004) immunized different groups of CD-1 mice with recombinant structural fimbrial subunits MrpA, PmfA and UcaA using two mucosal routes: nasal and transurethral. The animals were transurethrally infected with a uropathogenic P. mirabilis isolate, and antibody response to specific antigens in serum and urine and protection against a virulent P. mirabilis strain was assessed. Intranasally MrpA and UcaA immunized mice were protected against P. mirabilis ascending UTI, and both subunits significantly induced specific serum and urine antibodies. MrpA and PmfA transurethrally immunized animals were protected only at the kidney level, and only MrpA induced significant serum IgG.

The sequence of 99.97% of the P. mirabilis strain HI4320 chromosome (4.063 Mb) has been completed by the Wellcome Trust Sanger Institute and is available for searching and download (http://www.sanger.ac.uk/Projects/P_mirabilis/ – accessed on 18 April 2007). This will allow the identification of ORFs presenting homology with putative adhesins, as well as other virulence factors, thereby identifying new antigens with vaccine potential.

Conclusions

Proteus mirabilis is a common cause of UTIs in patients with structural abnormalities of the urinary tract or with urinary catheters. Proteus mirabilis fimbriae are important in the establishment of the colonization and pathogenesis of UTIs. Proteus mirabilis form biofilm on catheter material and the MR/P fimbriae are involved in such phenotype. Owing to the severe uropathogenesis of P. mirabilis, prophylactic vaccination in specific patients should be considered. An immunization strategy based on structural fimbrial proteins may be useful in preventing P. mirabilis UTIs.

References


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