



MAPPING OF CONFORMATIONAL B CELL EPITOPES WITHIN ALPHA-HELICAL COILED COIL PROTEINS

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Abstract—An approach to mapping antigenic B cell epitopes within α -helical coiled coil proteins has been developed and applied to two proteins: Streptococcal M protein and *C. elegans* paramyosin protein UNC-15. Overlapping peptides derived from an α -helical coiled coil conformational epitope were embedded between helical flanking peptides derived from the completely unrelated GCN4 leucine zipper peptide. The resulting chimeric peptides exhibited helical propensity. Chimeric peptides were tested for antigenicity (recognition by antibody) or immunogenicity (production of appropriate antibody response). A conformational epitope within the Streptococcal M protein recognised by three mAbs spanned 12 residues. Analysis of chimeric peptides based on *C. elegans* UNC-15 has enabled fine mapping of the minimal B cell epitope recognised by monoclonal antibody NE1-6B2 to seven non-contiguous residues (spanning 15 residues); the footprint of contact residues involved in antibody recognition being restricted to the hydrophilic face of the helix and covering five helical turns. This chimeric peptide epitope when coupled to diphtheria toxoid was highly immunogenic in mice and antisera recognised the conformationally dependent native peptide epitope. This approach has the potential to map conformational epitopes and design minimal epitopes for use as vaccine candidates. © 1997 Elsevier Science Ltd.

Key words: epitope, peptide, conformational, coiled coil, helix, vaccine.

INTRODUCTION

The identification of antigenic B cell epitopes is a major goal in the search for vaccine targets against disease. Many proteins which may be useful vaccine candidates against diseases will have surface exposed α -helical regions. This structural element is very common and in proteins approximately 32% of amino acids are found in helical motifs (Rost and Sander, 1993). A more complex arrangement of helices occurs in the α -helical coiled coil structure, an important structural and biologically abundant motif (Cohen and Parry, 1986, 1990). All coiled coil motifs share a characteristic seven amino acid residue repeat $(a-b-c-d-e-f-g)_n$ containing hydrophobic residues at positions *a* and *d* and hydrophilic residues generally elsewhere. The X-ray structure of several coiled coil domains have been solved and these include the leucine zipper portion of the yeast transcription factor GCN4 dimer (O'Shea *et al.*, 1991), several GCN4 leucine zipper mutants (Harbury *et al.*, 1993, 1994) and the repeat motif of α -spectrin (Yan *et al.*, 1993). More than 200 diverse

proteins have been predicted to contain coiled coil domains (Lupas *et al.*, 1991) and these include streptococcal protein A and M proteins, influenza hemagglutinin, HIV glycoprotein gp41, VSG of trypanosomes and schistosome paramyosin, all of which are of immunological interest. In these examples, protective epitopes may need to be presented in the correct conformation for immunological recognition.

In the design and development of a subunit vaccine based on protein α -helical regions, or more complex coiled coil structures, it is difficult to map neutralising or protective epitopes or to define a minimal epitope required for induction of a protective B cell response. While the correctly folded, intact protein may be an appropriate component in some vaccines, there are a number of situations where this may not be the case. These include situations where (1) the desired immune response is to a cryptic epitope, i.e. a region not naturally immunogenic; (2) the intact protein contains other epitopes, either antibody or T cell, which cause immunopathology; (3) it is technically difficult to manufacture correctly folded protein. In these cases, it is important to be able to map the minimal epitope and to devise ways of presenting it in the appropriate conformation.

Previous methods used to map minimal epitopes from

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antigens have used the PEPSCAN method of Mario Geysen (Geysen *et al.*, 1987). However the short peptides used will only indicate sequential or continuous epitopes. Methods to determine conformational epitopes have relied upon mimotope strategies such as peptides synthesised on polypropylene pins covering the total repertoire of 20⁸ octapeptides which can be made using the 20 common amino acids (Geysen *et al.*, 1987), or alternatively, epitope libraries consisting of a vast mixture of filamentous phage clones, each displaying one peptide sequence on the virion surface (Scott and Smith, 1990).

In this paper, we describe an approach to mapping epitopes in α -helical structures which overcomes these limitations by embedding peptides between helical flanking peptides derived from a completely unrelated GCN4 protein with a similar native conformation. Two proteins were studied: M protein, a principal Streptococcal bacterial surface virulence factor (Robinson and Kehoe, 1992) and the *C. elegans* paramyosin protein, UNC-15 (Waterston *et al.*, 1977), a core protein of the thick filament in many invertebrates, both of whose structures are thought to be an α -helical coiled coil. We generated mAbs to a region of the M protein recognised by opsonic polyclonal antibodies (Pruksakorn *et al.*, 1994a) and mapped the region recognised by three mAbs to a conformational B cell epitope. We also analysed an epitope within UNC-15 recognised by mAb NE1-6B2 previously mapped to a single amino acid substitution ⁸⁰⁹Q to R (Gengyo-Ando and Kagawa, 1991) within the coiled coil region. A series of chimeric peptides based on *C. elegans* UNC-15 has enabled fine mapping of the minimal B cell epitope and presentation as an immunogen. This approach has the potential to map conformational epitopes and design minimal epitopes for use as vaccine candidates.

