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Chemical and Immunochemical Characteristics of Tropomyosins from Striated and Smooth Muscle

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1. On electrophoresis in dissociating conditions the tropomyosins isolated from skeletal muscles of mammalian, avian and amphibian species migrated as two components. These were comparable with the α and β subunits of tropomyosin present in rabbit skeletal muscle. 2. The α and β components of all skeletal-muscle tropomyosins contained 1 and 2 residues of cysteine per 34000g respectively. 3. The ratio of the amounts of α and β subunit present in skeletal muscle tropomyosins was characteristic for the muscle type. Muscle consisting of slow red fibres contained a greater proportion of β-tropomyosin than muscles consisting predominantly of white fast fibres. 4. Mammalian and avian cardiac muscle tropomyosins consisted of α-tropomyosin only. 5. Mammalian and avian smooth-muscle tropomyosins differed both chemically and immunologically from striated-muscle tropomyosins. 6. Antibody raised against rabbit skeletal α-tropomyosin was species non-specific, reacting with all other striated muscle α-tropomyosin subunits tested. 7. Antibody raised against rabbit skeletal β-tropomyosin subunit was species-specific.

It has been shown in this laboratory that the subunits of tropomyosin from rabbit skeletal muscle can be fractionated into two main forms. These have been named the α and β subunits, and the tropomyosins formed from a single type of subunit have been designated the α- or β- tropomyosins respectively (Cummins & Perry, 1972, 1973). The two types of the tropomyosin dimer molecule, namely the α_2 and β_2 forms, possess all the biological properties of unfractionated tropomyosin, but the subunits differ slightly in electrophoretic mobility and amino acid composition, particularly in cysteine content. Although rabbit skeletal-muscle tropomyosin is composed of two main types of subunit, isoelectric focusing has indicated that each contains very small amounts of another component, which may be an additional polymorphic form of tropomyosin. This evidence of heterogeneity in the tropomyosin subunits agrees well with the sequence studies of Smillie and collaborators (Hodges & Smillie, 1970; Sodek *et al.*, 1972), which suggest that tropomyosin prepared by the standard procedures from mixed skeletal muscle of the rabbit consists of a number of forms differing slightly in amino acid sequence.

Our earlier studies (Cummins & Perry, 1972, 1973) strongly suggested that the relative amounts of the α and β forms of tropomyosin were constant for a given muscle, but varied with the species from which it was derived. The present work was to extend these findings and determine whether any correlation existed between muscle types and their respective tropomyosin subunit compositions. The results support the premise that in skeletal muscle the relative amount of β-tropomyosin increases as the speed of contraction decreases. The α- and β-tropomyosins have been shown to possess marked immunochemical differences in that antibody to the β-tropomyosin is species specific whereas that to the α-tropomyosin is not. It is also demonstrated that the tropomyosins of striated muscle differ chemically and immunologically from the tropomyosins present in smooth muscle.

Methods

Unless otherwise stated, all preparations and chromatographic separations were carried out at 0–4°C.

Tropomyosin

This protein was prepared from mammalian, avian and amphibian muscle by a procedure described previously (Cummins & Perry, 1973) which is based on the method of Bailey (1948). Tropomyosin from the adductor muscle of the mollusc *Pecten maximus* was prepared as described by Bailey & Ruegg (1960).

The tropomyosin preparations were fractionated into α and β components by chromatography on CM-cellulose equilibrated with 50mM-sodium form-

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ate buffer (pH4.0) – 8M-urea – 15mM-2-mercaptoethanol (Cummins & Perry, 1973). By this procedure 30.0mg of rabbit skeletal-muscle tropomyosin applied in 4.0ml of buffer to a column (12.0cm × 1.5cm) could be satisfactorily fractionated into the α and β components. Chromatography was repeated until the fractions were homogeneous as judged by polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate (see under 'Electrophoresis').

Electrophoresis

Electrophoresis was carried out as previously described (Cummins & Perry, 1973) on 10% polyacrylamide gel in 82.5mM-Tris–400mM-boric acid buffer (pH7.0) containing 0.1% sodium dodecyl sulphate. To detect traces of troponin T (mol.wt. 37000), which was not well-resolved from the tropomyosin components on electrophoresis in sodium dodecyl sulphate, 5M-urea was incorporated into the sodium dodecyl sulphate–polyacrylamide gels (Sender, 1971) as indicated in the text. Under these conditions the migration of the α- and β-tropomyosins was retarded with respect to troponin T. When examined by this procedure troponin T could not be detected in the preparations used for immunological studies when up to 200μg was applied to the gels.

