The solution structure of PapGII from uropathogenic *Escherichia coli* and its recognition of glycolipid receptors

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Uropathogenic *Escherichia coli* (UPEC) is the primary cause of symptomatic urinary tract infection. The P-pili, a bacterial surface organelle, mediates the bacterial host–cell adhesion. The PapG adhesin has generated much interest in recent years, not only because of its clinical value, i.e. in the prevention of microbial adherence, but also because of its ability to promote virulence. Using multidimensional nuclear magnetic resonance (NMR) and deuteration we have determined the solution structure of the adhesin domain from PapGII (PapGII-198). The novel structure of PapGII-198 is composed of a large elongated jellyroll motif. Despite an automated search of the structural database failing to reveal any similar proteins, PapGII adhesin shares some structural similarities with FimH. Furthermore, interpretation of NMR-titration data has enabled us to identify the putative binding site for the globoseries of oligosaccharides. This work provides insight into UPEC pathogenesis as well as aiding the development of preventative therapies and the guidance of future mutagenesis programmes.

INTRODUCTION

Symptomatic urinary tract infection (UTI) is a major infectious disease that causes considerable suffering and expense. UTI is also one of the most common infectious diseases amongst women and children. Pathogens responsible for UTI are typically enterobacteriaceae with uropathogenic *Escherichia coli* (UPEC) being the predominant causative agent. UPEC adheres to the host cell through the interaction of bacterial cell surface organelles and glycoconjugates on the plasma membrane of host cells. These organelles, termed pili or fimbriae, are exposed as long hair-like structures that protrude from the bacterial

surface for interaction with the target cell. Fimbriae are grouped into different classes due to their recognition of different glycoconjugates; type 1 fimbriae bind α -mannosyl groups on glycoproteins, P-fimbriae bind the globoseries of glycolipids, and S-fimbriae bind NeuNAc α (2-3)Gal β (Hanson *et al.*, 1997).

Until recently, it has been assumed that the primary role of adhesins was to bring the host cell in close proximity to the bacterium so that secondary interactions could take place and induce virulence. It is now believed that the adhesin alone can set up a complex signal cascade both inside the host and bacterium (Godaly *et al.*, 1998; Svanborg *et al.*, 1999). The P-pili are composed of six different protein subunits: PapA, PapH, PapG, PapE, PapF and PapK (Hull *et al.*, 1981; Hultgren *et al.*, 1993). They form heteropolymeric structures composed of a rigid stalk and a thin flexible extension (fibrillum) that is connected to the adhesive tip (Kuehn *et al.*, 1992). PapG, the adhesin of the P-pili, is situated at the tip and is a minor component of the whole pilus structure. Studies have shown that deletion of PapG has no effect on pilus formation but significantly impairs bacterial adhesion to the glycan receptors (Hultgren *et al.*, 1989), which are the Forsmann antigen (Gb₅), globotetraosylceramide (Gb₄) and globotriaosylceramide (Gb₃).

PapG has been typed into three classes according to its glycoconjugate-binding specificity. The most interesting from a biomedical perspective are the class II and III adhesins (PapGII, PapGIII), which are expressed within the pilus of *E. coli* isolates that infect humans and are critical for disease progression (Stromberg *et al.*, 1990). The PapGII adhesin dominates in acute pyelonephritis and binds preferentially to Gb₄ (GalNAc β 1-3Gal α 1-4Gal β 1-4GlcCer), which is abundant in the upper urinary tract of humans. P-pili presenting PapGIII are common in human cystitis, but rare in

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pyelonephritic isolates. They bind Gb₅ (GalNAcα1-3-GalNAc3Galα1-4Galβ1-4GlcCer), which carries an extension to the terminal GalNAc residue of globoside (Stromberg *et al.*, 1990). A two-domain structure has been postulated for PapG: a carbohydrate binding N-terminus and chaperone binding C-terminus (Hultgren *et al.*, 1989; Haslam *et al.*, 1994; Hansson *et al.*, 1995). The chaperone-binding domain is highly conserved across all pili subunits, and is essential for the correct assembly of the pili structure when aided by the chaperone molecule PapD. Structural studies of the PapD–PapK complex revealed that the chaperone functions by donating a terminal β-strand to complement an incomplete immunoglobulin-like fold of the pili subunits; a process termed donor strand complementation (Sauer *et al.*, 1999, 2000). The 198 amino acid carbohydrate-binding domain (in this study termed PapGII-198) interacts with the receptor glycan but is unable to form a complex with the chaperone.

