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Biochemistry

Presence of two distinct regions in the coiled-coil structure of the streptococcal Pep M5 protein: Relationship to mammalian coiled-coil proteins and implications to its biological properties

(heptad periodicity/internal homology/tropomyosin/myosin/antiphagocytic property)

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ABSTRACT **The complete amino acid sequence of Pep M5, a biologically active 197-residue fragment comprising nearly half of the group A streptococcal M5 protein, has structural features characteristic of an α-helical coiled-coil protein. Fourier analyses of the nonpolar residues show strong periodicities based on repeats of 7 residues (7/2 and 7/3). Except for the nonhelical NH_2-terminal 12-residue segment, the 7-residue periodicity in the distribution of nonpolar residues extends through the remainder of the Pep M5 molecule, with some discontinuities and irregularities. The molecule contains two distinct regions that differ in the pattern of distribution of the nonpolar and charged residues. The 7-residue pattern "a, b, c, d, e, f, g" in region 13–121 is atypical in that position "a" is predominantly occupied by asparagine, rather than nonpolar residues. On the other hand, the periodicity in region 122–196 is more typical of that found in other coiled-coil proteins, such as the myosin rod region, keratin, desmin, and vimentin, rather than tropomyosin. Although the periodicity in nonpolar residues is not highly regular, the predominance of basic and acidic residues in the inner "e" and "g" positions, respectively, suggests that ionic interactions between chains may contribute significantly to the stability of the coiled-coil. The distribution of charged residues in the outer positions within the two regions of the molecule is also distinct. The NH_2-terminal region carries a significantly higher net negative charge than the COOH-terminal region, suggesting that the former region may play an important role in some of the biological functions of the Pep M5 molecule.**

The M protein of the group A streptococcus, a fibrous protein on the bacterial cell surface, is a major virulence factor for the bacteria, by virtue of its property of impeding phagocytosis (1–4). Several serologically distinct variants of the M protein have been recognized over the years, and the immunity conferred by the induced antibodies to a given M type is essentially type specific (1, 2, 5).

Our previous studies have revealed that the partial amino acid sequences of three immunologically distinct M proteins—namely, M5, M6, and M24—are different but exhibit a 7-residue (heptad) periodicity in the distribution of nonpolar and charged amino acid residues, a characteristic of α-helical coiled-coil proteins (6–8). Our subsequent physicochemical studies have shown that a substantial part of the M protein exists in a coiled-coil conformation (4). Recently, we have determined the complete amino acid sequence of a biologically active 197-residue peptic fragment of the type 5 M protein—namely, Pep M5 (9, 10). Our other studies have shown that the Pep M protein is derived from the distal portion of the M protein fibrillae on the streptococcal cell wall and represents approximately the NH_2-terminal half of the native M molecule (4, 11).

In the present report, we describe a detailed analysis of the complete amino acid sequence of the Pep M5 protein to determine whether the heptad periodicity is continuous and regular throughout its entire length. This determination would further our understanding of both the relationship between the amino acid sequence of the Pep M5 molecule and its coiled-coil structure and the possible biological implications of this structure. In addition, the sequence of Pep M5 protein has been examined by dot matrix analysis (12) to assess the possible presence of internal homology in this molecule.

METHODS

Fourier Analysis. The Fourier periodicities in the amino acid sequence of the Pep M5 protein (10) were calculated as follows: The sequence was represented as a series of integers with the selected amino acid(s) as 1s and all others as 0s. The array was padded with 0s to a length of 700 in order to give finer sampling. The array was scaled, and a Fourier transform and probabilities were calculated as described by McLachlan (13).

Dot Matrix Analysis. Internal sequence homology in the Pep M5 protein was examined by dot matrix analysis (12). In this program, which is similar to the RELATE program (14), segments of a given length from one sequence are sequentially compared with segments of the same length of the other sequence, and the homologies between them are scored using the mutation data matrix (MDM 250 PAMs). The latter provides the probability of how often a given amino acid will either be conserved or mutated into another particular amino acid, based on studies of known protein families. The results of the analysis are represented by dots in a matrix plot and, hence, provide a visual display of the regions of highest similarity between the two sequences that are compared.

