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Isolation and Some Structural and Functional Properties of Macrophage Tropomyosin†

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ABSTRACT: Tropomyosin purified from rabbit lung macrophages is very similar in structure to other nonmuscle cell tropomyosins. Reduced and denatured, the protein has two polypeptides which migrate during electrophoresis in sodium dodecyl sulfate on polyacrylamide gels with slightly different mobilities corresponding to apparent M_r's of about 30 000. Following cross-linking by air oxidation in the presence of $CuCl_2$, electrophoresis under nonreducing conditions reveals a single polypeptide of M_r 60 000. Macrophage tropomyosin has an isoelectric point of 4.6 and an amino acid composition similar to other tropomyosins. It contains one cysteine residue per chain. In the electron microscope, macrophage tropomyosin molecules rotary shadowed with platinum and carbon are slender, straight rods, 33 nm in length. Macrophage tropomyosin paracrystals grown in high magnesium concentrations have an axial periodicity of 34 nm. On the basis of yields from purification and from two-dimensional electrophoretic analyses of macrophage extracts, tropomyosin comprises less than 0.2% of the total macrophage protein, a molar ratio of approximately 1 tropomyosin molecule to 75 actin monomers in the cell. Macrophage tropomyosin binds to actin filaments. Macrophage, skeletal muscle, and other nonmuscle cell tropomyosins inhibit the fragmentation of actin filaments by the Ca^{2+}–gelsolin complex. The finding implies that tropomyosin may have a role in stabilizing actin filaments in vivo.

The cytoplasm of most nonmuscle cells contains actin filaments and associated proteins (Weeds, 1982). One of these associated proteins is tropomyosin. It has been identified by immunofluorescence in epithelial cells (Lazarides, 1975) and has been purified from human platelets (Cohen & Cohen, 1972), brain (Fine et al., 1973), fibroblasts (Masaki, 1975), cultured mammalian cells (Schloss & Goldman, 1980), and porcine and equine platelets (der Terrossian et al., 1981; Côté & Smillie, 1981a,b; Côté et al., 1978a,b). Although tropomyosin is a component of the system that regulates the interactions between actin and myosin in striated muscles (Taylor, 1979; Mannherz & Goody, 1976), its role in nonmuscle cells is not clear.

As part of our endeavor to understand the structure and movement of cytoplasm in mammalian phagocytes, we have asked whether tropomyosin is present in rabbit lung macrophages and, if so, how it interacts with actin and actin-associated proteins. This paper documents the identification of tropomyosin in the macrophage and describes an inhibitory effect of this protein on the activity of gelsolin, a calcium-

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modulated protein of macrophages and other mammalian cells which shortens actin filaments (Yin & Stossel, 1979; Yin et al., 1981a,b). Ca^{2+} regulation of actin filament length is an efficient mechanism for controlling reversible cytoplasmic gel–sol transformations. Tropomyosin, through its effect on the gelsolin–actin interaction, may impose further control of the regulation of cell shape and motility.

Experimental Procedures

Purification of Muscle Proteins. (*A*) *Actin.* Actin was prepared from back and leg muscles of rabbit by a slight modification of the method of Spudich & Watt (1971).

(*B*) *Myosin.* Myosin was prepared from rabbit back muscles according to Kielley & Bradley (1956) and stored at −20 °C in 50% glycerol (v/v), 0.6 M KCl, and 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl),[1] pH 7.5.

(*C*) *Tropomyosin.* Rabbit skeletal muscle tropomyosin was prepared by the Bailey (1948) procedure with an additional purification step in which the Bailey tropomyosin was applied to a 1.5 × 15 cm column of hydroxylapatite and eluted with a sodium phosphate gradient in 1 M KCl according to Eisenberg & Kielley (1974). The purified tropomyosin was either stored at −20 °C after lyophilization or stored as a precipitate at 4 °C in 70% saturated $(NH_4)_2SO_4$. Before use, the tropomyosin was dissolved in and dialyzed against various solvents.