MATERIALS AND METHODS

Peptide synthesis

Peptides were synthesised by a manual solid-phase technique using the simultaneous multiple peptide syntheses "tea-bag" method of Houghten (1985). The starting resin was *p*-methylbenzhydrylamine hydrochloride and conventional *N*-tert-butyloxycarbonyl chemistry (Merrifield, 1963) was used, involving amino acid coupling with 1,3-diisopropylcarbodiimide and cleavage from the resin by treatment with hydrogen fluoride. Some peptides were synthesised on polyethylene pin crowns surface-grafted with poly-(2-hydroxyethyl methacrylate) (Valerio *et al.*, 1993). In these cases fluoren-9-ylmethoxycarbonyl chemistry was employed with cleavage by trifluoroacetic acid (Fields and Noble, 1990). Crude peptides were purified on a (10 mm $\times$ 250 mm) C18 reverse-phase HPLC column (Bio-Rad, CA, U.S.A.) using a linear gradient from 2% acetonitrile in water to 42% acetonitrile (both solvents containing 0.1% TFA). The purified peptides were homogeneous as determined by reversed phase HPLC and laser-desorption time-of-flight mass spectrometry (LaserMat, FinniganMat, U.K.).

Circular dichroism spectra

Circular dichroism spectra were obtained on a Jasco spectropolarimeter at 25°C with constant N₂ flushing. Peptides were at a concentration of between 50 and 100 mM in 25 mM phosphate, 75 mM NaCl, pH 7.0, 50% trifluoroethanol. Data were collected at 0.5 nm intervals from 250 to 190 nm.

Antibody production

Quackenbush mice (Animal Resources Centre, Willetton, Western Australia) were immunised *intra peritoneum* with the equivalent of 125 μ g of peptide, conjugated to diphtheria toxoid via an MCS linkage, emulsified in complete Freund's adjuvant. Boosts of 125 μ g equivalent peptide-diphtheria toxoid conjugate in incomplete Freund's adjuvant were administered at 4 week intervals. Serum samples were derived from blood from the tail artery taken 7 days after immunisation or boost, and stored at -20°C.

Monoclonal antibodies reactive with the streptococcal M protein were produced from Balb/C mice hyper-immunised *intra peritoneum* (Shibier *et al.*, 1988) with peptide p145 coupled, via an additional N-terminal cysteine residue, to diphtheria toxoid in increasing doses (10, 10, 10, 100, 200, 200, 200 μ g peptide). Spleen cells were fused with SP2 cells using the standard polyethylene glycol method and antibody producing hybridomas selected (Kohler and Milstein, 1975).

Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) was used to measure mouse monoclonal or polyclonal antibody reactivities against peptides. Peptides (1–10 μ g) were adsorbed to separate wells of 96-well microtitre plates by incubation in phosphate-buffered saline (PBS) for 2 hr at 37°C. Unbound peptide was washed out with PBS. Plates were incubated with 3% bovine serum albumin in PBS (BSA/PBS) for 1 hr at 37°C to prevent non-specific adsorption of mAb. Monoclonal antibody was bound to the peptides by incubating at 4°C for 18 hr using a 1:200 dilution of hybridoma supernatant in BSA/PBS. Alternatively, mouse sera was diluted 1:2000 in BSA/PBS. Unbound antibody was washed out with PBS prior to addition of horseradish peroxidase-conjugated sheep anti-mouse second antibody (Amersham, U.K.) at a 1:2000 dilution in BSA/PBS for 4 hr at 4°C. Excess conjugated second antibody was washed off with PBS and colorimetric detection was with *o*-phenylenediamine dihydrochloride (OPD) (Bethesda Research Laboratories, MD, U.S.A.) and hydrogen peroxide. Absorbance at 450 nm was read by a Multiskan PLUS spectrophotometer (Titertek, Finland). Antibody titres were considered significant if greater than three standard deviations above mean for control antibody.

SDS-polyacrylamide gel electrophoresis and immunoblotting

Total proteins from *C. elegans* worms were extracted into 1% Triton X-100, 150 mM sodium chloride, 50 mM

ethylenediamine tetraacetic acid, 50 mM Tris, pH 7.4. Aliquots of worm extract were added to SDS-PAGE loading buffer, fractionated on a 10% SDS-polyacrylamide gel and the separated proteins transferred to nitrocellulose membrane. Membrane strips corresponding to each sample lane were incubated with mouse monoclonal antibody (1:200 dilution) or antisera (1:2000) for 2 hr at 22°C followed by incubation with alkaline phosphatase conjugated goat anti-mouse IgG (Bio-Rad). Colour development used 5-bromo-4-chloro-3-indolyl phosphate (BCIP)/nitroblue tetrazolium (NBT) (Bio-Rad).