Carboxymethylation

Carboxymethylation was carried out at pH8.6 by using unlabelled or [2-^{14}C]-labelled iodoacetic acid as described by Cummins & Perry (1973).

Measurement of radioactivity

The radioactivity of [^{14}C]carboxymethylated tropomyosin was determined as described by Cummins & Perry (1973).

Determination of cysteine content

The cysteine contents of the tropomyosin subunits were determined by three different procedures.

(i) After separation of the iodo[2-^{14}C]acetate-labelled subunits by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis, slices containing individual subunits were dissolved by treatment with 0.7ml of H_2O_2 (100vol.) at 80°C for 4h and carboxymethylcysteine was determined by measurement of radioactivity. The relative amounts of protein present were determined by scanning the gels with a densitometer (Cummins & Perry, 1973), assuming that both components bound equivalent amounts of dye.

(ii) The iodo[2-^{14}C]acetate-labelled subunits were separated by CM-cellulose chromatography (see above) and the radioactivity was measured to determine the carboxymethylcysteine content. Protein was determined as described below and in addition the relative amounts were estimated by determination of the areas beneath the column profile peaks.

(iii) Cysteine contents were also determined as *S*-carboxymethylcysteine by amino acid analysis of the carboxymethylated isolated subunits.

Protein determinations

These were done as described by Schaub & Perry (1971).

Amino acid analysis

Amino acid analysis was performed as described by Cummins & Perry (1973).

Preparation of antisera to unfractionated and α and β tropomyosins

The purified proteins (approx. 1mg/ml) dissolved in buffered physiological saline (145mM-NaCl–10mM-sodium phosphate buffer, pH7.1) were diluted with an equal volume of Freund's Complete Adjuvant (Difco Laboratories, Detroit, Mich., U.S.A.) and samples containing 150μg of tropomyosin were injected subcutaneously at weekly intervals into the back regions of six guinea pigs. After 4 weeks, 1–2ml of blood was withdrawn from the animals by cardiac puncture and preliminary examination of the antisera carried out on agar plates by the Ouchterlony procedure.

When the antiserum was found to produce a satisfactory response, usually after four injections, approx. 5ml of blood was withdrawn from the animal by cardiac puncture, the blood clot removed and the antiserum separated (Hirabayashi & Perry, 1974). The samples of antisera were stored at –20°C.