RESULTS

Secondary Structural Characteristics. Analysis of the amino acid sequence of the Pep M5 molecule by the predictive algorithm of Chou and Fasman (15) revealed that most of the Pep M5 sequence exhibits a strong α-helical potential, with only three short segments exhibiting β-turn potential (Fig. 1). A few segments that exhibit α-helical as well as β-sheet potential are all short and are flanked by helical regions on either side. Apparently, the actual conformation of these latter segments may be determined by environmental conditions.

The Heptad Periodicity. The 7-residue periodicity in the distribution of the amino acid residues of the Pep M5 protein is shown in Fig. 2. α-Helical coiled-coil proteins are built with a characteristic heptapeptide repeat pattern "a, b, c, d,

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                10                  20                  30                  40
TVTRGTISDPQRAKEALDKYELENHDLKTKNEGLKTENEG
SSSSSSS    HHHHHHHHHHHHHHHHHHHHHHHHHHHHHH
   TTTT CTTTT

                50                  60                  70                  80
LKTENEGLKTENEGLKTEKSNLERKTAELTSEKKEHEAEN
HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH

                90                  100                 110                 120
DKLKQQRDTLSTQKETLEREVQNTQYNNETLKIKNGDLTK
HHHHHHHHHHHHHHHHHHHHHHHH HHHHHHHH  HHHHH
     SSSSSSSSS          SSSSSS   SSSSS
                                 TTTT   TTTT

                130                 140                 150                 160
ELNKTRQELANKQQESKENEKALNELLEKTVKDKIAKEQE
HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH
                                    SSSSSSS

