TROPOMYOSIN-LIKE SEVEN RESIDUE PERIODICITY IN THREE IMMUNOLOGICALLY DISTINCT STREPTOCOCCAL M PROTEINS AND ITS IMPLICATIONS FOR THE ANTIPHAGOCYTIC PROPERTY OF THE MOLECULE*

By B. N. MANJULA AND V. A. FISCHETTI‡

From The Rockefeller University, New York 10021

Downloaded from http://rup.silverchair.com/jem/article-pdf/151/3/695/1090805/695.pdf by guest on 25 March 2023

M proteins of the group A streptococcus, despite their well-recognized immunological variations, have the unique capacity of maintaining a common antiphagocytic function for the organism. Antibodies to M proteins, however, are capable of neutralizing the antiphagocytic activity of these molecules (1-3). Although, immunological cross-reactions between different M types have been observed (4-6), the opsonic activity of the M antibody is type specific (4, 7). Rarely does an antibody opsonic to one M type serve the same function for another.

In an attempt to understand the structural features governing the common biological function of the immunologically diverse M proteins, we have undertaken studies on the primary structure of these molecules. Our previous work (8) on the comparison of the partial sequence of M5 protein with M24, the only other M protein for which partial sequences have been reported (9), revealed certain similarities between them. In the present paper, we report the partial sequence of a third M protein; namely, M6, and additional sequence data on the M5 molecule. Comparison of these partial sequences with those of the M24 molecule (9) indicated that despite sequence variations certain amino acid identities were common to the three M molecules, which suggests a conservative sequence relationship between these proteins. In addition, secondary structural analysis of these known M protein segments revealed that they all exhibit high alpha-helical potential. Even more striking was the fact that they all contained regions exhibiting a repeating seven residue periodicity. Furthermore, the amino acid identities common to the homologous regions of the three M proteins occupied defined positions within the seven residue period.

In a recent report from our laboratory (10), we indicated that M protein has a close physicochemical resemblance to mammalian muscle tropomyosin. An examination of the partial sequence of the M24 protein (9) revealed significant similarity between this bacterial surface molecule and segments of mammalian muscle tropomyosin with up to 40% identical residues. In the present report, we have compared the partial sequence of two additional M molecules (M5 and M6) with tropomyosin to determine if the similarities observed with the M24 protein are a general feature of the M molecules. The results of these studies indicate that M5 and M6 proteins reveal even

* Supported in part by grants from the American Heart Association (78-1099), and the U. S. Public Health Service (AI-11822 and HL-03919), and from special funds from the Institut Merieux, Lyon, France.

‡ Recipient of a U. S. Public Health Service Research Career Development Award.

J. Exp. Med. © The Rockefeller University Press · 0022-1007/80/03/0695/14 $1.00

Volume 151 March 1980 695-708

closer structural similarities with regions of tropomyosin than the M24 molecule, with up to 50% identical residues. Furthermore, the seven residue periodicity found common to the three M proteins is a unique feature of the tropomyosin molecule. The significance of this marked structural and sequence similarities between M proteins and tropomyosin is discussed in relation to the biological and immunological properties of the M molecule.

Materials and Methods

M Proteins. M6 protein was isolated by extraction with nonionic detergent and purified as previously described (11) and will hereafter be referred to as Det-M6.[1] M5 protein was isolated from type 5 streptococci by pepsin digestion and purified as described recently (8), and will be referred to as Pep M5.

Cleavage of the Det-M6 Protein at Arginyl Peptide Bonds

CITRACONYLATION. Because end group analysis (12) of Det-M6 indicated a blocked amino terminus (13), sequence analysis of this molecule was carried out after cleavage with trypsin. In order that cleavage could be restricted to arginine residues, the lysine residues of purified Det-M6 protein were reversibly blocked with citraconic anhydride as outlined by Singhal and Atassi (14). Briefly, 5 mg of Det-M6 was dissolved in 4 ml of 0.05 M borate buffer (pH 8.5) that contained 6 M guanidine hydrochloride. While stirring, 13 aliquots (20-μl each) of citraconic anhydride were added at 15-min intervals while the pH was maintained at 8.3–8.5 with 3 N NaOH by a pH-stat (Radiometer Co., Copenhagen, Denmark). The solution was allowed to stand at room temperature for an additional 30 min and then dialyzed extensively against 0.2 M ammonium bicarbonate (pH 8.3) and lypophilized.

To determine the percentage of lysines blocked by citraconic anhydride, 200 μg of the citraconylated protein was dansylated (12) to irreversibly block the unreacted lysines. The dansylated protein was then dialyzed against 2% acetic acid (to deblock the citraconylated lysines), lyophilized, and analyzed for its lysine content after acid hydrolysis. It was thus estimated that 93% of the lysines in the M protein were citraconylated.

TRYPSIN DIGESTION. The citraconylated M protein was taken up in 3 ml of 0.2 M ammonium bicarbonate buffer, pH 8.1. Tosyl phenylalanine chloromethyl ketone-trypsin (50 μl of a 1 mg/ml solution) was added and the digestion was carried out at 37°C with the pH maintained at 8.1–8.3 with 1 N NaOH. After 4 h, a second aliquot of trypsin (50 μl) was added, and the digestion continued for an additional 6 h under nitrogen. The solution was then lyophilized and stored at −20°C. Deblocking was accomplished by dissolving the peptides in 20% acetic acid and maintaining the solution at room temperature for 30 min before gel filtration.

Purification of Two Tryptic Peptides of Det-M6. The tryptic digest of Det-M6 was fractionated on a column of Sephadex G-75 (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) in 20% acetic acid. The elution profile as monitored by the O-phthalaldehyde reaction (15) is shown in Fig. 1. Fractions were pooled as indicated and analyzed for amino terminal residues (12). Because region CT-3 revealed leucine as the sole amino terminus, it was sequenced directly. Of the other regions, CT-2 contained glutamic acid as the major amino terminal residue, along with a small amount of leucine, whereas the remaining pools revealed two or three major amino termini. Rechromatography of pool CT-2 on Sephadex G-50 in 20% acetic acid resulted in the purification of the peptide with glutamic acid as its amino terminal residue.

Sequence Analysis. Automated sequence analysis was carried out on 50–100 nmol of peptide (based on arginine content), or protein (based on Lowry et al. [16]), on a Beckman 890B sequencer (Beckman Instruments, Inc., Fullerton, Calif.) as described earlier (8).