The prevention of microbial adherence of PapGII to the host cell, and thus inhibition of colonization, is a possible strategy for therapeutic intervention. Rational drug design would require an in-depth knowledge of carbohydrate recognition by PapGII, from the atomic resolution standpoint. Using multidimensional nuclear magnetic resonance (NMR) combined with deuteration we have, for the first time, determined the solution structure of the adhesin domain from PapGII (PapGII-198). The structure of PapGII-198 is composed of one large elongated jellyroll motif, which shares some topological similarities with FimH (Choudhury *et al.*, 1999), the type 1 pili adhesin that mediates binding to mannose-containing glycoproteins, and plays a critical role in bladder colonization and disease (Mulvey *et al.*, 1998). Furthermore, interpretation of NMR-titration data has enabled us to identify the binding site for the globoseries of oligosaccharides, which is located on one side of the adhesion domain. This is in contrast to FimH where primary carbohydrate recognition occurs at the tip of the structure. These results provide significant understanding into the specificity of saccharide binding.

RESULTS AND DISCUSSION

The solution structure for PapGII-198

The PapGII-198 domain, comprising residues 21–219 of full-length PapGII, was produced as a recombinant protein in *E. coli* as previously described. For simplicity and clarity these residues are numbered as 1–198. Preliminary NMR studies revealed that PapGII-198 severely aggregated at millimolar concentrations, generating a molecular reorientation time consistent with a much larger protein. It was therefore necessary to introduce deuterium into the sample in order to facilitate sequential assignment (Sung *et al.*, 2001) and structure calculation. The structure determination of PapGII-198 was based on a total of 1724 restraints comprising 1238 nuclear Overhauser effect (NOE)-derived interproton distance constraints, 122 distance restraints for backbone hydrogen bonds, 212 dihedral angle restraints, and 148 backbone ¹⁵N–¹H residual dipolar couplings (see Methods). A final family of 10 structures was defined for PapGII-198 (shown in Figure 1A and Table I) based on agreement with the experimental data and overall structural quality; no NOE violations >0.5 Å, no torsion angle violations >5 degrees and no residual dipolar coupling violations >2 Hz (over a 70Hz range). The

Table I. Results from structure calculations for PapGII-198

Statistic	<SA> ^a	
Restraints		
medium/short range NOEs (1 < li-jl < 5)	832	0.058 ^b ± 0.003
long range NOEs (li-jl > 4)	400	0.058 ^b ± 0.003
hydrogen bonds	122	0.051 ^b ± 0.0031
dihedral angles	212	1.21 ^c ± 0.002
¹ D _{NH}	148	0.97 ^d ± 0.38
(total NOE restraints involving methyl group is 300)		
Idealized geometry		
bonds ^b		0.004 ± 0.0003
angles ^c		0.71 ± 0.06
improper angles ^c		0.71 ± 0.01
Coordinate positions		
backbone atoms for residues: 1–19, 25–70, 79–92, 96–119, 124–195		1.55 ± 1 ^e
backbone atoms for all residues 1–195		1.78 ± 0.1 ^e
Ramachandran statistics		
66% in the most favourable		
20% in the additionally allowed		
9% in the generously allowed		

^aThe average r.m.s.ds for the final 10 structures.

^bÅngstroms.

^cDegrees.

^dHertz.

^eThe average r.m.s.ds from the average structure.

overall root mean square deviation (r.m.s.d.) between the family and the mean co-ordinate position is 1.55 ± 0.1 Å for all the backbone atoms excluding loop A'B, loop CD and loop EF (1.78 ± 0.1 Å for all backbone atoms between 1 and 195).

