DIFFERENCE IN THE STRUCTURAL FEATURES OF STREPTOCOCCAL M PROTEINS FROM NEPHRITOGENIC AND RHEUMATOGENIC SEROTYPES

BY KIRAN M. KHANDKE,* THOMAS FAIRWELL,‡ AND
BELUR N. MANJULA*

From *The Rockefeller University, New York 10021; and the ‡Molecular Diseases Branch,
National Heart, Lung, and Blood Institute, Bethesda, Maryland 20205

M protein of the group A streptococcus, a fibrillar structure on the bacterial cell surface, functions as its antiphagocytic determinant (1–3). Over 75 different serotypes of group A streptococci have been recognized, the acquired immunity in humans to any particular serotype being type-specific (1). Two of the major sequelae of group A streptococcal infections are acute rheumatic fever and acute glomerulonephritis. It has been consistently found that some serotypes are more frequently associated with rheumatic fever, while others are more often associated with glomerulonephritis (4–8). However, neither the mechanism by which M protein impedes phagocytosis nor the role of the M proteins in the pathogenesis of these streptococcal diseases is fully understood at present.

Over the past decade, our knowledge about the structural features of the M proteins from the rheumatogenic serotypes has advanced considerably, derived essentially from studies on the M proteins of serotypes 5, 6, and 24 (3, 9–15). These studies have revealed that the streptococcal M protein is a dimeric molecule, and exists as a highly flexible α-helical coiled-coil structure, with its NH_2-terminal region being distal to the bacterial cell surface. Sequence comparisons of the biologically active NH_2-terminal halves of the M5, M6, and M24 proteins, namely PepM5, PepM6, and PepM24 (9, 12, 14, 16), have revealed that the three M protein serotypes exhibit homology with each other in their NH_2-terminal region. However, PepM5 and PepM6 show greater homology between each other in the COOH-terminal region, but differ significantly from the COOH-terminal region of PepM24 (12, 13). Thus, the PepM5 and PepM6 proteins are more closely related to each other than either is to the PepM24 protein. Clearly, there are similarities as well as differences among the various M protein serotypes.

While our present structural studies on the rheumatogenic M protein serotypes are limited to three, the nephritogenic M protein serotypes are even less well studied. With a view to understanding the structural relatedness among M proteins of the nephritogenic and rheumatogenic serotypes, we undertook studies on the type 49 M protein, a nephritogenic serotype. Our studies have revealed that PepM49, a peptic fragment of the M49 protein, retains the opsonic antibody

epitope of the native molecule and is dimeric like the other PepM proteins. However, its NH₂-terminal sequence differs significantly from the NH₂-terminal sequences of the rheumatogenic M protein serotypes PepM5, PepM6, and PepM24. Nevertheless, like the 5, 6, and 24 M proteins, the NH₂-terminal sequence of PepM49 exhibits a heptad periodicity in its nonpolar amino acid residues, a characteristic of α-helical coiled-coil proteins.

During the course of our investigations, the NH₂-terminal sequence of another M protein serotype, PepM1, was reported (17). PepM1 also does not exhibit homology with the NH₂-terminal regions of the PepM5, PepM6, and PepM24 proteins. Our analysis of the reported sequence of the PepM1 protein has revealed the presence of a heptad periodicity of the nonpolar amino acids in this M protein as well. Moreover, we have found that the pattern of heptad periodicity in the PepM49 and PepM1 proteins is more similar to each other than to those in the PepM5, PepM6, and PepM24 proteins. PepM1 protein is also a nephritogenic serotype. Thus, our studies suggest that the rheumatogenic and the nephritogenic M protein serotypes bear distinct structural features despite conservation of the overall conformational characteristics.

Materials and Methods

Streptococcal Strain. Type 49 streptococcal strain B915 was from The Rockefeller University's collection. It was an isolate from a patient suffering from acute glomerulonephritis. Before growth for M protein extraction, the cells were passed several times in normal human blood to select for M protein–rich organisms (18).

Culture Conditions. Overnight cultures of the type 49 streptococci were grown in Todd-Hewitt broth (BBL Microbiology Systems, Cockeysville, MD), supplemented with 10% yeast extract (Difco Laboratories, Detroit, MI), and 1.7% glucose. The pH was maintained at 7.0 with 5 N NaOH. The culture was concentrated using a Pellicon ultrafiltration unit (Millipore, Bedford, MA), and the cells harvested by centrifugation. The yield of the bacteria varied from 575 to 680 g (wet wt) per 60 liters of broth.

