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Identification and Quantification of Actin Isoforms in Vertebrate Cells and Tissues

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The cytoskeletal protein actin exists in vertebrates as six different isoforms, which are difficult to identify conclusively because of a high degree (> 90%) of overall sequence homology. We have used IEF immunoblotting in combination with a panel of isoform-specific and -selective antibodies to analyze the actin isoform composition of nine tissues from adult rat. In three nonmuscle tissues (lung, spleen, and testis), we detected a previously unreported isoform that we identified as smooth muscle α. The IEF immunoblot technique was also used to quantify the proportions of the isoforms expressed in these nine rat tissues.

Key words: isoactins, isoelectric focusing, quantitative immunoblots

Actin is a highly conserved cytoskeletal protein that exists in vertebrate cells in at least six different isoforms, each encoded by a separate gene [1]. The isoactins are expressed in a tightly regulated, tissue-specific pattern, which has generated much interest in actin as a model for the study of multigene families. Changes in actin isoform expression have been correlated with transformation [2–6], differentiation [7–9], and acquisition of drug resistance [10]. As with other proteins that exist as multiple isoforms produced by multigene families, such a collagen and tubulin, it is important to determine the member of the gene family expressed as well as the level of this expression in each cell or tissue. However, one problem encountered with studies of actin isoform expression is that, while the existence of six isoforms has been demonstrated [11], individual isoforms are not easy to identify conclusively (eg, the six isoforms can be resolved into only three bands on isoelectric focusing gels). Many researchers have made use of cDNA probes and the Northern blot technique, measuring the ratios of isoform-specific mRNAs as a means of detecting actin isoforms and quantifying their levels of expression [12–15]. Unfortunately, the information obtained in this way is limited, since it is not possible to infer a relationship between the amount of detectable message and the amount of accumulated protein because of differing translational efficiencies of the mRNAs or differing half-lives of the proteins.

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Many investigations in which actin isoform synthesis or accumulation was monitored during development and differentiation [9,16,17], transformation [2–6], or among various cellular compartments [18] have made use of two-dimensional electrophoresis or isoelectric focusing. One limitation associated with these studies is the poor resolution of individual actin isoforms on two-dimensional or isoelectric focusing (IEF) gels. In at least one study, a minor isoform (< 10% of the total) was obscured by the more predominant species [19,20]. In addition, the absolute identification of an isoform based on its isoelectric point is not feasible, since two species focus at the position of the γ isoform (nonmuscle and smooth muscle γ) and three focus at the position of the α isoform (striated, cardiac, and smooth muscle α). This ambiguity has prohibited the identification of novel isoforms [4,6,21].

A considerable contribution to the identification and quantification of accumulated isoform levels has been made by Vandekerckhove and coworkers [22–27]. These researchers have made extensive use of a method that involves digestion of the actins and "fingerprinting" of the resultant peptides. Since the N-terminal peptide of each isoform is highly acidic and is unique in mobility, an unambiguous identification and quantification of actin isoforms present in a crude cell lysate can be made in this way. However, this method has not been widely adopted, possibly because of problems with differential lability of the actins, potential artifacts of derivatization, or a general reluctance to attempt the time-consuming chemistry. Also, the sensitivity of this method is variable. Under optimal conditions of high radioactive incorporation and low background, species that comprise as little as 1% of the total actin can be detected, although this sensitivity is reduced to > 5% under less favorable conditions.

Because of the limitations associated with the existing techniques for quantifying the isoactins, we previously developed a procedure for Western blotting and immunostaining of actin isoforms resolved by IEF [28]. In the present study, we have used a panel of isoform-specific and -selective antibodies, in combination with the IEF blot technique, to determine the actin isoform composition of nine rat tissues. We also demonstrate the use of this method to identify unknown isoforms and to quantify the isoform ratios in crude extracts.