Computer Comparison of Sequences. Sequences of the M5 and M6 proteins were compared with those of other proteins with the computer program SEARCH, in which homology scores were

[1] *Abbreviations and nomenclature used in this paper:* Det-M6, M6 protein isolated by extraction with nonionic detergent; k-m-e-f, keratin-myosin-epidermin-fibrinogen; Pep M5, M5 protein isolated from type 5 streptococci by pepsin digestion.

Downloaded from http://rup.silverchair.com/jem/article-pdf/151/3/695/1090805/695.pdf by guest on 25 March 2023

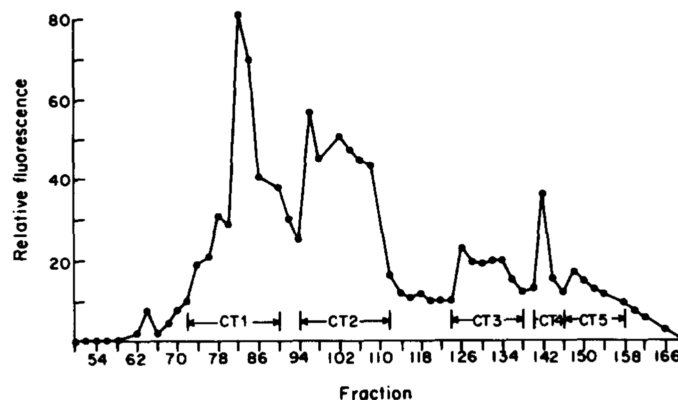

FIG. 1. Gel filtration of the tryptic digest of citraconylated Det-M6 on Sephadex G-75 (1.5 × 100 cm) in 20% acetic acid. Fraction volume: 1.3 ml. Fractions were monitored by fluorometry after alkaline hydrolysis followed by reaction with O-phthalaldehyde (15) and pooled as indicated.

obtained by the mutation data matrix (17). This analysis was performed by Doctors M. O. Dayhoff, C. W. Barker, and L. T. Hunt (National Biomedical Research Foundation, Georgetown University, Washington, D. C.).

Results

Sequence Analysis of Det-M6 Peptides. The sequences of the amino terminal region of peptides CT-3 and CT-2 are illustrated in Fig. 2. Comparison of these sequences with the reported amino terminal sequence of Pep M6(I)[2] (18) revealed that residues 14–20 of peptide CT-2 are identical to residues 1–7 of Pep M6(I) (Fig. 2). These results suggest that the Leu-Gln linkage in the M6 molecule may be one of the peptide bonds susceptible to the action of pepsin at pH 5.8 (the condition used in the pepsin digestion method for M protein isolation from the streptococcus [18]). In addition, these results place peptide CT-2 near the amino terminal region of the Det-M6 molecule. Because Det-M6 (11) is about 8,000 daltons greater than Pep M6 (18) (B. N. Manjula and V. A. Fischetti. Unpublished observations.), the peptide bond cleaved by pepsin at pH 5.8 appears to be located about 70 residues from the amino terminus of the Det-M6 molecule.

Sequence Repeats in Pep M5. Fig. 3 illustrates the first 50 residue sequence of the amino terminal region of the Pep M5 molecule. A salient feature of this sequence is that beginning at Leu-27 there is an internally repeating heptapeptide, Leu-Lys-Thr-Glu-Asn-Glu-Gly, which essentially repeats consecutively three times (with the exception of Lys for Glu at position 30 and Lys for Gly at position 47) with the suggestion of a fourth repeat beginning at Leu-48. The occurrence of a heptapeptide repeat in the partial sequence of Pep M24[3] (9) was observed earlier in our laboratory (10). The

[2] M6 protein isolated by pepsin digestion (Pep M6) was found to be a mixture of two components, Pep M6(I) and Pep M6(II). Pep M6(I) has been purified to homogeneity and shown to have type-specific precipitating activity (18).

[3] Pep M24: M24 protein isolated by pepsin digestion. The sequence of this molecule has been shown to be highly repetitious. Cyanogen bromide cleavage of Pep M24 yields seven peptides, CB-1, through CB-7. Partial sequence analyses of these peptides revealed that CB-1 and CB-2 are identical to each other and to the amino terminal region of the uncleaved molecule, at least through residue 27. On the other hand, the amino terminal sequences of CB-3 through CB-7 were identical to each other through residue 20, but differed from CB-1 and CB-2 (9).

Downloaded from http://rup.silverchair.com/jem/article-pdf/151/3/695/1090805/695.pdf by guest on 25 March 2023

	1				5					10					15					20
Det M6/CT-3	Leu	Thr	Lys	Glu	Asn	Lys	Gly	Leu	(Ser)	Lys	Lys	Leu	Ser	Glu	Ala	Glu	Glu	Glu	Ala	Ala
Det M6/CT-2	Glu	Leu	Leu	Asn	Lys	Tyr	Asp	Val	Glu	Asn	(Ser)	Met	Leu	Gln	Ala	Asn	Asn	Asp	Lys	Leu
														1				5		
Pep M6(I)														Gln	Ala	Asn	Asn	Asp	Lys	Leu

FIG. 2. Amino terminal sequences of two tryptic peptides; namely, CT-2 and CT-3, of Det-M6. Residues in parentheses indicate tentative identifications. A portion of the amino terminal sequence of Pep M6(I) (18) is aligned to show identity with peptide CT-2.

1 5 10 15 20 25
Thr Val Thr Arg Gly Thr Ile Ser Asp Pro Gln Arg Ala Lys Glu Ala Leu Asp Lys Tyr Glu Leu Glu Asn His Asp

30 35 40 45 50
[Leu Lys Thr (Lys) Asn Glu Gly] [Leu Lys Thr Glu Asn Glu Gly] [Leu Lys Thr Glu Asn Glu (Lys] [Leu) Lys Thr

FIG. 3. Amino terminal 50 residue sequence of Pep M5. Residues in parentheses are tentative identifications. The nearly identical heptapeptide repeats are boxed.

sequence, Leu-Glu-Ala-Glu-Lys-Ala-Ala, was found to occur essentially twice within a segment of this molecule (but for the seventh residue in the second repeat, which is presently unknown). Thus, Pep M5 is the second M protein in which heptapeptide sequence repeats have been demonstrated, which suggests that the occurrence of such repeats could be a common feature of the M molecules. It is, however, interesting to note that in the regions of Pep M5 and Pep M24 examined thus far, the repeating heptapeptide sequences are not identical.

