FIMBRIAL COLONIZATION FACTOR CFA/1 PROTEIN FROM HUMAN ENTEROPATHOGENIC *ESCHERICHIA COLI* STRAINS

Purification, characterization and N-terminal sequence

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This work presents a new and easy means of purifying CFA/1 fimbriae in good yield, which gives rise to one band in sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoreses, indicating a subunit app. mol. wt 14 500. Furthermore, amino acid analyses have been carried out on the fimbriae. Manual Edman degradation of the 22 N-terminal amino acid residues as well as investigation of the C-terminal sequence by carboxypeptidase Y, indicates the sequence of the CFA/1 fimbrial subunit to be different from hitherto known fimbrial primary structures.

1. Introduction

Fimbriae (also called pili) are long thread-like proteinaceous appendages found on the surfaces of many bacteria. These structures have been found widely distributed among bacterial genera [1].

A typical fimbriated bacterium carries 100–300 fimbriae peritrichously arranged on its surface. Most fimbriae are 0.5–1.5 μm long with ~ 7 nm diam. [1]. Each fimbria consists of ~ 100 identical subunits with mol. wts 14–26 × 10^3.

Fimbriae are known to confer specific adhesion of pathogenic bacteria to host tissues. For example, fimbriated *Neisseriae gonorrhoeae* strains adhere to epithelial cells in the human urogenital tract [2].

An interesting group of plasmid-mediated fimbriae from enteropathogenic strains of *Escherichia coli*, known to confer adhesive and colonizing properties for the intestinal epithelium of several domestic animals and man to the host strain, encompasses the K88 antigen (pig), the K99 antigen (cow and sheep) and the colonization factor antigens CFA/1 and CFA/2 from human *E. coli* strains.

The CFA/1 is commonly found on pathogenic *E. coli* strains isolated from adults or children with naturally acquired diarrhea [3–5], and has been shown to have a widespread geographic occurrence [6]. CFA/1 has been isolated by isoelectric precipitation and ultracentrifugation techniques, and purified fimbriae can be seen electron microscopically as thin threads of 7 nm × 1 μm [3]. Furthermore, the CFA/1 fimbriae appears to be specified by a 60 × 10^6 mol. wt plasmid [7].

2. Experimental

2.1. *Bacterial strain and purification of fimbriae*

The bacterial strain used for the isolation of CFA/1 was H-10407, originally isolated from a patient in Bangla-desh [8]. Cultivation of bacteria took place on solid medium in order to suppress the appearance of type 1 fimbriae [9]. Cells from confluent growth on 50 broth-agar plates (14 cm) were harvested and suspended in 300 mol 0.1 M sodium phosphate (pH 7.0).

The suspension was heated to 60°C for 20 min and sheared in a Turrax-blender, in order to detach the fimbriae. The cells were spun down by centrifugation (50 min at 16 500 × *g*), and the supernatant filtered (0.80 μm pore size; Millipore Filter Corp.). The filtered supernatant was then applied to a Sepharose 2B column (5 × 84 cm) and eluted with 0.1 M NH_4HCO_3 (pH 7.5) (fig.1). The pooled material was lyophilized.

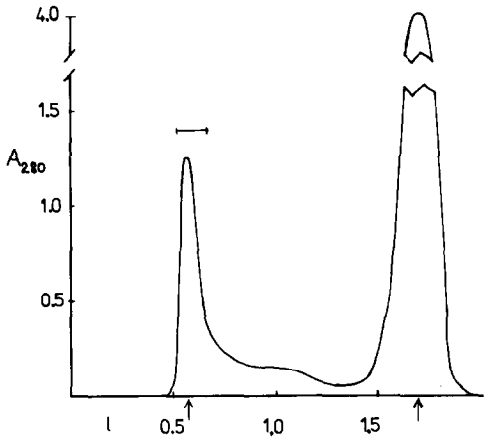

Fig.1. Fractionation of a crude extract of CFA/1 fimbriae on a Sepharose 2B column (5 × 84 cm). CFA/1 suspension (100 ml) in 0.1 M sodium phosphate (pH 7.0) was applied to the column. The column was eluted with 0.1 M NH_4HCO_3 at 75 ml/h flowrate. Fractions (18.5 ml) were collected. The bar indicates the pooled fractions, and the arrows indicate V_o and V_t.

2.2. *SDS–polyacrylamide electrophoresis*

Purity was assessed by SDS–polyacrylamide gel electrophoresis of fluorescamine-treated CFA/1-fimbriae [10].

2.3. *Amino acid analysis*

Amino acid analysis was performed on a Durrum D-500 amino acid analyser. Samples in duplicate, were hydrolysed for 24, 48 and 72 h at 110°C in 6 N HCl containing 0.1% phenol. Furthermore, samples of performic acid oxidized protein were hydrolysed for 24 h to determine cysteine as cysteic acid. For serine and threonine, values extrapolated to zero-time were used, whereas the values for 72 h hydrolysis were used in the case of valine and isoleucine.

2.4. *Determination of the N-terminal sequence*

The primary structure of the N-terminal part of the CFA/1 fimbrial subunit was determined by manual Edman degradation as in [11]. Identification of phenylthiohydantoin (PTH)-amino acid derivatives was performed by high-performance liquid chromatography on a Dupont model 830 liquid chromatograph, equipped with a Waters Microbondapak column [12].

Analyses of the parent amino acids were performed on a Durrum D-500 analyser after conversion with 66% hydrogen iodide in evacuated ampules at 130°C for 20 h.