MATERIALS AND METHODS

Preparation of Cell and Tissue Extracts

Organs were removed from freshly killed, adult Sprague-Dawley rats (two males and two females), freed of residual connective tissue and blood vessels, and placed in cold phosphate-buffered saline (PBS) containing the following as protease inhibitors: 0.2 mM phenylmethysulfonyl fluoride, 0.1 mM benzamidine-HCl, 10 μg/ml aprotinin, 1 μg/ml o-phenanthroline, 1 μg/ml pepstatin, and 1 mM EDTA (all from Sigma Chemicals, St. Louis, MO). The tissues were minced with a scalpel, homogenized in a ground-glass homogenizer containing 2–5 ml (per whole organ) of the above buffer, and centrifuged at 3,000g for 10 min at 0°C. The protein concentration of each supernatant was determined by the method of Lowry et al [29]. Aliquots of extract were stored at −80°C until use. Extracts of rabbit aorta and chicken gizzard were prepared as described, except that the gizzards were obtained from Vons Grocery Store (Los Angeles, CA).

HeLa cells, strain S3, were grown in suspension as previously described [30]. Approximately 10^7 cells were centrifuged at 1,200 rpm for 5 min, washed twice in

PBS, and resuspended in IEF sample dilution buffer (9.5% urea, 5% β-mercaptoethanol, and 1.6% Triton X-100; a ratio of 150 μl of buffer per 10 μl cell pellet). DNase purification of actin from extracts of HeLa or from tissues was performed as described by Witt et al [3].

Assay of Actin Isoforms

IEF electrophoresis and blotting were described previously [28]. For the quantification of actin ratios, unstained IEF gels were blotted and all actin isoforms were immunostained with the C4 antibody (see below). Parallel gels from the same gel run were protein-stained (0.4% Coomassie Brilliant Blue G-250 [Sigma Chemicals] in 5% perchloric acid). The immunostained blots and the protein-stained gels were scanned on a Bio Rad Model 620 Video Scanning Densitometer and integrated on a Bio Rad Model 3329A Reporting Integrator (Bio Rad, Richmond, CA). Data presented are the average values obtained from at least three IEF immunoblot analyses of each of two preparations of each type of extract. To identify unequivocally the immunostained actin isoforms, blots that had been immunostained with an isoform-specific antibody were photographed, then marked at the position of the immunoreactive band or bands, and restained with the C4 general actin antibody. In this way, the isoelectric position of the original stained band(s) could be determined by comparison with the other actin species present in the extract.

Actin Antibodies

For immunostaining of Western blots, the C4 monoclonal actin antibody, which is reactive with all isoforms (we will call this the "C4 general actin antibody"; it was the generous gift of J.L. Lessard, Children's Hospital Research Foundation, Cincinnati, OH, and has been characterized previously [31]), was used at a 1:2,000 dilution of ascites fluid. The B4 monoclonal antibody, which is selective for muscle isoforms [31], was used at a 1:500 dilution of ascites fluid, and the γ peptide antibody, which is reactive with the N-terminal sequence of nonmuscle γ actin [20], was used at a 1:40,000 dilution of ammonium sulfate-purified antibody. The Sα peptide antibody, which is specifically reactive with sarcomeric α actins [32], was affinity-purified on a cyanogen bromide-Sepharose 4B column (Pharmacia, Piscataway, NJ); muscle actin (1 mg/ml of resin) was used as the ligand. Antibodies were eluted with 5 M $MgCl_2$, dialyzed against PBS, and used at a concentration of 0.67 μg/ml.

Preparation of β Peptide Antibody

A synthetic peptide whose sequence corresponded to the 14 residues at the amino terminus of nonmuscle β actin was used to prepare a peptide antibody as previously described for the nonmuscle γ peptide [20]. To remove from the antiserum antibodies reactive with both β and γ actins, an IgG fraction of β peptide antibody [33] was preabsorbed with 10^{-4}M γ peptide for 1 hr at 37°C, and the preabsorbed IgG was affinity-purified on a Sepharose-4B peptide column containing as its ligand a heptapeptide linker terminating in a tyrosine residue and covalently attached to the β peptide by reaction with bis-diazobenzidine [34]. β-Specific antibody was eluted with 5 M $MgCl_2$, dialyzed against PBS, and used at a concentration of 3.4 μg/ml.