Purification of Macrophage Tropomyosin. Twenty milliliters of packed cells was obtained from the lungs of New Zealand white rabbits by intratracheal lavage (Myrvik et al., 1961) and treated with DFP as reported by Amrein & Stossel (1980). All steps of the purification procedure were carried out at 4 °C. The packed cells were suspended in 20 volumes of a 100% ethanol, 2 mM DTT, and 10 mM EDTA solution and homogenized by mixing for 2 min at high speed in a Waring blender followed by disruption with 20 strokes of a Dounce homogenizer with a type B pestle. A precipitate was collected by centrifugation at 6000*g* and suspended in 20 volumes of anhydrous ethyl ether, 2 mM DTT, and 10 mM EDTA solution. Extractions of the pellet were repeated twice with ethanol–DTT–EDTA. The resulting powder was allowed to dry and then extracted overnight in solution containing 1 M KCl, 10 mM EDTA, and 2 mM DTT, pH 7.0. The insoluble residue was removed by centrifugation at 12000*g* and the viscous extract centrifuged 1 h at 100000*g*. The supernatant fluid was immersed in a boiling water bath for 10 min. A flocculent precipitate formed which was centrifuged at 12000*g* and discarded. The clear supernatant fluid was concentrated in a dialysis tube placed in Sephadex G-200, saturated to 85% with solid ammonium sulfate, and stirred for 30 min. The precipitate was then collected by centrifugation, dissolved in a minimal quantity of a solution of 0.5 M NaCl, 2 mM DTT, and 10 mM EDTA, pH 7.0, and dialyzed overnight against 4% formic acid (pH 2.1). The pH was adjusted to 4.6 with 1 M NaOH and the resulting precipitate collected by centrifugation for 30 min at 100000*g*. The precipitate was suspended with 3 mL of a solution of 10 mM imidazole hydrochloride, 0.15 M NaCl, 10 mM EDTA, and 2 mM DTT, pH 7.5. After the solution was stirred for 2 h, solid ammonium sulfate was added to 40% saturation. The preparation was immediately centrifuged and the precipitate discarded. The ammonium sulfate saturation of the resulting supernatant was increased to 55%. The suspension was left standing for 12 h at 4 °C. The precipitate was collected by centrifugation for 1 h at 37000*g*, dissolved in a minimal volume of a solution of 1 M KCl, 2 mM DTT, 10 mM EDTA, and 1 mM sodium phosphate buffer, pH 7.0, and dialyzed 2 h against the same solution. The sample was applied to a column (1.5 × 15 cm) of hydroxylapatite equilibrated with the same solution in which the sample was dissolved and eluted with a linear, 1–200 mM sodium phosphate gradient. Fractions eluting with an absorbance at 280 nm were analyzed by electrophoresis on polyacrylamide gels in the presence of sodium dodecyl sulfate. Rabbit macrophage and skeletal muscle tropomyosins eluted at the same phosphate concentration from the hydroxylapatite column. Peaks containing either the 30000- or the 35000-dalton polypeptide subunit of macrophage or muscle tropomyosin, respectively, were dialyzed against water, lyophilized, and stored at −20 °C.

Brain and Platelet Tropomyosin. Ethanol/ether powders from human platelets and rabbit brain were prepared as previously described (Cohen & Cohen, 1972; Fine et al., 1973). The tropomyosins were purified from these powders as described above for macrophage tropomyosin.

Analytical Procedures. (*A*) *Total Protein.* Protein concentrations were measured by the Folin procedure (Lowry et al., 1951) or by the dye-binding method of Bradford (1976) with bovine serum albumin as a standard.

(*B*) *Polyacrylamide Gel Electrophoresis and Isoelectric Focusing.* The discontinuous pH, 5–15% polyacrylamide gradient slab gel system of Laemmli (1970) was used. The intensity of the Coomassie blue stained protein bands was determined by scanning the gels with a densitometer (E-Z gel scanner). Isoelectric focusing was carried out in 5% polyacrylamide–0.13% bis(acrylamide) slab gels containing 2% ampholine, pH 4–6, and 0.33% ampholine, pH 3.5–10, as described by Winter & Anderson (1977).

(*C*) *Amino Acid Analysis.* Samples of purified tropomyosins were dialyzed extensively against glass-distilled water, lyophilized, and hydrolyzed with 6 N HCl at 105 °C in vacuo for 24 h. Amino acid analysis was performed with a Beckman 121 MB analyzer.

(*D*) *Paracrystal Formation.* Paracrystals were grown from a 0.5 mg/mL solution of macrophage or muscle tropomyosin by dialyzing overnight at 4 °C against 5 mM DTT, pH 8.0, and then for 1 week against 50 mM Tris-HCl–50 mM $MgCl_2$, pH 8.2. The resulting tactoids were placed on carbon–formvar-coated copper grids, negatively stained with 1% uranyl acetate, and examined in a Phillips 301 electron microscope operated at 60 kV.

(*E*) *Low-Angle Rotary Shadowing.* To visualize molecules of tropomyosin, we used the rotary shadowing technique of Tyler & Branton (1980). Solutions (50–100 μg/mL) of either muscle or macrophage tropomyosin in 0.02 mM $CaCl_2$, 0.05 mM ATP, 0.05 mM 2-mercaptoethanol, and 0.2 mM Tris-HCl, pH 7.5, were mixed with an equal volume of 99.7% glycerol and sprayed on mica. The mica was dried under vacuum at room temperature and rotary shadowed with platinum and carbon at a 5° platinum shadow angle. The replicas were examined in a Phillips 301 electron microscope at 80 kV. Rotary-shadowed specimens were photographed in the electron microscope at a magnification of 45000× and photographically enlarged to 135000× on photography paper. The length of molecules was determined by using an Apple computer equipped with a graphic tablet.