RESULTS

Rationale for design of chimeric peptides

If an epitope is known to reside within an α -helix protein structural conformation, then a model peptide can be synthesised to fold to this conformation. We designed a model α -helical coiled coil peptide based on the structure of the GCN4 leucine zipper (O'Shea *et al.*, 1991). The first heptad contains the sequence MKQLEDK, which includes several of the features found in a stable coiled coil heptad repeat motif (*a-b-c-d-e-f-g*)_n (Cohen and Parry, 1990). These include large apolar residues in the *a* and *d* positions, an acid/base pair (glu/lys) at positions *e* and *g* (usually favouring interchain ionic interactions), and polar groups in positions *b*, *c*, *f* (consistent with the prediction of Lupas *et al.* (1991)). The GCN4 peptide also contains a consensus valine in the *a* position. It has also been noted that when positions *a* and *d* are occupied by V and L, a coiled coil dimer is favoured (Harbury *et al.*, 1994). A model heptad repeat was derived from these consensus features of the GCN4 leucine zipper peptide: (VKQLEDK)_n, with the potential to form a α -helical coiled coil. This peptide became the framework peptide. Overlapping fragments of a conformational epitope under study were embedded within the model coiled coil peptide to give a chimeric peptide. Amino acid substitutions, designed to ensure correct helical coiled coil conformations (Cohen and Parry, 1990), were incorporated into the chimeric peptides whenever an identical residue was found in both the helical model peptide and the epitope sequence. The following substitutions were typically used: position *a*, V to I; *b*, K to R; *c*, Q to N; *d*, L to A; *e*, E to Q; *f*, D to E; *g*, K to R. All of these replacement residues are commonly found at their respective position in coiled coil proteins (Lupas *et al.*, 1991).

Chimeric peptide epitope mapping of M protein

Chimeric peptides J1 to J4 were synthesised based on 12-mer peptide fragments spanning the peptide p145 region of Streptococcal M protein, offset by one residue, embedded into the α -helical coiled coil framework by addition of octamer helical flanking peptides (Table 1). The sliding window of 12 residues would contain three complete turns of an α -helix (3.5 residues per turn). The helical flanking peptides were based on the model α -helical coiled coil peptide (VKQLEDK)_n and were added

in frame with the coiled coil protein heptad repeat periodicity of M protein.

Three monoclonal antibodies raised against peptide p145, coupled to diphtheria toxoid via an added N-terminal cysteine residue, recognised chimeric peptides J1 and J2, and native peptides p144 and p145. The overlap between the sequences of J1 and J2 indicates an 11-mer epitope. Shorter peptides containing the insert sequences alone from J1 and J2 (J₁ and J₂, respectively) were recognised poorly by monoclonal antibody.

Chimeric peptide epitope mapping of UNC-15

Chimeric peptides containing 15-mer peptide fragments spanning the *C. elegans* UNC-15 protein, offset by one residue, and embedded in the framework peptide were synthesised. Peptides C2 to C7 span the region containing the mAb NE1-6B2 epitope and contain the essential residue ⁸⁰⁹Q (Gengyo-Ando and Kagawa, 1991). Maximum ELISA reactivity with mAb NE1-6B2 was obtained for peptide C4 (Fig. 1). Control peptide C_{con}, based on the (VKQLEDK)_n framework, and peptide J41, containing an unrelated insert (Relf *et al.*, 1996), were not recognised by mAb NE1-6B2. Activity was minimal after removal of the ⁸⁰⁹Q residue (peptide C1) to the C terminus and the ⁷⁹⁸R residue (peptide C6) to the N terminus of the UNC-15 protein. Based on the overlap between the UNC-15 peptides embedded within the chimeric peptides, this indicates that the minimal epitope resides between residues ⁷⁹⁸R and ⁸⁰⁹Q, a 12-mer peptide: RLTEKLNIQKRQ.

Overlapping 21-mer peptides, derived from UNC-15 and offset by one amino acid residue, were also synthesised and assayed by ELISA (Fig. 2). Peptide N4 had the highest ELISA reactivity suggesting that a 21-mer peptide is long enough for recognition of the epitope. Monoclonal antibody reactivity was restricted to peptides N2 through to N9. The negative reactivity of mAb for peptide N1 delineates the extent of the epitope towards the C terminus of the protein and confirms the requirement of the ⁸⁰⁹Q residue within the epitope (Gengyo-Ando and Kagawa, 1991). Based on the overlap between peptides N2 and N9, the minimal epitope resides in the 14-mer peptide ADRLTEKLNIQKRQ. However, maximal reactivity is found with peptide N4, a much longer 21-mer peptide which could be considered the optimal native epitope.