RESULTS

We have prepared a peptide antibody reactive with nonmuscle β actin by following the scheme illustrated in Figure 1. Rabbits immunized with a synthetic

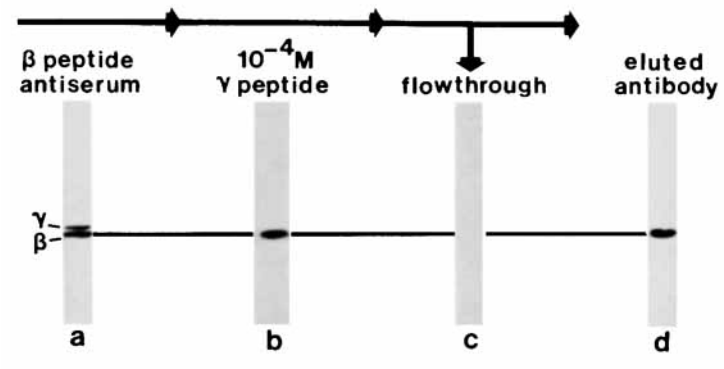

Fig. 1. Affinity purification of β peptide antiserum. Rat brain extract (50 μg) was electroblotted on quadruplicate blots and immunostained with **a**) untreated β peptide antiserum diluted 1:1,000; **b**) β peptide antiserum diluted 1:1,000, preincubated with 10^{-4} M γ peptide; **c**) flowthrough from Sepharose-β peptide affinity column (the sample was concentrated to the original volume and diluted 1:1,000); and **d**) β-specific antibodies eluted from the Sepharose-β peptide column at a concentration of 3.4 μg/ml. β and γ indicate the IEF positions of β and γ actins, respectively.

peptide corresponding to the N-terminal 14 residues of β actin produced an antibody that reacted with both the nonmuscle β and γ isoforms of actin. To remove antibodies cross-reactive with γ actin, the antiserum was preincubated with a γ actin N-terminal peptide (γ peptide) and then affinity-purified on a Sepharose-β-peptide column in the presence of excess γ peptide. The cross-reactive species, still bound to the γ peptide, flowed through the column, and β-specific antibodies were eluted from the column.

In addition to the β-specific actin antibody, we used other actin antibodies with known isoform specificities in our analysis of the isoform composition of rat tissues: C4, a mouse monoclonal reactive with all six vertebrate actins [31]; B4, a mouse monoclonal that reacts selectively with muscle actins [31]; γ peptide antibody, a rabbit polyclonal reactive with N-terminal sequence of nonmuscle γ actin [20]; and Sα peptide antibody, a rabbit polyclonal specific for the cardiac α and skeletal α isoforms [32]. The isoform composition of nine tissues obtained from adult rats was determined by assaying immunoblots of IEF gels with the C4 general actin antibody and the β and γ peptide antibodies. Figure 2 demonstrates that in each tissue we examined, at least two isoforms, with electrophoretic positions corresponding to the β and γ isoforms, were present. Additionally, the β peptide antibody demonstrated slight reactivity with a few other protein bands in some types of extracts; these other bands may represent reactivity of the β peptide antibody with either unacetylated actins or with nonactin proteins containing sequences similar to that recognized by the β peptide antibody. Alternatively, these other bands may be attributable to nonspecific binding of the antibody at the low dilution of affinity-purified antibody that we used.

