

N-Terminal Amino Acid Sequence of Pilin Isolated from *Pseudomonas aeruginosa*

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The amino-terminal amino acid sequence of the pili protein from *Pseudomonas aeruginosa* K pili is presented. The sequence is compared with those reported by others for pilin obtained from *Neisseria gonorrhoeae* and *Moraxella nonliquefaciens*. All three sequences are highly homologous, contain only two hydrophilic residues in the first 22 positions, and contain an unusual amino acid, N-monomethylphenylalanine, at the amino terminus.

The polar PSA pili of *Pseudomonas aeruginosa* K (PAK) mediate the infectious process of several *Pseudomonas* bacteriophages (2, 4). A pilus retraction stage is evidently involved in the phage infectious process since Bradley (2) has isolated a nonretractile mutant (PAK/2Pfs) which is both phage resistant and multipiliated. In an earlier publication (10), we demonstrated that PAK/2Pfs pili are identical to the wild-type PAK pili and that they comprise a single protein subunit of molecular weight 17,800. The pilin subunit was found to lack histidine and to contain 43% hydrophobic amino acids. The N-terminal residue was shown to be N-monomethylphenylalanine (11).

The present communication describes the sequence of the first 22 amino acids of the amino-terminal region of PAK pilin and compares this to the amino-terminal sequences of pilin obtained from *Neisseria gonorrhoeae* (12) and *Moraxella nonliquefaciens* (9). Automated Edman degradations were performed on 1 to 5 mg of protein with a Beckman model 890B Sequencer, utilizing either the standard 1 M Quadrol buffer system of Edman and Begg (8), or the 0.1 M Quadrol system of Brauer et al. (5). All reagents used were "Sequanal" grade (Pierce Chemical Co.), and all solvents were "Distilled-in-glass" grade (Burdick and Jackson Laboratories Inc., Muskegon, Mich.). Residues were identified by a combination of three methods. Portions of the sequencer products, the anilinothiazolinone derivatives, were converted to the corresponding phenylthiohydantoin by exposure to 1 M HCl at 80°C for 10 min (8) in order to permit identification by gas-liquid chromatography on a column (1.2 m by 2 mm) of 10% SP-400 on 100/120 Supelcoport (Supelco, Inc., Bellefonte, Pa.), using a Beckman GC-45 gas chromatograph (14). Thin-layer chromatography of these phenylthiohydantoin derivatives

on silica gel plates (F254, Merck and Co., Inc., Rahway, N.J.) utilizing the solvent systems chloroform-ethanol, 98:2 (vol/vol), and chloroform-ethanol-methanol, 88.2:1.8:10 (vol/vol) was also employed (6). In addition, amino acid analysis by a Durrum D-500 analyzer after hydrolysis of the thiazolinone derivatives with hydroiodic acid (Ultrapure grade, Alfa Chemicals Ltd., Montreal, Quebec) at 127°C for 20 h was used (15). Identification of the amino-terminal residue by these methods, together with mass spectroscopy and proton magnetic resonance spectroscopy, has already been described (11).

The first 22 amino acid residues were cleaved and identified as:

1		5		10								
MePhe	-Thr	-Leu	-Ile	-Glu	-Leu	-Met	-Ile	-Val	-Val			
	15			20								
	Ala	-Ile	-Ile	-Gly	-Ile	-Leu	-Ala	-Ala	-Ile	-Ala	-Ile	-Pro

It is of interest to compare the foregoing sequence of PAK pilin to corresponding amino-terminal sequences obtained for pilin derived from *M. nonliquefaciens* (9) and *N. gonorrhoeae* (12). As shown in Fig. 1, all three amino-terminal sequences are highly homologous. The only differences are Val-Ile exchanges in positions 10, 13, and 19, and an Ile-Leu exchange in position 21. Whereas Frøholm and Sletten (9) did not identify the N-terminal residue, their evidence suggests that it is N-methylphenylalanine. It is of interest that both *M. nonliquefaciens* and *P. aeruginosa* K pilin yielded a single N-terminal residue, suggesting that 100% of the pilin subunits contained N-methylphenylalanine. In *N. gonorrhoeae* pili, on the other hand, about half the pilin molecules lacked the N-methylphenylalanine amino-terminal residue (12).

To our knowledge, N-methylphenylalanine has not yet been detected in nonpilus proteins,

	1				5					10					
PAK:	Met	Phe	Thr	Leu	Ile	Glu	Leu	Met	Ile	Val	Val	Ala	Ile	Ile	Gly
NEISSERIA:	Met	Phe	Thr	Leu	Ile	Glu	Leu	Met	Ile	Val	Ile	Ala	Ile	Val	Gly
MORAXELLA:	X	-	Thr	Leu	Ile	Glu	Leu	Met	Ile	Val	Ile	Ala	Ile	Ile	Gly

	15				20									
PAK:	Ile	Leu	Ala	Ala	Ile	Ala	Ile	Pro						
NEISSERIA:	Ile	Leu	Ala	Ala	Val	Ala	Leu	Pro	Ala	Tyr	Gln			
MORAXELLA:	Ile	Leu	Ala	Ala	Ile	Ala	Leu	Pro	Ala	Tyr	Gln			

FIG. 1. PAK: amino-terminal amino acid sequence of the pili protein of *P. aeruginosa* K. NEISSERIA: amino-terminal amino acid sequence of the pili protein of *N. gonorrhoeae*, from Hermodson et al. (12). MORAXELLA: amino-terminal amino acid sequence of the pili protein of *M. nonliquefaciens*, from Frøholm and Sletten (9).

although it has been shown to be a constituent of the peptide antibiotic Staphylomycin S (16). It is worth noting that Pettigrew and Smith (13) have reported the finding of dimethylproline at the amino terminus of *Crithidia oncopelti* cytochrome C557, whereas Chen et al. (7) have shown that certain ribosomal proteins of *Escherichia coli* contain N-monomethylalanine or N-monomethylmethionine as amino-terminal residues. Unfortunately, the biological significance of α -methylated N-terminal amino acid residues is not understood.

