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Tropomyosin from Smooth Muscle of the Uterus*

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ABSTRACT: Tropomyosin was isolated from smooth muscle of human and pregnant sheep uterus. The tropomyosin preparations were shown to be essentially free of other proteins. The sedimentation constant and the intrinsic viscosity of human uterus tropomyosin were similar to those of rabbit skeletal tropomyosin but those of sheep uterus tropomyosin were higher. Other methods revealed differences between the uterus tropomyosins and rabbit and sheep skeletal tropomyosins, but no differences between human and sheep uterus tropomyosins. In starch gel electrophoresis at pH 7.6 the smooth muscle tropomyosin migrated faster toward the positive pole than the skeletal tropomyosin. Quantitative amino acid analyses showed significant differences in their amino acid composition. Peptide maps of tryptic digests of human and sheep uterus tropomyosins, though resembling those of skeletal tropomyosin, consisted of fewer peptide spots.

Physiological differences between uterine smooth muscle and skeletal muscle may be a reflection of chemical differences in the contractile proteins of the myofibril. There are three major proteins recognized in the myofibril: (1) actin, (2) myosin, and (3) tropomyosin. While it has been established that actin and myosin interact in muscle contraction, the function of the third protein of the myofibril is still not completely understood; it has been suggested that tropomyosin is implicated in sensitizing actomyosin to EGTA or calcium control when combined with the cofactor troponin (Ebashi and Kodama, 1966).

Tropomyosin found in skeletal and heart muscle (Bailey, 1948) appeared essentially identical (Katz and Converse, 1964). Whether the proteins of smooth muscle are identical with those of skeletal and heart muscle is still largely unknown because of the difficulty in preparing the smooth muscle proteins in adequate yield and purity for extensive characterization. Actin from uterus was found highly similar if not identical with skeletal muscle actin (Carsten, 1965). Myosin from uterus muscle has not been prepared in pure form. Differences between tropomyosin from smooth and skeletal muscle have been suggested (Kominz *et al.*, 1957a,b) but no extensive investigation has been made. In the present communication tropomyosin was obtained from human and from pregnant sheep uterus muscle. It was compared with rabbit skeletal tropomyosin because this is best characterized in the literature. We are referring to tropomyosin or tropomyosin B of vertebrate smooth muscle in contrast to tropomyosin A or paramyosin of invertebrate smooth muscle. Some analyses of sheep skeletal tropomyosin are included in order to be certain that any differences encountered are due to organ specificity and not to the class of animal.

Experimental Procedures

The human uteri were surgical specimens removed because of uterine prolapse and showed no signs of pathology. The sheep uteri were from pregnant sheep near term. They were obtained after removal of the fetus but with the placenta still attached to the walls. The placental cotyledons and major blood vessels were resected and the uterine muscle strips were frozen. The sheep skeletal muscle was from a fetus at term.

The procedure of Bailey (1948) for tropomyosin preparation was used with some modification (Katz and Converse, 1964). After the third precipitation in 70% $(NH_4)_2SO_4$, the protein was dissolved in H_2O and dialyzed against three changes of 0.09 M KCl–0.01 M HCl for 72 hr. Protein concentration was determined by the biuret method, standardized with a freeze-dried sample of tropomyosin corrected for moisture.

To minimize aggregation the same solvent (Tsao *et al.*, 1951) was used for sedimentation and viscosity studies. The former were carried out in a Spinco Model E ultracentrifuge equipped with RTIC temperature control and the latter in an Ostwald-type viscometer at

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25 ± 0.02°. Starch gel electrophoresis was carried out in Veronal buffer (pH 7.6). The remaining protein solution was centrifuged, dialyzed against water, freeze dried, and the starch gel electrophoresis was repeated.

To test for the presence of actomyosin, approximately 20 mg of the freeze-dried preparation was dissolved in 2 ml of 0.1 M phosphate buffer (pH 7.0) and heated in a water bath at 60° for 5 min (Hamoir and Laszt, 1962).

Alkylation with iodoacetate and amino acid analysis were carried out as described for actin (Carsten, 1963; Carsten and Katz, 1964; Carsten, 1965). Tryptic digestion of the alkylated protein was carried out as previously described (Katz and Carsten, 1963; Carsten and Katz, 1964; Carsten, 1965) in an automatic titrator (Radiometer 11, Copenhagen) with a ratio of protein: trypsin of 50:1 until alkali uptake ceased (90 min). Two-dimensional chromatography and high-voltage electrophoresis were carried out as before (Katz and Carsten, 1963; Carsten and Katz, 1964). Natural actomyosin was prepared from skeletal myofibrils and desensitized according to the method of Perry *et al.* (1966). Mg^{2}-activated ATPase[1] activity was assayed in the presence of increasing amounts of tropomyosin. Assays were carried out simultaneously in the presence and in the absence of EGTA. The reaction was terminated by addition of trichloroacetic acid.