                170                 180                 190
NKETIGTLKKILDETVKDKLAKEQKSKQNIGALKQEL
HHHHH HHHHHHHHHHHHHHHHHHHHH HHHHHHH
   SSSSSSSSSS                SSSSSS   C
```

FIG. 1. Secondary structural characteristics of the Pep M5 amino acid sequence, as determined by the Chou–Fasman predictive analysis (15). Amino acid residues are indicated by single letter code. Secondary structural characteristic assigned to each residue indicates the potential of that residue, taking into account the influence of neighboring 5 amino acid residues. The assignments H, S, C, and T represent α-helical, β-sheet, random coil, and β-turn potential, respectively.

e, f, g" in which the nonpolar residues preferentially occupy positions "a" and "d" and form a closely packed hydrophobic interface between the two strands of the coiled-coil

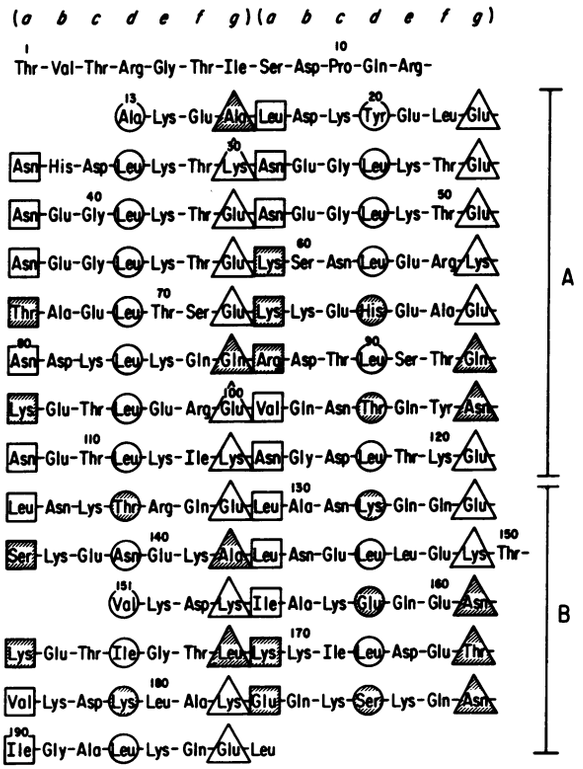

FIG. 2. Seven-residue periodicity in the complete amino acid sequence of the Pep M5 protein. Positions of residues in each period are indicated by letters "a"–"g" (16). Residues at position "a" are boxed, whereas those at positions "d" and "g" are circled and triangled, respectively. Exceptions at these positions are indicated by hatch marks (see text for details). The two structurally distinct regions in Pep M5 molecule are indicated by vertical bars on the right marked A and B.

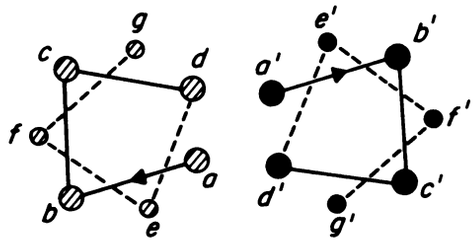

FIG. 3. Cross section of the two helices of the coiled-coil showing relative positions of residues "a"–"g" viewed from the amino end [adapted from McLachlan and Stewart (16)].

(16). The inner positions "e" and "g" lie next to the core, while amino acid side chains in the outer positions "b," "c," and "f" point away from the core and are free to interact with the surrounding molecules. These spatial relationships are illustrated in Fig. 3. Such a distribution of amino acids, originally predicted by Crick (17), was first detected in tropomyosin (16, 18). Subsequent studies have shown that it is also prominent in other fibrous proteins, such as keratin (19, 20), fibrinogen (21), myosin rod region (22–24), hemagglutinin HA2 light chain (25), *Escherichia coli* lipoprotein (26), desmin (27), vimentin (28), and the COOH-terminal subsets of *Trypanosoma brucei* variable surface glycoproteins (29).

As can be seen in Fig. 2, with the exception of the short nonhelical NH_2-terminal segment, the periodicity in the Pep M5 protein begins at residue 13 and extends fairly regularly through residue 149 of the molecule. In addition, if one allows for a skip residue, as in the myosin rod sequence (24), the skip residue in the Pep M5 protein being threonine-150, and a reversal in the polarity of the "a" and "d" heptad designation, then the periodicity virtually extends from alanine-13 through glutamic acid-196, and consists of 183 residues. However, as a result of the reversal in polarity of the "a" and "d" designations at valine-151, the core "a" and "d" residues in region leucine-146 to isoleucine-155 are now separated by intervals of 4–4 rather than the usual 3–4–3–4, etc., as seen in other regions of the Pep M5 protein. Such discontinuity in the heptad periodicity of the core "a" and "d" residues is also known as "stutter," and has been previously seen in the sequences of mouse epidermal keratin subunit (20), chicken desmin (27), vimentin (28), fibrinogen (30), and myosin rod (24). As suggested earlier for these proteins, the discontinuity in the periodicity probably results in a rotation of the backbone of the coiled-coil at this point. Alternatively, residues 150–154 may loop out entirely and the periodicity resume in phase starting at residue 155. In either instance, the discontinuity in the periodicity is clearly indicative of an irregularity in the coiled-coil structure of the Pep M5 protein. If the first alternative is correct, then, with an axial rise of 1.5 Å per residue, the entire α-helical portion of the Pep M5 molecule, consisting of 183 residues, may be calculated to be $\approx$275 Å long.