The occurrence of a highly hydrophobic, common sequence at the amino terminus of pilin produced by three relatively unrelated microorganisms suggests this common sequence may be important to pilus function. For example, the finding by Beard and Connolly (1) that F pilin subunits are found in the outer membrane of *E. coli* suggests that other types of pili may also be assembled in this region of the cell. It is therefore conceivable that the highly hydrophobic amino-terminal portion of the pilus protein may provide a pilot function which facilitates the transport of pilin subunits from the cell interior to the outer membrane. Once the pilin subunits are assembled into pili, the highly hydrophobic amino-terminal sequence would presumably be buried within the subunit or involved in subunit-subunit interactions.

Although it is tempting to suggest that the foregoing ideas may be applicable to many other pilus systems, it is noteworthy that the amino-terminal sequence (12) of type 1 pili of *E. coli* (previously called common pili) bears no homology to the three sequences discussed in the present communication, and contains no N-methylphenylalanine. Clearly, further studies on

the structure of a wide variety of pilus types are needed to elucidate the structure-function relationships of these interesting filamentous structures.

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LITERATURE CITED

1. Beard, J. P., and J. C. Connolly. 1975. Detection of a protein, similar to the sex pilus subunit, in the outer membrane of *Escherichia coli* cells carrying a derepressed F-like R factor. *J. Bacteriol.* **122**:59-65.
2. Bradley, D. E. 1966. The structure and infective process of the *Pseudomonas aeruginosa* bacteriophage containing ribonucleic acid. *J. Gen. Microbiol.* **45**:83-96.
3. Bradley, D. E. 1974. The adsorption of *Pseudomonas aeruginosa* pilus-dependent bacteriophages to a host mutant with nonretractile pili. *Virology* **58**:149-163.
4. Bradley, D. E., and T. L. Pitt. 1974. Pilus-dependence of four *Pseudomonas aeruginosa* bacteriophages with non-contractile tails. *J. Gen. Virol.* **24**:1-15.
5. Brauer, A. W., M. N. Margolies, and E. Haber. 1975. The application of 0.1 M Quadrol to the microsequence of proteins and the sequence of tryptic peptides. *Biochemistry* **14**:3029-3035.
6. Bridgen, J., A. P. Graffe, B. L. Karger, and M. D. Waterfield. 1975. The identification of PTH amino acids, p. 111-145. In R. N. Perham (ed.), *Instrumentation in amino acid sequence analysis*. Academic Press, London.
7. Chen, R., J. Brosius, and B. Wittmann-Liebold. 1977. Occurrence of methylated amino acids as N-termini of proteins from *E. coli* ribosomes. *J. Mol. Biol.* **111**:173-181.
8. Edman, P., and G. Begg. 1967. A protein sequenator. *Eur. J. Biochem.* **1**:80-91.
9. Frøholm, L. O., and K. Sletten. 1977. Purification and N-terminal sequence of a fimbrial protein from *Moraxella nonliquefaciens*. *FEBS Lett.* **73**:29-32.
10. Frost, L., and W. Paranchych. 1977. Composition and molecular weight of pili purified from *Pseudomonas aeruginosa* K. *J. Bacteriol.* **131**:259-269.
11. Frost, L. S., M. Carpenter, and W. Paranchych. 1978. N-Methylphenylalanine at the N-terminus of pilin isolated from *Pseudomonas aeruginosa* K. *Nature (London)* **271**:87-89.
12. Hermodson, M. A., K. C. S. Chen, and T. M. Buchanan. 1978. *Neisseria* pili proteins: amino-terminal amino acid sequences and identification of an unusual amino acid. *Biochemistry* **17**:442-445.
13. Pettigrew, G. W., and G. M. Smith. 1977. Novel N-terminal protein blocking group identified as dimethylproline. *Nature (London)* **265**:661-662.
14. Pisano, J. J., and T. J. Bronzert. 1969. Analysis of amino acid phenylthiohydantoins by gas chromatography. *J. Biol. Chem.* **244**:5597-5607.
15. Smithies, O., D. Gibson, E. M. Fanning, R. M. Goldfiesch, J. G. Gilman, and D. L. Ballantyne. 1971. Quantitative procedures for use with the Edman-Begg sequenator. Partial sequences of two unusual immunoglobulin light chains, Rzf and Sac. *Biochemistry* **10**:4912-4921.
16. Vanderhaeghe, J., and G. Parmentier. 1960. The structure of factor S of Staphylomycin. *J. Am. Chem. Soc.* **82**:4414-4422.