Inorganic phosphate was determined by the method of Rockstein and Herron (1951), tryptophan by the method of Graham *et al.* (1947), and hydroxyproline by the method of Troll and Cannan (1953). Free sulfhydryl groups were determined with *N*-ethylmaleimide (Alexander, 1958). Ammonium sulfate (Mann Analyzed), special enzyme grade, free from heavy metal impurities (Mann Research Laboratories), was used throughout. The urea was recrystallized. For comparision some experiments with SH-tropomyosin (Mueller, 1966) prepared from sheep uterus are included; these have to be considered as preliminary.

Results

After the final (third) precipitation, approximately 1.5 and 1.1 mg of tropomyosin were obtained from 1 g of human and sheep uterus muscle (wet weight), respectively. No protein precipitated between 26 and 36% saturation with ammonium sulfate, in the one instance tried, in a preparation from sheep uterus. At this ammonium sulfate saturation tropomyosin A or paramyosin should precipitate (Kominz *et al.*, 1957a). On heating the tropomyosin solution at 60° there was no visible precipitate, turbidity, or change in protein concentration, attesting to the absence of actomyosin (Hamoir and Laszt, 1962).

Starch gel electrophoresis showed that both the sheep and the human uterus tropomyosin migrated faster toward the positive electrode than skeletal tropomyosin (Figure 1). Only one well-defined band was seen in the sheep uterus tropomyosin, but the human tropomyosin was somewhat spread and showed a fast-moving component. More of the fast-moving component was present in preparations not dialyzed extensively in acid. No difference was observed in electrophoretic patterns obtained before or after freeze drying. Polyacrylamide gel electrophoresis (Williams and Reisfeld, 1964) in 4 M urea was kindly performed by Dr. F. Norman Briggs of the University of Pittsburgh (Figure 2). A and B are samples of SH-tropomyosin isolated in the presence of Cleland's (1964) reagent and of troponin, respectively, both prepared by Dr. Briggs and run at the same time for comparison. The mobility of the main band of rabbit skeletal tropomyosin (C) agrees closely with the tropomyosin in Dr. Brigg's SH-tropomyosin (A). The sheep (D) and the human (E) uterus tropomyosins moved considerably faster toward the positive pole but not as fast as Dr. Briggs' troponin (B). The several slow-moving bands seen in all preparations may be artifacts due to oxidation with persulfate used to polymerize the gels. Increased electrophoretic heterogeneity has been observed in urea-containing acrylamide gels when persulfate was used as polymerizing agent (Brewer, 1967).

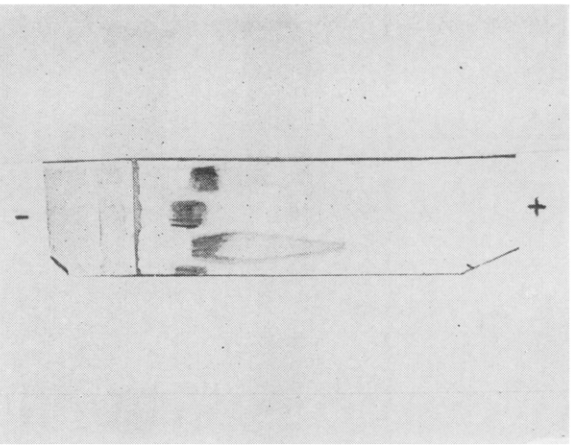

FIGURE 1: Starch gel electrophoresis of tropomyosin preparations. Top to bottom: sheep uterus, rabbit skeletal, human uterus, and sheep skeletal tropomyosins. Veronal buffer (pH 7.6), ionic strength, 0.012; at 8 V/cm of gel, 4 hr; protein concentration, 10 mg/ml.

Sedimentation in the ultracentrifuge showed only one boundary for sheep as well as for human uterus tropomysosin. In Figure 3 the sedimentation velocities are plotted as a function of protein concentration and compared with rabbit skeletal tropomyosin. All values were corrected for density and viscosity of the solvent and for a partial specific volume of 0.71 g/ml (Bailey *et al.*, 1948). The extrapolated value for the sedimentation constant ($s^{0}_{20,w}$) calculated by the method of least squares, was 2.48 S for human uterus tropomyosin in excellent agreement with 2.51 S of rabbit skeletal tropomyosin. Sheep uterus tropomyosin, however, had a sedimentation constant of 2.82 S.

Reduced viscosities of tropomyosin are plotted as a function of protein concentration in Figure 4. The reduced viscosities of rabbit and sheep skeletal and of human uterus tropomyosins were similar to one another but those of sheep uterus tropomyosin were higher

[1] Abbreviations used: ATPase, adenosine triphosphatase; ATP, adenosine 5′-triphosphate; EGTA, ethyleneglycol bis(β)-aminoethyl ether *N,N*-tetraacetic acid.