Using the chimeric peptide approach, the optimal epitope contained within peptide C4 was truncated to better define the minimal epitope recognised by mAb NE1-6B2. Truncation of the embedded epitope from the C terminus (peptides C8, C9) decreased ELISA reactivity as did truncation from the N terminus (peptide C10) (Fig. 1). Further truncation of residues from the N terminus of the epitope (peptides C11, C12) was not well tolerated. Thus, although overlapping fragment epitope mapping would suggest RLTEKLNIQKRQ to be the minimal epitope, truncation mapping indicates that residues ⁷⁹⁷D, ⁸¹⁰L and ⁸¹¹A flanking this region are critical. Furthermore, a 15-mer peptide consisting of the optimal epitope

Table 1. Chimeric peptide epitope mapping of M protein

	Peptide sequence										Monoclonal antibody titre		
	a	d	a	d	a	d	a	d	a		IIE10	IIF10	3C8
p145					LRRDLASREAKKQVEKALE						12800	12800	12800
p144			NKISEASRKGLRRDLASRE								204800	204800	204800
J1			<u>QLEDKVKQ</u> LRRDLASREAK <u>EELQDKVK</u>								102400	102400	204800
J2			<u>LEDKVKQA</u> RRDLASREAK <u>ELQDKVKQ</u>								204800	204800	204800
J3			<u>EDKVKQA</u> ERDLASREAKKQ <u>LQDKVKQL</u>								—	—	—
J4			<u>DKVKQAED</u> DLASREAKKQV <u>QDKVKQLE</u>								—	—	—
J _{con}			<u>DKVKQAEDKVKQ</u> LEDKVEELQDKVKQLE								—	—	—
J ₁					LRRDLASREAK						6400	6400	6400
J ₂					RRDLASREAKK						6400	6400	6400
J ₃					RDLASREAKKQ						—	—	—
J ₄					DLASREAKKQV						—	—	—

Chimeric peptides J1 to J4 containing overlapping fragments of Streptococcal M protein embedded in model helical peptide were synthesised. Native peptide fragment shown in bold face, helical flanking regions in normal face. Putative heptad repeat positions *a* and *d* indicated above sequence. Peptides were coated on microtitre plates (2 µg per well), incubated with mAbs IIE10, IIF10 and 3C8 and bound antibody detected with anti-mouse antibody and OPD colorimetric assay at 450 nm. —, ELISA titre less than cutoff of the mean + 3 SD of unrelated hybridoma supernatant. Control peptide J_{con} contains model helical peptide residues only.

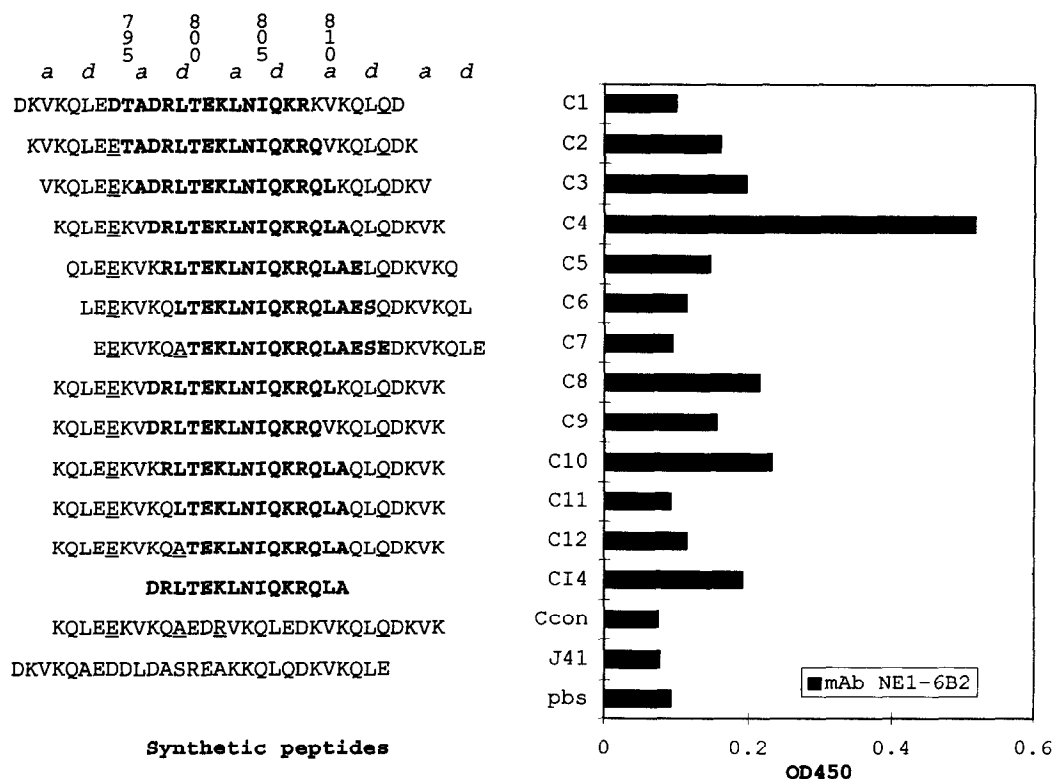


Fig. 1. Chimeric peptide epitope mapping of UNC-15. Chimeric peptides containing overlapping or truncated fragments of *C. elegans* UNC-15 embedded in model helical peptide were synthesised. Native peptide fragment shown in bold face, helical flanking regions in normal face. Conservative replacements in helical flanking regions shown underlined. Putative heptad repeat positions *a* and *d* indicated above chimeric peptide sequence. Peptides were coated on microtitre plate (2 µg per well), incubated with mAb NE1-6B2 and bound antibody detected with anti-mouse antibody and OPD colorimetric assay at 450 nm. Control peptides C_{con} and J41 contain model helical peptide residues only.