Fourier Analysis. Many fibrous proteins contain linear periodicities that reflect their secondary and tertiary structural characteristics as well as mode of aggregation (13, 20, 23, 24). Fourier transforms are often used to analyze such periodic features in amino acid sequences (30, 31). Fourier analysis on the complete amino acid sequence of the Pep M5 protein revealed that its nonpolar residues exhibit significant periodicities at 7/2 and 7/3 (Table 1). Such periodicities are clearly harmonics of a 7-residue repeat and are characteristic of α-helical coiled-coil proteins (30). In addition, the acidic and basic residues of the Pep M5 protein also exhibit distinct periodicities. While the acidic residues exhibited a periodicity of 7/3, which is also a harmonic of the 7-residue repeat, the basic residues exhibited a significant periodicity at 5/2.

Table 1. Significant peaks in the Pep M5 sequence revealed by Fourier analysis

Residue type	Period	Rational approximation	Probability
Nonpolar			
Ala, Val, Leu, Ile, Tyr	2.33	7/3	0.2301×10^{-4}
	3.54	7/2	0.7318×10^{-3}
Basic			
Lys, Arg	2.50	5/2	0.9307×10^{-5}
Acidic			
Glu, Asp	2.34	7/3	0.1124×10^{-3}

Fourier analysis was done on the complete sequence of the Pep M5 protein. Fourier methods evaluated selected types of amino acids for nonrandom periodicities. Probabilities of <5% (0.05) are considered statistically significant. Only the strongest periodicities are shown.

The significance of these periodicities in the charged amino acid residues is not apparent at present. However, it is probable that the charged groups contribute to the stabilization of the coiled-coil structure of the Pep M5 protein by forming salt bridges between the two chains.

The sequence was also modeled with a perfect heptad repeat and two blanks after residue 150, as in the case of the myosin rod sequence (24). However, this did not appear to significantly alter the observed periodicities.

Amino Acid Distributions Within the Heptad. The distribution of some of the selected amino acid residues within the 7 positions of the coiled-coil pattern in region 13–196 of the Pep M5 molecule, with the exception of threonine-150, is shown in Fig. 4. This region, comprising 183 of the 197 residues of the Pep M5 molecule, contains 25 complete and 2 partial heptads (see Fig. 2). It may be seen from Fig. 4 that, as in the case of other coiled-coil proteins, position "d" in the Pep M5 heptad is predominantly occupied by nonpolar residues (more often leucine). In contrast, although nonpolar residues are present in position "a," an equal number of asparagine residues are also found in this position. In addition, whereas 3 of the 4 valines occupy the core "a" and "d" positions, only 1 of the 9 alanines (alanine-13) is present at a

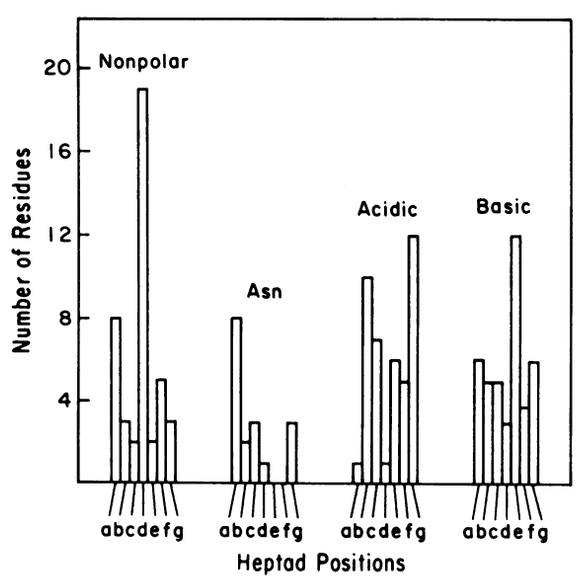

FIG. 4. Distribution of amino acid residues within the 7-residue repeats of the Pep M5 molecule. The histogram shows the total number of amino acid(s) of a particular type at each position within the 7-residue period for the region 13–196, with the skip residue threonine-150 omitted.

core "d" position, the remainder being present in the outer "b," "c," and "f" positions. This is similar to the placement of alanine residues in the S-2 fragment of heavy meromyosin (23).

As in other coiled-coil fibrous proteins, the charged residues of the Pep M5 protein occur less frequently in the core positions "a" and "d." While the positively charged residues, especially lysine, are found in some of these positions (5 lysines and 1 arginine in position "a," 2 lysines and 1 histidine in position "d"), there is only a single negatively charged residue (glutamic acid) in each of the "a" and "d" positions. It has been suggested previously that the tolerance of positively charged residues in core positions may be due to the nonpolar nature of the long hydrocarbon side chains of lysine and arginine, which may reinforce the core and will also hold their charged end groups at a distance from the helix surface (24, 32). Furthermore, at two of the core positions that are occupied by charged residues, the adjacent position is occupied by a nonpolar leucine residue. Thus, lysine-169 at an "a" position is preceded by leucine-168, and lysine-179 occupying a "d" position is followed by leucine-180. Thus, the presence of leucine in these positions may compensate for the influence of the charge. Similar occurrences have been seen in the sequence of the myosin rod (24).