Comparison of the Sequences of M Peptides. To determine if sequence similarities exist among M proteins that could suggest a mechanism for their common biological function, a comparison was made of the partial sequence of Pep M5 and Det-M6 with partial sequences of Pep M6(I) (18) and Pep M24 (9). As can be seen in Fig. 4, segments of M5, M24, and M6 exhibit considerable sequence homology with each other. An outstanding feature of this homology is that six amino acid residues (corresponding to Glu-15, Glu-23, Asn-24, Leu-27, Asn-31, and Leu-34 of Pep M5) are common to the segments of Pep M5, Pep M24, and Det-M6. In addition to these common identities, several other identities as well as conservative substitutions (17) between these segments can also be seen in Fig. 4. Furthermore, because of the overlap in the sequences of Det-M6/CT-2 and Pep M6(I) (see Fig. 2), if segment 8–19 of Pep M6(I) can be considered as an extension of the Det-M6/CT-2 peptide, then, as can be seen in Fig. 4, the identity between M6 and Pep M5 extends to 13 of 32 residues.

Thus, it is apparent that despite the fact that the segments of Pep M5, Pep M24, and Det-M6 shown in Fig. 4 were derived from the amino terminal region of their respective molecules, they are not identical to each other; however, certain residues appeared to be conserved. Because a common feature of M proteins is their characteristic amino acid composition (3) in which there is a predominance of alpha-helix-promoting amino acid residues, and only negligible amounts of helix breakers (Pro and Gly) (19), it appears likely that the M molecules may be predominantly alpha-helical proteins. It is therefore conceivable that conservation of certain residues in conformationally similar M molecules may be necessary for the common antiphagocytic function of these proteins. Data in support of this idea are presented below.

Conformational Analysis of M Peptides. To determine if conformational similarities do indeed exist between M molecules, the secondary structure of the sequenced regions of the M proteins were analyzed by a computer program based on the predictive

Downloaded from http://rup.silverchair.com/jem/article-pdf/151/3/695/1090805/695.pdf by guest on 25 March 2023

```
Pep M5        15      20                  25                30                      35         40                   45
              Glu Ala Leu Asp Lys Tyr Glu Leu Glu Asn His Asp Leu Lys Thr(Lys) Asn Glu Gly Leu Lys Thr Glu Asn Glu Gly Leu Lys Thr Glu Asn Glu
Pep M24/CB-2  15          20              25            30
              Glu Arg Ala Asp Ser Phe Glu Ile Glu Asn Asn Thr Leu Lys Leu X Asn Ser Asp Leu
Det M6/CT-2   1           5           10          15         20
              Glu Leu Leu Asn Lys Tyr Asp Val Glu Asn Ser Met Leu Gln Ala Asn Asn Asp Lys Leu
Pep M6(I)                                         1           5            10                    15
                                                  Gln Ala Asn Asn Asp Lys Leu Thr Thr Glu Asn Lys Asn Lys X Asp Gln Asn Lys
```

FIG. 4. Comparison of the partial sequences of Det-M6/CT-2 and Pep M5 with the reported partial sequences of Pep M24/CB-2 (9) and Pep M6(I)(18). In the case of Pep M5 and Pep M24/CB-2, only the regions exhibiting maximal homology are illustrated. Identical residues are boxed, and conservative substitutions are underlined.

Pep M5

	1				5					10					15					20					25	
Residue	T	V	T	R	G	T	I	S	D	P	Q	R	A	K	E	A	L	D	K	Y	E	L	E	N	H	D
Secondary structure	S	S	S	S	S	S	S	C			H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
			T	T	T	T			T	T	T	T														

			30					35					40					45					50
L	K	T	(K)	N	E	G	L	K	T	E	N	E	G	L	K	T	E	N	E	(K)	(L)	K	T
H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H

Pep M24/CB-2

	1				5					10					15					20					25				
Residue	V	A	T	R	S	Q	T	D	T	S	E	K	V	Q	E	R	A	D	S	F	E	I	E	N	N	T	L	K	L
Secondary structure				H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
	S	S	S	S	S	S	S	S	S																				
			T	T	T	T																							

Pep M24/CB-5

	1				5					10					15					20					25
Residue	N	F	S	T	A	D	S	A	K	I	K	T	L	E	A	E	K	A	A	L	E	A	E	K	A
Secondary structure	C	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
								S	S	S	S	S	S												

Det-M6/CT-3

	1				5					10					15					20
Residue	L	T	K	E	N	K	G	L	(S)	K	K	L	S	E	A	E	E	E	A	A
Secondary structure	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H

Det-M6/CT-2

	1				5					10					15					20
Residue	E	L	L	N	K	Y	D	V	E	N	(S)	M	L	Q	A	N	N	D	K	L
Secondary structure	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	C	C	C
						S	S	S	S	S	S	S	S	S	S					
				T	T	T	T													

FIG. 5. Predictive analysis (19) of the secondary structure of the partial sequences of Pep M5, Det-M6, and Pep M24 (9). To conserve space, amino acid residues are indicated by single-letter code (20). The secondary structural characteristic assigned to each residue indicates the potential of that residue, taking into account the influence of neighboring residues. The assignments H, S, and C represent alpha-helical, beta-sheet, and random coil regions, respectively, whereas T represents regions with beta-turn potential.

algorithm of Chou and Fasman (19). A composite result of the computer analysis is illustrated in Fig. 5. It can be seen that, in general, all the M sequences contain regions that exhibit a high alpha-helical potential. With the exception of Det-M6/CT-3, which appears to be completely alpha-helical, the other M segments contain some regions that exhibit beta-sheet and/or beta-turn potential, either alone or in addition to alpha-helical potential. When a similar analysis was performed on tropomyosin, a molecule reported to be nearly 100% alpha-helical (21, 22), it was found that even this molecule contains a few regions having beta-sheet as well as

Downloaded from http://rup.silverchair.com/jem/article-pdf/151/3/695/1090805/695.pdf by guest on 25 March 2023