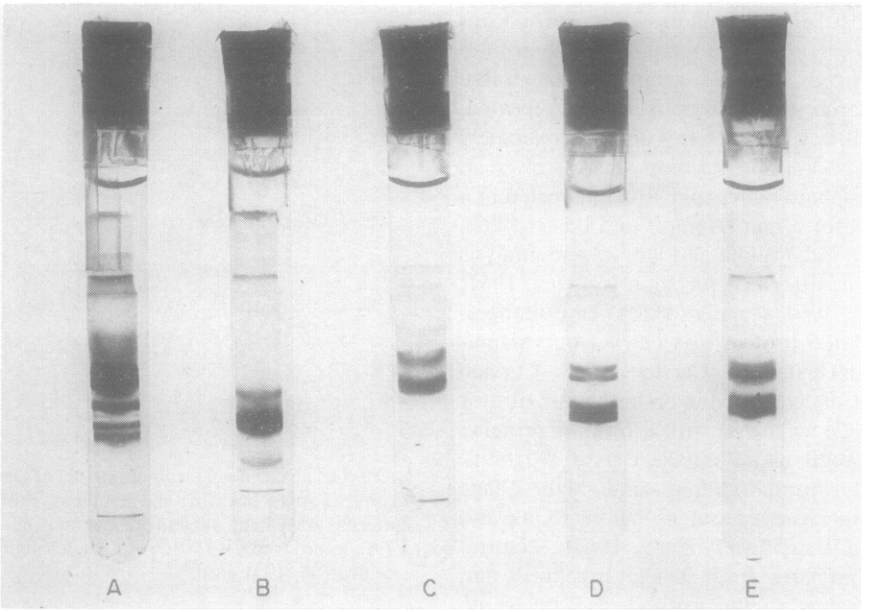

FIGURE 2: Polyacrylamide gel electrophoresis in 4 M urea. (A) SH tropomyosin (Briggs); (B) troponin (Briggs); (C) rabbit skeletal tropomyosin; (D) sheep uterus tropomyosin; (E) human uterus tropomyosin.

throughout. Intrinsic viscosities, calculated by the method of least squares were 0.213, 0.183, and 0.426 dl/g for the skeletal, human uterus, and sheep uterus preparations, respectively. Molecular weights calculated from sedimentation and viscosity data using the Scheraga and Mandelhem (1953) equation as given by Schachman (1957) were 55,400 for rabbit skeletal tropomyosin, 50,400 for human uterus, and 93,200 for the sheep uterus tropomyosins. This equation involves certain assumptions as to the β function for the hydrodynamic model and hence molecular weights cannot be regarded as definitive.

The amino acid composition of human and of sheep tropomyosins from uterus is shown in Table I and compared with analyses of rabbit and sheep skeletal tropomyosins carried out simultaneously. All results were calculated assuming a molecular weight of 55,000. Results were corrected for a moisture content of 11.8%; there was no ash (Elek Microanalytical Laboratories). Our results for rabbit skeletal tropomyosin agree reasonably well with results in the literature (Katz and Converse, 1964) with the possible exception of glycine–alanine and isoleucine–leucine, areas in which chromatographic resolution is poor. Owing to our correction to dry weight of the protein (88.2% of the weighed amount of protein), all our values appear lower than those in the literature which were arbitrarily corrected to 95%. Amino acid analysis of the same preparation of rabbit skeletal tropomyosin carried out after 70-hr hydrolysis revealed a decline in nearly all amino acid values by about 14–15%. When extrapolated to zero time, the values agree very closely with those arrived

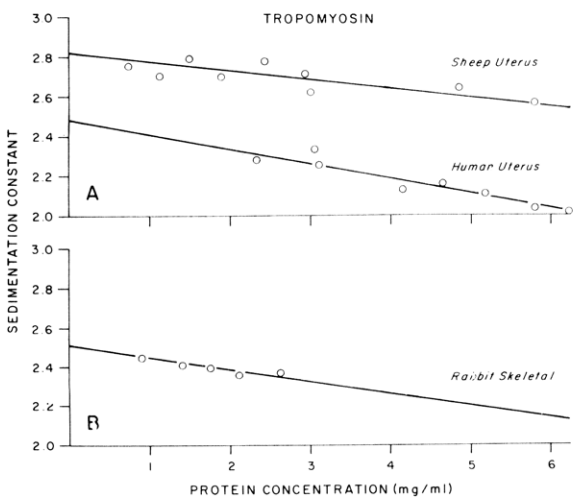

FIGURE 3: Sedimentation velocity of human uterus, sheep uterus, and rabbit skeletal tropomyosins as a function of protein concentration. An-E rotor, 50,740 rpm, 30-mm centerpiece at 3-mg/ml protein concentration or less; An-D rotor, 59,780 rpm, 12-mm centerpiece at higher protein concentrations.