PapGII-198 forms an open and elongated eight-stranded β-sandwich with long loops and a short helical section connecting the strands (Figure 1B). PapGII-198 is ~80 Å in length and highly asymmetric in solution, as determined by ¹⁵N relaxation data (not shown). The β-strands follow the topology of two Greek key-like motifs linked by two connections and resemble the jellyroll barrel (Figures 1B and 2A), a motif that is found in many carbohydrate-binding and viral-coat proteins (Ito *et al.*, 1991; Logan *et al.*, 1993; Bizebard *et al.*, 1995; Johnson *et al.*, 1996). However, the PapGII-198 jellyroll is extremely elongated and NOE data indicate that the hydrogen-bonding pattern of many principal strands is likely to be interrupted. Additionally, there is a strand insertion between strands A' and H, which is connected by a short helical section from strand H' (see Figures 1B and 2A). This breaks the jellyroll topology and projects the N- and C-terminus to opposite ends of the molecule. Furthermore, PapGII-198 contains a disulfide bridge between residues Cys31 and Cys120 as identified by neighbouring NOE data.

An automated search of the structure database revealed no significantly similar proteins (Holm and Sander, 1993). Despite this result, the FimH adhesin from type 1 pili, which exhibits little sequence homology with PapGII, shares several structural

Fig. 1. The solution structure of PapGII-198. **(A)** Stereo-image backbone traces for the 10 lowest energy structures of PapGII-198 superimposed onto the mean coordinate position. Superimposition is based on the backbone atoms of 1–19, 25–70, 79–92, 96–195. The average structure is shown in orange. **(B)** Schematic diagram illustrating the arrangement of secondary structure elements for PapGII-198. β -strands are shown as blue arrows, helices as red cylinders and the strand insertion to the jellyroll motif is shaded magenta. **(C)** Sequence alignment and topology for the PapG family of adhesins. The residues highlighted in red and green represent identity and conservation with the PapG class II sequence, respectively. The approximate location of secondary structure elements is also indicated; helices delineated as tubes and β -strands as arrows. The strand insertion to the jellyroll motif is shaded magenta. The amino acid positions are shown for PapGII from DS17 and correspond to residues 21–219 in full-length PapGII. Asterisks represent amide resonances that are significantly perturbed upon the addition of galabiose.

similarities, which is suggestive of common evolutionary origins. The lectin domain of FimH is also formed by an elongated β -barrel with an interrupted jellyroll-like motif. In addition to these

apparent similarities there are some intriguing differences between the FimH and PapGII lectin domains. First, the PapGII adhesin contains over 30% more amino acids than FimH and

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therefore the overall structure is further elongated (see Figure 2). Secondly, FimH starts with a 15 amino acid β -hairpin extension that is not present in PapGII-198, in which the N-terminus starts at the third FimH strand. These two features further exaggerate the difference in lengths of the two molecules; FimH being $\sim 50\text{\AA}$ and PapGII $80\text{\AA}$. Finally, PapGII-198 contains a short helical section, which precedes the C-terminal strand insertion. This comparison is illustrated in Figure 2.

The carbohydrate-binding site of PapGII-198

Although the optimal glycolipid receptor for PapGII has been localized to the tetrasaccharide Gb_4 (Stromberg *et al.*, 1990), it has been shown that soluble $\text{Gal}\alpha(1-4)\text{Gal}\beta$ (galabiose) can effectively inhibit the agglutination between uropathogenic *E. coli* and human cells (Bock *et al.*, 1985). We therefore used the readily available galabiose to map the carbohydrate-binding site of PapGII by using NMR chemical shift perturbation studies. The amides of surface-exposed residues that exhibit large chemical shift changes and/or line broadening in the presence of the carbohydrate reveal residues likely to be involved in binding. A comparison of the ^1H - ^{15}N HSQC spectra with and without saturating amounts of galabiose is shown in Figure 3A. The binding of galabiose to PapG is in fast exchange limit on the NMR timescale therefore making the assignment of perturbed resonance straightforward. Twelve significantly perturbed amide resonances were observed whilst the rest of the spectrum remained unchanged, which indicates the formation of a specific complex between PapGII-198 and galabiose (see Figure 3B). The affected residues are illustrated on the PapG structure in Figure 3C, in which they are highlighted on a contact surface representation of PapGII-198. The residues that exhibit the largest chemical changes include Ile173, Lys172, Tyr166, Glu109, Leu102 and Ser89. These residues are largely surface exposed and lie on one side of the PapG molecule. Intriguingly, the conservative variations in residue type for these positions may have implications for PapG class specificity.

