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MOLECULAR ANALYSIS OF THE ENDOFLAGELLA OF THE PATHOGENIC TREPONEMES.

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A thesis submitted for the degree of Doctor of Medicine

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Abbreviations

2D-PAGE	two dimensional-polyacrylamide gel electrophoresis
AIDS	acquired immunodeficiency syndrome
A_n	Absorbance at n nm
ATP	adenosine triphosphate
cpm	counts per minute
dATP	deoxyadenosine triphosphate
dCTP	dexoycytidine triphosphate
dGTP	deoxyguanosine triphosphate
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
EDTA	(ethylenedinitrilo)tetraacetic acid
FlaA	the mature endoflagellar sheath protein
*fla*A	the gene encoding the endoflagellar sheath protein
FlaB1	a *T. pallidum* endoflagellar core protein of M_r 34.5 kD
*fla*B1	the gene encoding FlaB1
FlaB2	a *T. pallidum* endoflagellar core protein of M_r 33 kD
*fla*B2	the gene encoding FlaB2
FlaB3	a *T. pallidum* endoflagellar core protein of M_r 30 kD
*fla*B3	the gene encoding FlaB3

HEPES	N-2-hydroxyethylpiperazine-N′-2-ethane-sulphonic acid
HIV	human immunodeficiency virus
HPLC	high pressure liquid chromatography
IEF	isoelectric focusing
Ig	immunoglobulin
IPTG	isopropyl-β-D-thiogalactopyranoside
kb	kilobase pairs
kD	kilodalton
min	minute
M_r	relative molecular mass
NP-40	Nonidet 40
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PEG 6000	polyethylene glycol 6000
PMSF	phenyl methyl sulphonyl fluoride
pre-FlaA	the precursor protein of FlaA
RBS	ribosomal binding site
RIP	radioimmunoprecipitation
RNA	ribonucleic acid
RNase	ribonuclease

rpm	revolutions per minute
sarkosyl	N-lauroyl sarcosinate
SDS	sodium dodecyl sulphate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
sec	second
ssDNA	single stranded DNA
subsp.	subspecies
Tris	2-amino-2-(hydroxymethyl)-1,3-propanediol
UV	ultraviolet
vol	volume
wt	weight
X-Gal	5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside

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Abstract

1. Motility of *Treponema pallidum* is mediated by endoflagella located within the periplasmic space. The endoflagella comprise a core surrounded by a sheath, with the sheath being composed of a 37-kD antigen (FlaA).

2. Treponemal endoflagella were isolated and component proteins were purified by reverse-phase high pressure liquid chromatography (HPLC). N-terminal amino acid sequence analysis was performed using the sheath protein and two core proteins from *T. pallidum* subsp. *pallidum*, *T. pallidum* subsp. *pertenue*, and *T. phagedenis*. For all three proteins, the N-terminal sequences found for *T. pallidum* and *T. pertenue* were identical but differed from the sequence of *T. phagedenis*. The sheath proteins from the three treponemes were also compared by HPLC tryptic peptide maps: maps for *T. pallidum* and *T. pertenue* were very similar but differed from *T. phagedenis*.

3. A λgt11 clone, λA34, that expressed a portion of FlaA was selected from a *T. pallidum* genomic library using a murine monoclonal antibody. The insert from λA34 provided a probe with which a chimeric plasmid, pRI4, encoding all but the nine N-terminal amino acids of FlaA was selected from a pBR322 *T. pallidum* genomic library.

4. The nucleotide sequence of *fla*A upstream of amino acid 10 could not be determined by routine cloning strategies.

Instead, the remaining sequence was determined using the inverse- and asymmetric-polymerase chain reaction methods. *fla*A contains a consensus *Escherichia coli* promoter and a ribosome binding site. A single open reading frame encodes a precursor protein, pre-FlaA, of 350 amino acids with a calculated M_r 38,860. A 20 amino acid signal sequence with a typical signal peptidase I cleavage site immediately precedes the known N-terminus of FlaA. Twenty six per cent (91/350) of the DNA-derived amino acid sequence was confirmed by N-terminal sequence analysis of ten tryptic peptides derived from native FlaA.

5. Polymerase chain reaction-derived constructs lacking the native *T. pallidum* promoter were cloned downstream of a T7 promoter. T7 polymerase transcription was under the control of a P_L λ promoter. When the λ repressor was inactivated at 42°C, FlaA was expressed at relatively low levels in *E. coli*. Native and recombinant FlaA were identical as assessed by antibody reactivity and electrophoretic mobility in both sodium dodecyl sulphate- and two dimensional-polyacrylamide gels.

6. Although some pre-FlaA is processed to FlaA in *E. coli*, pre-FlaA is accumulated indicating inefficient processing of pre-FlaA in *E. coli*. Soluble FlaA was not detected in either the cytoplasmic or the periplasmic fractions of *E. coli* transformants. Fractionation of *E. coli* cell envelopes unexpectedly revealed that pre-FlaA and FlaA were associated

with both the inner and outer membranes.

7. Two gene fusions, *cro-lac*Z-*fla*A and *lac*Z'-*fla*A, were constructed in an attempt to increase expression of the recombinant antigen; a bovine protease X_a site was engineered into the hybrid proteins immediately upstream of the N-terminus of FlaA to allow for subsequent proteolytic cleavage of the purified hybrid proteins. The LacZ'-FlaA protein was expressed in *E. coli* in increased amounts as compared with FlaA, but like recombinant FlaA, associated with both the inner and outer bacterial membranes. LacZ'-FlaA can be released from the membranes by treatment with 1% [wt/vol] sarkosyl, 6 M urea, or 6 M guanidine hydrochloride.