Four of the tissues (brain, kidney, liver, and thymus) contained the β and γ isoforms as their only actins; these could be identified as authentic β and γ actins by their immunological cross-reactivity. The other five tissues contained an additional, more acidic, band at an IEF position corresponding to that of an α isoform. To determine which of the three α isoforms might be expressed in these tissues, we examined reactivity of extract proteins with several antibodies of known isoform specificity. In three cases (lung, spleen, and testis), this more acidic actin was immunostained with the γ peptide antibody. We wanted to know if this band repre-

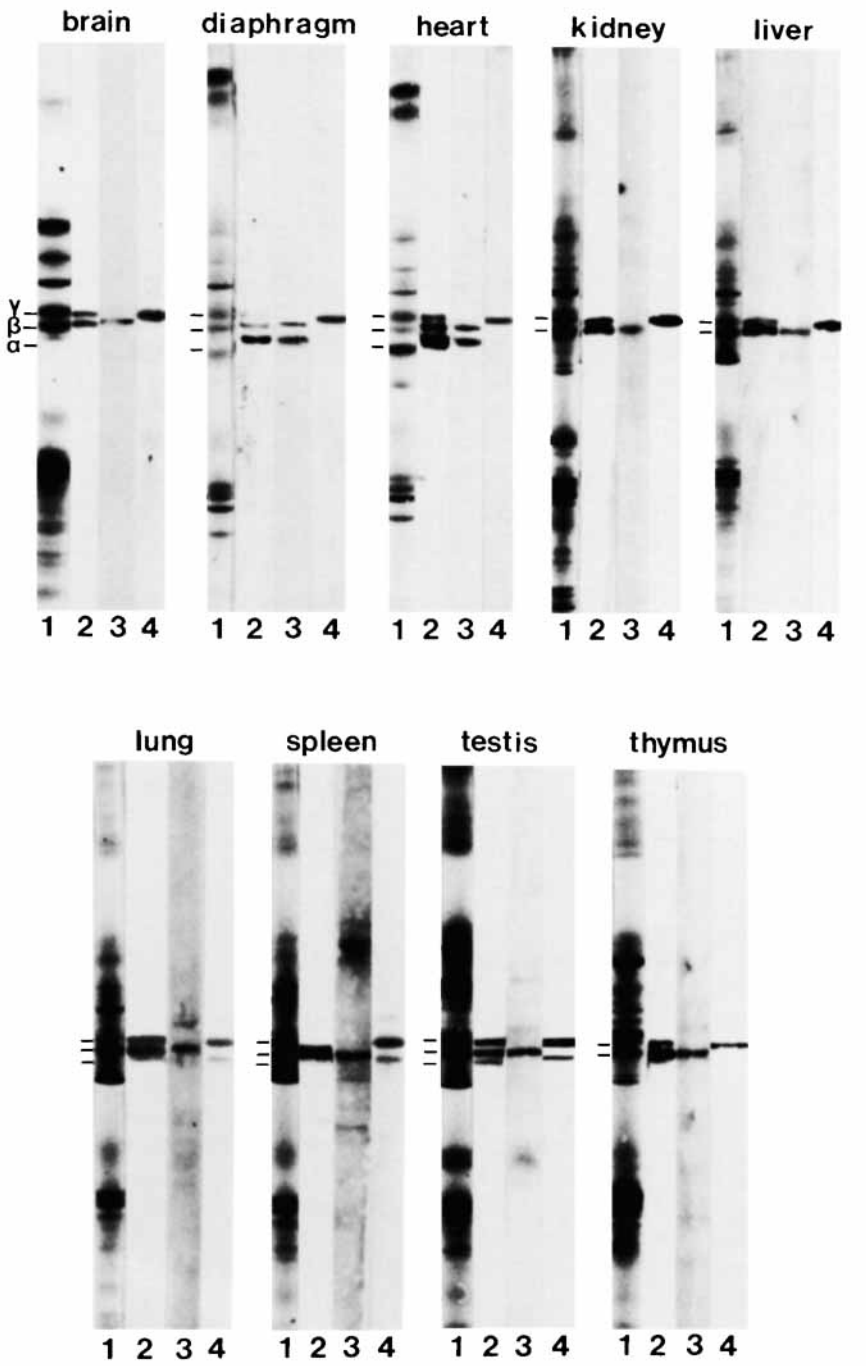

Fig. 2. Actin isoform composition of rat tissues. IEF Western blotting was performed with extracts from the following nine rat tissues: brain, diaphragm, heart, kidney, liver, lung, spleen, testis, and thymus. In each set lane 1 shows an IEF gel stained with Coomassie Brilliant Blue, lane 2 shows an IEF blot stained with C4 general actin monoclonal antibody, lane 3 shows an IEF blot stained with γ peptide antibody. Extract protein (100 μg) was electrofocused for each tissue except diaphragm, in which 200 μg was focused. α, β, and γ indicate the IEF positions of the α, β, and γ actin isoforms.