Immunodiffusion

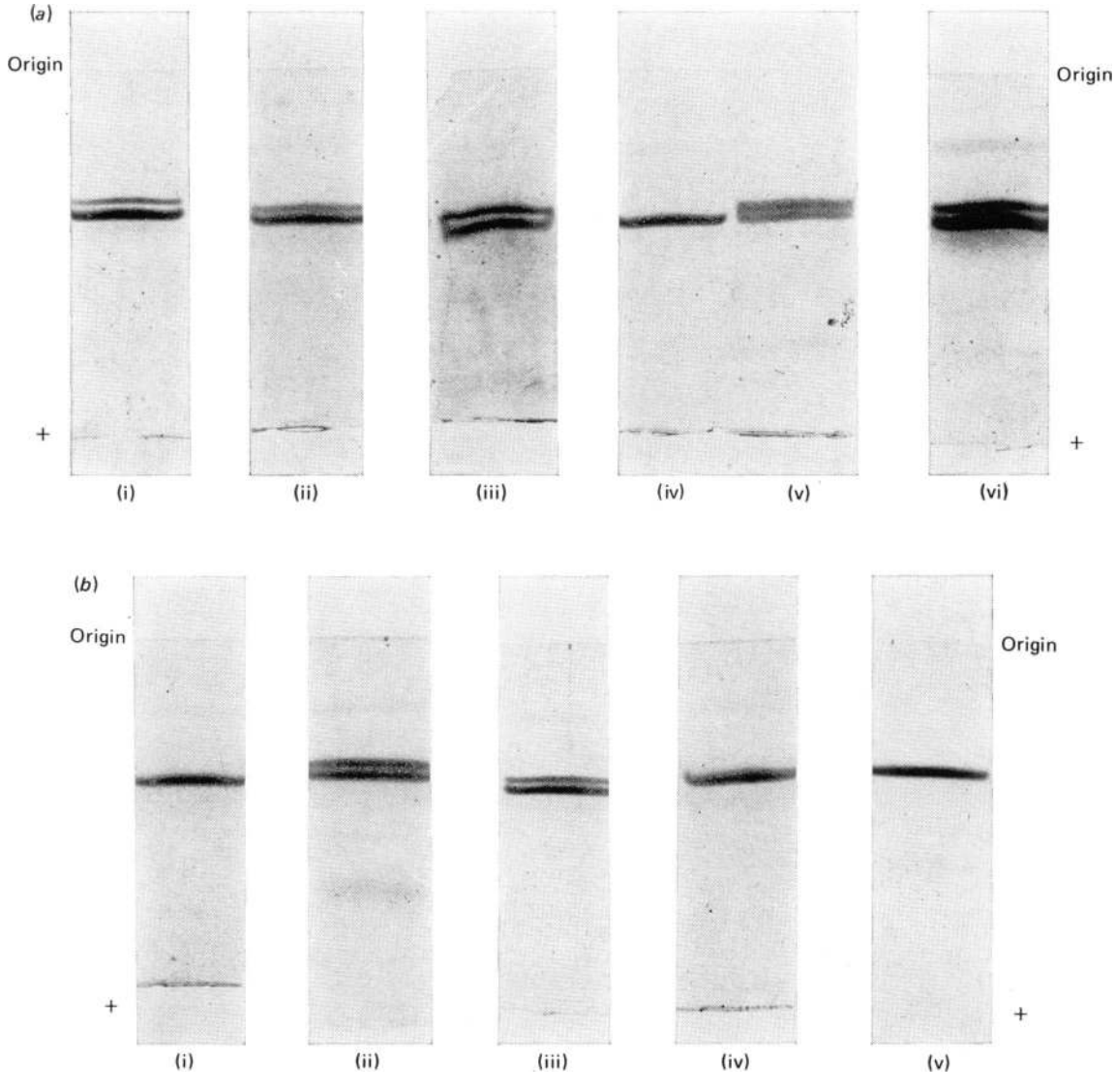
Ouchterlony immunodiffusion tests were carried out in general as described by Hirabayashi & Perry (1974). $CdCl_2, 2.5H_2O$ (0.01%) was incorporated into the 145mM-NaCl–10mM-sodium phosphate buffer (pH7.1), and 0.01% thimerosal (Koch–Light Laboratories Ltd., Colnbrook, Bucks., U.K.) was incorporated into the agar gel to prevent bacterial growth.

The reaction was followed at 4°C for 1–5 days and stopped by immersion of the plates in the buffered physiological saline. Photographs were taken with dark-field illumination.

Results

Subunit composition of tropomyosin from different muscle types

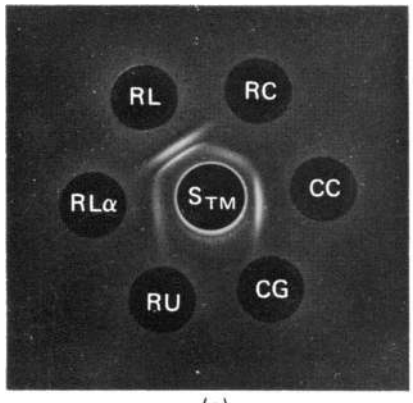
Striated muscle. All samples of highly purified tropomyosin isolated from skeletal muscles of mammalian, avian and amphibian species migrated as two



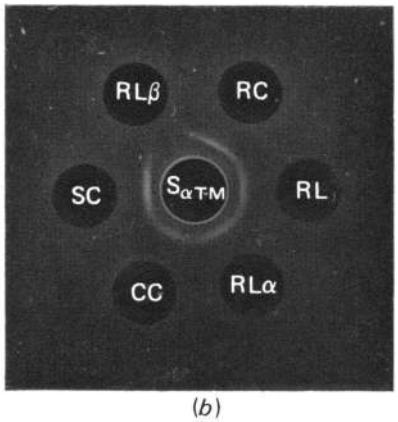
EXPLANATION OF PLATE 1

Polyacrylamide-gel electrophoresis of tropomyosins from striated muscle and smooth muscle