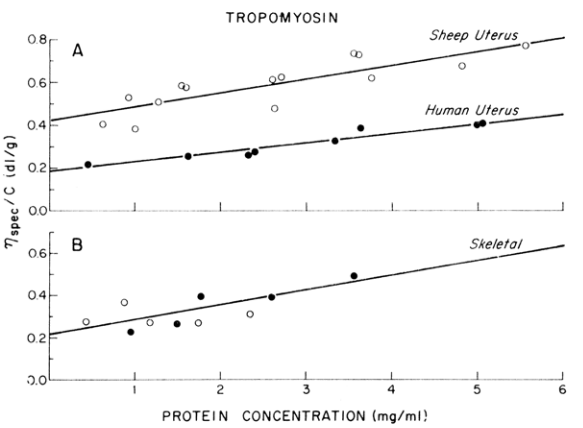

FIGURE 4: Reduced viscosities of tropomyosin as a function of protein concentration. (A, ●) Human and (○) sheep uterus tropomyosins; (B, ●) rabbit and (○) sheep skeletal tropomyosins; solvent 0.09 M KCl–0.01 M HCl.

TABLE I: Amino Acid Composition of Uterus Tropomyosin Preparations.

Amino Acid	Number of Amino Acid Residues/55,000 g of Protein			
	Uterus Muscle		Skeletal Muscle	
	Human	Sheep	Rabbit	Sheep
Lysine	*48.3 ± 1.6*[a,b]	*52.8 ± 1.9*	58.4	57.2
Histidine	*4.9 ± 0.5*	*5.4 ± 0.6*	3.1	2.9
Arginine	*26.9 ± 0.9*	*25.7 ± 0.5*	22.6	23.0
SCM-cysteine[c]	1.3 ± 0.1	2.9 ± 1.4	1.5	3.5
Aspartic acid	*39.1 ± 0.8*	*41.8 ± 0.6*	44.4	43.0
Threonine	*13.5 ± 0.1*	*13.8 ± 0.8*	11.7	12.1
Serine	18.4 ± 0.1	17.7 ± 1.0	20.2	17.9
Glutamic acid	*114.1 ± 1.1*	*112.9 ± 1.9*	106.0	110.0
Proline	0.8 ± 0.2	0.3 ± 0.04	1.0	2.3
Glycine	7.0 ± 0.6	5.7 ± 0.3	7.8	7.8
Alanine	51.2 ± 0.3	52.1 ± 0.5	51.7	51.4
Valine	*14.9 ± 0.0*	*15.5 ± 0.5*	13.2	12.9
Methionine	10.0 ± 1.4	10.0 ± 2.1	10.1	8.2
Isoleucine	*12.0 ± 0.1*	*12.1 ± 0.8*	14.4	15.1
Leucine	*48.3 ± 1.2*	*47.7 ± 0.2*	44.7	43.6
Tyrosine	*5.7 ± 0.3*	*5.0 ± 0.2*	7.4	7.0
Phenylalanine	2.2 ± 0.4	2.0 ± 0.3	2.5	3.0
Tryptophan	0.0	0.0	0.0	0.0
Total	418.6	423.4	420.7	420.9

[a] The variation is expressed as the average of the deviations from the mean. [b] Italicized figures indicate significant differences between smooth and skeletal tropomyosins. [c] SCM, *S*-carboxymethyl.

at by correction for moisture. There were three exceptions to this. Serine decomposed to a greater extent, *i.e.*, between 22- and 70-hr hydrolysis 26% of the serine present at 22-hr hydrolysis was destroyed. Extrapolation to zero time would bring the value for serine to 21.3 μmoles/55,000 g of protein (increase of 5.5% over value given in Table I). Valine stayed constant on prolonged hydrolysis, hence should not have been corrected for moisture content, and would be 12.4 μmoles at zero time. Finally, only 2.4% of the isoleucine present at 22-hr hydrolysis was destroyed after 70 hr. Extrapolation to zero time will give a value of 13.8 μmoles of isoleucine/55,000 g of protein. These corrections are small and have not been made in Table I. If one wishes to apply them, corrections of similar magnitude ought to be made to all analyses. Analyses for small amounts of proline are notably poor and hence show considerable variation. Therefore, one can merely conclude that there are between 0 and 2 moles of proline per 55,000 g of tropomyosin. Rabbit and sheep skeletal tropomyosins were identical in their amino acid composition except for glutamic acid; sheep skeletal tropomyosin analyzed more like uterus tropomyosin.

Two analyses of human and four analyses of sheep uterus alkylated tropomyosins, respectively, were averaged and presented in Table I. Agreement between individual amino acid analyses especially in the human was excellent. The human and the sheep uterus tropomyosins are highly similar to one another, but quite different from rabbit and sheep skeletal tropomyosins (Table I). Values for each amino acid for all analyses performed on the uterus tropomyosins were averaged and the standard deviation was computed. Whenever the average values for the skeletal tropomyosins differed from the mean of the values for the uterus tropomyosins by more than two standard deviations, significant differences in protein structure were considered to occur. In these instances the analytical values for the amino acids of uterus tropomyosin were italicized in Table I. Notably the uterus tropomyosin contained more arginine, histidine, threonine, glutamic acid, valine, and leucine, and less lysine, aspartic acid, isoleucine, and tyrosine than skeletal tropomyosin.

