Localization of antigenic domains on the major subunits of *Bordetella pertussis* serotype 2 and 3 fimbriae

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Antibody-binding domains on the major subunits of *Bordetella pertussis* serotype 2 (Fim2) and 3 fimbriae (Fim3) have been identified using synthetic peptides which were screened for recognition by anti-protein monoclonal antibodies (mAbs). The presence of non-contiguous fimbrial epitopes was demonstrated by both anti-Fim2 and anti-Fim3 mAbs, several of which recognized at least two peptides that were discontinuous in the amino acid sequence of the corresponding subunits. The specificity of one mAb, 51/24, directed against Fim2, was investigated by replacement-set analysis of a 10-residue peptide, and revealed that antibody binding to the peptide was dependent on the sequence N⁹⁴PQ⁹⁶ which is non-conserved in Fim3. Furthermore, proline at residue 95 was found to be essential for mAb 51/24 binding. The specific anti-Fim3 mAb, AG3A, was found to recognize the 10-residue carboxy-terminal peptide from both Fim3 and, unexpectedly, from Fim2. This result suggests that mAb AG3A serospecificity at the protein level is determined by a conformational constraint which prevents mAb AG3A binding to the Fim2 C-terminal domain. Several free peptides containing amino acid residues which comprise part of the Fim2 and Fim3 epitopic domains were prepared as immunogens. One of these peptides was immunogenic in the mouse, indicating the location of a T-helper cell epitope within the peptide sequence, and induced a strong anti-peptide antibody response. The other peptides each required immunization as a conjugate with a carrier protein for anti-peptide antibody stimulation. All four anti-peptide antibody preparations only weakly recognized fimbriae-coated ELISA plates. The results of this investigation demonstrate that although short linear peptides can mimic sub-domains of non-contiguous fimbrial epitopes, they are, however, poor candidate antigens for stimulating an anti-fimbrial antibody response.

Keywords: *Bordetella pertussis*, fimbriae, antigenic domain

INTRODUCTION

Bordetella pertussis, the aetiological agent of whooping cough, can express two separate fimbrial proteins (Fim2 and Fim3) which are important for the colonization of the host's respiratory tract (Robinson *et al.*, 1991). Fim2 and

Fim3 are each assembled into helical structures (Steven *et al.*, 1986) from multiple copies of their distinct major subunits, which possess molecular masses of 22·5 kDa and 22·0 kDa, respectively (Irons *et al.*, 1985). Recent evidence indicates that the fimbriae are also comprised of accessory subunits which may serve as scaffolding or adhesion proteins (Willems *et al.*, 1992). The DNA-derived amino acid sequences of the major subunits of Fim2 (Livey *et al.*, 1987) and Fim3 (Mooi *et al.*, 1990) display 67% overall amino acid homology, and are particularly conserved at the amino and carboxy termini. Despite the high amino acid sequence identity of these subunits, the fimbriae are

Abbreviations: cBSA, cationized bovine serum albumin; KLH, keyhole limpet haemocyanin; mAb, monoclonal antibody; MBS, *m*-maleimido-*N*-hydroxysuccinimide ester; SMCC, sulpho-succinimidyl *trans*-4-(*N*-maleimidomethyl)-cyclohexane-1-carboxylate; TFE, trifluoroethanol.

serospecific, with only weak cross-reactivity being demonstrated by antibodies which have been raised to either the purified proteins (Ashworth *et al.*, 1985; Robinson, *et al.*, 1989) or induced during the disease process (Preston, 1985; Robinson *et al.*, 1989). Both fimbriae are regarded as important components for vaccines designed to prevent bacterial colonization (Robinson *et al.*, 1989). An understanding of the antigenic structure and the localization of the serospecific fimbrial epitopes could lead to the development of simpler acellular vaccines designed to protect infants from whooping cough.