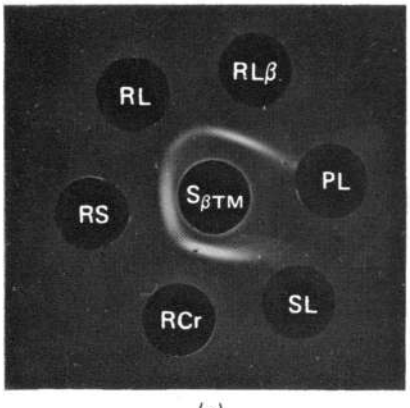
Samples of carboxymethylated tropomyosin (10–15μg) were applied to 10% (w/v) polyacrylamide gels in 82.5mM-Tris–400mM-borate buffer (pH 7.0)–0.1% sodium dodecyl sulphate–5M-urea. (*a*) Striated muscle: (i) rabbit longissimus dorsi; (ii) rabbit psoas; (iii) rabbit crurius; (iv) sheep cardiac; (v) rabbit soleus; (vi) frog whole leg. (*b*) Smooth and striated muscle: (i) chicken cardiac; (ii) chicken gizzard; (iii) chicken breast; (iv) rabbit uterus; (v) pig uterus.


RL
RC
RLα
S_{TM}
CC
RU
CG

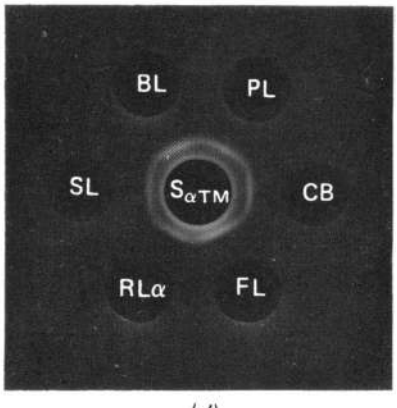
(*a*)


RLβ
RC
SC
$S_{\alpha TM}$
RL
CC
RLα

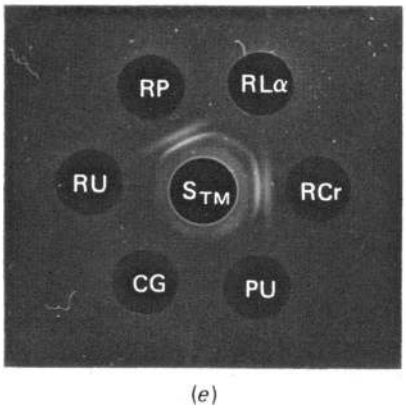
(*b*)


RL
RLβ
RS
$S_{\beta TM}$
PL
RCr
SL

(*c*)


BL
PL
SL
$S_{\alpha TM}$
CB
RLα
FL

(*d*)


RP
RLα
RU
S_{TM}
RCr
CG
PU

(*e*)

components on electrophoresis in sodium dodecyl sulphate at pH7.0 in both the presence and the absence of 5 M-urea (Plates 1*a* and 1*b*). The mobilities of the two forms were very similar to the α- and β-tropomyosins present in the longissimus dorsi muscle of the rabbit (Cummins & Perry, 1973). The cysteine contents of the faster and slower components, measured by labelling with iodo[2-^{14}C]acetic acid, were consistently in the ratio of 1:2 respectively. It could be deduced from the total cysteine contents of the unfractionated tropomyosins and the relative proportions of the two tropomyosin subunits present that the faster of the two components observed on sodium dodecyl sulphate–polyacrylamide-gel electrophoresis contained 1 mol of cysteine, and the slower component 2 mol per 34000 g. This was confirmed by analysis of the separated purified subunits (Table 1). It was therefore concluded that the two components present in skeletal muscles of other species were very similar to the α and β forms described for rabbit skeletal muscle, and the nomenclature was extended to the corresponding subunits of tropomyosin from striated muscles of other species.

The data presented in Table 2 extend and confirm our earlier suggestions that the ratio of the amount of α to β fractions present in tropomyosin is characteristic of the muscle type. Skeletal muscles consisting predominantly of white fibres contained tropomyosin in which the α/β ratio was appreciably greater than 1:1, ranging from 1.3:1 in the longissimus dorsi of the sheep to 3.8:1 in longissimus dorsi muscle of the rabbit. In the slower red muscles the relative amounts of β-tropomyosin were greater than in fast white skeletal muscle, the ratio falling to about 1:1 (Table 2). It is noteworthy that β-tropomyosin has not been detected in cardiac muscle from the species studied to date, i.e. rabbit, chicken and sheep.