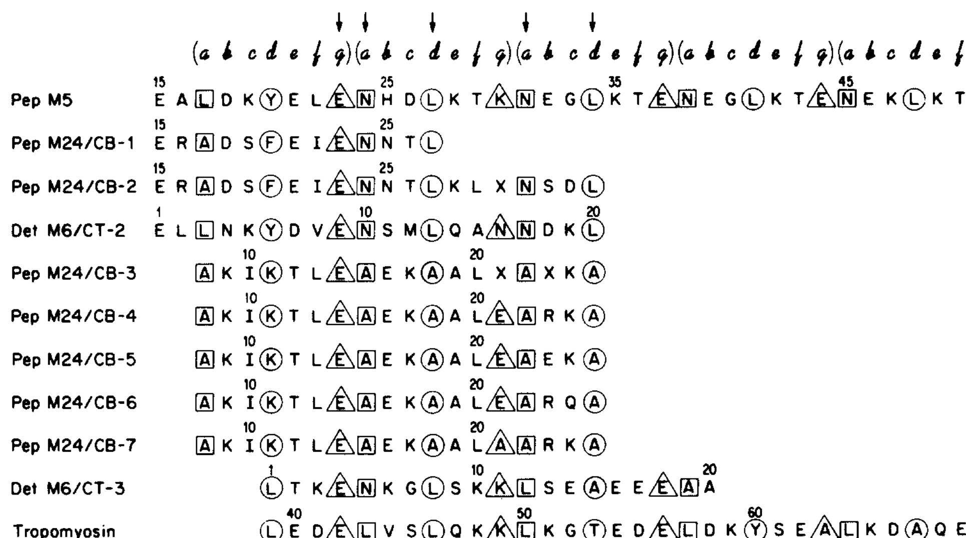

FIG. 6. Seven residue periodicity within the partial sequence of M proteins. The positions of residues within each period are indicated by letters *a* through *g*. Residues at position *a* are boxed, whereas those at position *d* and *g* are circled and triangled, respectively (for details, see text). The first four sequences are aligned to indicate not only periodicity but also homology, as in Fig. 4. Arrows indicate the positions of identical residues in these four sequences. A region of rabbit skeletal alpha-tropomyosin (24) is shown for comparison. Sequences of Pep M5, Det-M6/CT-2, and CT-3 are from this study, whereas those of Pep M24/CB-1 through CB-7, the cyanogen bromide fragments of the M24 molecule, are from (9).

alpha-helical potential. Recent x-ray analysis of tropomyosin points to the existence of regions with less stable conformation within it (23). Apparently, those segments exhibiting both sheet and helix potential in the secondary structural analysis represent areas within the molecule that are unstable, and environmental conditions will dictate the preferred conformation. Therefore, in view of the nature of their amino acid composition and the secondary structural features observed in their partial sequences, it appears likely that the M molecules are predominantly helical.

Periodicity in M-Protein Segments. A closer look at the sequenced segments of the three M proteins revealed that all contained regions that exhibit a repeating seven residue periodicity. An alignment of those regions is shown in Fig. 6. It should be emphasized that, in this figure, the sequences of the first four segments (Pep M5, Pep M24/CB-1 and CB-2, as well as Det-M6/CT-2) are aligned to indicate not only periodicity but also homology, as in Fig. 4. If the seven residues within a period are assigned letters *a* through *g*, then it can be seen that within each period, the amino acid residue at position *a* (boxed) is either hydrophobic or asparagine, that at position *d* (circled) is most often hydrophobic (often Leu or Ala), and that at position *g* (triangled) is nearly invariably charged (often Glu[4]). It should be noted here that of the six amino acid residues that are common to Pep M5, Det-M6, and Pep M24 (Fig. 4), five occur in positions corresponding to *g*, *a*, *d*, *a*, and *d*, respectively, in the seven residue period (indicated by arrows in Fig. 6), placing them in defined positions

[4] Lys-11 in Pep M24/CB-3 to CB-7 is an exception for position *d*, and Asn-16 in Det-M6/CT-2 and Ala-21 in Pep M24/CB-7 are exceptions for position *g*.

Downloaded from http://rup.silverchair.com/jem/article-pdf/151/3/695/1090805/695.pdf by guest on 25 March 2023

within the sequence. Furthermore, because the known sequence of Pep M24 (9) accounts for nearly 50% of the molecule, it is clear that the observed periodicity extends over a significant length in this molecule. Whether the regular periodicity found within the three M molecules extends throughout their entire length must await further sequence results. However, the existence of a common periodicity within the sequence of the three M proteins examined suggests that common conformational features could certainly exist within these molecules.

The seven residue periodicity observed in M proteins was found to be similar to that found in mammalian tropomyosin (24, 25). Tropomyosin exhibits a nearly regular seven residue periodicity throughout its entire length, making 40 complete periods and a final short period (24, 26). Within each seven residue period, amino acid residues at position *a* and *d* are normally hydrophobic (often Leu or Ala), whereas position *g* is usually a charged residue. In the tropomyosin alpha-helix, the nonpolar residues at positions *a* and *d* form a twisted zig-zag band on one side of the helix (27), as illustrated schematically in Fig. 7. These hydrophobic residues interact with those of a second tropomyosin molecule forming a thermodynamically stable double helical coiled-coil structure (27, 28).

To illustrate the periodicity of tropomyosin with relation to the M molecules, tropomyosin segment 39–69 is also shown in Fig. 6. This region of tropomyosin was chosen for comparison because residues 39–58 exhibit significant homology with Det-M6/CT-3 (see Fig. 8A). Thus, it can be seen that the seven residue periodicity observed in M proteins is similar to, but not identical with, that of tropomyosin. While residues at positions *d* and *g* bear the same relationship in both tropomyosin and M proteins (hydrophobic and charged, respectively),[5] the residue at position *a* in tropomyosin is hydrophobic whereas it is either hydrophobic or asparagine in the M proteins. For instance, in segments CB-3 to CB-7, the residue at position *a* is invariably hydrophobic (Ala); while the same relationship is seen in Det-M6/CT-3 (but for Asn-5), in the other M segments position *a* is most often occupied by Asparagine, an uncharged polar residue. As illustrated in Fig. 7, the occurrence of hydrophobic groups at positions *a* and *d* of the seven residue period along the whole length of the tropomyosin molecule has been suggested to be responsible for the alpha-helical coiled-coil structure of this molecule (27, 28). Because M protein segments exhibit high alpha-helical potential (Fig. 5) and because the periodicity observed in Det-M6/CT-3 and Pep M24/CB-3 to CB-7 is virtually the same as in tropomyosin, it is apparent that these regions of the M6 and M24 molecule could certainly participate in coiled-coil interactions. However, a helical folding of the other M segments would result in an alternate arrangement of a hydrophobic residue and asparagine on one side of the helix. Although asparagine residues can participate in hydrogen bonding, their role on the stabilization of coiled-coil interactions in these M segments is not clear at present.

Comparison of the Partial Sequences of Pep M5 and Det-M6 with Tropomyosin. It was demonstrated earlier from our laboratory (10) that the reported partial sequences of Pep M24 (9) exhibited significant homology with segments of rabbit skeletal alpha-tropomyosin. To determine if this observation represents an isolated incident or

[5] Lys-11 in Pep M24/CB-3 to CB-7 is an exception for position *d*, whereas Thr-53 and Ala-63 in tropomyosin are exceptions for positions *d* and *g*, respectively.