In this study, sets of multiple overlapping peptides which consecutively spanned the amino acid sequences of the two major subunits were screened with various monoclonal antibodies (mAbs) in order to identify subunit epitopes. The peptides were synthesized and used for antibody screening on polyethylene pins as previously described for the epitope mapping technique (Geysen *et al.*, 1984). This procedure has successfully been used to identify many protein epitopes, including those of bacterial pili (Cassels *et al.*, 1992). Several of the peptides which were recognized by the mAbs were re-synthesized as 'free' peptides and prepared as immunogens to assess their potential for inducing a fimbrial-recognizing antibody response.

METHODS

Materials. The following were purchased from Sigma: anti-mouse immunoglobulin enzyme conjugates, bovine serum albumin (BSA), glutaraldehyde, keyhole limpet haemocyanin (KLH), *m*-maleimido-*N*-hydroxysuccinimide ester (MBS), trifluoroethanol (TFE) and Tween 20. Reagents for pin-peptide synthesis were obtained from Cambridge Research Biochemicals (ICI). The co-purified preparation of Fim2 + Fim3 was derived from *B. pertussis* strain Wellcome 28 by the method of Robinson *et al.* (1989).

Epitope mapping. Consecutive overlapping peptides, synthesized on polyethylene pins and corresponding, respectively, to the Fim2 and Fim3 major subunit amino acid sequences (Livey *et al.*, 1987; Mooi *et al.*, 1990), were prepared using the protocol already described (Tallet *et al.*, 1993). Both 10- and 8-residue peptides, overlapping by 5 and 6 residues, respectively, were synthesized for each subunit amino acid sequence. The 10-amino-acid-residue peptide 19 from Fim2 was also prepared as a series of substitution peptides in a replacement-set synthesis (Getzoff *et al.*, 1987). This involved synthesizing (10 × 19) 10-residue analogue peptides of the parent peptide, in which a different residue of the parent peptide was iteratively replaced by the 19 alternative amino acids. Antibody preparations were screened, either as a dilution of the polyclonal sera or as purified monoclonal immunoglobulin (20–50 µg ml⁻¹), for binding to the pin-peptides as previously described (Tallet *et al.*, 1993), but with species-specific peroxidase antibody conjugates used as the second antibody.

Synthesis of 'free' peptides and peptide conjugation to carrier proteins. Free peptides 1Fim2 and 2Fim2 were each manually synthesized by *t*-Boc chemistry (Kent, 1988) on *N*^ε-Boc-*S*-4-methylbenzyl-L-cysteine phenylacetamidomethyl resins (Bachem), and free peptides 1Fim3 and 2Fim3 were prepared by Applied Biosystems FastMoc chemistry (Kent, 1988) on an

automated synthesizer using 4-[2',4'-dimethoxyphenyl-*N*-(9-fluorenyl methoxy carbonyl)-amino methyl] phenoxy resins (Novabiochem). Following peptide-resin deprotection and cleavage, the peptides were purified by gel filtration and reverse-phase HPLC on a Vydac C₈ column. Peptide composition was confirmed by fast-atom-bombardment mass spectrometry. The purified peptides were conjugated to carrier proteins as follows: (1) cross-linking to KLH via the peptidyl cysteine was achieved with MBS using the method of Green *et al.* (1982); (2) glutaraldehyde was used to couple peptides to BSA as previously described (Seabrook *et al.*, 1990); and (3) conjugation to cationized BSA (cBSA) (Supercarrier, Pierce Chemical) was obtained through the peptidyl cysteine with sulphonosuccinimidyl *trans*-4-(*N*-maleimidomethyl)-cyclohexane-1-carboxylate (SMCC) according to the manufacturer's instructions (Pierce Chemical).