Isolation of PepM49. PepM49 was isolated from the type 49 streptococci by limited proteolysis with pepsin as described (16). Cells (260 g wet wt) were washed in 0.067 M phosphate buffer, pH 5.8, and treated with pepsin (1 mg pepsin per 10 g cells) at 37°C for 60 min, with slow stirring. The digest was recovered by centrifugation and concentrated by ultrafiltration using a YM-10 membrane (Amicon Corp., Danvers, MA), dialyzed against 0.05 M ammonium bicarbonate, and lyophilized. The pepsin digestion was repeated twice to recover most of the PepM49 protein from the cells. Typical yields of the crude PepM49 protein were 190, 150, and 130 mg, respectively, from the three successive digests.

Purification Procedures. Chromatography on DEAE–Sephadex A-25 was carried out in 0.01 M sodium phosphate buffer, pH 8.0, using a 0–0.3 M linear NaCl gradient. Gel filtration was carried out on an Ultrogel AcA44 column in 0.2 M ammonium bicarbonate. The elution of protein was monitored at 230 nm and the purity of the fractions was monitored by SDS-PAGE.

PAGE and Immunoblots. SDS-PAGE was carried out on a 12% polyacrylamide gel essentially according to the procedure of Laemmli (19). Protein standards were run concurrently to obtain estimates of molecular mass of the M protein preparations. Immunoblots were carried out as described (20, 21).

Coupling of PepM49 to an Insoluble Support. PepM49 (1 mg) in 0.5 ml PBS, pH 7.4, was added to 175 mg of glutaraldehyde-activated affinity absorbent (Boehringer Mannheim Biochemicals, Indianapolis, IN). The reaction mixture was gently rotated for 6 h at room temperature. SDS-PAGE of the postreaction supernatant showed complete absence of protein, indicating efficient coupling of the PepM49 to the silicate carrier matrix. The
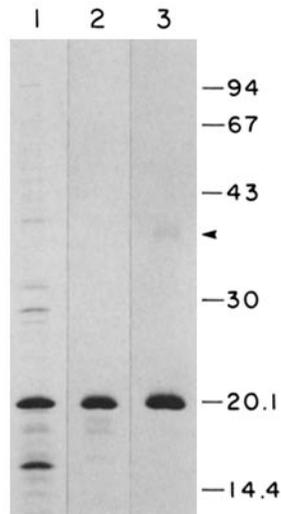
FIGURE 1. SDS-PAGE of the pepsin digest of type 49 streptococci (lane *1*); PepM49 preparation after fractionation on DEAE-Sephadex (lane *2*); PepM49 preparation after ultrogel fractionation (lane *3*). Arrowhead indicates the position of the 39,500 M_r band. The positions of the molecular mass (kD) standards on the same gel are indicated.

unreacted sites on the matrix were blocked with ethanolamine, and the gel was extensively washed with PBS, pH 7.4. A control gel without the protein was prepared under identical conditions.

Antisera. Type-specific sera used in these studies were prepared in rabbits by inoculation with whole heat-killed streptococcal vaccines as previously described (22).

Absorption of Type 49 Opsonic Antibodies. The affinity gel carrying the PepM49 protein, as well as the control gel were incubated with 250 μl of type 49 opsonic serum and rotated for 16 h at 4°C. The gel was removed by centrifugation and the supernatant serum was used to test for opsonic antibodies by the indirect bactericidal test.

Indirect Bactericidal Assay. This test was carried out according to the procedure of Lancefield (23) as previously described (24).

Other Methods. 3–5 nmol of the protein were hydrolyzed with 6 N HCl for amino acid analysis. Amino acid analysis and amino acid sequencing were carried out as previously described (10).

Results

Isolation and Purification of PepM49. Limited proteolysis of type 49 streptococci with pepsin resulted in the release of a major band of M_r 20,000 and several minor bands (Fig. 1, lane *1*). On immunoblots, the major 20,000 M_r band reacted strongly with type-specific antiserum to the type-49 streptococci. For isolation of this major component, designated hereafter as PepM49, the crude pepsin digest of the type-49 cells was first fractionated on a DEAE Sephadex column in 0.01 M sodium phosphate, pH 8.0. The PepM49 protein was adsorbed on the column and eluted in the NaCl gradient at a salt concentration of 0.13 M. SDS-PAGE revealed that this fraction was free of most of the other associated proteins in the digest (Fig. 1, lane *2*). Furthermore, this fraction was also free of UV-absorbing nonproteinaceous material, which eluted at higher concentrations of NaCl. The ratio of the absorbance at 230/260 nm increased from a value of ~1.0 in the crude digest to ~10.0 in this 0.13 M NaCl fraction. The small amount of the lower-molecular-mass bands in this preparation was removed by gel filtration on an Ultrogel AcA44 column eluted with 0.2 M ammonium bicarbonate. The PepM49 protein eluted at a position corresponding to 44,000
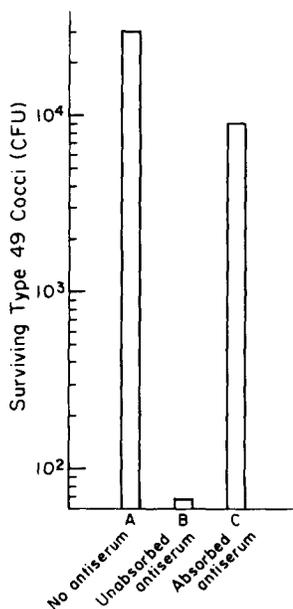
FIGURE 2.   Indirect bactericidal assay for the type 49 streptococci in presence of no antiserum (*A*), unabsorbed type 49 antiserum (*B*), and type 49 antiserum after absorption on PepM49-coupled affinity gel (*C*). Heparinized whole blood from nonimmune human donors was used as the source of phagocytes. Dilutions of the log-phase cultures of the type 49 streptococci (100 μl) were mixed with human blood (400 μl) in the presence or absence of the test serum (100 μl) and rotated at 37°C at 16 rpm for 3 h. The surviving colony forming units (CFUs) were determined by pour-plate method. Dilutions of the reaction mixtures were plated to obtain an exact count of the CFUs. Stationary controls without the test serum were run to ensure the ability of the organisms to grow in the donors blood. Inoculum, 256 CFU. The data presented represent CFU from a 100-μl aliquot of the assay mixture at the end of the assay period.