Downloaded from http://rup.silverchair.com/jem/article-pdf/151/3/695/1090805/695.pdf by guest on 25 March 2023

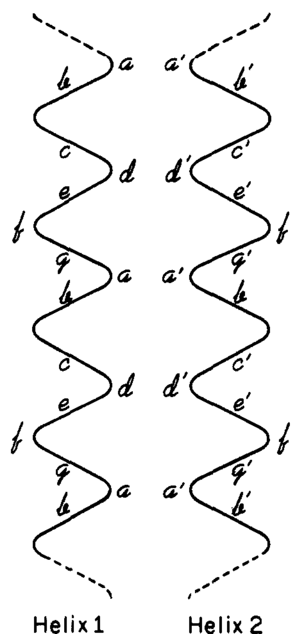

FIG. 7. A schematic diagram illustrating the arrangement of residues along the alpha-helix in a molecule with tropomyosin-like periodicity. The letter notation used for the seven residue repeats is the same as in Fig. 6. Primed letters represent residues in a second alpha-helix. Because of their relative positions in the seven residue period, hydrophobic residues *a*, *d* and *a′*, *d′* are arranged along one side of the helix. Hydrophobic residues of Helix 1 interact with those of Helix 2 to form a thermodynamically stable two-stranded alpha-helical coiled-coil.

whether it is a common characteristic of M proteins, the partial sequences of M6 and M5 proteins were compared with proteins of known sequences, using the computer program SEARCH (17).

When Det-M6/CT-3 was analyzed in this manner, the first and second highest scoring segments retrieved by the computer from the nearly 100,000 segments of comparable length in the protein sequence data bank were two separate regions of tropomyosin, namely, residues 39–58 and 221–240 (Fig. 8A). Alignment of peptide CT-3 with tropomyosin segment 39–58 revealed 8 amino acid identities (40% identity), whereas segment 221–240 exhibited 10 identities (50% identity). In addition to these identities, a number of conservative substitutions (17) were also noted between the segments compared. Furthermore, both tropomyosin segments showed identical residues in positions corresponding to 4, 8, 11, 12, and 16 of peptide CT-3.

Analysis of peptide CT-2 (Fig. 8B) also revealed a certain degree of homology with tropomyosin; segment 87–106 was retrieved by the computer, but with lower homology than that obtained for the CT-3 fragment. Although the number of identities between the sequences compared are limited, as many as 10 conservative substitutions were noted. Curiously, of the residues in CT-3 and CT-2 that are identical with those of tropomyosin segments, leucine, glutamic acid, and lysine appear to predominate.

For the purposes of computer analysis, the 50 residue amino terminal sequence of Pep M5 was divided into two segments; namely, 1–29 and 27–50. Pep M5/(1–29) exhibited homology with the tropomyosin segments 122–150 (eight identities and

Downloaded from http://rup.silverchair.com/jem/article-pdf/151/3/695/1090805/695.pdf by guest on 25 March 2023

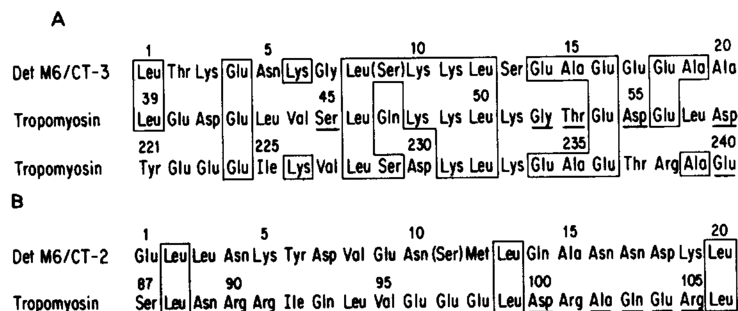

FIG. 8. (A) Comparison of the amino terminal region of Det-M6/CT-3 with two regions of rabbit skeletal alpha-tropomyosin (24). Residues identical between CT-3 and tropomyosin segments are boxed, and conservative substitutions are underlined. (B) Comparison of the amino terminal region of Det-M6/CT-2 with a region of rabbit skeletal alpha-tropomyosin (24). Identical residues are boxed, and conservative substitutions are underlined.

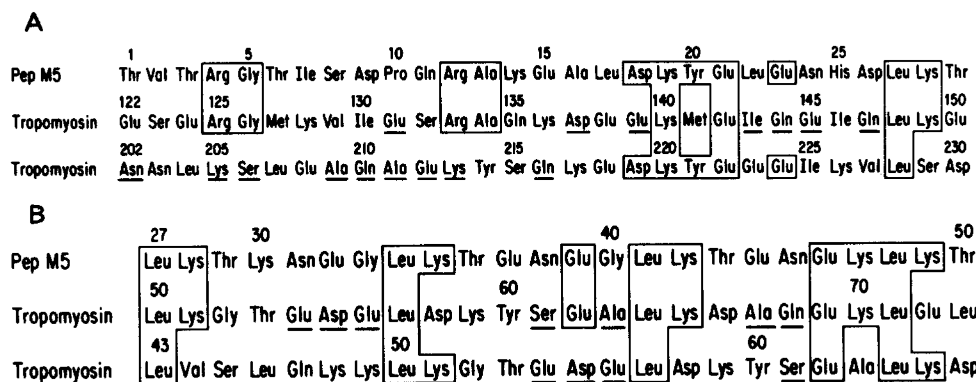

FIG. 9. (A) Comparison of Pep M5/(1–29) with two regions of rabbit skeletal alpha-tropomyosin (24). Residues identical between Pep M5 and tropomyosin are boxed, and conservative substitutions are underlined. (B) Comparison of Pep M5/(27–50) with two regions of rabbit skeletal alpha-tropomyosin (24). Residues identical between Pep M5 and tropomyosin are boxed, and conservative substitutions are underlined.

seven conservative substitutions) and 202–230 (six identities, which included a tetrapeptide, and eight conservative substitutions) (Fig. 9A). On the other hand, analysis of Pep M5/(27–50) revealed that, as in the case of Det-M6/CT-3, the highest homology was obtained for a segment of tropomyosin, namely residues 50–73. Alignment of Pep M5/(27–50) with tropomyosin/(50–73) revealed that 9 of the 24 residues are identical (37% identity) and 7 substitutions are conservative (Fig. 9B). Based on a normal distribution of SEARCH scores (29), the probability that the homology between tropomyosin/(50–73) and Pep M5/(27–50) occurred by chance is $< 10^{-7}$. Generally, only 5% of the searches of unrelated segments with the mutation data matrix get this high a score (M. O. Dayhoff. Personal communication.). Therefore, the observed homology between Pep M5/(27–50) and tropomyosin/(50–73) appears to be highly significant.