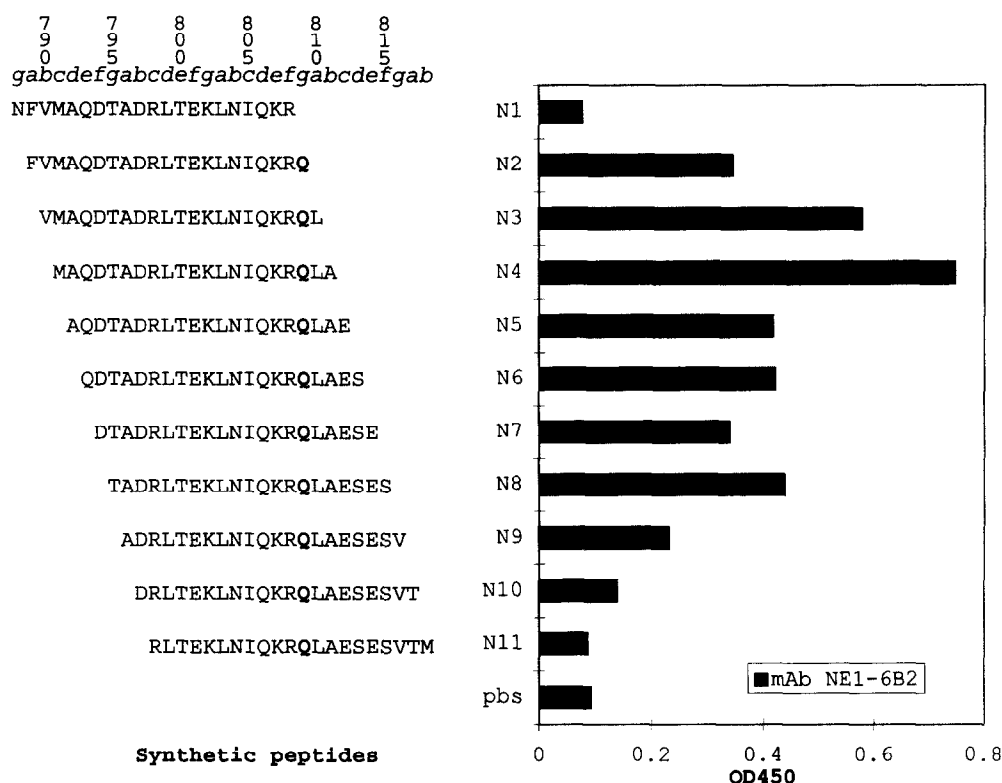


Fig. 2. Native epitope mapping of UNC-15. Overlapping peptides derived from native *C. elegans* UNC-15 were synthesised. Putative heptad repeat positions *abcdefg* indicated above native UNC-15 sequence. Numbering of amino acid residues according to Gengyo-Ando and Kagawa (1991). Essential gln residue ⁸⁰⁹Q shown in bold. Peptides were coated on microtitre plates (2 µg per well), incubated with mAb NE1-6B2 and bound antibody detected with anti-mouse antibody and OPD colorimetric assay at 450 nm.

⁷⁹⁷D to ⁸¹¹A (peptide C₄) was recognised to a lesser degree than the same peptide embedded in the chimeric peptide C4. This emphasises the need for flanking regions to ensure maximum reactivity.

Chimeric peptide epitope substitution mapping of UNC-15

Mapping of critical residues required in the mAb NE1-6B2 epitope for ELISA reactivity was accomplished by replacement of individual residues from within the peptide C4 optimal epitope. Helical framework residues were substituted for epitope residues according to the model peptide (VKQLEDK)_n design rationale (see Section 3.1.). ELISA reactivity was abrogated when substitutions were made for residues ⁷⁹⁸R, ⁸⁰¹E, ⁸⁰⁵I, ⁸⁰⁸R and ⁸⁰⁹Q (Fig. 3a). A decrease in epitope reactivity also was found for two other substitutions, namely ⁷⁹⁷D and ⁸¹¹A. When the sequence of UNC-15 containing the mAb NE1-6B2 epitope was shown pictorially as a cylindrical net (Fig. 3b), all the critical residues were found on the hydrophilic face of the helix.