No significant amounts of hydroxyproline were detactable on amino acid analysis. Colorimetric analyses for hydroxyproline (Troll and Cannan, 1953) and for tryptophan (Graham *et al.*, 1947) were negative in all tropomyosin preparations. After alkylation with iodoacetate 1–4 moles of cysteine was recovered as *S*-carboxymethylcysteine. Direct analysis of free SH groups with *N*-ethylmaleimide after denaturation of the protein with Dupanol yielded 1.3 moles of SH groups/55,000 g of tropomyosin throughout.

The peptide map of rabbit skeletal tropomyosin was essentially similar to that obtained by other investigators (Katz and Converse, 1964), but due to the longer time allowed for chromatography, resolution was improved and two fast-moving peptides (32 and 33) were

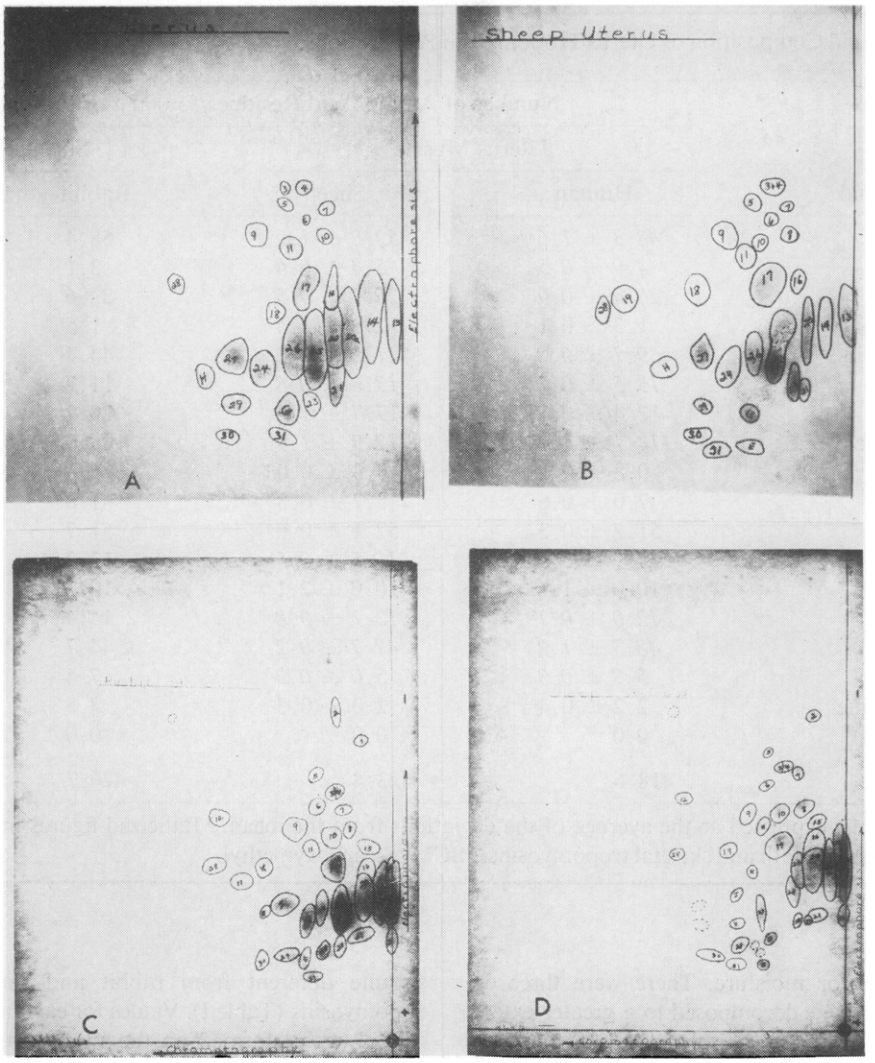

FIGURE 5: Peptide maps of tryptic digests of (A) human uterus, (B) sheep uterus, (C) rabbit skeletal, and (D) sheep skeletal tropomyosins. Descending chromatography was from right to left in butanol–acetic acid–water (4:1:5), 24 hr; electrophoresis in the vertical direction in pyridine–acetic acid–water (10:100:2890), pH 3.7, 1 hr at 2000 V. Stained with 0.5% ninhydrin in acetone.

not seen. Sheep skeletal tropomyosin showed only slight differences from rabbit skeletal tropomyosin (Figure 5). One peptide was missing, either 1 or 2, and there was one extra peptide, labeled "E." Peptides 18 and H appear to move more slowly in the chromatographic run. A total of 35 peptides were seen in the skeletal tropomyosin preparations. In contrast, the sheep and human uterus tropomyosin preparations showed only 29 and 27 peptides, respectively (Figure 5). Peptides 1, 2, 12, 15, 20a, and 22 were missing in both; in addition, peptides 19 and E were missing in the human uterus tropomyosin.