A second segment of tropomyosin exhibiting homology with Pep M5/(27–50) is residues 43–66 (Fig. 9B). It is interesting to note that the difference between this segment and segment 50–73 represents a seven residue shift within the tropomyosin molecule.

Downloaded from http://rup.silverchair.com/jem/article-pdf/151/3/695/1090805/695.pdf by guest on 25 March 2023

The fact that multiple regions of tropomyosin are retrieved by the computer in response to an M protein segment may be explained by the fact that the sequence of tropomyosin is highly repetitious. The molecule consists of seven nearly regular 42 residue segments which, though not identical, are highly homologous to each other (26). Therefore, the homology of a given M protein segment with multiple segments of tropomyosin is in keeping with the pseudorepetitious nature of the latter.

Thus, the results presented above indicate that the sequence homology with tropomyosin observed earlier for the M24 protein (10) was not unique to that molecule, but appears to be a common characteristic of M proteins.

Discussion

The results of this study reveal that the partial sequences of three immunologically distinct M proteins; namely, M5, M6, and M24, while not identical, are highly homologous to each other. Although the degree of homology between any two M protein segments varies, some amino acid residues appeared to be conserved within the three molecules. As will be seen below, it may be the relative juxtaposition of these common residues within the conformation of the M protein molecules that determines their common biological function.

In addition to homology between each other, the partial sequences of the M5 and M6 proteins also exhibited significant homology with different regions of mammalian tropomyosin. A relationship between M proteins and tropomyosin was suggested earlier from our laboratory (10), based on similarities in physicochemical properties and homology with the partial sequence of Pep M24 (9). The homologies with tropomyosin observed in this study for M5 and M6 proteins are even closer than that observed with Pep M24 (10). Thus, up to now partial sequences of three immunologically unrelated M proteins have been demonstrated to exhibit significant sequence homology with different segments of mammalian tropomyosin.

Secondary structural analyses of the sequenced regions of the three M proteins by the Chou and Fasman method (19) revealed that they all exhibit a high alpha-helical potential. Even more striking is the fact that they all contain regions with a common seven residue periodicity. This subsequence periodicity observed in M proteins was similar to that found in mammalian tropomyosin. Because it has been shown that the seven residue periodicity in tropomyosin is responsible for its coiled-coil alpha-helical conformation (27, 28), it is plausible that, in addition to homologies in primary structure, similarities may also exist in the secondary and possibly tertiary structure of M protein and tropomyosin.

The existence of a tropomyosin-like seven residue periodicity has been recently demonstrated in two members of the keratin-myosin-epidermin-fibrinogen (k-m-e-f) group of proteins; namely, keratin (30) and fibrinogen (31). Although this periodicity extends throughout the molecule in tropomyosin, in keratin and fibrinogen it appears to be restricted only to certain regions. However, participation of these regions in coiled-coil interactions within these proteins has been suggested based on an atomic scale model (31) and computational analysis (30). Therefore, the tropomyosin-like periodicity observed in the partial sequences of the streptococcal M proteins is highly suggestive of their involvement in intra- and/or intermolecular coiled-coil interactions.

The results of this study are in support of our earlier suggestion that a common conformation of the M molecules may be the basis for their common antiphagocytic

Downloaded from http://rup.silverchair.com/jem/article-pdf/151/3/695/1090805/695.pdf by guest on 25 March 2023

function (6, 8). Because of the constraints imposed by the repeating seven residue periodicity on the folding of the protein, certain residues could occupy spatially equivalent positions in the tertiary structure of the different M molecules. It may be the similarity in the juxtaposition of these residues that governs, in an as yet unknown way, the common biological function of the M molecule. The observation that the amino acid residues conserved within the three M protein sequences occupy defined positions in the seven residue periodicity supports this idea. Type-specificity may then be determined by those residues not involved in maintaining the tropomyosin-like periodicity. Therefore, as suggested previously by Fischetti (6), it is possible that through immunological pressure, the antigenic character of the M molecule can change, as long as the conformation necessary for its biological function is not altered.

Several bacterial proteins have been compared with contractile proteins to understand either their structure and/or function. Flagellar protein, a component of the motile organelle of bacteria, has long been known to belong to the k-m-e-f family of fibrous proteins and has been compared to F-actin (32). *Escherichia coli* ribosomal protein L7/L12, involved in translocation during peptide synthesis, also exhibits structural similarities with certain contractile proteins (33, 34). In addition, regions of staphylococcal protein A have recently been shown by this laboratory to exhibit sequence homology with actin and myosin (10). Curiously, the sequence of *E. coli* lipoprotein, which is highly repetitious (35), has been shown to exhibit a tropomyosin-like seven residue periodicity throughout its entire length (36). Analysis of the secondary structure of this molecule has indicated that, like tropomyosin, *E. coli* lipoprotein can also attain a coiled-coil helical structure (36). Therefore, from these results and those reported in this study, one could speculate that *E. coli* lipoprotein and M protein may have evolved from a common ancestral gene. For instance, in the case of tropomyosin, it has been suggested that the almost regular 42 residue pattern found in the 7 regions of the molecule probably arose from gene duplication, a reasonable common ancestor being the heptapeptide Leu-Lys-Val-Leu-Glu-Glu-Lys, with the alternative of alanine at position 4 (26). In this regard, it is interesting to note that the observed seven residue repeat in Pep M5, namely Leu-Lys-Thr-Glu-Asn-Glu-Gly, has identity with this segment in three of the seven positions (i.e., at positions 1, 2, and 6).