Immunogenicity of UNC-15 chimeric peptides

Mice were immunised with UNC-15 chimeric peptides to determine the ability to induce epitope specific antibody response. Chimeric peptides C4 and C_{con} were resyn-

thesised with an N terminal cysteine residue, for coupling to diphtheria toxoid (DT) via a maleimidocaproic acid *N*-hydroxysuccinimide ester (MCS) linkage, to give peptides C4' and C_{con}', respectively. Peptide N12', consisting of residues ⁷⁹⁰V–⁸¹⁸M from UNC-15, was similarly synthesised and coupled to DT. Antisera raised against peptide C4' recognised in ELISA both chimeric peptide C4 and the native peptide N4 but only weakly peptide C₄ (Fig. 4a). An ELISA titre of greater than 10⁶ was demonstrated (data not shown). Antisera raised against control chimeric peptide C_{con}', based solely on the model helical peptide, recognised peptide C4 but not native peptide N4 or peptide C₄. Paramyosin protein, from total *C. elegans* worm extracts separated by SDS-PAGE, also was recognised by anti-peptide C4' sera in immunoblot analysis (Fig. 4B). Thus the antibody response raised with the chimeric peptide C4' was to a conformational epitope found within native UNC-15, and functionally equivalent in chimeric peptide C4 and native peptide N4.

Structural determination of chimeric peptides

Circular dichroism spectra from UNC-15 chimeric peptides C4 and C_{con}, in 50% trifluoroethanol, contained double minima at 208 and 222 nm and maximum at 195 nm indicating helical propensity (Fig. 5). A similar spectrum was obtained from the native 21-mer peptide N4.

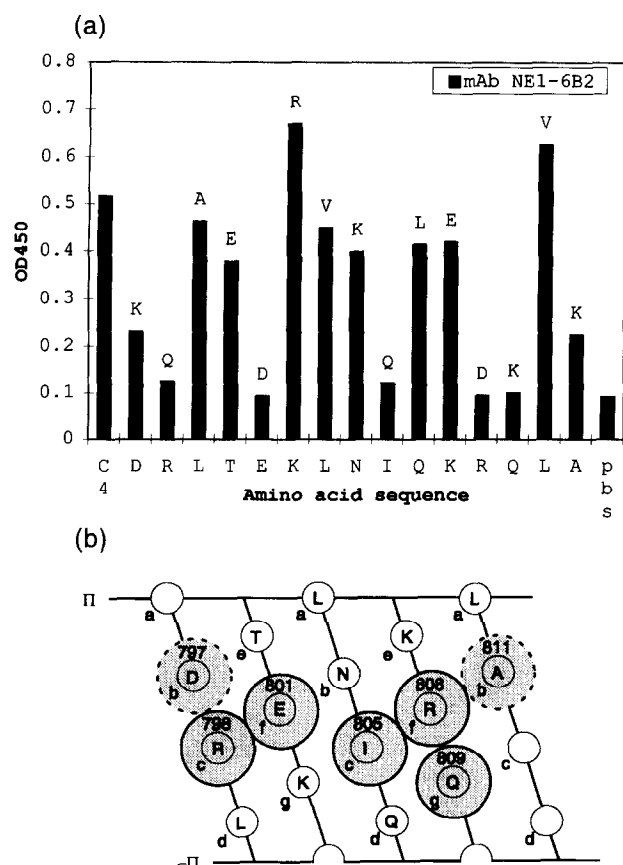


Fig. 3. (a) Chimeric epitope substitution mapping of UNC-15. Chimeric peptides, derived from peptide C4 with each residue substituted in turn by an alternative replacement (see Materials and Methods), were coated on microtitre plates ($2 \mu\text{g}$ per well), incubated with mAb NE1-6B2 and bound antibody detected with anti-mouse antibody and OPD colorimetric assay at 450 nm. (b) Cylindrical net representation of UNC-15 helix containing mAb NE1-6B2 epitope. Helix with 3.5 residues per turn showing the polar face. Helix runs from left to right. Shaded residues interact with mAb NE1-6B2; solid circle is a critical residue; dashed circle is a less critical residue.

DISCUSSION

We have demonstrated an approach to mapping the minimal region of an α -helical coiled coil conformationally dependent B cell epitope recognised by monoclonal antibody. This was accomplished, in part, by extending the traditional PEPSCAN approach (Geysen *et al.*, 1987) by incorporation of truncated overlapping fragments of a protein region within an unrelated peptide, known to form an α -helical coiled coil. These chimeric peptides contained typically 12–15 residue long fragments embedded in register with respect to helical coiled coil heptad periodicity between flanking regions (7–8 residues long) from the yeast transcription factor GCN4 leucine zipper (O'Shea *et al.*, 1991). The helical propensity of all these chimeric peptides, derived from *C. elegans* paramyosin protein UNC-15, has been demonstrated by circular dichroism and confirms the helical potential previously determined for Streptococcal M protein chimeric peptides (Relf *et al.*, 1996). Using chimeric peptides offset by one residue we were able to map the conformational epitopes recognised by mAbs within