(*F*) *Flow Birefringence Measurements.* The effect of tropomyosin on the ability of gelsolin to shorten actin filaments

[1] Abbreviations: EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; DTT, dithiothreitol; DFP, diisopropyl fluorophosphate; Ellman reagent (Nbs_2), 5,5'-dithiobis(2-nitrobenzoic acid).

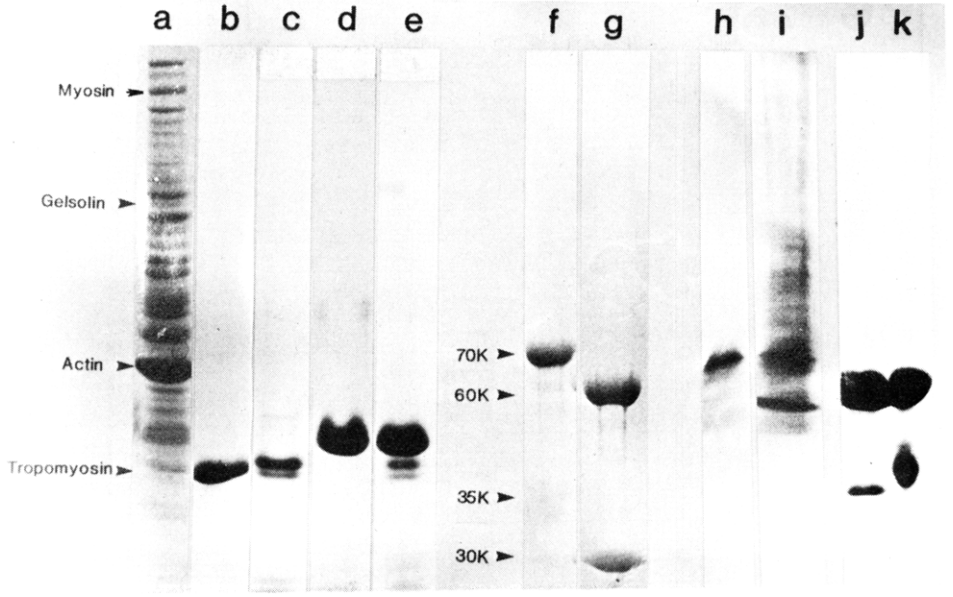

FIGURE 1: 5–15% gradient polyacrylamide gels after electrophoresis in the presence of sodium dodecyl sulfate and staining with Coomassie blue. (a) Soluble protein extract of rabbit lung macrophages. (b) Purified macrophage tropomyosin. The α and β components of macrophage tropomyosin are not resolved. (c) α and β forms of macrophage tropomyosin resolved by extended electrophoresis. (d) Purified rabbit skeletal muscle tropomyosin. (e) Mixture of macrophage and muscle tropomyosins. (f) $CuCl_2$-treated muscle tropomyosin. (g) $CuCl_2$-treated macrophage tropomyosin. (h) Isoelectric focusing gel of purified macrophage tropomyosin. (i) Isoelectric focusing gel of M_r 30 000 material in macrophage extract. (j) and (k) show that muscle (k) and macrophage (j) tropomyosins bind to and pellet with actin after centrifugation at 100000*g* for 1 h.

was determined by means of flow birefringence measurements. Five milliliters of 6 μM actin in a solution of 0.2 mM $CaCl_2$, 0.15 mM ATP, 0.5 mM 2-mercaptoethanol, and 20 mM Tris-HCl, pH 7.5, was polymerized in the presence or absence of muscle and nonmuscle tropomyosins for 2 h at 25 °C by the addition of KCl and $MgCl_2$ to final concentrations of 100 and 5 mM, respectively. The molar ratio of actin to tropomyosin was either 4 to 1 or 7 to 1. Gelsolin was then added at different molar ratios to actin and the flow birefringence of the solution measured after 20 min at 25 °C in an Edsall-type apparatus (Rao Instrument Co., Brooklyn, NY) as described by Maruyama (1964).