Immunization and antibodies. Murine polyclonal antibodies were induced following intraperitoneal immunization of Porton NIH mice (H-2^a haplotype) with 10 µg of peptide or peptide-carrier conjugate. Each immunogen was pre-adsorbed onto 25% (v/v) alhydrogel (Superfos) as adjuvant. Mice were boosted at 3 weeks and sera collected at 6 weeks after primary immunization. The anti-Fim2 mAbs, 51/21 (IgG), 51/24 (IgG), AG2A (IgM) and AG2B (IgM), and the IgM anti-Fim3 mAbs AG3A, AG3B and AG3C were derived previously (Ashworth *et al.*, 1985). Purified immunoglobulin was obtained either by gel filtration with Superose 6 (Pharmacia) or by affinity chromatography with protein A Sepharose (Pharmacia).

ELISA. Plates were coated with either synthetic peptides or native protein as described before (Seabrook *et al.*, 1990). Whole-cell ELISA plates were prepared by diluting a formalin-inactivated culture of *B. pertussis* (strain Wellcome 28; Fim2⁺ Fim3⁺), grown on charcoal agar containing 10% (v/v) defibrinated horse blood, to an OD₆₇₀ of 0.9 and 100 µl aliquoted to the wells of the ELISA plate. After overnight evaporation at 37 °C, these plates were washed with phosphate-buffered saline, pH 7.2, containing 0.1% (v/v) Tween 20. Primary antibodies were employed in each ELISA as a serial dilution and the colour and endpoint titre, calculated as the antibody dilution which would yield an *A*₄₅₀ of 1.0, were obtained as before (Seabrook *et al.*, 1990).

Circular dichroism. Far-UV circular dichroism spectra (250–190 nm) of peptide 1Fim2 in TFE/H₂O mixtures were measured with a Jasco-J 600 spectropolarimeter at a peptide concentration of 17 µM in a 0.05 cm pathlength cell. Four spectra, measured for each solution at a scan speed of 10 nm min⁻¹ and time constant of 4 s, were computer-averaged. Similarly averaged blank spectra were subtracted, and corrected spectra converted to mean residue ellipticities [*θ*] using a mean residue weight of 110.5 for the peptide.

RESULTS

Fim2 epitope mapping

Four purified mAbs, AG2A, AG2B, 51/24 and 51/21, which are effectively serospecific for the Fim2 protein, were initially screened for recognition of 10-amino-acid-residue peptides, overlapping by five amino acids, which consecutively spanned the complete amino acid sequence of the mature Fim2 subunit. The only antibody able to demonstrate unequivocal binding to a synthetic peptide was 51/24 (Fig. 1a). This antibody recognized peptide 19 (Y⁹¹ATNPQTQLS¹⁰⁰) and did not bind the correspond-

Fig. 1. Epitope mapping with mAb 51/24 of (a) consecutive 10-residue peptides of Fim2 overlapping by five amino acids, (b) as (a) with Fim3 peptides and (c) consecutive 8-residue Fim2 peptides overlapping by six amino acids.

ing Fim3 peptide, peptide 18 ($Y^{90}NASGNGQLS^{99}$), which shows 40 % sequence homology (Fig. 1b). When this mAb was screened with Fim2 peptides of eight amino acids overlapping by six residues (Fig. 1c), antibody binding was found to be directed against the same protein domain as that delineated by the 10-residue peptides, but had been dispersed over three overlapping peptides. These were peptides 45 ($M^{89}VYATNPQ^{96}$), 46 ($Y^{91}ATNPQTQ^{98}$) and 47 ($T^{93}NPQTQLS^{100}$), which share the consensus sequence $T^{93}NPQ^{96}$. Other anti-Fim2 mAbs reacted less strongly with these peptides.