Contamination with nucleic acid was assessed by measuring the optical density at 260 and 280 mμ. The ratio of optical density at 260 mμ to that at 280 mμ was 0.575 for sheep uterus, 0.626 for human uterus, 0.582 for rabbit skeletal, and 0.873 for sheep skeletal tropomyosins. It appeared that prolonged time of dialysis of the protein solution against the acid KCl decreased the ratio.

The Mg^{2}-activated ATPase activity of natural actomyosin is strongly inhibited by EGTA, whereas that of reconstituted or of desensitized actomyosin is not inhibited (Perry and Grey, 1956; Perry *et al.*, 1966). Inhibition occurs, however, in the presence of natural tropomyosin, a complex of tropomyosin and troponin (Ebashi and Kodama, 1966) or of SH-tropomyosin (Mueller, 1966). Our tropomyosin preparations did not inhibit the ATPase activity of desensitized actomyosin as demonstrated in Figure 6A. Hence it is concluded that no appreciable amounts of troponin were present. On the other hand, sheep uterus SH-tropomyosin inhibited the ATPase activity of desenitized actomyosin consistently (Figure 6B).

Discussion

A comparison of proteins from various sources with respect to their chemical or biological properties is valid only if based on relatively pure protein preparations. The tropomyosin preparations used here were free from tryptophan. Tropomyosin was previously

shown not to contain tryptophan (Kominz *et al.*, 1954). Furthermore, the tropomyosin preparations were free from actomyosin often found as an impurity (Hamoir and Laszt, 1962). If any collagen was present in the tropomyosin preparations, it must be less than 1.0% as inferred from the hydroxyproline analyses. The tropomyosin preparations also appear free from troponin with which tropomyosin is closely associated. The slow-moving bands in the polyacrylamide gel electrophoresis are thought to be oxidation products arising in the electrophoretic procedure. The lack of EGTA-sensitizing activity in the tropomyosin preparations further demonstrates the absence of troponin. Moreover, the absence of paramyosin or tropomyosin A in uterine smooth muscle (Needham and Williams, 1963a) is confirmed.

The yield of 1.5 and 1.1 mg of tropomyosin obtained from 1 g (wet weight) of human and pregnant sheep uteri, respectively, agrees well with 1.5 mg obtained from uterus by Needham and Williams (1963a). The lower yields obtained from uterus compared with rabbit skeletal muscle (4.7 mg/g) or pig cardiac muscle (2.1 mg/g wet weight) (Bailey, 1948) confirm the concept of a lower content of contractile proteins in uterus smooth muscle than in skeletal muscle, observed with respect to actin (Carsten, 1965) and to myosin (Needham and Williams, 1963a). The reason has to be sought in the relatively large myometrial content of proteins other than those of the myofibril, such as collagen and still unidentified proteins (Needham and Williams, 1963b,c).

Tropomyosin from smooth muscle often is extracted in the form of nucleotropomyosin, a complex of tropomyosin with nucleic acid (Hamoir, 1951a). Differences between tropomyosin and nucleotropomyosin have been found in their ultracentrifugal (Hamoir, 1951a) and electrophoretic patterns (Needham and Williams, 1963a), the nucleotropomyosin being inhomogeneous, but becoming homogeneous upon removal of nucleic acid; the protein component of both appears to be identical (Hamoir, 1951a). The nucleic acid content of skeletal and uterus tropomyosins varies with the method of preparation (Needham and Williams, 1963a; Hamoir, 1951b): prior preparation of dried muscle powder assures lower nucleic acid content in the tropomyosin. Using the ratio of optical density at 260 mμ to that at 280 mμ as a criterion of nucleic acid content, our preparations have a considerably lower nucleic acid content than previously reported for uterus tropomyosin (Needham and Williams, 1963a), and are in good agreement with those of approximately 0.6 and 0.55 reported by Kominz *et al.* (1957a) for their purest tropomyosin preparations. The apparently high nucleic acid content of the sheep skeletal tropomyosin is unexplained. The appearance of only one boundary in the sedimentation pattern suggests the absence of nucleotropomyosin from our preparations. The fast-moving component in the starch gel electrophoresis pattern of human tropomyosin, however, may indicate a small amount of nucleotropomyosin (or some nucleic acid) in our preparation, particularly since the optical density ratio for the human preparation was 0.626,