M_r, as determined by gel filtration of protein standards (BSA, ovalbumin, chymotrypsinogen, and ribonuclease) under the same conditions. This result indicates that the PepM49 protein is present as a dimer under nondissociating conditions. SDS-PAGE of the column fractions revealed a clear separation of the minor low-M_r bands from the major PepM49 band. The fractions free of the minor low-M_r bands were pooled and lyophilized. On SDS-PAGE, this preparation revealed, in addition to the major 20,000 M_r band, a faint band at ~39,500 M_r (Fig. 1, lane *3*). The amount of the latter component varied from preparation to preparation. These results suggest that a small amount of the PepM49 protein remains in dimerized state even after SDS-mercaptoethanol treatment. Similar dimeric forms have been previously observed for other M proteins (3). Electroblotting of this PepM49 preparation, and subsequent reaction with type-specific antiserum to the type 49 streptococci confirmed immunoreactivity of this protein preparation. Typically the final yield of the purified PepM49 protein was of the order of 10% (by weight) of the crude digest.

Immunologic Properties of PepM49.   The retention of the opsonic antibody epitope in PepM49 was tested by determining its ability to absorb opsonic antibodies from type 49 antiserum. For this, the PepM49 protein was immobilized on a glutaraldehyde-activated affinity absorbent as described in Materials and Methods. Rabbit anti–type 49 antiserum was incubated with the immobilized PepM49, the reaction mixture was spun to remove antibody-bound immobilized PepM49, and the supernatant (absorbed type 49 antiserum) was compared with unabsorbed type 49 antiserum for its ability to opsonize type 49 streptococci in the indirect bactericidal assay. The results are presented in Fig. 2. As can be seen, the cocci multiply readily in the absence of antiserum (Fig. 2*A*). However, the unabsorbed type 49 antiserum is highly opsonic to the type 49 cocci, and the cocci are ingested and killed (Fig. 2*B*). In contrast, opsonic activity of the type

TABLE I

Amino Acid Composition of the PepM49 Protein. Comparison with Other M Proteins

Amino acid	Residues per 100				
	PepM49	PepM1	PepM5	PepM6	PepM24
Asx	7.73	10.74	13.20	16.67	14.63
Thr	1.30	4.52	10.67	12.72	4.52
Ser	5.03	7.35	3.05	2.63	6.65
Glx	33.75	23.16	23.86	18.86	14.36
Pro	—	0.57	0.51	0.88	—
Gly	1.53	2.83	4.06	3.07	1.86
Ala	8.19	10.17	4.57	5.70	19.42
Val	5.11	1.70	2.03	3.07	1.06
Cys	—	—	—	—	—
Met	—	0.57	—	0.44	1.60
Ile	2.08	3.39	1.03	4.39	1.86
Leu	8.68	13.56	12.18	10.53	12.50
Tyr	3.15	2.26	1.02	0.44	—
Phe	—	1.13	—	0.44	2.66
His	2.00	1.13	1.02	—	0.80
Lys	13.03	10.74	17.77	17.54	14.89
Arg	8.45	6.22	3.05	2.63	3.19

The references for the amino acid compositions of the other M proteins are: PepM1 (17), PepM5 (10), PepM6 (12, 13), and PepM24 (14).