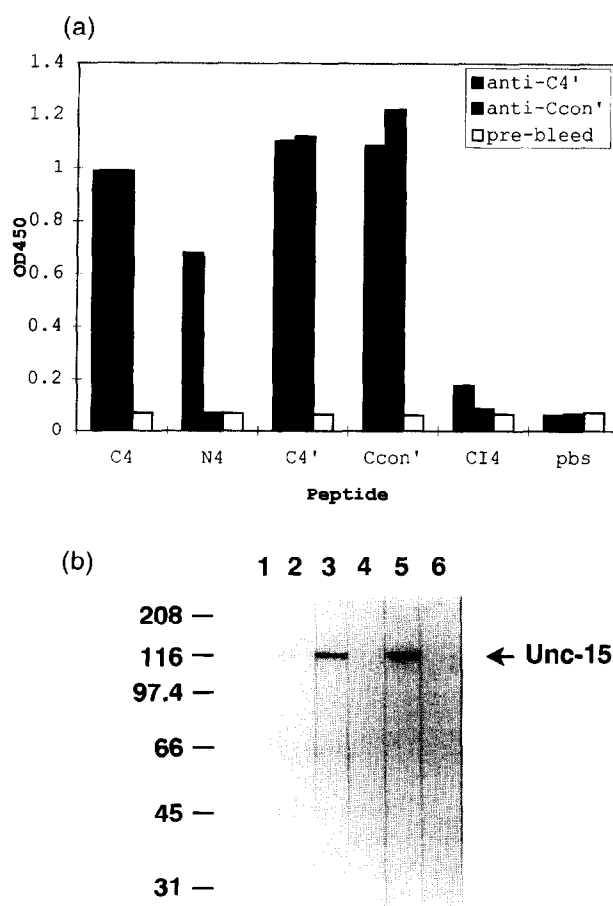


Fig. 4. (a) Antibody response to UNC-15 chimeric peptide. Chimeric peptides C4, N4, C4', Ccon' and peptide C₄ were coated on a microtitre plate ($2 \mu\text{g}$ per well), and incubated with mouse antisera (diluted 1:2000) to C4' (anti-C4') or Ccon' (anti-Ccon'), or mouse pre-bleed sera (pre-bleed). Bound antibody was detected with anti-mouse antibody and OPD colorimetric assay at 450 nm. (b) Immunoblotting of UNC-15 chimeric peptide antisera. *C. elegans* worm extracts were separated by SDS-PAGE and transferred to nitrocellulose membrane. Strips were immunoblotted with monoclonal antibodies NE1-5B5 (lane 1) and NE1-6B2 (lane 2); antisera (diluted 1:2000) raised against chimeric peptides C4' (lane 3), Ccon' (lane 4), or native peptide N12' (lane 5); or pooled mouse pre-immune sera (lane 6). Bound antibody was detected with alkaline phosphatase conjugated anti-mouse antibody and BCIP/NBT colour development.

these two proteins. These epitopes spanned between 12 residues (M protein) and 15 residues (UNC-15); however, only chimeric peptides were effectively recognised by mAb, shorter peptides consisting of insert peptide sequence alone did not bind antibody. These shorter peptide fragments had little helical conformation as expected; it has been reported that at least eight helical turns (calculated from a sequence length of 29 residues) are required for a stable coiled coil structure (Lau *et al.*, 1984). Thus, although longer peptides may allow the epitope to be located, the short peptides needed to map the minimal epitope will not be recognised by antibody.

Application of the chimeric peptide approach to fine mapping of the minimal epitope recognised by a monoclonal antibody was demonstrated with *C. elegans* UNC-15. Previous studies with large recombinant pro-

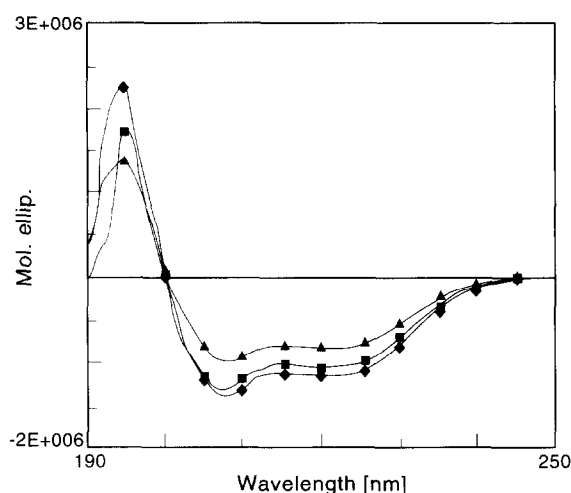


Fig. 5. Circular dichroism spectra of UNC-15 chimeric peptides. Spectra obtained for UNC-15 chimeric peptides C_{con} (▲) and C4 (◆), and native peptide N4 (■) in 25 mM phosphate, 75 mM NaCl, pH 7.0, 50% trifluoroethanol.