(*G*) *Actin Sedimentation.* The effect of tropomyosin on the binding of gelsolin to F-actin was determined by sedimentation in the presence of calcium. A 150-μL sample of 47 μM G-actin was polymerized in the presence or absence of tropomyosin (molar ratio of actin to tropomyosin of 4 to 1) as described above. Gelsolin was added at a molar ratio of 1 to 50 actin monomers. This solution was incubated for 20 min at 25 °C and then centrifuged in a Beckman airfuge at 30 psi for 3 h. Equivalent samples of the supernatant and pellet fractions were subjected to electrophoresis on polyacrylamide gels in the presence of sodium dodecyl sulfate. The relative amount of gelsolin in the two fractions was determined by densitometry of these gels after being stained with Coomassie blue.

(*H*) *Cross-Linking.* Disulfide bond formation was induced by air oxidation in the presence of $CuCl_2$ as described by Stewart (1975). Lyophilized tropomyosin samples were dissolved and stirred in 1 M NaCl, 25 mM $CuCl_2$, and 25 mM sodium borate, pH 9.3, for 1 h at 25 °C. The products of these reactions were subjected to polyacrylamide gel electrophoresis in sodium dodecyl sulfate in the absence of disulfide reducing reagents.

(*I*) *Nbs_2 Assay.* Disulfide exchange and determination of cysteine content were carried out according to Cowgill (1974) and der Terrossian et al. (1981) by using the Ellman reagent (1959).

Results

Purity and Composition of Macrophage Tropomyosin. Macrophage tropomyosin, like other tropomyosins of nonmuscle origin, is a dimer with a molecular weight of 60 000. It migrated as a single polypeptide band of 30 000 daltons when electrophoresed in polyacrylamide gels in the presence of sodium dodecyl sulfate with the same mobility as those of rabbit brain and human platelet tropomyosins, but faster than rabbit skeletal muscle tropomyosin (Figure 1). When it was treated with a large excess of reducing agent and subjected to extended electrophoresis, two polypeptide bands were resolved (Figure 1c). This indicated that it contained subunits of slightly different molecular weights which we designate α (faster moving component) and β forms, as has been done for other tropomyosins. As revealed by densitometry, 24% of the macrophage tropomyosin was α and 76% was β.

Mixtures of macrophage and muscle tropomyosins migrate on polyacrylamide gels electrophoresed in the presence of sodium dodecyl sulfate as well-separated bands (Figure 1e), and when treated with $CuCl_2$, in the absence of reducing agent, major bands with molecular weights of 60 000 and 70 000, respectively, were resolved. The presence of some 30 000 and 35 000 molecular weight material in copper-treated samples indicated the subunits were not quantitatively cross-linked.

The amino acid composition of macrophage tropomyosin was virtually identical with that of tropomyosin isolated from other sources (Table I). The macrophage protein contained 2.3 mol of cysteine per mol of dimer, and serine and threonine were found in higher amounts than in the other tropomyosins. In general, it contained large amounts of charged residues. The low amount of proline detected is consistent with the α-helical structure of tropomyosins.

The average yield of tropomyosin was about 50 μg/g of macrophage extracts desiccated with solvents. Because of this low yield, we quantitated the amount of 30 000-dalton polypeptide in macrophage extracts by densitometry of Coomassie blue stained polyacrylamide gels electrophoresed in the

Table I: Comparison of the Amino Acid Composition (mol/10^5 g) of Rabbit Macrophage and Rabbit Skeletal Muscle Tropomyosins with the Composition of Other Nonmuscle Tropomyosins Reported in the Literature

amino acid	rabbit skeletal muscle[d] A	rabbit skeletal muscle[d] B	rabbit macrophage	human platelets[a]	pig platelets[b]	BHK-21 cells[c]
aspartic acid	105	89	85	88	78	88
threonine	26	24	40	26	24	30
serine	40	39	69	25	28	41
glutamic acid	229	216	231	256	230	245
proline			1.5			6
glycine	21	11	35	33	34	37
alanine	103	106	102	105	103	94
valine	30	30	36	36	31	36
methionine	20	19	21	20	14	12
isoleucine	32	34	40	37	34	30
leucine	98	96	106	106	113	98
tyrosine	16	16	16	8	10	11
phenylalanine	4	4.3	8	7	4	7
half-cystine	4.6[e]	4.1	3.8[e]	5	3	
histidine	6	5.7	7	4	3	8
lysine	125	116	88	79	77	87
arginine	48	42	66	61	62	59

[a] From Cohen & Cohen (1972). [b] From der Terrossian et al. (1981). [c] From Schloss & Goldman (1980). [d] The data for rabbit skeletal muscle are (A) determined on a Beckman 121 MB analyzer under the same conditions as macrophage tropomyosin or (B) from Hodges & Smillie (1970). [e] Cysteine content was determined by NbS_2 reaction.

presence of sodium dodecyl sulfate. Polypeptides of this molecular weight represented 1% of the total protein in the macrophage extract. The 30 000-dalton polypeptide was further analyzed by isoelectric focusing and the resultant Coomassie blue stained pattern compared to that of purified macrophage tropomyosin (Figure 1h,i). Only 20% of the material which migrated at a molecular weight of 30 000 had the same (4.6) isoelectric point as purified macrophage tropomyosin. The maximal estimate of the tropomyosin content in these extracts is therefore 0.2% of the total protein.