The biological and/or pathological implications of the resemblance of M proteins to tropomyosin is not clear at present. However, the structural and sequential resemblance of M protein to a muscle protein may possibly explain some of the immunological cross-reactions observed between mammalian muscle and streptococcal components in the sera of rheumatic fever patients (37–39). In addition, M protein's resemblance to tropomyosin, in conjunction with the capacity to maintain a specific tertiary structure, may be responsible for the common antiphagocytic property of this immunologically diverse molecule. In this regard, there is a good deal of evidence to suggest that the biochemical pathway for the generation of energy for phagocytosis is similar to that of muscle contraction (40, 41). The presence of actin and myosin in phagocytic cells and their resemblance to their muscle counterparts has been clearly demonstrated (42–45). Although tropomyosin-like regulatory proteins have not as yet been isolated from leukocytes, they have been tentatively identified in fibroblasts (46) and have been isolated from other nonmuscle cells like human and horse platelets (47, 48), as well as chicken and calf brain (49, 50). Furthermore,

Downloaded from http://rup.silverchair.com/jem/article-pdf/151/3/695/1090805/695.pdf by guest on 25 March 2023

troponin-tropomyosin complex from muscle, in the presence of actin, is able to confer Ca^{++}-regulated Mg^{++}-ATPase activity upon myosins isolated from mouse fibroblast and guinea pig polymorphonuclear leukocyte (43, 51). Therefore, it is conceivable that, because of their striking resemblance to tropomyosin, a regulatory protein in muscle contraction, M proteins may also play a regulatory role in the contractile machinery of leukocytes. Answers to these and many other questions obviously require a more-detailed knowledge of the sequence of M proteins as well as their physiological and immunochemical relationship to tropomyosin. These are currently being investigated.

Summary

Partial sequences of three immunologically distinct group A streptococcal M proteins (M5, M6, and M24) revealed significant homology with each other, certain amino acid residues being conserved within the three molecules. In addition, a common feature of the sequenced regions of these M proteins was their high alpha-helical potential and the presence of a repeating seven residue periodicity that is characteristic of the double helical coiled-coil molecule, tropomyosin. The existence of a tropomyosin-like seven residue periodicity strongly suggests that regions of these three M proteins may participate in intra- and/or intermolecular coiled-coil interactions. Because of the constraints imposed by such a repeating periodicity, certain conserved residues within the M proteins would occupy spatially equivalent positions in the tertiary structure of these molecules. This common characteristic could play an important role in the common antiphagocytic property of the immunologically diverse M molecules. In addition to similarities in the secondary structure of M proteins and tropomyosin, significant sequence homology has also been observed between certain regions of these molecules with up to 50% identical residues. As a result of the striking structural similarity with tropomyosin, M proteins may play a regulatory role in the contractile mechanisms involved in phagocytosis.

The authors wish to thank Doctors J. M. Manning and A. S. Acharya for their comments on the manuscript; Doctors C. Cohen and G. N. Phillips, Jr. (Brandeis University, Waltham, Mass.) for helpful discussions; Doctors M. O. Dayhoff, W. C. Barker, and L. T. Hunt for their computer "SEARCH" analysis; and Doctors M. McCarty and E. C. Gotschlich for their encouragement and continued interest in these studies. They also wish to acknowledge Ms. S. McManus for her expert assistance in sequence analysis, Mr. M. Simont for his invaluable help in computer analyses, and Ms. J. Maier for her excellent assistance in the preparation of the manuscript.

Received for publication 21 November 1979.

References

1. Lancefield, R. C. 1959. Persistence of type-specific antibodies in man following infection with group A streptococci. *J. Exp. Med.* **110:**271.
2. Lancefield, R. C. 1962. Current knowledge of type-specific M antigens of group A streptococci. *J. Immunol.* **89:**307.
3. Fox, E. N. 1974. M-proteins of group A streptococci. *Bacteriol. Rev.* **35:**57.
4. Wiley, G. G., and P. N. Bruno. 1968. Cross-reactions among group A streptococci. I. Precipitin and bactericidal cross-reactions among types 33, 41, 43, 52, and Ross. *J. Exp. Med.* **128:**959.
5. Wiley, G. G., and P. N. Bruno. 1969. Cross-reactions among group A streptococci. II. Further analysis of antigens related to type-specificity and protection. *J. Immunol.* **103:**149.

Downloaded from http://rup.silverchair.com/jem/article-pdf/151/3/695/1090805/695.pdf by guest on 25 March 2023

6. Fischetti. V. A. 1978. Streptococcal M protein extracted by Nonionic detergent. III. Correlation between immunological cross-reactions and structural similarities with implications for antiphagocytosis. *J. Exp. Med.* **147:**1771.
7. Bergner-Rabinowitz, S., I. Ofek, and M. D. Moody. 1972. Cross-protection among serotypes of group A streptococci. *J. Infect. Dis.* **125:**339.
8. 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.
9. Beachey, E. H., J. M. Seyer, and A. H. Kang. 1978. Repeating covalent structure of streptococcal M protein. *Proc. Natl. Acad. Sci. U. S. A.* **75:**3163.
10. Hosein, B., M. McCarty, and V. A. Fischetti. 1979. Amino acid sequence and physicochemical similarities between streptococcal M protein and mammalian tropomyosin. *Proc. Natl. Acad. Sci. U. S. A.* **76:**3765.
11. Fischetti, V. A., E. C. Gotschlich, G. Siviglia, and J. B. Zabriskie. 1976. Streptococcal M-protein extracted by nonionic detergent. I. Properties of the antiphagocytic and type-specific molecules. *J. Exp. Med.* **144:**32.
12. Woods, K. R., and K.-T. Wang. 1967. Separation of dansyl amino acids by polyamide layer chromatography. *Biochim. Biophys. Acta.* **133:**369.
13. Fischetti, V. A., E. C. Gotschlich, G. Siviglia, and J. B. Zabriskie. 1973. The subunit structure of streptococcal M-protein. *Bacteriol. Proc.* 81.
14. Singhal, R. P., and M. Z. Atassi. 1976. Immunochemistry of sperm whale of myoglobin. IX. Specific interaction of peptides obtained by cleavage at arginine peptide bonds. *Biochemistry.* **10:**1756.
15. Benson, J. R., and P. E. Hare. 1975. O-phthalaldehyde: fluorogenic detection of primary amines in the picomole range. Comparison with fluorescamine and ninhydrin. *Proc. Natl. Acad. Sci. U. S. A.* **72:**619.
16. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:**265.
17. Dayhoff, M. O. 1976. Atlas of Protein Sequence and Structure. National Biomedical Research Foundation, Washington, D. C. **5**(Suppl. 2):3–8 and 311.
18. Beachey, E. H., J. M. Seyer, and A. H. Kang. Studies of the primary structure of streptococcal M-protein antigens. *In* Streptococcal Diseases and the Immune Response. J. B. Zabriskie and S. E. Read, editors. Academic Press, Inc., New York. In press.
19. Chou, P. Y., and G. D. Fasman. 1974. Conformational parameters for amino acids in helical, Beta-sheet, and random coil regions calculated from proteins. *Biochemistry.* **13:**211.
20. IUPAC-IUB. Commission on Biochemical Nomenclatures. 1968. A one-letter notation for amino acid sequences, tentative rules. *J. Biol. Chem.* **243:**3557.
21. Cohen, C., and A. G. Szent-Györgyi. 1957. Optical rotation and helical polypeptide chain configuration in alpha-proteins. *J. Am. Chem. Soc.* **79:**248.
22. Woods, E. F. 1969. Comparative physicochemical studies on vertebrate tropomyosins. *Biochemistry.* **8:**4336.
23. Phillips, Jr., G. N., E. E. Lattman, P. Cummins, K. Y. Lee, and C. Cohen. 1979. Crystal structures and molecular interactions of tropomyosin. *Nature (Lond.).* **278:**413.
24. Stone, D., J. Sodex, P. Johnson, and L. B. Smillie. 1975. Tropomyosin: correlation of amino acid sequence and structure. *In* Proceedings of the IXth Federation of European Biochemical Societies Meeting. E. N. A. Biro, editor. Elsevier North-Holland, Inc., New York. **31:** 125.
25. McLachlan, A. D., and M. Stewart. 1975. Tropomyosin coiled-coil interactions: evidence for an unstaggered structure. *J. Mol. Biol.* **98:**293.
26. McLachlan, A. D., M. Stewart, and L. B. Smillie. 1975. Sequence repeats in alpha-tropomyosin. *J. Mol. Biol.* **98:**281.
27. Hodges, R. S., J. Sodek, L. B. Smillie, and L. Jurasek. 1972. Tropomyosin: amino acid