The charged residues themselves exhibit a preferential distribution. Among the inner "e" and "g" positions, lysine is more predominantly found in position "e," while glutamic acid shows a strong preference for the "g" positions. Furthermore, the number of basic residues in position "e" is equal to the number of acidic residues in position "g" and vice versa. The distribution of charged residues in positions "e" and "g" found in the Pep M5 protein is the reverse of that found in the sequence of the myosin rod region (23, 24) and tropomyosin (16, 32), but it is similar to that found for the two fragments of α-keratin (19, 30). It may, therefore, be envisaged that, as in the case of these proteins (16, 19, 30, 32), salt bridges between opposite charges in position "e" of one helix and position "g" of a second helix may contribute significantly to the stability of the coiled-coil structure of the Pep M5 protein.

The only other amino acid residue that shows a preference for its distribution is threonine, being present predominantly in the "f" position.

The data on the percentage of amino acids in region 13–196 of the Pep M5 protein that belong in the core, inner, and outer positions is presented in Table 2. In the entire sequence of the Pep M5 protein, there is an equal number of both the negative and the positive charges, suggesting zero net charge for this molecule. However, an examination of the charged residue distribution within each class reveals that there are a relatively higher number of negative charges than positive charges in the outer "b," "c," and "f" positions, suggesting a net negative charge for the outer surface of the molecule. Among other coiled-coil proteins, a relatively higher negative charge distribution in the outer positions has been found for the keratin molecule (24).

Structurally Distinct Regions in the Pep M5 Molecule. A closer examination of the heptad periodicity shown in Fig. 2 revealed the presence of two structurally distinct regions in the Pep M5 molecule (indicated by vertical bars A and B in the figure). The pattern of distribution of nonpolar and charged residues in region 13–121 is significantly different from that found in region 122–196.

With regard to the core "a" and "d" positions, in the region 13–121 (region A), there is a predominance of asparagine at position "a" and leucine at position "d." On the other hand, in the region 122–196 (region B), asparagine is totally absent in the "a" position and, although not highly regular, there is a predominance of nonpolar residues

Table 2. Percentage of amino acids in different regions of the Pep M5 protein that belong to core, inner, and outer positions

Residue type	Core "a" and "d"	Inner "e" and "g"	Outer "b," "c," and "f"	Total
Region 13–196*				
Nonpolar (LIVYA)	14.75	2.73	5.46	22.95
Asparagine	4.92	1.64	2.73	9.29
Positive charges (HKR)	4.92	9.84	7.65	22.41
Negative charges (DE)	1.09	9.84	12.02	22.95
Positive + negative	6.01	19.68	19.67	45.36
Region 13–121				
Nonpolar (LIVYA)	14.68	0.92	4.6	20.20
Asparagine	7.34	0.92	1.84	10.10
Positive charges (HKR)	4.59	10.09	6.42	21.10
Negative charges (DE)	0	11.93	12.84	24.77
Positive + negative	4.59	22.02	19.26	45.87
Region 122–196*				
Nonpolar (LIVA)	14.86	5.41	6.76	27.03
Asparagine	1.35	2.70	4.05	8.10
Positive charges (KR)	5.41	9.46	9.46	24.33
Negative charges (DE)	2.70	6.76	10.81	20.27
Positive + negative	8.11	16.22	20.27	44.60

*The skip residue threonine-150 is not included. Amino acid residues are denoted by single letter code. L, leucine; I, isoleucine; V, valine; Y, tyrosine; A, alanine; H, histidine; K, lysine; R, arginine; D, aspartic acid; E, glutamic acid.