Replacement-set analysis of the mAb 51/24 antigenic peptide 19

To identify the amino acids of the 10-residue peptide, $Y^{91}ATNPQTQLS^{100}$, which contribute to binding the mAb 51/24 paratope, each residue was iteratively substituted with the 19 alternative naturally occurring L-amino acids in a replacement-set (Getzoff *et al.*, 1987). When the resultant substitution peptides were screened

Fig. 2. Replacement-set analysis of (a) N^{94} , (b) P^{95} and (c) Q^{96} from Fim2 peptide 19, $Y^{91}ATNPQTQLS^{100}$. Peptides were screened with mAb 51/24.

for mAb 51/24 binding (Fig. 2), it was found that the residues $Y^{91}AT^{93}$ and $T^{97}QLS^{100}$ could be substituted without significant detriment to antibody binding, but residues comprising the sequence $N^{94}PQ^{96}$, which are part of the previously deduced consensus sequence, were essential for paratope recognition. For example, N^{94} could only be substituted with the conserved amino acid glutamine and by the hydrophobic amino acids tyrosine and phenylalanine (Fig. 2a). There was an absolute requirement for P at residue 95, and replacement of this residue by any of the other amino acids used in the replacement-set resulted in a loss of mAb recognition (Fig. 2b). Interestingly, Q^{96} could not be replaced by an asparagine residue, but substitution with methionine was possible without complete loss of mAb recognition (Fig. 2c).

Fim3 epitope mapping

Three Fim3 serospecific mAbs, AG3A, AG3B and AG3C, were screened for their ability to recognize 10-amino-acid-residue peptides which consecutively spanned the complete amino acid sequence of the mature Fim3 subunit (Mooi *et al.*, 1990). Only two of the purified mAbs, AG3A and AG3B, demonstrated significant binding to any of the synthetic peptides, which were principally those of the central and C-terminal domain (Fig. 3a and

Fig. 3. Epitope mapping with mAb AG3A of 10-residue peptides overlapping by five amino acids which were derived from (a) Fim3 or (b) Fim2.

Fig. 4. Epitope mapping with mAb AG3B of 10-residue peptides overlapping by five amino acids which were derived from (a) Fim3 or (b) Fim2.

Fig. 4a). For AG3A, recognition of peptides 17 (G⁸¹DLIAKYKTQTY⁹⁰) and 29 (T¹⁴¹NGSKSYTLR¹⁵⁰) was most marked. There was also appreciable cross-reaction of AG3A with the C-terminal Fim2 peptide 36 (T¹⁹⁸YVGFSVVYP²⁰⁷) (Fig. 3b). Fim3 peptide 15 (E⁷¹PGITTNYDT⁸⁰) produced the strongest binding with mAb AG3B (Fig. 4a), and there was no significant recognition of a specific Fim2 peptide (Fig. 4b) by this mAb.

Antibody recognition of 'free' linear peptides

Several peptides, consisting of 19 amino acid residues or greater, were chemically synthesized with their amino acid sequences corresponding to some of the antigenic regions identified from the above epitope mapping procedures (Table 1). Peptide 1Fim2 incorporated the Fim2 antigenic residues recognized by mAb 51/24 (Figs. 1a and 1c); the amino acid sequence of peptide 2Fim2 was

derived from the C-terminal 20 amino acids of Fim2, which is 66 % homologous with the C-terminus of Fim3. The amino acid sequence of 1Fim3 spans the antigenic central domain of the Fim3 subunit (e.g. Figs. 3a and 4a), and peptide 2Fim3 represents the Fim3 antigenic peptides 29–31 (Figs. 3a and 4a). When these peptides were coated onto the solid-phase of ELISA plates for use as antigens and subsequently incubated with various anti-protein antibody preparations (Tables 2 and 3), mAb 51/24 was found to recognize peptide 1Fim2 strongly and 2Fim2 weakly (Table 2). Interestingly, mAb 51/21 was also found to recognize 2Fim2 (Table 2), although this antibody did not demonstrate any significant binding to the 10-residue peptides previously used for the epitope mapping of Fim2 (data not shown). Both peptides 1Fim2 and 2Fim2 were recognized by polyclonal anti-native Fim2 sera in this ELISA configuration (Table 2). Of the two peptides derived from Fim3, only the shorter peptide, 2Fim3, was recognized by mAbs AG3A and AG3C

Table 1. Free peptides

Peptide	Subunit	Amino acid sequence
1Fim2*	Fim2	G ⁸² DLRAYKMVYATNPQTQLSN ¹⁰¹
2Fim2*	Fim2	K ¹⁶¹ NGDVEASAITTYVGFSVVYP ¹⁸¹
1Fim3†	Fim3	Q ⁶¹ ALGALKLYFEPGITTNYDTGDLIAKYKTYNASGN ⁹⁵
2Fim3*	Fim3	K ¹⁴⁰ VTNGSKSYTLRYLASVVK ¹⁵⁸

* GC sequence was added at the C-terminus for protein cross-linking.