Smooth muscle. Chicken gizzard-muscle tropomyosin displayed a somewhat different picture on

Table 1. *Cysteine content of tropomyosins and their subunits from different muscles*

For experimental details see the text. The values given are expressed as amino acid residues/68000 g of protein. Analyses are for unfractionated tropomyosins where there is no entry in the tropomyosin subunit column. All samples were analysed by direct carboxymethyl cysteine determinations, procedure (iii). In addition the ratios of cysteine content were verified by procedure (i), for all tropomyosins that contained more than one subunit. Procedure (ii) was used for the analysis of tropomyosins from white striated muscle and chick gizzard muscle. Values obtained by the different methods were averaged.

Source	Tropomyosin subunit	Content (mol/68000 g)
White striated		
Rabbit skeletal		2.7
Rabbit psoas		2.6
Rabbit psoas	α	1.5
Rabbit psoas	β	3.1
Frog skeletal		2.5
Frog skeletal	α	1.6
Frog skeletal	β	3.3
Sheep cardiac		1.6
Chicken cardiac		1.7
Chicken skeletal		2.6
Chicken skeletal	α	1.7
Chicken skeletal	β	3.5
Red striated		
Rabbit crurius		2.8
Rabbit soleus		2.5
Smooth muscle		
Chicken gizzard		2.1
Chicken gizzard*	Fast	1.8
Chicken gizzard*	Slow	1.7
Rabbit uterus		2.0
Pig uterus		1.8

* Fast and slow are used to describe the relative electrophoretic mobilities of the two components of chicken gizzard tropomyosin on electrophoresis in sodium dodecyl sulphate at pH7.0.

EXPLANATION OF PLATE 2

Reactions of guinea-pig antisera raised against tropomyosins of rabbit striated muscle

For details of agar diffusion procedure see the Methods section. Portions (50 μl) of antigen or antisera were added to wells. Antigen solutions were in the range 40–60 μg of tropomyosin/ml, but results were similar with antigen concentrations up to 3000 μg/ml. RL, RLα and RLβ represent unfractionated, α- and β-tropomyosins of rabbit longissimus dorsi muscle respectively. S_{TM}, $S_{\alpha TM}$ and $S_{\beta TM}$ represent guinea-pig antisera raised against unfractionated, α- and β-tropomyosins of rabbit longissimus dorsi muscle respectively. (*a*) Reactions of tropomyosin from different muscle types with antiserum raised against unfractionated tropomyosin from rabbit longissimus dorsi muscle. RC, rabbit cardiac; CC, chicken cardiac; CG, chicken gizzard; RU, rabbit uterus. (*b*) Reactions of antiserum raised against α-tropomyosin of rabbit longissimus dorsi muscle with tropomyosins from cardiac and skeletal muscles. RC, rabbit cardiac; CC, chicken cardiac; SC, sheep cardiac. (*c*) Reactions of antiserum raised against β-tropomyosin of rabbit longissimus dorsi muscle with tropomyosins of skeletal muscle. PL, pig longissimus dorsi; SL, sheep longissimus dorsi; RCr, rabbit crurius; RS, rabbit soleus. (*d*) Reactions of antiserum raised against α-tropomyosin of rabbit longissimus dorsi muscle with tropomyosins of skeletal muscles of other species. PL, pig longissimus dorsi; CB, chicken breast; FL, mixed leg muscles of frog; SL, sheep longissimus dorsi; BL, cow longissimus dorsi. (*e*) Reactions of antiserum raised against unfractionated tropomyosin of rabbit longissimus dorsi muscle with tropomyosins from smooth muscle. RCr, rabbit crurius; PU, pig uterus; CG, chicken gizzard; RU, rabbit uterus; RP, rabbit psoas.