(leucine, isoleucine, and valine) in both the "a" and "d" positions. The fact that asparagine is found more predominantly in the "a" position within region 13–121 clearly points to some unique features in the Pep M5 protein compared to other coiled-coil molecules. However, the strongly hydrophobic leucine residues are present more regularly within this region, more or less at intervals of 7 and, hence, may have a major influence on the stability of the coiled-coil structure in this part of the Pep M5 molecule. In the remainder of the sequence—i.e., residues 122–196—the periodicity is more typical of that found in the coiled-coil proteins such as the myosin rod region.

In both regions 13–121 and 122–196, there are some segments in which the nonpolar residues at the core positions occur at intervals >7, the intervals being 14 in segments 69–83, 129–143, and 176–190, and 10 in segments 101–111 and 155–165. These segments probably represent potentially weak spots in the coiled-coil structure. Thus, it appears that the strength of the interaction of the nonpolar residues between the two chains within the Pep M5 protein coiled-coil (i.e., the seam of nonpolar residues) is considerably less in relation to the other coiled-coil proteins. However, as described above, because the residues in the inner "e" and "g" positions are readily capable of participating in interchain ionic interactions, these may also contribute to the strength of the coiled-coil.

The results of an analysis of the charged residue distribution in regions 13–121 and 122–196 are also included in Table 2. As can be seen here, in region 13–121, there are nearly twice as many acidic residues in the outer "b," "c," and "f" positions as there are basic residues; in contrast, in the region 122–196, the number of acidic and basic residues in the outer "b," "c," and "f" positions are essentially equal. Thus, it is apparent that the higher negative charge of the outer "b," "c," and "f" positions described above for the whole protein is localized to the NH_2-terminal region of the Pep M5 protein.

Internal Sequence Homology. Pep M5 protein contains some essentially identical sequence repeats (10, 33). In view of this and the repeating periodicity in the distribution of certain amino acid residues described above, the Pep M5 sequence was examined for the possible presence of internal homology by dot matrix analysis. As can be seen in Fig. 5, the Pep M5 sequence indeed exhibits significant internal homology. However, these homologies are not random; they are localized in two distinct regions of the molecule—i.e., in the NH_2-terminal half and in the COOH-terminal quarter of the sequence. Multiple segments within the NH_2-terminal half of the molecule are homologous to one another. Similarly, segments within the COOH-terminal quarter of the sequence are homologous to one another but are distinct from the segments in the NH_2-terminal region. Furthermore, these two distinct regions containing internal homologies are essentially the same as the two distinct regions recognized on the basis of the nonpolar and charged amino acid distributions (Fig. 2).

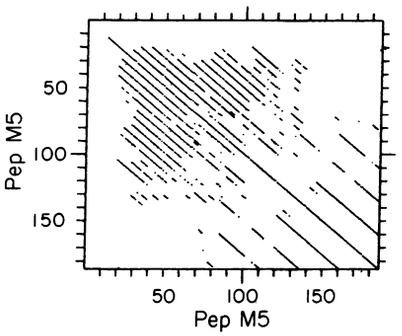

FIG. 5. Self-comparison of the Pep M5 sequence by dot matrix analysis (12). The complete amino acid sequence of Pep M5 protein is presented on both horizontal and vertical axes. Numbers on each axis represent residue positions in the corresponding sequence. Scores are accumulated from a sequential comparison of segments of 25 residues from the vertical axis with segments of the same length from the horizontal axis (no gaps allowed), using MDM (14). Scores greater than or equal to the MDM score of 25 are displayed by dots in a matrix plot, with the residue positions as the two axes. Dots are plotted relative to the middle of each segment in any given pair. The continuous diagonal line represents the line of identity as a result of comparison of the sequence with itself. Internal homology within the sequence is revealed by the series of diagonals offset from the major diagonal.

While Pep M5 exhibits significant internal homology, extensive identical sequence repeats have been found in the partial sequence of Pep M24, another M protein serotype (34, 35). It is, therefore, conceivable that gene duplication may have been an important event during the evolution of M protein.