Morphology of Macrophage Tropomyosin. Electron micrographs of negatively stained macrophage tropomyosin paracrystals revealed a banding pattern having a 34-nm periodicity (Figure 2). This repeat length is 6 nm shorter than is observed in skeletal muscle tropomyosin paracrystals (Casper et al., 1969; Figure 2a) and is near that reported for other nonmuscle tropomyosins (Fine et al., 1973; Fine & Blitz, 1975; der Terrossian et al., 1981; Côté & Smillie, 1981b). The shorter paracrystal repeat indicates that the macrophage protein is approximately 15% shorter than its muscle counterpart. This difference in size was directly confirmed in the electron microscope. Both macrophage and muscle tropomyosin molecules could be visualized and their length measured in the electron microscope of samples coated with platinum and carbon at low angles (Figure 3). The lengths of individual macrophage and muscle tropomyosin molecules were 33.2 ± 4.5 nm (mean ± SD, n = 80) and 38.7 ± 7.2 nm (n = 80), respectively. Linear aggregates of both tropomyosins were observed in the electron microscope but were not considered in our length determinations.

Functional Properties of Macrophage Tropomyosin. Figure 1j,k demonstrates that both macrophage and muscle tropomyosins bound actin filaments, pelleting with them when centrifuged at 100000g for 1 h.

Macrophage, brain, and platelet tropomyosins increase the equilibrium value of flow birefringence of actin filament solutions (Figure 4). However, when compared at similar concentrations to skeletal muscle tropomyosin, the nonmuscle proteins were less effective in increasing the flow birefringence of actin. The equilibrium flow birefringence value of a 6 μM solution of actin was 100°. Muscle and macrophage tropomyosins increased this value to 120 and 110°, respectively. This difference may reflect the lower binding affinities of the

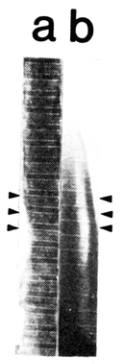

FIGURE 2: Electron micrographs of tropomyosin paracrystals formed as described under Experimental Procedures after staining with 1% uranyl acetate. (a) Muscle tropomyosin magnesium paracrystal; (b) macrophage tropomyosin magnesium paracrystal. The magnification is 51000×.

nonmuscle molecules to actin relative to muscle tropomyosin (Fine et al., 1973; Côté & Smillie, 1981b). This increase in the equilibrium flow birefringence value of actin filament solutions by tropomyosin has been interpreted as either filament stiffening and/or increases in length (Maruyama & Ohashi, 1978). We observed that the binding of tropomyosin to actin filaments did not increase their susceptibility to fragmentation by shear. Increasing the shear in the apparatus by increasing its velocity from 10 to 200 s^{-1} did not affect the relative increase in birefringence caused by tropomyosin.

Actin filaments formed in the presence of tropomyosin were protected in part from the shortening action of gelsolin. The gelsolin–Ca^{2+} complex decreases, in a concentration-dependent fashion, the equilibrium flow birefringence of F-actin solutions (Yin et al., 1981b). The flow birefringence of actin polymerized in the presence of muscle tropomyosin was only minimally affected by gelsolin (Figure 4). In the presence of calcium, 60 nM gelsolin decreased the equilibrium flow birefringence of a 6 μM F-actin solution by 30% in the absence of tropomyosin, but by only 2% in the presence of tropomyosin (1 molecule of tropomyosin to 4 actin monomers in filaments). This represents a 93% inhibition of gelsolin activity by muscle tropomyosin. All of the nonmuscle tropomyosins examined also diminished the effect of gelsolin–Ca^{2+} on actin filaments although they were less effective than the muscle molecule.