Table 2. *Ratios of amounts of protein and carboxymethylcysteine present in the α and β subunits of tropomyosins from different species and muscle types*

Tropomyosin carboxymethylated with iodo[2-^{14}C]acetate was subjected to electrophoresis (see the Methods section) on polyacrylamide gel in the presence of sodium dodecyl sulphate and Tris–borate buffer, pH 7.0. Protein ratios were determined by densitometric gel scanning assuming that both components bound equivalent amounts of dye. Numbers of determinations are given in parentheses. Ratio of carboxymethylcysteine contents is given by ratio of specific radioactivity of bands.

Muscle source				Ratio of α/β	
	Species	Muscle		Protein	Carboxymethylcysteine
Skeletal white	Rabbit*	Longissimus dorsi	(4)	3.83:1	1:2.06
	Rabbit	Psoas	(4)	3.53:1	1:2.07
	Sheep*	Longissimus dorsi	(2)	1.31:1	1:1.98
	Pig*	Longissimus dorsi	(2)	1.78:1	1:1.93
	Cow*	Longissimus dorsi	(2)	1.57:1	1:1.98
	Chicken	Breast	(4)	2.34:1	1:2.02
	Frog	Whole leg	(3)	2.23:1	1:1.93
Skeletal red	Rabbit	Soleus	(2)	1.09:1	1:2.10
	Rabbit	Crurius	(4)	1.07:1	1:2.08
Smooth muscle	Chicken	Gizzard	(4)	1.04:1	1:0.99

* Data from Cummins & Perry (1973).

electrophoresis compared with that obtained with unfractionated tropomyosin from skeletal muscle of the same species. On electrophoresis in sodium dodecyl sulphate in both the presence and the absence of urea, chicken gizzard tropomyosin migrated as two bands (Plate 1*b*). However, the faster of the two components of chicken gizzard tropomyosin migrated with a velocity very similar to that of the β component of chicken breast muscle. The components of gizzard tropomyosin were not strictly comparable with the α and β components of striated muscle tropomyosin, for analysis after fractionation both by electrophoresis and by chromatography demonstrated that both components contained equal amounts of cysteine. The faster and slower components on electrophoresis could, however, be separated by the procedures described for the fractionation of skeletal-muscle tropomyosin and were eluted at NaCl concentrations of 0.13 and 0.11M respectively, i.e. similar to those of the α and β components of chicken breast tropomyosin. Tropomyosins from rabbit uterus and pig uterus muscle both migrated on gel electrophoresis in sodium dodecyl sulphate as single bands with a velocity very similar to that of the faster component of chicken gizzard tropomyosin and of β-tropomyosin of striated muscle. No component corresponding in migration rate to the α component of striated-muscle tropomyosins was observed in any of the smooth-muscle tropomyosins studied.

Carboxymethylated chicken gizzard, rabbit and pig uterus tropomyosins all contained approx. 2mol of carboxymethylcysteine per 68000g of tropomyosin (Table 1). Non-identity with β-tropomyosin was indicated from amino acid analyses of the unfractionated smooth-muscle tropomyosins. The amounts of histidine, glutamic acid and valine were greater in these tropomyosins, whereas the amounts of lysine, isoleucine and tyrosine were lower than in striated-muscle tropomyosin (Table 3).

Immunochemical reactions of the tropomyosins

α- and β-tropomyosins of striated muscle. Guinea-pig antisera produced against unfractionated tropomyosin from longissimus dorsi muscle of the rabbit gave rise to two precipitin lines in agar gels when tested against the antigen (Plate 2*a*). During the early stages of immunization the antibody that was responsible for the precipitin line nearest the antigen well was often produced before the antibody responsible for the other precipitin line, but this was not always the case. Once the antisera gave a satisfactory reaction against both antigens, however, both bands appeared after a similar period of time in the agar diffusion tests. Guinea-pig antisera produced against purified α subunit of rabbit skeletal-muscle tropomyosin gave rise to one precipitin band when tested against either the α-tropomyosin used as antigen (Plate 2*b*) or unfractionated tropomyosin from rabbit skeletal muscle. This band corresponded to that nearest to the antiserum well in the reaction between unfractionated tropomyosin and the antiserum raised against it. The antiserum to rabbit α-tropomyosin did not react with the purified β-subunit of rabbit skeletal-muscle tropomyosin.

Antiserum directed against purified β subunit of tropomyosin from longissimus dorsi muscle of rabbit also gave rise to a single precipitin line when tested against the β-tropomyosin used as antigen (Plate 2*c*). When the antiserum to β-tropomyosin was tested

Table 3. *Amino acid composition of tropomyosins from different species and muscle types*

For experimental details see the text. The values are expressed as amino acid residues/68000g of protein.