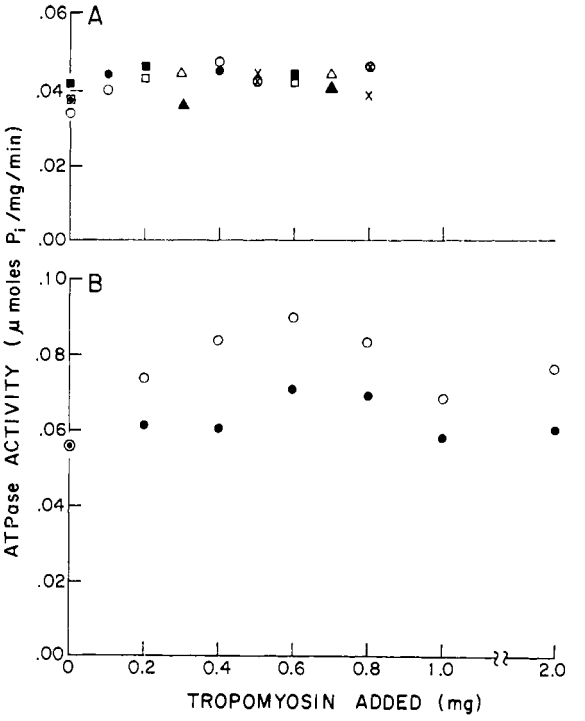

FIGURE 6: Effect of EGTA on the Mg^{2+}-activated ATPase activity of desensitized actomyosin in the presence of tropomyosin preparations. (A, ■, □) Human uterus tropomyosin; (●, ○) sheep uterus tropomyosin; (▲, △) rabbit skeletal tropomyosin; and (⊗, ×) sheep skeletal tropomyosin; closed symbols: EGTA (1 mM) added; open symbols: no addition. (B) Sheep uterus SH tropomyosin.

somewhat higher than that of the sheep uterus or rabbit skeletal tropomyosins.

Furthermore, it was noticed that with a decrease in the 260:280 mμ optical density ratio the fast-moving component decreased. It appears that the nucleic acid is split off the nucleotropomyosin in the acid dialysis medium or precipitates in the form of an acid-insoluble nucleotropomyosin as suggested for fish nucleotropomyosin (Hamoir, 1951a,b).

Although a single peak in the ultracentrifuge in itself is no proof of homogeneity of a protein, it is one of many necessary criteria of homogeneity. The tropomyosin preparations studied showed a single sedimenting boundary. Tropomyosin shows a tendency to aggregate in solutions of decreasing ionic strength at neutral pH. At acid pH (Tsao *et al.*, 1951), however, as used in the present experiments, tropomyosin should be in its monomeric form. The extrapolated sedimentation constant $s^0_{20,w}$ of 2.48 S for rabbit skeletal tropomyosin is in excellent agreement with values found in the literature, such as 2.60 (Bailey *et al.*, 1948), 2.53 (Katz and Converse, 1964), and 2.59 S (Holtzer *et al.*, 1965). The sedimentation constant of the human uterus tropomyosin of 2.51 S agreed very well with the above values. The higher sedimentation constant obtained for sheep uterus tropomyosin (2.82 S) does not lend itself to a simple interpretation. A difference in shape of the molecule or in aggregation is indicated; possibly with a low nucleic acid content aggregation is more likely to occur.

The high intrinsic viscosity of the sheep uterus tropomyosin further attests to aggregation and the calculated molecular weight is approximately double that of the skeletal and human uterus tropomyosins. Molecular weights for tropomyosin, however, are somewhat equivocal, values in the literature ranging from 53,000 to 90,000 (Tsao *et al.*, 1951; Kay and Bailey, 1960; Katz and Converse, 1964; Woods, 1967; Holtzer *et al.*, 1965; Bailey *et al.*, 1948), in general molecular weights between 53,000 and 55,000 being obtained in acid solvents. As our calculated results for rabbit skeletal and human uterus tropomyosins are in this range within experimental error, the amino acid analyses were calculated on the basis of a molecular weight of 55,000.

Amino acid analyses given in Table I revealed significant changes in the acidic and the basic amino acid contents in the preparations from uterus smooth muscle was compared to rabbit skeletal muscle. These changes appeared to lead to an increase of approximately eight in the number of net negative charges and thus explained the greater electrophoretic mobility of the preparations from uterus at pH 7.6. In spite of the difference of four glutamic acid residues, the electrophoretic mobility of rabbit and sheep skeletal tropomyosins was the same. One may surmise that these four residues occur in the form of glutamine in the native protein. On this basis the net negative charge would change by four from skeletal to uterus smooth muscle tropomyosin. The differences in threonine, glutamic acid, valine, and leucine are in addition to previously noted differences (Kominz *et al.*, 1957a).