The carbohydrate recognition region implicated by the results of our NMR-based PapGII-galabiose titration contains both hydrophilic and aromatic residues, which are usually responsible for carbohydrate recognition. This is consistent with previous observations and a proposed model in which hydrogen-bonding partners are required together with hydrophobic stacking surfaces (Kihlberg *et al.*, 1989). Furthermore, the recognition of Gb_3 by the verotoxin from pathogenic *E. coli* has been reported to require an aromatic side chain for stacking to the $\text{Gal}\beta$ moiety and an array of charged and hydrophilic side chains for hydrogen bonding interactions (Shimizu *et al.*, 1998; Thompson *et al.*, 2000). The putative binding site for mannose on FimH has been located at its N-terminus, where it resides on the tip of the structure at the opposite end to the pilin-binding domain. In PapGII-198 the carbohydrate-binding surface lies on one side of the molecule. This has several implications for the recognition of carbohydrate by PapGII-198. First, a larger binding surface would be required for recognition of the tetrasaccharide receptor of PapGII, which is much larger than a single mannose moiety. Also, it has been postulated that these oligosaccharides are kinked about the galabiose junction particularly when tethered to the membrane (Magnusson *et al.*, 1993; Gronberg *et al.*, 1994) making the galabiose moiety more accessible for an

Fig. 2. The structural comparison between PapGII and the FimH adhesins. (A) Ribbon representation of PapGII-198. (B) Ribbon representation of the FimH adhesin domain. β -strands are shown as blue arrows, helices as red cylinders and the strand insertions to the jellyroll motif are shaded magenta.

intimate interaction. The side-on binding pockets could be perfectly adapted to accommodate such a kinked oligosaccharide, providing an optimal surface area for interaction.

This study provides the first structural insight into the molecular basis of the P-pili and host-cell interactions in UPEC infection. The results also provide insight into UPEC pathogenesis as well as aiding the development of preventative therapies and the guidance of future mutagenesis programmes. This work also further demonstrates the usefulness of combined perdeuteration/protonation approaches and NMR spectroscopy in the rapid generation of structural information with clear functional implications.

METHODS

Protein expression. The DNA encoding PapGII-198 (*E. coli* strain DS17) was cloned into the expression vector pET21b and corresponds to residues 660–939 in intact PapG-II. Over-expression and purification of $\text{U-}^2\text{H}$, ^{13}C , ^{15}N PapGII-198 samples have been described elsewhere (Sung *et al.*, 2001). Pure PapGII-198 was dialysed into 20 mM sodium acetate buffer pH 5.2 and concentrated for NMR. An anisotropic liquid crystal sample of PapGII-198 was prepared in 3.0% C12E5/hexanol ($r = 0.96$) (Ruckert and Otting, 2000).

NMR spectroscopy. Sequence-specific backbone $^1\text{H}_\text{N}$, ^{15}N , $^{13}\text{C}\alpha$, $^{13}\text{C}\beta$ and C' assignments for PapGII-198 have been reported previously (Sung *et al.*, 2001). Identical solution conditions, i.e. ~ 0.5 mM protein, 20 mM sodium acetate buffer pH 5.2, 37°C were employed in the current work. NMR experiments

Fig. 3. Identification of the binding site for galabiose in PapGII-198. **(A)** Region of the ^1H - ^{15}N HSQC NMR spectrum for ^2H , ^{13}C , ^{15}N -labelled PapGII-198 with the identical region of the spectrum for ^2H , ^{13}C , ^{15}N -labelled PapGII-198 with equimolar amounts of galabiose overlaid (red). Peaks corresponding to amides that are affected upon addition of galabiose are labelled with their residue number. **(B)** Column chart of summed $^1\text{H}/^{15}\text{N}$ chemical shift perturbations in the presence of galabiose (weighted n according to ^1H and ^{15}N chemical shift ranges for PapGII-198 i.e. $4.7\delta_{^1\text{H}} + \delta_{^{15}\text{N}}$). Contact-surface representation **(C)** for the structure of PapGII-198. Residues shown in red and yellow experience $^1\text{H}/^{15}\text{N}$ chemical shift perturbations in excess of 1 p.p.m. and between 0.35 and 1 p.p.m., respectively. Only solvent-accessible residues are annotated, as these are most likely to be directly involved in binding.

were performed at 500 MHz proton frequency. The $^1\text{H}/^{13}\text{C}$ -edited NOESY-HSQC spectrum was also repeated at 800 MHz.