DISCUSSION

The detailed analyses presented here reveal that, with the exception of the 12-residue nonhelical segment at the NH_2-terminal end of the molecule, a 7-residue periodicity characteristic of α-helical coiled-coil proteins essentially extends throughout the Pep M5 molecule. However, the periodicity in the distribution of nonpolar residues in the core "a" and "d" positions is much less regular than that found in tropomyosin, the archetypical coiled-coil protein, but it exhibits certain features that are seen in other coiled-coil proteins such as the myosin rod, α-keratin, and the intermediate filament proteins desmin and vimentin. Thus, the streptococcal M5 protein appears to be more similar to members of the keratin-myosin-epidermin-fibrinogen (k-m-e-f) family other than tropomyosin. In addition, although the Pep M5 protein exhibits significant sequence homology with tropomyosin as well as myosin, the homology with myosin is substantially higher than that with tropomyosin (unpublished observations). Knowing whether this is true for other

streptococcal M proteins requires more complete sequence data on these molecules.

Earlier electron microscopic studies have shown that the M protein molecules are highly flexible structures rather than rigid rods (3, 4). The discontinuities in the periodicity of the residues in the core positions of the M5 protein observed in the present study suggest interruptions in its coiled-coil structure, which in turn may explain the flexibility in its structure. Interruptions in the periodicities were also noted previously (7) in a 92-residue segment of the Pep M24 protein (34). In addition to the flexibility in the overall structure of this fibrous molecule, the variations in the discontinuities are highly suggestive of variability in the stability of the coiled-coil along its length. This in turn may have an influence on the arrangement of the coiled-coil on the cell surface, as well as have relevance to the biological properties of the molecule.

A salient feature of the Pep M5 protein is the presence of two distinct regions within its coiled-coil structure, as a result of distinct differences in the pattern of distribution of its amino acid residues. The COOH-terminal region (residues 122–196) has characteristics more similar to coiled-coil proteins, such as the myosin rod region, and has a net neutral charge on its outer surface. In contrast, the NH_2-terminal region (residues 13–121), having a 7-residue periodicity quite distinct from the COOH-terminal region, has a significantly higher negative charge on its outer surface. Human neutrophils have a net negative surface charge at physiological pH (36). Hence, it is conceivable that being distal to the cell surface, the net negative charge of the NH_2-terminal region of the M5 protein may hinder the contact between the streptococcus and the phagocyte due to electrostatic repulsion. This effect may thus play a role, at least in part, in the biological function of the M protein molecule.

A recent study by Fischetti (37) has shown that the opsonic M antibodies to the type 6 streptococci are relatively more basic than the nonopsonic M antibodies. Hence, it is presumable that the net negative charge of the outer surface of the M protein may have relevance to the opsonogenic determinant of the M protein molecule. Dale *et al.* (38) have demonstrated that antibodies produced against region 1–20 of the strain Manfredo M5 protein are protective antibodies. Region 1–20 of the M5 protein from strain Manfredo, although not identical, is highly homologous to the corresponding region of the Pep M5 protein reported here (10, 39). It is, therefore, conceivable that the antibodies to region 1–20 may function by sterically masking the negative charge in the NH_2-terminal region of the Pep M5 molecule. These results suggest that neutralization of the antiphagocytic function of the M protein may perhaps be achieved by more than one mechanism.

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