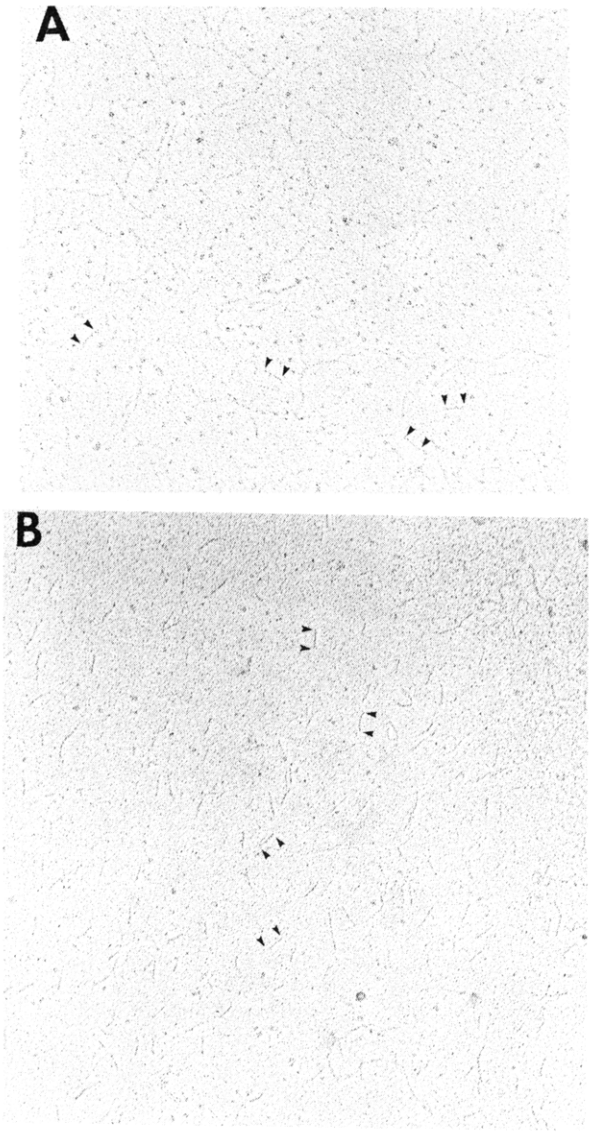

FIGURE 3: Electron micrographs of tropomyosin molecules revealed by spraying onto mica in 50% glycerol and rotary shadowing with platinum. (A) Macrophage tropomyosin; (B) muscle tropomyosin. The magnification is 67500×. Note the presence of end to end aggregates in both samples. The arrows define single molecules.

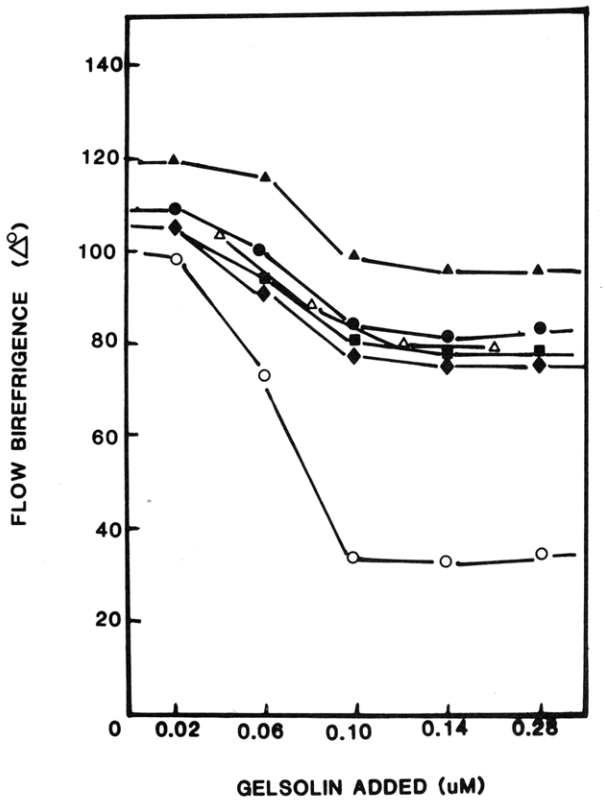

FIGURE 4: Effect of macrophage gelsolin on the equilibrium flow birefringence of actin filaments in the presence and absence of tropomyosins. Actin (6 μM) was polymerized in the presence of tropomyosin. Increasing concentrations of gelsolin were added to the actin in the presence of the following: no tropomyosin (○); skeletal muscle tropomyosin, molar ratio of 1 to 4 actin monomers (▲); brain tropomyosin, molar ratio of 1 to 7 actin monomers (●); human platelet tropomyosin, molar ratio of 1 to 7 actin monomers (■); macrophage tropomyosin, molar ratios of 1 to 7 actin monomers (♦) and 1 to 4 actin monomers (△).

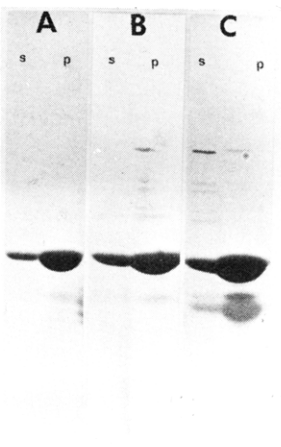

FIGURE 5: Effect of tropomyosin on the binding of gelsolin to actin filaments. The protein samples were centrifuged at 100000g for 1 h and the resulting supernatants and pellets analyzed by electrophoresis on polyacrylamide gels in the presence of sodium dodecyl sulfate. (A) Actin alone; (B) actin treated with gelsolin; (C) actin–tropomyosin treated with gelsolin. The supernatant (s) and pellets (p) are indicated.