Amino acid	Rabbit skeletal	Rabbit psoas	Rabbit crurius	Rabbit soleus	Frog skeletal	Sheep cardiac	Chicken cardiac	Chicken skeletal	Chicken gizzard	Rabbit uterus	Pig uterus
Cys	2.7	2.6	2.8	2.5	2.5	1.6	1.7	2.6	2.1	2.0	1.8
Asx	63.8	66.8	60.3	64.3	67.5	61.4	61.0	60.8	57.0	56.4	59.3
Thr	16.7	15.4	17.8	17.1	18.4	17.3	19.3	18.3	17.5	17.0	16.3
Ser	28.0	28.0	28.2	29.4	25.8	26.5	25.4	26.3	28.1	25.3	26.4
Glx	150	153	153	156	157	151	171	169	182	178	177
Pro	0.5	0	0.1	0	0	0.3	0	0	0	0.4	1.0
Gly	8.9	12.4	7.3	9.0	11.3	6.5	10.3	10.1	9.9	11.3	10.8
Ala	76.4	76.7	75.3	73.8	75.6	77.8	74.1	75.8	73.4	71.4	73.1
Val	20.2	21.1	18.4	19.4	22.8	15.9	20.2	20.2	26.3	27.4	28.3
Met	11.7	13.8	13.8	12.2	15.0	16.5	13.9	12.8	15.0	13.9	12.2
Ile	23.9	20.7	21.3	21.6	18.6	20.4	22.4	20.3	17.7	17.1	15.8
Leu	68.0	66.5	67.2	67.0	61.0	68.9	60.8	62.1	64.8	63.4	61.6
Tyr	10.7	10.4	10.1	9.9	10.6	11.4	9.1	10.4	6.3	7.9	5.4
Phe	2.9	3.6	2.8	2.7	4.8	1.9	3.4	3.1	2.4	3.2	3.4
His	4.4	5.1	4.2	4.8	6.1	4.1	4.2	4.6	5.5	6.8	7.0
Lys	78.5	73.5	82.1	76.6	74.9	81.6	70.7	74.0	60.9	61.3	67.4
Arg	30.7	29.5	31.4	31.0	26.4	31.4	30.3	26.7	30.0	33.8	31.6
No. of preparations ...	8	3	2	2	2	2	1	3	3	1	2

against unfractionated tropomyosin from rabbit skeletal muscle the same single precipitin band was obtained. This line was continuous with the outer of the two observed with antisera to unfractionated tropomyosin. No reaction was observed when the β-tropomyosin antiserum was tested against purified α-tropomyosin from skeletal muscle or rabbit cardiac tropomyosin.

When guinea-pig antiserum against unfractionated tropomyosin was tested against the unfractionated tropomyosins from the skeletal muscles of other vertebrate species one precipitin band was obtained in all cases (Plate 2*d*). This band corresponded to that obtained in the reaction of α-tropomyosin from rabbit skeletal muscle with its corresponding antiserum. An identical picture was observed when the unfractionated skeletal-muscle tropomyosins from different vertebrate species were tested against the antiserum to rabbit skeletal α-tropomyosin. No reaction at all was observed, however, when antiserum to rabbit skeletal β-tropomyosin was tested against the unfractionated tropomyosins from skeletal muscles of other species (Plate 2*c*).

On testing the reaction of the anti-(α-tropomyosin) serum against rabbit cardiac tropomyosin one precipitin band, continuous with the inner band corresponding to α-tropomyosin, was obtained (Plate 2*b*). This was confirmed by the reaction of rabbit cardiac tropomyosin against the antiserum to unfractionated rabbit skeletal tropomyosin (Plate 2*a*). It was concluded that the subunit of rabbit cardiac tropomyosin was immunochemically indistinguishable from the α subunit of skeletal-muscle tropomyosin.

Sheep cardiac and chicken cardiac tropomyosins also gave a single identical precipitin band when tested against antisera to both unfractionated tropomyosin and α-tropomyosin from rabbit skeletal muscle (Plates 2*a* and 2*b*). No reaction was observed between these cardiac tropomyosins and the rabbit skeletal β-tropomyosin antisera.