49 antiserum is virtually lost upon absorption with the PepM49-coupled affinity gel (Fig. 2C). In control experiments, the opsonic activity of the type 49 antiserum absorbed on gel without the PepM49 protein was comparable to that of the unabsorbed antiserum. Similar results were obtained when the assay was carried out using a 10-fold higher inoculum. These results clearly demonstrate that the opsonic antibody epitope of the M49 molecule is retained in the purified PepM49 protein.

Amino Acid Composition of PepM49. The amino acid composition of the PepM49 protein is presented in Table I, along with those of PepM5 (10), PepM6 (13), PepM24 (14), and the more recently reported composition of PepM1 (17). As can be seen, the amino acid composition of the PepM49 protein is quite similar to the composition of other Pep M proteins, in that it is rich in acidic, basic, and aliphatic nonpolar amino acids, but poor in aromatic amino acids. It may also be seen from Table I that, although the amino acid composition of the different M proteins is generally similar, there is also a certain degree of variation among the different serotypes. The striking feature of the PepM49 protein in comparison with the PepM5, PepM6, and PepM24 proteins is the relatively higher content of glutamic acid and arginine. A relatively higher content of arginine is also found in the PepM1 protein (17).

Amino Acid Sequence of PepM49 Protein. The sequence of the NH₂-terminal 60 residues of the PepM49 protein was determined by automated Edman degradation, and the results are presented in Fig. 3A. Unlike the PepM5 and PepM6 proteins, no sequence repeats are present within the NH₂-terminal region of the PepM49 protein. This sequence was compared with the previously reported sequences of M5, M6, and M24 proteins (10, 12–15), and was found to
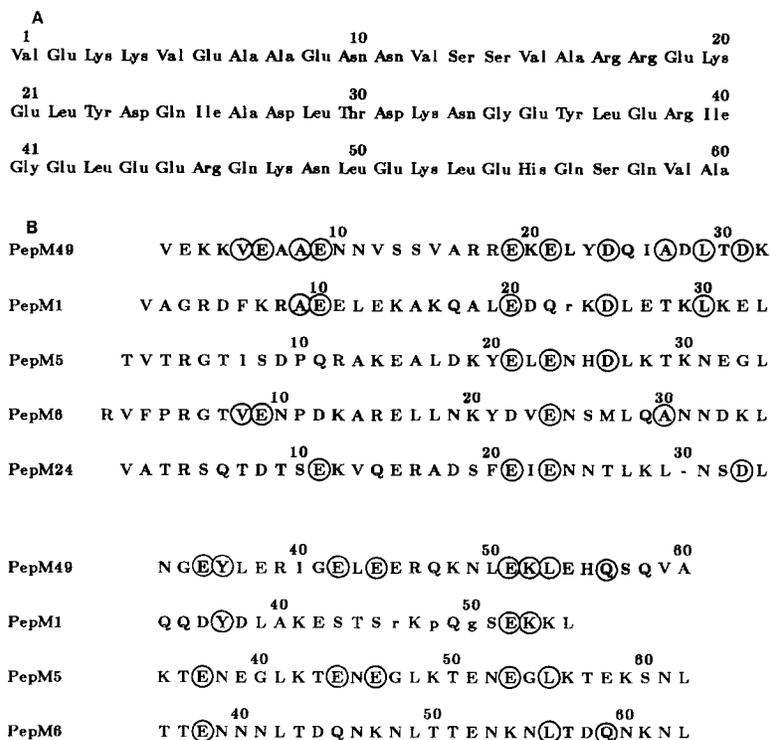
A

1   10   20
Val Glu Lys Lys Val Glu Ala Ala Glu Asn Asn Val Ser Ser Val Ala Arg Arg Glu Lys

21   30   40
Glu Leu Tyr Asp Gln Ile Ala Asp Leu Thr Asp Lys Asn Gly Glu Tyr Leu Glu Arg Ile

41   50   60
Gly Glu Leu Glu Glu Arg Gln Lys Asn Leu Glu Lys Leu Glu His Gln Ser Gln Val Ala

B

FIGURE 3. (A) NH₂-terminal amino acid sequence of the PepM49 protein. (B) Comparison of the NH₂-terminal sequence of the PepM49 protein with PepM1 (17), PepM5 (10), PepM6 (12, 13, 21), and PepM24 (14) proteins. Residues in the PepM1, PepM5, PepM6, and PepM24 proteins that are identical with the PepM49 sequence are circled. The sequences are represented in single-letter code.

bear very little homology with the NH₂-terminal regions of these molecules (Fig. 3B). During the course of the present studies, the NH₂-terminal sequence of the PepM1 protein was reported (17). It may also be seen from Fig. 3B that the NH₂-terminal region of the PepM49 protein shows very little homology with the reported NH₂-terminal sequence of the PepM1 protein (17).