At a molar ratio of 1 tropomyosin molecule to 4 or 7 monomers of actin, the effect of gelsolin, at concentrations of 60 and 100 nM, on actin birefringence was decreased by 87 and 50%, respectively.

Tropomyosin inhibits the binding of gelsolin–Ca^{2+} to actin filaments. The binding of gelsolin to actin was determined by cosedimentation with actin in the presence of calcium (Yin & Stossel, 1979). At a molar ratio of 1 gelsolin to 50 actin monomers, 75% of the added gelsolin sedimented with F-actin in the presence of calcium (Figure 5). In contrast, when actin was assembled in the presence of muscle tropomysoin, only 20% of the gelsolin was sedimentable with the actin. The total amount of actin sedimented remained the same in both cases. This indicates that tropomyosin blocked the binding of gelsolin–Ca^{2+} to the actin filaments. This blockage of binding by tropomyosin is likely its basis in diminishing gelsolin's filament shortening activity.

Discussion

The existence of tropomyosin in nonmuscle cells has been known for a decade (Cohen & Cohen, 1972). Recent work has analyzed the structure of platelet tropomyosin in considerable detail and established that it is very similar in structure to skeletal muscle tropomyosin, although slightly smaller in mass (Côté & Smillie, 1981a,b). The extent to which we have characterized the structure of macrophage tropomyosin permits us to conclude with reasonable confidence that this molecule is an authentic tropomyosin. Macrophage tropomyosin, like other nonmuscle tropomyosins, is smaller than its muscle

counterpart. This size difference was directly demonstrated in the electron microscope, and macrophage tropomyosin molecules were found to be approximately 15% shorter than molecules of muscle tropomyosin. The existence of tropomyosin in rabbit lung macrophages and the similarity of its chemical composition and morphology to other nonmuscle cell tropomyosins are not surprising.

Two features of our work concerning macrophage tropomyosin are new in relation to earlier studies. First, the amount of tropomyosin in macrophages is low relative to that in platelets. It comprises about 2% of the total platelet protein (Côté & Smillie, 1981a–c; der Terrossian et al., 1981) but only 0.2% of the macrophage protein. Second, we have established that macrophage, as well as other tropomyosins, protects actin filaments against the fragmenting activity of the gelsolin–calcium complex. We determined that tropomyosin decreased the binding of gelsolin to actin filaments and diminished the ability of gelsolin–calcium to fragment these filaments. When the potency of muscle and nonmuscle tropomyosins was compared, the muscle protein was more effective in preventing filament fragmentation by gelsolin. This is in keeping with its greater binding affinity and the cooperative nature of its binding to actin filaments relative to the nonmuscle tropomyosin. The basis for the interference of gelsolin binding to actin by tropomyosin is not known, but the binding of tropomyosin to the filament grove may sterically block the gelsolin binding sites on actin.

The protection of actin filaments by tropomyosin against the fragmenting action of gelsolin–calcium bears on the recent observation that gelsolin is present in muscle and localizes by immunofluorescence in the I-band region of the sarcomere (Yin et al., 1981a). Because a rise in myoplasmic free calcium initiates the interaction of myosin and actin filaments to produce tension during the contraction of skeletal muscle, a concomitant destruction of actin filaments by gelsolin–calcium would be counterproductive. The protection of actin filaments by tropomyosin described herein would prevent such degradation. In this manner, tropomyosin can override the effect of calcium-activated gelsolin and stabilize actin filaments to gelsolin irrespective of the ambient calcium concentration.

Tropomyosin may be important for maintaining the stability of certain actin fibers in nonmuscle cells. Regions of nonmuscle cells that are highly motile, such as the ruffling membranes at the leading edge of cells during locomotion, have actin filaments organized in a relatively isotropic state (Hartwig et al., 1982). This architecture contrasts with the anisotropic bundles of actin filaments found in "stress fibers" of cultured cells and in filopodia of certain cells such as activated platelets (Nachmias, 1979) and fertilized marine eggs (Begg & Rebhun, 1979). Tropomyosin, which has been identified by immunofluorescence analysis in stress fibers (Lazarides, 1975; Wehland & Weber, 1980), might influence the architecture of actin fibers in the cell by means of its stabilizing action. Cytochalasin B, a fungal metabolite, fragments actin filaments in vitro (Hartwig & Stossel, 1979; Estes et al., 1981; Maruyama et al., 1980; Schliwa, 1982) and disrupts stress fibers in living cells (Wessells et al., 1971; Rathke et al., 1977; Schliwa, 1982). The action of cytochalasin B on actin in vitro is blocked by tropomyosin (Spudich, 1972). By analogy, tropomyosin in cells would be expected to protect actin filaments from the fragmenting action of gelsolin–calcium. It therefore provides yet another mechanism to modulate cell shape and motility.