When unfractionated tropomyosins isolated from the psoas, crurius and soleus muscles of the rabbit were tested against the antiserum to unfractionated tropomyosin from longissimus dorsi muscle of the rabbit, two continuous precipitin lines, identical with those obtained with α- and β-tropomyosins, were observed in each case (Plate 2*e*). The tropomyosins isolated from these three different rabbit muscles gave a single precipitin line corresponding to α- or β-tropomyosin when tested against antisera specific for each of the two types of tropomyosin subunit (Plate 2*c*).

Smooth-muscle tropomyosins. In contrast with the results obtained with striated muscle, the tropomyosins isolated from chicken gizzard or uterus muscles of the pig and rabbit failed to give any reaction when tested against guinea-pig antisera to unfractionated, α- or β-tropomyosins from rabbit skeletal muscle (Plates 2*a* and 2*e*). No reaction was observed between tropomyosin isolated from the adductor muscle of the mollusc *Pecten maximus* and antisera to the unfractionated tropomyosin of rabbit skeletal muscle.

Discussion

In all the vertebrate striated muscles investigated, two types of tropomyosin subunit were present. These are similar to the α and β forms of rabbit skeletal muscle as judged by their relative electrophoretic mobilities and cysteine contents. Apart from their cysteine contents only minor differences could be observed in the amino acid analyses of unfractionated tropomyosins from skeletal muscles of different species when they were compared with the composition of α- and β-tropomyosins from rabbit skeletal muscle. Although they were not analysed separately this suggests that the amino acid composition of the α and β subunits of tropomyosin from sheep, pig and cow skeletal muscles are probably very similar to their rabbit counterparts. For the α-tropomyosin subunit the structure must be very similar if not identical in different species, for the antibody to α-tropomyosin of rabbit skeletal muscle gave a reaction of identity with the α-tropomyosins from the five other species studied. Thus it may be concluded that the antibody obtained to α-tropomyosin probably corresponds to the genus- and organ-non-specific component in the antibody preparation of Hirabayashi & Hayashi (1970), at least insofar as striated muscle is concerned. In contrast the antibody to β-tropomyosin, which was specific for the species studied, but which reacted with all the types of skeletal muscle from the rabbit that were tested, probably corresponds to the genus- and organ-specific antibody reported by Hirabayashi & Hayashi (1970).

It is noteworthy that the α- and β-tropomyosins, which are apparently so similar in amino acid composition and both of which are largely in the α-helical form (see Sodek *et al.*, 1972), should be so different antigenically. This property would suggest that the antigenic site is restricted to a very limited part of the molecule. It also follows that it may be possible to identify the antigenic sites when the sequence data for the two forms of tropomyosin become available.

In striated skeletal muscles of the rabbit there appears to be a good correlation between the ratio of the amounts of the α and β subunits present and the speed of contraction, the ratio ranging from 3.8:1 in longissimus dorsi to 1:1 in the soleus and crurius. Some correlation was also apparent between species in that the tropomyosin from longissimus dorsi muscles of larger animals, the muscles of which are usually slower and contain more red fibres (Close, 1972), also contained more β component.

The position with cardiac muscle, however, is anomalous in that, whereas myosin from this tissue is more like that isolated from slow skeletal muscle, cardiac muscle contains no β-tropomyosin, but only the α form, a high content of which is present in fast skeletal muscle. Although the final decision as to whether α-tropomyosin and cardiac tropomyosin from a given species are identical will depend on the determination of amino acid sequences of the two proteins, there is strong presumptive evidence that they are, from amino acid analysis, cysteine content and immunochemical properties.

The investigation of the tropomyosins of smooth muscle has reached a less definitive stage, but our findings confirm those of other workers (Cohen & Longley, 1966; Carsten, 1968; Woods, 1969; M. E. Hilburn, P. Cummins & S. V. Perry, unpublished work), who have reported differences between tropomyosin from striated and smooth muscle. It can be concluded that in the mammalian and avian species smooth muscle tropomyosin is clearly different from the α and β forms found in striated muscle. Whether the smooth-muscle tropomyosin corresponds to one of the two minor bands seen after isoelectric focusing of tropomyosin from skeletal muscle cannot be determined at this stage. Nevertheless it can be concluded that the production of tropomyosin by muscle cells is under the control of at least three structural genes in the mammal and four in the chicken.

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