† C added to the N-terminus for protein cross-linking.

Table 2. Antibody ELISA titres obtained with peptide, fimbriae and whole-cell antigens for anti-Fim 2 and anti-Fim 2 peptide antibody preparations

Antibody preparation	Solid-phase antigen			
	1Fim2	2Fim2	Fim2+3	<i>B. pertussis</i> cells
51/21*	< 20	70	213 000	31 000
51/24*	59 500	900	102 400	110 800
AG2A*	< 20	< 20	192 000	54 200
AG2B*	< 20	< 20	122 000	30 800
Anti-Fim2†	600	60	78 000	67 800
Anti-1Fim2/KLH†	13 200	ND	50	< 20
Anti-2Fim2/cBSA†	ND	10 000	600	100

ND, Not determined.

* mAb ascites fluids.

† Murine polyclonal antisera.

Table 3. Antibody ELISA titres obtained with peptide, fimbriae and whole-cell antigen for anti-Fim 3 and anti-Fim 3 peptide antibody preparations

Antibody preparation	Solid-phase antigen			
	1Fim3	2Fim3	Fim2+3	<i>B. pertussis</i> cells
AG3A*	< 20	50	87 300	6 400
AG3B*	< 20	< 20	53 600	49 400
AG3C*	< 20	1 600	79 850	13 900
Anti-Fim3†	90	100	66 000	28 000
Anti-1Fim3†	29 700	ND	90	30
Anti-2Fim3/BSA†	ND	4 200	70	20

ND, Not determined.

* mAb ascites fluid.

† Murine polyclonal antisera.

(Table 3). Anti-native Fim3 sera recognized both of the Fim3 peptides (Table 3). It is interesting that two mAbs, 51/21 and AG3C, were found to be able to recognize linear peptides in the solid-phase ELISA but not the peptides used in the epitope mapping procedure above. This discrepancy is most likely due to the longer length of peptides 2Fim2 and 2Fim3, compared to the 10-residue peptides employed in the pin-peptide mapping.

Anti-peptide antibodies

To assess whether the peptides listed in Table 1 can induce anti-peptide antibodies which can also recognize the native fimbrial protein, different peptide-carrier protein conjugates were prepared for immunization. The strongest anti-peptide antibody responses were obtained with the following peptide immunogens: 1Fim2 con-

Fig. 5. Far-UV circular dichroism spectra of peptide 1Fim2 as a function of TFE concentration: (a) 0 %, (b) 20 %, (c) 30 %, (d) 40 %, (e) 50 %, (f) 70 % and (g) 90 % (v/v) TFE. The inset shows the effect of increasing concentrations of TFE on the mean residue ellipticity of peptide 1Fim2 measured at 222 nm.

jugated to KLH via the peptide C-terminal cysteine using MBS as the cross-linker; 2Fim2 conjugated to cBSA, using SMCC; 1Fim3 employed as a free peptide immunogen; and 2Fim3 conjugated to BSA using glutaraldehyde. Each of these immunogens induced an anti-peptide antibody response (Tables 2 and 3) which recognized the homologous peptide as antigen. Furthermore, each of these anti-peptide antibodies were able weakly to recognize, in an ELISA, a mixed co-purified preparation of native Fim2+3 (Table 2 and 3). However, only anti-peptide antibodies induced by 2Fim2, 1Fim3 and 2Fim3 were able to weakly recognize whole-cell ELISA fimbriae presented on the surface of *B. pertussis* (Tables 2 and 3).