Heptad Periodicity in PepM49. Our earlier studies have revealed that a common characteristic of the previously studied rheumatogenic M protein serotypes 5, 6, and 24 is the presence of a repeating heptad periodicity in the distribution of their nonpolar and charged residues, a characteristic of α-helical coiled-coil proteins (9). If the repeating heptad periods of an α-helical coiled-coil protein can be represented by the generalized sequence *abcdefg*, then residues at positions *a* and *d* are generally nonpolar, and form a closely packed hydrophobic interface between the two strands of the coiled-coil. The residues at the *a* and *d* positions thus constitute the core residues. The inner positions *e* and *g* lie next to the core, while the amino acid side chains in the outer *b*, *c*, and *f* positions point away from the core and are free to interact with the surrounding molecules (25).

The NH₂-terminal sequence of the PepM49 protein was analyzed to determine whether the heptad periodicity of the nonpolar residues previously observed for the M5, M6, and M24 proteins is also present in this molecule. As can be seen
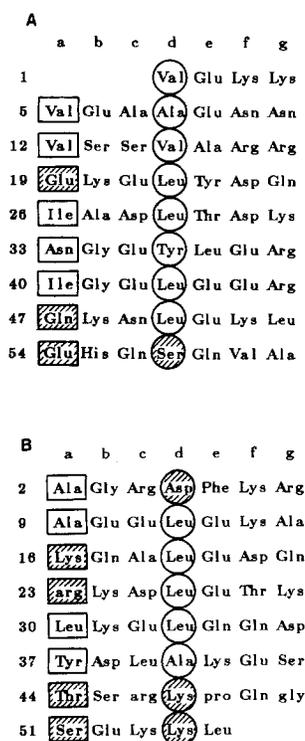
FIGURE 4. Heptad periodicity in PepM49 (A) and PepM1 (B). The residues within each heptad are designated by letters a through g. The residues at position a and d are boxed and circled respectively. Residues other than nonpolars and asparagines at these positions are hatched (see text for details). PepM1 sequence is from Moravek et al. (17).

in Fig. 4A, like the other M protein serotypes, PepM49 also exhibits a repeating heptad periodicity in the linear disposition of its nonpolar amino acid residues that is characteristic of α-helical coiled-coil proteins. However, the distribution of the nonpolar residues at positions a and d within the NH_2-terminal region of the PepM49 protein is certainly more regular than is found in PepM5, PepM6, and PepM24 proteins (9, 11, 26). Within the NH_2-terminal regions of the PepM5 and PepM6 proteins, there is a predominance of asparagine at the a positions and leucine at the d positions. This trend is also seen in PepM24. In contrast, within the NH_2-terminal region of the PepM49 protein, there is only one asparagine in the a position and nonpolar residues predominate in both the a and the d positions. Thus, the coiled-coil structure of the NH_2-terminal region of the PepM49 protein may be expected to be more stable relative to the NH_2-terminal regions of the PepM5, PepM6, and PepM24 proteins.

Heptad Periodicity in PepM1. Like the type M49 protein, type 1 M protein is also a nephritogenic serotype. In addition to the PepM49 sequence, we have also analyzed the reported sequence of the PepM1 protein (17) for the presence of special features in it. This analysis revealed that, as in the PepM49 protein, sequence repeats are absent in the NH_2-terminal region of the PepM1 molecule. Furthermore, PepM1 protein also exhibits a heptad periodicity in its nonpolar amino acids (Fig. 4B). Like the PepM49 protein, positions a and d of the PepM1 sequence are occupied predominantly by nonpolar residues and asparagine is completely absent in these core positions. Thus, the pattern of nonpolar residue

distribution within the NH$_2$-terminal region of the PepM1 protein is more similar to that of the PepM49 protein than to those of the PepM5, PepM6, and PepM24 proteins.

Discussion

The present study has demonstrated that limited pepsin digestion, previously used for the isolation of biologically active fragments of the rheumatogenic M protein serotypes (9, 12, 14, 16), can also be successfully used for the isolation of a biologically active fragment of the type 49 M protein, a nephritogenic serotype. These results suggest that, like the M proteins of the rheumatogenic serotypes, the M49 protein in its native state contains a region that is readily susceptible to proteolysis with pepsin, and hence permits the isolation of a functional domain of the molecule. Thus, there appears to be a similarity in the anatomy of the M proteins of the rheumatogenic and the nephritogenic serotypes.

The PepM49 protein, isolated from a nephritogenic M protein serotype has the general characteristics of M proteins. Its amino acid composition is similar to those of the other M proteins, and gel filtration studies indicate that, like the other M proteins, the PepM49 protein exists as a dimeric molecule. Moreover, it exhibits the heptad periodicity characteristic of α-helical coiled-coil proteins, suggesting conservation of the overall tertiary structural features between the rheumatogenic and the nephritogenic M protein serotypes.