tein fragments have shown that the epitope within UNC-15 recognised by mAb NE1-6B2 lies within a 113 residue coiled coil region containing ⁸⁰⁹Q (Kagawa and Gengyo, 1988). ELISA reactivity of overlapping native fragments of UNC-15 suggested that a 14 residue overlap between ⁷⁹⁶A and ⁸⁰⁹Q contained the NE1-6B2 epitope. The alternative chimeric approach using overlapping fragments suggested that the minimal epitope involved only the 12 residues ⁷⁹⁸R to ⁸⁰⁹Q. This is substantially shorter than that obtained by mapping with native peptides. However, a major difference between epitope mapping with native versus chimeric peptides is the better discrimination between adjacent peptides in ELISA analysis. With chimeric mapping, the highest ELISA reactivity was only found in peptide C4, suggesting the optimal epitope to include the 15-mer peptide ⁷⁹⁷D to ⁸¹¹A, whereas in native peptides the signal was broadly similar over some seven peptide fragments. Presumably in the native peptides, two competing processes lead to substantial discrimination: helix formation (perhaps as a consequence of binding to the solid phase support) versus individual recognition by mAb. Furthermore, chimeric inserts less than 15 residues long had decreased ELISA reactivity; thus prediction of a minimal epitope based on peptide overlap, either by native or chimeric mapping, is not as useful as expected. It appears that immunoreactive epitopes found within an α -helical coiled coil may be up to at least 15 residues in length and helical flanking regions are required to maintain conformation.

An extension to our goal of mapping the minimal epitope within a coiled coil protein recognised by mAb was to determine the critical amino acid residues involved in antibody interaction. Homolog- and alanine-scanning mutational analysis of proteins has been used to map side-chains important in antibody binding (Jin *et al.*, 1992) and linear B cell epitopes have been mapped using PEPSCAN replacement set analysis (Geysen *et al.*, 1987). The former method uses recombinant protein whilst the later could be performed on the longer native peptides

used in this study; however, chimeric peptides would simplify the procedure. Furthermore, not all amino acid replacements would be tolerated based purely on the need to ensure correct helical coiled coil conformation. When all 15 residues within the insert of UNC-15 chimeric peptide C4 were substituted in turn with alternative, coiled coil promoting replacements, we found that only half of the residues within the mAb NE1-6B2 epitope were involved in antibody recognition. This could be either as a direct requirement for a particular functional group or as a consequence of a different functional group sterically or electrostatically interfering with antibody interaction and is consistent with other studies using human growth hormone (Jin *et al.*, 1992) or recombinant influenza virus neuraminidase (Nuss *et al.*, 1993). Other residues may be contacts but a complete replacement net would be required to demonstrate this. It is assumed that amino acid replacements had no effect on the conformation since all favour a coiled coil structure (Lupas *et al.*, 1991). Importantly, all contact residues were on the hydrophilic face of the helix and included residues at the extremities of the mapped epitope. In a coiled coil dimer, as proposed for paramyosin, the hydrophobic faces would interact and not be recognised by antibody. The footprint of contact residues also covers five helical turns which are involved in recognition by the antibody combining site. Since no crystal structure of an antibody complexed to a helical peptide has yet been solved, the chimeric peptides used here may be useful for such a study.

A goal of mapping a minimal B cell epitope from an antigen is in the design of a subunit vaccine. When embedded in the GCN4 model helical coiled coil framework and following conjugation to diphtheria toxoid, which has been used as a carrier for other peptide vaccines (Jones *et al.*, 1988), antibodies to the UNC-15 peptide insert (residues ⁷⁹⁷D to ⁸¹¹A) recognised native peptide and intact protein conformations. Thus we have been able to elicit a high titre antibody response to a chimeric peptide which recognises the native peptide conformation. Although an antibody response to the flanking helical regions was observed in ELISA analysis of the control chimeric peptides, it did not cross-react with the native peptide sequence in immunoblotting analysis. Thus an antibody response to this completely unrelated protein, a yeast transcription factor, should not interfere with generation of an appropriate immune response when used as a vaccine. Furthermore, no ill effects were observed in the animals immunised with the chimeric peptide immunogens.

Synthetic peptide-based vaccines are attractive because they will contain only the minimal region of an antigen required for an effective protective response. The α -helical coiled coil chimeric peptide approach obviates the problems which are associated with the need to present the immunogen in a α -helical conformation. Chimeric peptides were shown to be very immunogenic both with carrier protein (this study) and without (Relf *et al.*, 1996). These constructs will help in an understanding, at the molecular level, of the immune response required for

protection chiefly by offering the potential for fine-tuning or improvement of an immunogen's peptide sequence. The end result would be the design of a vaccine candidate or therapeutic agent which excludes deleterious auto-immune responses or epitopic suppression due to neighbouring immunodominant regions of the antigen. The former problem was recently identified in analysis of peptide p145 within the Streptococcal M protein recognised by opsonic antibodies (Pruksakorn *et al.*, 1994a). This 20-mer peptide also contained a T cell epitope which cross-reacted with heart muscle myosin (Pruksakorn *et al.*, 1994b). Such cross-reactions with the Streptococcal M protein have been proposed as the basis for later development of rheumatic heart disease (Robinson and Kehoe, 1992), necessitating determination of the minimal B cell epitope. We have shown in this study that the minimal antibody epitope, within a coiled coil protein, can be as short as 12 residues. The success of the chimeric approach in delineating such coiled coil conformational epitopes and presenting these epitopes as an immunogen emphasises the potential of chimeric peptides as potential vaccine candidates.

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