Peptide structure

Peptide 1Fim2, which was strongly recognized by mAb 51/24, was investigated by circular dichroism to deduce its propensity to fold into a secondary structure. In the far-UV, the circular dichroism spectrum of the peptide in aqueous buffer, with a negative maximum at about 197 nm (Fig. 5), was characteristic of a conformation in which no regular secondary structure predominates. However, as the dielectric constant of the solvent was decreased by successive additions of TFE, changes were observed in the circular dichroism spectrum of the peptide (Fig. 5) which were consistent with the formation of α -helical secondary structure. The increase in mean residue ellipticity at 222 nm induced by TFE was used to construct a titration curve which appeared triphasic (Fig. 5). Triphasic TFE titration curves have previously been obtained for a 20-residue peptide representing the immunodominant domain of the foot-and-mouth disease protein VP1 and were interpreted in terms of the ability of the peptide to adopt three different

conformational states (Siligardi *et al.*, 1991). Thus, although peptide 1Fim2 does not appear to possess a regular secondary structure in aqueous buffer, ordered conformations may still be adopted by segments of the peptide sequence in an appropriate environment, which could include the antibody paratope (Stanfield *et al.*, 1990; Scherf *et al.*, 1992).

DISCUSSION

The anti-protein mAbs used in this study do not recognize either SDS- or guanidinium-hydrochloride-denatured fimbriae (Pearce *et al.*, 1992), indicating that the epitopes of the major subunits are either non-contiguous or conformationally dependent. The presence of non-contiguous fimbrial epitopes was confirmed by peptide mapping using mAb 51/24, directed against Fim2, and mAbs AG3A and AG3B, which are both directed against Fim3. Specifically, mAb 51/24 strongly recognized free peptide 1Fim2 but also weakly recognized free peptide 2Fim2, whose amino acid sequence is located 60 residues distal to 1Fim2 in the amino acid sequence of the subunit. The epitopic mapping profiles derived for antibodies AG3A and AG3B identified the location of epitopic regions throughout the central and C-terminal domains of the Fim3 subunit. An earlier study of ours (Pearce *et al.*, 1992) suggested that mAbs 51/24, AG3A and AG3B do not bind to epitopes dependent on quaternary structure. Thus, it is likely that these non-contiguous epitopes are dependent on tertiary structure and are formed by the juxtaposition of the subunit C-terminal and central domains.

The serospecificity of the two fimbrial antigens was shown by the inability of mAbs 51/24 and AG3B, directed against Fim2 and Fim3, respectively, to show significant recognition of peptides derived from the heterologous subunit. In contrast, mAb AG3A, which has been shown to be specific for Fim3 by different laboratories (Ashworth *et al.*, 1985; Frederiksen *et al.*, 1987), possessed strong binding to the C-terminal 10-residue peptide of Fim2 (peptide 36). The last 9 residues of this peptide are identical to the corresponding Fim3 amino acids (Mooi *et al.*, 1990). This cross-reaction at the peptide level suggests that the serospecificity at the protein level may be due to a structural constraint.

The basis for mAb 51/24 serospecificity for Fim2, displayed at the peptide level, was investigated by replacement-set analysis of Fim2 peptide 19 (Y⁹¹ATNP-QTQLS¹⁰⁰). Replacement of those residues in peptide 19 which shared sequence homology with Fim3 peptide 18 did not significantly affect antibody binding, indicating that these amino acids have no direct role in paratope interaction. A similar finding was also obtained for amino acids A⁹², T⁹³ and T⁹⁷ of the Fim2 peptide which are not present in Fim3 peptide 18. In contrast, the sequence N⁹⁴PQ⁹⁶ was crucial for 51/24 recognition, and a single substitution of any of these residues with the corresponding residue of Fim3 (i.e. N⁹⁴ → S, P⁹⁵ → Q or Q⁹⁶ → N) resulted in a loss of antibody binding. This is an interesting result, since shortening the Q⁹⁶ side-chain by a