A significant difference between the PepM49 protein in comparison with the PepM5, PepM6, and PepM24 proteins is the absence of sequence repeats. The presence of sequence repeats is a common characteristic of the 5, 6, and 24 M proteins, although the size of the repeats is different in each M protein (9, 10, 12–14, 27). However, no sequence repeats are present within the NH$_2$-terminal region of the PepM49 protein.

Although 75 M protein serotypes have been recognized to date, complete or partial sequence information is available only for 4 other M protein serotypes, namely M1 (17), M5 (10), M6 (12, 13), and M24 (14, 15). PepM5, PepM6, and PepM24 NH$_2$-terminal sequences are distinct, yet homologous to one another (10). Among these, PepM5 and PepM6 proteins are more closely related to each other than either one of them to the PepM24 protein, both as seen by sequence homology (12, 13), as well as by immunological crossreactivity (12, 28). The NH$_2$-terminal sequence of the PepM1 protein, on the other hand, exhibits very little homology with the NH$_2$-terminal sequences of the PepM5, PepM6, and PepM24 proteins. However, it exhibits some homology with the internal regions of the PepM24 protein (17).

Comparison of the structural features of the PepM49 protein to the other M protein serotypes has revealed that the PepM49 protein is more closely related to PepM1 than to PepM5, PepM6, and PepM24 proteins. Within the NH$_2$-terminal regions of the PepM5 and PepM6 proteins, the residues at position a are predominantly asparagine, whereas those at position d are predominantly nonpolar, most often leucine (9, 11, 12, 26). A similar trend is also seen in the M24 protein (9, 26). The presence of asparagines at a significant number of the a positions may be expected to render the coiled-coil structure of this region of the molecule more flexible (9, 11). Thus, the presence of asparagines at the a

positions, a feature that is less typical of other coiled-coil proteins, appears to be a common characteristic of the rheumatogenic M protein serotypes. In contrast, the occurrence of nonpolars at the a as well as the d positions within the NH_2-terminal regions of both the PepM1 and the PepM49 proteins is relatively more regular, and more similar to that seen in other coiled-coil proteins. Asparagine is found only in one of the a positions in the PepM49 protein and is completely absent in both the a and d positions in PepM1. 12 of 17 of the a and d positions within the NH_2-terminal 60 residues of the PepM49 protein are occupied by nonpolar residues. In the PepM1 protein, 9 of 16 of the a and d positions are occupied by the nonpolars; two of the a positions in the PepM1 sequence are occupied by lysine and arginine. Occurrence of lysine and arginine in the a position has been observed before in other coiled-coil proteins (29–31). The coiled-coil features are even more regular within the first 53 and 43 residues of PepM49 and PepM1 proteins, respectively. While the nonpolar residues occupy 12 of 15 of the core positions in region 1–53 of the PepM49 protein, they occupy 9 of 12 of these positions in region 1–43 of the PepM1 protein. Such higher percentages of the nonpolar residues has not been seen within the NH_2-terminal regions of the PepM5, PepM6, and PepM24 proteins (9, 11, 26). These results are suggestive of a more stable α-helical coiled-coil structure for the NH_2-terminal regions of the PepM1 and PepM49 proteins. Thus, although PepM1 and PepM49 proteins are not homologous to each other, the patterns of heptad periodicity in the two proteins are more similar to each other than to those in the PepM5, PepM6, and PepM24 proteins.

Our previous studies have suggested that the streptococcal M protein and the *E. coli* lipoprotein may have evolved from a common ancestral gene (9, 32). The pattern of heptad periodicity observed here in the PepM49 and PepM1 proteins is even more similar to that of the *E. coli* lipoprotein than previously observed for the M5, M6, and M24 proteins (12, 13).

While PepM5 and PepM6 proteins are more closely related to each other than either one to the PepM24 protein, PepM1 and PepM49 proteins appear to be more related to each other than to PepM5, PepM6, and PepM24 proteins. We have also observed that the NH_2-terminal sequence of PepM30, a rheumatogenic serotype, is highly homologous to the PepM5, PepM6, and PepM24 proteins, but not to PepM1 and PepM49 proteins (our unpublished observations). These findings indicate that a high degree of sequence variation in the M protein is tolerated with the features necessary to maintain a coiled-coil structure being conserved. These results suggest that, despite conservation of the overall tertiary structure, the M proteins of the nephritogenic serotypes 1 and 49 are quite distinct, and may be evolutionarily further apart from the rheumatogenic serotypes 5, 6, and 24, and possibly type 30. These findings point to the complexity in the M protein antigenic variation and are suggestive of the existence of several subclasses of the M protein serotypes.