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Composition of the *Escherichia coli* 70S Ribosomal Interface: A Cross-Linking Study†

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ABSTRACT: 70S tight-couple ribosomes from *Escherichia coli* were cross-linked by using the bifunctional reagent phenyldiglyoxal (PDG). The reaction was stopped after 4-h incubation while still in the linear range. In comparison with untreated ribosomes, 30% of those treated with PDG were shown, by sucrose gradient experiments, not to be separable into their subunits, but remained as 70S particles. There was no detectable change in the structure of the reacted particles when their sedimentation behavior was compared with that of native 70S controls. When the cross-linking reaction was performed in the presence of $tRNA^{Phe}$ and poly(U), the reacted ribosomes retained 40–50% of their tRNA binding activity. The reaction leads predominantly to the formation of RNA–protein cross-links but protein–protein as well as RNA–RNA cross-links could also be detected. Cross-linked material was extracted, and the individual RNAs were separated into 23S, 16S, and 5S RNAs. Proteins were identified electrophoretically after reversal of the RNA–protein cross-links. Proteins were found to be cross-linked to RNAs within and across the ribosomal subunits; the latter are considered to be close to or at the 70S subunit interface. The arrangement of RNA and protein at the subunit interface is discussed.

There is growing evidence that the interacting domains of the two ribosomal subunits, the so-called subunit interface, are of central importance in protein synthesis. In a series of affinity labeling and cross-linking studies (Ofengand et al., 1980; Girshovich et al., 1981; Maassen & Möller, 1981; Gimautdinova et al., 1981), the interface has been shown to harbor binding sites for substrates such as tRNA, mRNA, and the initiation, elongation, and termination factors of protein synthesis. Furthermore, the reversible association–dissociation of the ribosomal subunits is an important step during the initiation and termination of the protein synthesis cycle (Grunberg-Manago & Gros, 1977; Weissbach, 1980).

A number of interface components have already been identified by using different approaches. Important information has been obtained from immune electron microscopy studies (Stöffler et al., 1980; Kahan et al., 1981), partial nuclease digestion studies (Santer & Shane, 1977), chemical modification (Herr & Noller, 1979; Herr et al., 1979), and direct chemical cross-linking (Lambert & Traut, 1981; Bäumert et al., 1978).

Although these studies yielded valuable information, there is still a lack of information about which components are involved in subunit association and how these components are arranged topographically. In fact, little is known about how much RNA and/or protein contribute(s) to the formation of the interacting domain of the two ribosomal subunits. Clearly, more data have to be collected if a detailed picture of the topography of the subunit interface is to be constructed.

To gain a better understanding of how RNA and protein are arranged between the interacting sites of the two subunits, we have undertaken a cross-linking study using the bifunctional reagent phenyldiglyoxal (PDG). This reagent seems to be especially suitable for topographical studies of particles consisting of both RNA and protein: (i) the reaction can be performed under conditions optimal for protein synthesis; (ii) the reagent does not noticeably affect the structure of the particles, and reacted ribosomes retain 40–50% of their tRNA binding activity; (iii) the reaction with RNA is specific for guanosine while proteins react through their lysine and arginine residues; consequently, the reaction yields defined cross-linking products between RNA–protein, protein–protein, and RNA–RNA components of the ribosome; (iv) the reaction with RNA (guanosine) is completely reversible under mild alkaline conditions which allows a direct analysis of proteins cross-linked to RNA (Wagner et al., 1980; Wagner & Garrett, 1978).

In this study, we describe the identification of proteins cross-linked to the 23S, 16S, and 5S RNAs of the *Escherichia coli* ribosome. Cross-linking occurred within and across the individual subunits and allowed us to draw conclusions about the arrangement of the proteins and RNA in the free and associated states of the subunits, and which components are involved in the formation of the ribosomal interface.

Materials and Methods

Materials. PDG was synthesized as described (Wagner & Garrett, 1978). Acrylamide, *N,N'*-methylenebis(acrylamide), and sodium dodecyl sulfate ($NaDodSO_4$) were from Bio-Rad; sucrose (ribonuclease free) and $AgNO_3$ were from Merck. Urea (ultra pure) was from BRL. Dithiothreitol was from

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