PapGII-198 exhibited a molecular reorientation time consistent with a much larger protein; it was therefore necessary to introduce deuterium into the sample in order to facilitate sequential assignment and structure calculation. Only a limited NOE data set is available from perdeuterated material, which precludes the calculation of high-resolution structures. Partial assignment of the protonated spectra, particularly hydrophobic residues, can yield significantly more NOE data and facilitate significant improvement in precision. This was the chosen strategy for PapGII-198 and ~90% of all methyl group assignments were achieved. The side chain assignments were determined using a combination of HCCH-total correlation (TOCSY) spectroscopy (Bax *et al.*, 1990) and HBHA(CO)NH experiments (Grzesiek and Bax, 1992a,b; Kay *et al.*, 1994; Muhandiram and Kay, 1994). Distance restraints for use in structure calculation were obtained from $^1\text{H}/^{15}\text{N}$ - and $^1\text{H}/^{13}\text{C}$ -edited NOESY-HSQC experiments (Fesik and Zuurweg, 1988; Norwood *et al.*, 1990), as well as $^{15}\text{N}/^{15}\text{N}$ -, $^{15}\text{N}/^{13}\text{C}$ - and $^{13}\text{C}/^{13}\text{C}$ -edited HMQC-NOESY-HSQC experiments (Vuister *et al.*, 1993). NOE mixing times of 80 and 200 ms were employed. Amide protons involved in hydrogen

bonds were identified by the presence of NH resonances in HSQC spectra recorded 12 h after dissolving in D_2O . For PapGII-198 surface mapping experiments, a ^{15}N -labelled sample was prepared in 20 mM sodium acetate pH 5.2 and split into two. Galabiose was introduced into one at saturating amounts and then the two samples were mixed in stepwise fashion; this ensured that pH and buffer effects were reduced to a minimum. ^1H - ^{15}N HSQC spectra were recorded in order to follow the titration. $^1\text{D}_{\text{NH}}$ values were measured using interleaved ^{15}N - ^1H IPAP-HSQC spectra with carbonyl decoupling (Ottiger *et al.*, 1998). All experiments incorporated gradient sensitivity enhancement. NMR data were processed using XWINNMR and nmrPipe, and analysed using AURELIA.

Structure determination. NOE cross-peak intensities were measured at both 80 and 200 ms mixing times. The structure calculations utilized a hybrid torsion angle and Cartesian co-ordinate dynamics protocol, executed with CNS (Brunger *et al.*, 1998), and were based on 1238 NOE and 122 H-bond distance restraints, 212 dihedral angles and 148 dipolar couplings. The distance restraints were calibrated internally using known sequential distances. In deuterated material NOEs observed at a mixing time of 200 ms were placed into four categories on the basis of

estimated NOE cross-peak intensity: strong (<3.0 Å), medium (<5.0 Å) weak (6.8 Å) and very weak (<7.5 Å). In protonated spectra NOEs observed at 80 ms mixing time were grouped into strong (<3.7 Å), medium (<3.5 Å) and weak (5.0 Å). Hydrogen bonds were introduced into the calculation as two distance restraints $d_{\text{HN-O}} = 1.8\text{--}2.6$ Å and $d_{\text{N-O}} = 1.8\text{--}3.5$ Å. Two hundred and twelve approximate ϕ , ψ dihedral angle restraints were estimated using the backbone torsion angle prediction package TALOS (Cornilescu et al., 1999), in which $^{13}\text{C}\alpha$, $^{13}\text{C}\beta$ and $^{13}\text{C}'$ shifts were corrected for deuterium isotope effects (Venters et al., 1996). Five degrees was added to all estimated dihedral angle errors. No distance violation >0.5 Å, no dihedral violation $>5.0^\circ$ and no dipolar coupling violation >2 Hz (over a 70 Hz range) has been found.

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