Downloaded from http://rup.silverchair.com/jem/article-pdf/151/3/695/1090805/695.pdf by guest on 25 March 2023

sequences and coiled-coil structure. *Cold Spring Harbor Symp. Quant. Biol.* **37**:299.
28. Crick, F. H. C. 1953. The packing of alpha-helices: simple coiled-coils. *Acta Crystallogr.* **6**: 689.
29. Dayhoff, M. O. 1978. Atlas of Protein Sequence and Structure. National Biomedical Research Foundation, Washington, D. C. 5(Suppl. 5):374.
30. Parry, D. A. D., W. G. Crewther, R. D. B. Fraser, and T. P. MacRae. 1977. Structure of alpha-keratin: structural implication of the amino acid sequences of the type I and type II chain segments. *J. Mol. Biol.* **113**:449.
31. Doolittle, R. F., D. M. Goldbaum, and L. R. Doolittle. 1978. Designation of sequences involved in the "coiled-coil" interdomainal connections in fibrinogen: construction of an atomic scale model. *J. Mol. Biol.* **120**:311.
32. Seifler, S., and P. M. Gallop. 1966. The structure of proteins: flagellin. *In* The Proteins. H. Neurath, editor. Academic Press, Inc., New York. 359.
33. Kischa, K., and W. Möller. 1971. Reconstitution of a GTPase activity by a 50S ribosomal protein from *E. coli. Nat. New Biol.* **233**:62.
34. Amons, R., A. Van Agthoven, W. Pluijons, and W. Möller. 1978. A comparison of the alanine-rich sequences of the L7/L12-ribosomal proteins from rat liver, *Artemia salina* and *E. coli*, with the amino terminal region of the alkali light-chain A, from rabbit myosin. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **86**:282.
35. Braun, V., and V. Bosch. 1972. Repititive sequences in the murein-lipoprotein of the cell wall of *E. coli. Proc. Natl. Acad. Sci. U. S. A.* **69**:970.
36. McLachlan, A. D. 1978. The double helix coiled-coil structure of murein lipoprotein from *E. coli. J. Mol. Biol.* **122**:493.
37. Stollerman, G. H., editor. 1975. Rheumatic Fever and Streptococcal Infection. Grune & Stratton, Inc., New York. 123.
38. Kaplan, M. H. 1967. Multiple nature of the cross-reactive relationship between antigens of group A streptococci and mammalian tissue. *In* Cross-reacting Antigens and Neoantigens. J. J. Trentin, editor. The Williams & Wilkins Co., Baltimore, Md. 48.
39. Zabriskie, J. B., and E. H. Freimer. 1966. An immunological relationship between the group A streptococcus and mammalian muscle. *J. Exp. Med.* **124**:661.
40. Stossel, T. P. 1975. Phagocytosis: recognition and ingestion. *Semin. Hematol.* **12**:83.
41. Silverstein, S. C., R. M. Steinman, and Z. A. Cohn. 1977. Endocytosis. *Annu. Rev. Biochem.* **46**:669.
42. Pollard, T. D., and P. A. Weihing. 1974. Actin and myosin and cell movement. *CRC Crit. Rev. Biochem.* **2**:1.
43. Stossel, T. P., and T. D. Pollard. 1973. Myosin in polymorphonuclear leukocytes. *J. Biol. Chem.* **248**:8288.
44. Hartwig, J. H., and T. P. Stossel. 1975. Isolation and properties of actin, myosin, and a new actin-binding protein in rabbit alveolar macrophages. *J. Biol. Chem.* **250**:5696.
45. Boxer, L. A., E. T. Hedley-Whyte, and T. P. Stossel. 1974. Neutrophil actin dysfunction and abnormal neutrophil behavior. *N. Engl. J. Med.* **291**:1093.
46. Lazarides, E. 1975. Tropomyosin antibody: the specific localization of tropomyosin in non-muscle cells. *J. Cell Biol.* **65**:549.
47. Cohen, C., and A. L. Cohen. 1972. A tropomyosin-like protein from human platelets. *J. Mol. Biol.* **68**:383.
48. Cote, G., W. G. Lewis, and L. B. Smillie. 1978. Non-polymerisability of platelet tropomyosin and its NH_2- and COOH-terminal sequences. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **91**:237.
49. Fine, R. E., A. L. Blitz, S. Hichcock, and B. Kaminer. 1973. Tropomyosin in brain and growing neurons. *Nat. New Biol.* **245**:182.
50. Fine, R. E., and A. L. Blitz. 1975. A chemical comparison of tropomyosins from muscle and non-muscle tissues. *J. Mol. Biol.* **95**:447.
51. Adelstein, R. S., M. A. Conti, and G. S. Johnson. 1972. Isolation and characterization of myosin from cloned mouse fibroblasts. *Proc. Natl. Acad. Sci. U. S. A.* **69**:3693.

Downloaded from http://rup.silverchair.com/jem/article-pdf/151/3/695/1090805/695.pdf by guest on 25 March 2023