2.5. *Carboxypeptidase Y digestion*

Digestion of 20 nmol CFA/1 protein in the presence of SDS with carboxypeptidase Y was performed as in [13].

3. Results and discussion

The yield of purified CFA/1 fimbriae was 115 mg, corresponding to 2.3 mg/plate. Fimbriae isolated in the manner described proved to be pure as estimated by SDS–polyacrylamide gel electrophoresis and sequence analysis. The subunit was app. mol. wt. 14 500 as estimated by SDS–polyacrylamide gel electrophoresis in 15% acrylamide gels (fig.2), although a slightly higher value was obtained by electrophoresis in 10% gels.

The amino acid composition of the CFA/1 fimbrial protein was calculated from the average and/or extra-

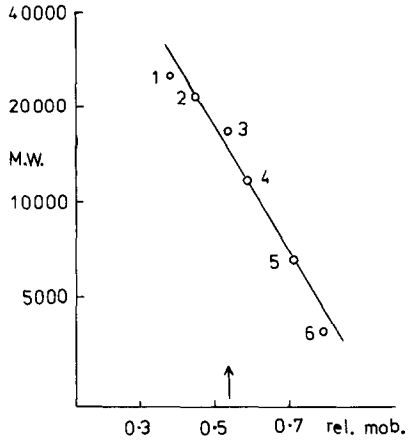

Fig.2. Determination of the molecular weight of the CFA/1 subunit. The relative mobilities to bromophenol blue of 6 marker proteins are plotted against their respective molecular weights. The arrow indicates the relative mobility of the CFA/1 subunit. The following marker proteins were used (mol. wt): (1) chymotrypsinogen A (25 000); (2) trypsin inhibitor, soy bean (21 500); (3) myoglobin, whale (16 900); (4) cytochrome *c*, bovine heart (11 700); (5) aprotinin (6500); (6) insulin B-chain (3800). The extrapolated value for the CFA/1 subunit was 14 500.

Table 1
Amino acid composition of the CFA/1 protein

Amino	Residues/fimbrial subunit	
Asx	18.1	(18)
Thr	12.7	(13)
Ser	10.9	(11)
Glx	12.2	(12)
Pro	4.0	(4)
Gly	10.7	(11)
Ala	16.2	(16)
Cys	0.0	(0)
Val	13.0	(13)
Met	2.1	(2)
Ile	5.8	(6)
Leu	11.3	(11)
Tyr	3.8	(4)
Phe	2.9	(3)
His	0.7	(1)
Lys	7.8	(8)
Arg	3.3	(3)
Trp	n.d.	
Total		136

polated values, and on the basis of mol. wt 14 500 (table 1). The protein has a rather high content of hydrophobic amino acid residues, and contains no cysteine residues, resembling the K88 fimbrial protein [14].

The N-terminal sequence of the first 22 residues of the CFA/1 protein is shown in fig.3 with the N-terminal sequences from the K88 fimbrial protein (mol. wt 25 000), the type 1 fimbrial protein (mol. wt 17 500) of *E. coli* [13,15] and the highly homologous fimbrial proteins of *Moraxella nonliquefaciens*, *Neisseria gonorrhoeae* and *Pseudomonas aeruginosa* [15–17].

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-Met-(Val,Thr,Gly)-Ser-Leu-COOH
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Fig.4. The amino acid sequence of the C-terminal part of the CFA/1 protein.

The sequence of the 6 C-terminal amino acid residues of CFA/1 indicated by carboxypeptidase Y results is depicted in fig.4. The mutual position of the Gly, Thr and Val could not be assessed by their release rates.

The N-terminal sequence of the CFA/1 protein does not show any homology with the other presented N-terminal sequences. Nor does the composition of the C-terminal show any features in common with the only C-terminal sequence available from a fimbrial protein, namely that of the K88 antigen [13]. The sequence can therefore be concluded to the new solution to fimbrial subunit design.

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                1               5                  10                  15                  20
CFA/1:          Val-Glu-Lys-Asn-Ile-Thr-Val-Thr-Ala-Ser-Val-Asp-Pro-Val-Ile-Asp-Leu-Leu-Gln-Ala-Asp-Gly
K88:            Trp-Met-Thr-Gly-Asp-Phe-Asn-Gly-Ser-Val-Asp-Ile-Gly-Gly-Ser-Ile-Thr-Ala-Asp-Gly-Tyr-Gly
Type 1:         Ala-Ala-Thr-Thr-Val-Asn-Gly-Gly-Thr-Val-His-Phe-Lys-Gly-Glu-Val-Val-Asn-Ala-Ala
Neisseria:   MePhe-Thr-Leu-Ile-Glu-Leu-Met-Ile-Val-Ile-Ala-Ile-Val-Gly-Ile-Leu-Ala-Ala-Val-Ala-Leu-Pro
Moraxella:   MePhe-Thr-Leu-Ile-Glu-Leu-Met-Ile-Val-Ile-Ala-Ile-Ile-Gly-Ile-Leu-Ala-Ala-Ile-Ala-Leu-Pro
Pseudomonas: MePhe-Thr-Leu-Ile-Glu-Leu-Met-Ile-Val-Val-Ala-Ile-Ile-Gly-Ile-Leu-Ala-Ala-Ile-Ala-Ile-Pro
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Fig.3. N-terminal amino acid sequence of the CFA/1 fimbrial protein. The N-terminal sequences of the K88 antigen, the type 1 fimbrial protein from *E.coli*, and the fimbrial proteins from *Neisseria*, *Moraxella* and *Pseudomonas* are given for comparison.

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