single carbon atom was sufficient to disrupt paratope recognition of the peptide. Alternative amino acid substitutions at the N⁹⁴ and Q⁹⁶ positions were found to be tolerable for antibody binding. At position 94, Q, F and Y could each replace N without a significant decrease in antibody binding. In the case of the two hydrophobic side-chains, this may be permissible because of their potential for both hydrogen bonding and hydrophobic contacts with aromatic residues. The latter often predominate in antigen-combining sites (Stanfield *et al.*, 1990; Scherf *et al.*, 1992). The Q⁹⁶ replacement with M is difficult to explain without structural information. However, since N was ineffective at this position, it suggests that M may be making fortuitous contacts with the paratope which are different to those made by the wild-type Q. Proline at position 95 was found to be essential for paratope interaction, a result which has been found for many protein epitopes (e.g. Stanfield *et al.*, 1990; Scherf *et al.*, 1992).

When peptides representing various antigenic domains of the two fimbriae (Table 1) were used as carrier-protein conjugates to immunize, 2Fim2 induced a strong anti-peptide antibody response, whereas the anti-peptide responses induced by 1Fim2 and 2Fim3 were weaker. 'Free' peptide 1Fim3 was found to be immunogenic and it is likely that the T-helper cell epitope(s) contained within the peptide may include amino acids G⁶⁴ALK⁶⁷, which represent a pattern of amino acids that has been found within many T-helper cell epitopes (Lamb *et al.*, 1987). Each of the anti-peptide antibody preparations were able to recognize Fim2+3 coated onto ELISA plates, and, with the exception of serum raised to 1Fim2-KLH, were able weakly to recognize native Fim2+3 in a whole-cell ELISA. It is particularly interesting that peptide 1Fim2, prepared as either a peptide-KLH or as peptide-cBSA, -BSA or -thyroglobulin conjugates (coupled using MBS, SMCC, glutaraldehyde and 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide, respectively) was poor at inducing antibodies capable of recognizing native fimbriae (data not shown), since this free peptide, when used as an antigen, was strongly recognized by the anti-Fim2 mAb 51/24. Peptide recognition by the mAb cannot be attributed to this antibody being induced to a denatured form of the protein (Pearce *et al.*, 1992), as has been indicated for other peptide-reactive anti-protein antibodies (Jemmerson & Blankenfeld, 1989), since this mAb strongly agglutinates *B. pertussis* cells. This finding with peptide 1Fim2 clearly shows that a peptide which serves as a good mimic of a native protein epitope will not necessarily induce a protein-recognizing response when prepared as an immunogen.

Peptide 1Fim2 was investigated by circular dichroism and was found not to possess an ordered conformation in aqueous solution. However, the TFE titration curve that was obtained for 1Fim2 indicated that when exposed to a suitable environment, segments of the peptide can adopt a secondary structure conformation (Siligardi *et al.*, 1991). In the case of 1Fim2, such an environment may be the 51/24 paratope, which could induce the peptide into a conformation that allows it to mimic the corresponding

region of the native protein epitope. However, because this conformation is not assumed by the peptide cross-linked to a carrier protein, the anti-peptide antibodies generated fail to recognize the peptide sequence in the fimbriae. Paratope-induced conformational changes in peptide antigens have previously been proposed (Crump-ton, 1986) and recently demonstrated by both NMR (Scherf *et al.*, 1992) and crystallographic studies (Stanfield *et al.*, 1990).

In this study we have shown that peptides can be used to locate parts of non-contiguous fimbrial epitopes, but, when these antigenic peptides are prepared as immunogens, the resultant antibodies do not recognize the native protein antigen with high affinity. We have also shown that anti-protein antibodies which do not recognize denatured forms of the fimbriae can bind to peptide fragments of the protein subunits. This may be explained by a requirement for the epitope to be in the correct local conformation, which is lost in the subunit under denaturing conditions, but can be induced in a peptide fragment by the anti-protein antibody paratope.

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