In amino acid analyses 3.3 and 3.6 half-cystine residues have been found previously per 55,000 g of rabbit skeletal tropomyosin (Bailey, 1948; Kominz *et al.*, 1957a). Discussion in the literature has centered on how many of these are free SH groups. Variable results have been reported with values ranging from 0 to 4 when calculated for 55,000 g of protein (Kominz *et al.*, 1957a); Drabikowski and Nowak, 1965; summary in Jen *et al.*, 1965). The results varied with the method of analysis and the method of preparation. Apparently the SH groups are autooxidizable during preparation and storage in the dry state. This process can be prevented or slowed by removing metal impurities from the ammonium sulfate used in salting out the tropomyosin, by the addition of reducing agents during preparation, and speedy analysis (Drabikowski and Nowak, 1965; Mueller, 1966). Our present results were obtained by alkylation of free SH groups with iodoacetate followed by amino acid analysis and show values of 1.3 residues of *S*-carboxymethylcysteine for the human uterus, 2.9 for the sheep uterus tropomyosin, 1.5 for rabbit skeletal, and 3.5 for sheep skeletal tropomyosin. These values are within the range of those obtained for rabbit skeletal tropomyosin discussed above and there appear to be no fundamental differences between the preparations from uterus and from skeletal muscle. In the case of the sheep uterus tropomyosin two individual analyses showed 4.3 and 3.5 *S*-carboxymethylcysteine residues, respectively. The lower values (1.3) obtained throughout with *N*-ethylmaleimide in the presence of a denaturing agent are unexplained, but even lower values were recorded by others using this method (see summary in Jen *et al.*, 1965). It seems that partial oxidation took place in our tropomyosin preparations in spite of the use of ammonium sulfate free of metal impurities, and performance of the analyses within minimum time after preparation. Similar autooxidation of sulfhydryl groups was encountered with actin (Carsten, 1966).

The differences in amino acid composition are verified by differences in the peptide maps. In the present study resolution of the peptides was improved by extending the time for chromatography. In the skeletal tropomyosin preparations at least 35 peptide spots appeared not counting the two that moved off the paper (32 and 33, Katz and Converse, 1964). Fewer peptides (27–29) were obtained in the maps of tropomyosin digests from uterus. Differences between the two preparations from skeletal muscle (rabbit and sheep) were minor; differences between the two preparations from uterus muscle (sheep and human) were also small. On the other hand, the preparations from the uterus differed considerably from those of skeletal muscle, namely by six to eight peptides. This is in accordance with the amino acid composition. Our amino acid analyses showed approximately six residues of arginine plus lysine less in the uterus tropomyosin than in the skeletal tropomyosin. Trypsin is known to split the peptide chain at the carboxyl end of the lysine and arginine residues. The observation of approximately 35 peptide spots in skeletal tropomyosin against approximately 81 arginine plus lysine residues is due either to incomplete digestion or suggests the presence of two largely similar peptide chains. A ninhydrin-staining spot was seen at the origin in all peptide maps and alkali uptake during digestion was only 59–64% of the arginine plus lysine residues. End-group analysis of rabbit skeletal tropomyosin indicated two C-terminal and two N-terminal residues (Kominz *et al.*, 1957a; Saad and Kominz, 1961; Alving *et al.*, 1966). Further evidence for two peptide chains has been obtained recently from measurement of the molecular weight by equilibrium sedimentation in urea in the presence of mercaptoethanol (Woods, 1965) or after reaction with *N*-ethylmaleimide (Woods, 1966). Subunit molecular weights were calculated to be between 30,000 and 35,000. One may conjecture that in uterus smooth muscle tropomyosin with about 77 arginine plus lysine residues and 27–29 peptide spots there would also be two peptide chains, but that in skeletal as well as in uterus muscle the peptide chains are not identical.

Differences in amino acid composition between uterus smooth muscle and skeletal muscle tropomyosins were found in 10 out of 17 amino acids. Approximately 34 residues out of 421 are different. Many changes appear to involve a pair of similar amino acids such as glutamic and aspartic acid, or arginine and lysine or leucine and isoleucine. Furthermore, the net negative charge is increased in the uterus tropomyosin. As the charged amino acids contribute to maintain the tertiary structure by electrostatic interaction, the double-stranded α-helical structure of skeletal tropomyosin (Cohen

and Holmes, 1963) might be somewhat different in smooth muscle tropomyosin.

Tropomyosin is thus different from actin, the other myofibrillar protein studied in this laboratory (Carsten, 1965), in as much as there are differences in the primary structure between skeletal and uterus smooth muscle tropomyosins but not in actin within the limits of sensitivity of the methods used. Similar to actin, no significant differences were found between the uterus tropomyosins of the two mammals studied. The extent of the variations in the tropomyosin molecule may prove greater than indicated here, at a time when more sensitive methods, such as sequential analysis, will be applicable to a molecule as large as tropomyosin.

The observed differences between skeletal and uterus smooth muscle tropomyosins may well relate to differences in physiological behavior of these muscles, such as changes in length–tension relationships and slowness of contraction of smooth muscle. If indeed tropomyosin is proven to be one of the components necessary for calcium activation of muscle contraction, the role of calcium in the mechanism of smooth muscle contraction will have to be reevaluated.

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