Numerous investigators, most notably Rammelkamp (7) and Stollerman (33) have established that acute poststreptococcal glomerulonephritis results from infection with a limited number of serological types of group A streptococci. M type 1 has been clearly related to epidemic nephritis following pharyngitis (6, 8). The Red Lake strain, type 49, has been repeatedly associated with pyoderma

nephritis epidemics not only at Red Lake but throughout the world (6, 8). Thus, it is interesting to note that the M serotypes associated with nephritis clearly show distinct differences in the pattern of their heptad periodicity in comparison to the M serotypes associated with rheumatic fever. The biologic and the pathologic significance of the observed structural differences in the M proteins of the rheumatogenic and the nephritogenic serotypes is not readily apparent, and must await further investigation.

Summary

The association of only certain M protein serotypes of group A streptococci with acute glomerulonephritis is very well recognized. Structural information on the M protein, a dimeric α-helical coiled-coil molecule, has come so far from three rheumatogenic serotypes, 5, 6, and 24. However, M proteins from the nephritogenic serotypes have not been well characterized. In the present study, we have isolated a biologically active 20,000 M_r pepsin fragment of type 49 M protein (PepM49), a nephritogenic serotype, and purified it to homogeneity using DEAE-Sephadex and gel filtration. The amino acid composition of PepM49 is similar to those of the rheumatogenic M protein serotypes PepM5, PepM6, and PepM24. However, the sequence of the NH_2-terminal 60 residues of PepM49 shows little homology to any of these M protein serotypes, although the latter have significant homology among themselves. Nevertheless, PepM49 exhibits a strong heptad periodicity in its nonpolar residues, suggesting its overall conformational similarity with the other M molecules. During the course of the present studies, Moravek et al. (17) reported the NH_2-terminal sequence of another M protein serotype, PepM1, which also does not exhibit much homology with the PepM5, PepM6, and PepM24 proteins. Our analysis of this sequence revealed that the PepM1 protein also exhibits a heptad periodicity of the nonpolar amino acids. A closer examination has revealed that the pattern of heptad periodicity in PepM49 and PepM1 proteins is more regular and more similar to each other than has been previously seen for the PepM5, PepM6, and PepM24 proteins. PepM1 is also a nephritogenic serotype. Taken together, these findings indicate an underlying conservation of the tertiary structure of the various M protein serotypes, despite the complexity in their antigenic variation and suggest that the nephritogenic M protein serotypes M1 and M49 may be further apart evolutionarily from the rheumatogenic serotypes 5, 6, and 24. The distinct differences in the structural features of the PepM1 and PepM49 proteins relative to the PepM5, PepM6, and PepM24 proteins are also suggestive of a correlation with the earlier broader classification of the group A streptococci into rheumatogenic and nephritogenic serotypes.

References

1. Lancefield, R. C. 1962. Current knowledge of the type specific M antigens of group A streptococci. *J. Immunol.* 89:307.

2. Swanson, J., K. C. Hsu, and E. C. Gotschlich. 1969. Electron microscopic studies on streptococci. I. M antigen. *J. Exp. Med.* 130:1063.

3. Phillips, G. N., P. F. Flicker, C. Cohen, B. N. Manjula, and V. A. Fischetti. 1981. Streptococcal M protein: alpha-helical coiled-coil structure and arrangement on the cell surface. *Proc. Natl. Acad. Sci. USA.* 78:4689.

4. McCarty, M. 1972. Theories of pathogenesis of streptococcal complications. *In* Streptococci and Streptococcal Diseases. L. W. Wannamaker and J. M. Matsen, editors. Academic Press, New York. 517.

5. Zabriskie, J. B. 1967. Mimetic relationships between group A streptococci and mammalian tissues. *Adv. Immunol.* 7:147.

6. Dillon, H. C. 1972. Streptococcal infections of the skin and their complications: impetigo and nephritis. *In* Streptococci and Streptococcal Diseases. L. W. Wannamaker and J. M. Matsen, editors. Academic Press, New York. 571–587.

7. Rammelkamp, C. H. 1980. Acute poststreptococcal glomerulonephritis. *In* Streptococcal Diseases and the Immune Response. S. E. Read and J. B. Zabriskie, editors. Academic Press, New York. 43–51.

8. Wannamaker, L. W. 1970. Differences between streptococcal infections of the throat and of the skin (second of two parts). *N. Engl. J. Med.* 282:78.

9. Manjula, B. N., and V. A. Fischetti. 1980. Tropomyosin-like seven residue periodicity in three immunologically distinct streptococcal M proteins and its implication for the antiphagocytic property of the molecule. *J. Exp. Med.* 151:695.

10. Manjula, B. N., A. S. Acharya, S. M. Mische, T. Fairwell, and V. A. Fischetti. 1984. The complete amino acid sequence of a biologically active 197-residue fragment of M protein from type 5 group A streptococci. *J. Biol. Chem.* 259:3686.

11. Manjula, B. N., B. L. Trus, and V. A. Fischetti. 1985. Presence of two distinct regions in the coiled-coil structure of the streptococcal PepM5 protein: relationship to mammalian coiled-coil proteins and implications to its biological properties. *Proc. Natl. Acad. Sci. USA.* 82:1064.

12. Manjula, B. N., A. S. Acharya, T. Fairwell, and V. A. Fischetti. 1986. Antigenic domains of the streptococcal PepM5 protein: localization of epitopes crossreactive with type 6 M protein and identification of a hypervariable region of the M molecule. *J. Exp. Med.* 163:129.

13. Hollingshead, S. K., V. A. Fischetti, and J. R. Scott. 1986. Complete nucleotide sequence of type 6 M protein of the group A streptococcus: repetitive structure and membrane anchor. *J. Biol. Chem.* 261:1677.

14. Beachey, E. H., J. M. Seyer, and A. H. Kang. 1978. Repeating covalent structure of streptococcal M protein. *Proc. Natl. Acad. Sci. USA.* 75:3163.

15. Beachey, E. H., J. M. Seyer, J. B. Dale, and D. L. Hasty. 1983. Repeating covalent structure and protective immunogenicity of native and synthetic polypeptide fragments of type 24 streptococcal M protein. *J. Biol. Chem.* 258:13250.

16. Manjula, B. N., and V. A. Fischetti. 1980. Studies on group A streptococcal M proteins: purification of type 5 M-protein and comparison of its amino terminal sequence with two immunologically unrelated M-protein molecules. *J. Immunol.* 124:261.

17. Moravek, L., O. Kuhnemund, J. Havlicek, P. Kopecky, and M. Pavlik. 1987. Type 1 M protein of streptococcus pyogenes. N-terminal sequence and peptic fragments. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 208:435.

18. Becker, C. G. 1964. Selection of group A streptococci rich in M-protein from populations poor in M-protein. *Am. J. Pathol.* 44:51.

19. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680.

20. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* 76:4350.

21. Fischetti, V. A., K. F. Jones, B. N. Manjula, and J. R. Scott. 1984. Streptococcal M6 protein expressed in *Escherichia coli*. Localization, purification and comparison with streptococcal-derived M protein. *J. Exp. Med.* 159:1083.

22. Rotta, J., R. C. Krause, R. C. Lancefield, W. Everly, and H. Lackland. 1971. New approaches for the laboratory recognition of M types of group A streptococci. *J. Exp. Med.* 134:1298.

23. Lancefield, R. C. 1959. Persistence of type specific antibodies in man following infection with group A streptococci. *J. Exp. Med.* 110:271.

24. Manjula, B. N., M. L. Schmidt, and V. A. Fischetti. 1985. Unimpaired function of human phagocytes in the presence of phagocytosis-resistant group A streptococci. *Infect. Immun.* 50:610.

25. McLachlan, A. D., and M. Stewart. 1975. Tropomyosin coiled-coil interactions: Evidence for an unstaggered structure. *J. Mol. Biol.* 98:293.

26. Fischetti, V. A., and B. N. Manjula. 1982. Biologic and immunologic implications of the structural relationship between streptococcal M protein and mammalian tropomyosin. *In* Seminars in Infectious Disease, Vol. IV, Bacterial Vaccines. J. B. Robbins, J. C. Hill, and J. C. Sadoff, editors. Thieme-Stratton, Inc., New York. 411–418.

27. Manjula, B. N., S. M. Mische, and V. A. Fischetti. 1983. Primary structure of streptococcal PepM5 protein: Absence of extensive sequence repeats. *Proc. Natl. Acad. Sci. USA.* 80:5475.

28. Dale, J. B., and E. H. Beachey. 1985. Multiple, heart-cross-reactive epitopes of streptococcal M proteins. *J. Exp. Med.* 161:113.

29. Parry, D. A. D. 1975. Analysis of the primary sequence of alpha-tropomyosin from rabbit skeletal muscle. *J. Mol. Biol.* 98:519.

30. Parry, D. A. D. 1982. Coiled-coils in alpha-helix-containing proteins: analysis of the residue types within the heptad repeat and the use of these data in the prediction of coiled-coils in other proteins. *Biosci. Rep.* 2:1017.

31. McLachlan, A. D., and J. Karn. 1983. Periodic features in the amino acid sequence of nematode myosin rod. *J. Mol. Biol.* 164:605.

32. Manjula, B. N., and V. A. Fischetti. 1986. Sequence homology of group A streptococcal PepM5 protein with other coiled-coil proteins. *Biochem. Biophys. Res. Commun.* 140:684.

33. Stollerman, G. H. 1980. Pathogenetic issues in the clinical features of rheumatic fever. *In* Streptococcal Diseases and the Immune Response. S. E. Read and J. B. Zabriskie, editors. Academic Press, New York. 3–11.