sented an actin degradation product, an unrelated protein with a γ-like amino acid sequence, or another actin isoform with a γ-like N-terminus. In Western blots of SDS gels the material immunoreactive with either the C4 or γ peptide antibodies was identical in gel mobility to bona fide actin; this rendered the possiblity of an acidic degradation product of the γ actin unlikely. In addition, the possibility that it was a nonactin protein was discounted because the band was reactive with the C4 general actin antibody, which reacts with all known actin types and no nonactin proteins [31]. We reasoned that this more acidic actin band might be smooth muscle α actin, since this isoform has an N-terminal sequence (Ac-Glu-Glu-Glu-Asp—) that closely resembles that of the nonmuscle γ peptide (Ac-Glu-Glu-Glu-Ile—) against which the γ peptide antibody was elicited. It would be equally logical to expect that the γ peptide antibody would react with the smooth muscle γ isoform (Ac-Glu-Glu-Glu-Thr—). Similarly, the more acidic actin band that was detected with the β peptide antibody in diaphragm and heart probably indicates cross-reactivity of this antibody with one or both of the sarcomeric α actins because of a similarity between the N-terminal sequences of these and the nonmuscle β isoforms (cardiac α = Ac-Asp-Asp-Glu-Glu—; striated α = Ac-Asp-Glu-Asp-Glu—; nonmuscle β isoform = Ac-Asp-Asp-Asp-Ile—).

To test these predicted cross-reactivities, we first analyzed two tissues known to contain smooth muscle actins [24,25]. Extracts of rabbit aorta and chicken gizzard were resolved on IEF gels, blotted, and immunostained with all five of the actin antibodies: the C4 general actin monoclonal, the Sα, β, and γ peptide antibodies, and the B4 monoclonal, as shown in Figure 3A. No immunostaining with the Sα peptide antibody was observed in either the aorta or gizzard smooth muscle extract, indicating that neither of the sarcomeric α isoforms was present in these smooth muscle tissues. Two bands, with α-like and γ-like IEF positions, were stained with the B4 muscle-selective actin antibody in the aorta extact, indicating that both smooth muscle α and smooth muscle γ isoforms are expressed in this tissue. As predicted, the γ peptide antibody reacted with both of these species. Similarly, the B4 antibody reacted with a smooth muscle γ band in chicken gizzard extract, as did the γ peptide antibody.

We next determined the extent of cross-reactivity of the γ peptide antibody with the smooth muscle isoforms: if the antibody were completely cross-reactive it would provide a sensitive means of detecting these isoforms, while if it showed selective reactivity, the γ peptide antibody might prove useful in specific detection or immunostaining of nonmuscle γ actin in tissues containing the smooth muscle α or γ forms. We tested the cross-reactivity of the γ peptide antibody by determining how sensitively the antibody could detect each isoform. IEF immunoblots of nonmuscle actin purified from rat brain extract by DNase affinity chromatography demonstrated that the γ peptide antibody gave detectable immunoreactivity with as little as 15 ng of nonmuscle γ actin (data not shown). Greater amounts (82 and 60 ng, respectively) of smooth muscle α and γ actins purified from extracts of aorta and gizzard were required for immunodetection by γ peptide antibody under the same conditions. The degree of cross-reactivity of the γ peptide antibody with the smooth muscle α and γ actin isoforms is consistent with the degree of homology between the N-terminal amino acid sequences of these three proteins and the peptide used to produce the antibody. Although from the sensitivity of detection of γ actin in gizzard extract we can be certain that the γ peptide antibody reacts significantly with smooth muscle γ actin, we cannot determine from this type of assay whether a small proportion of the

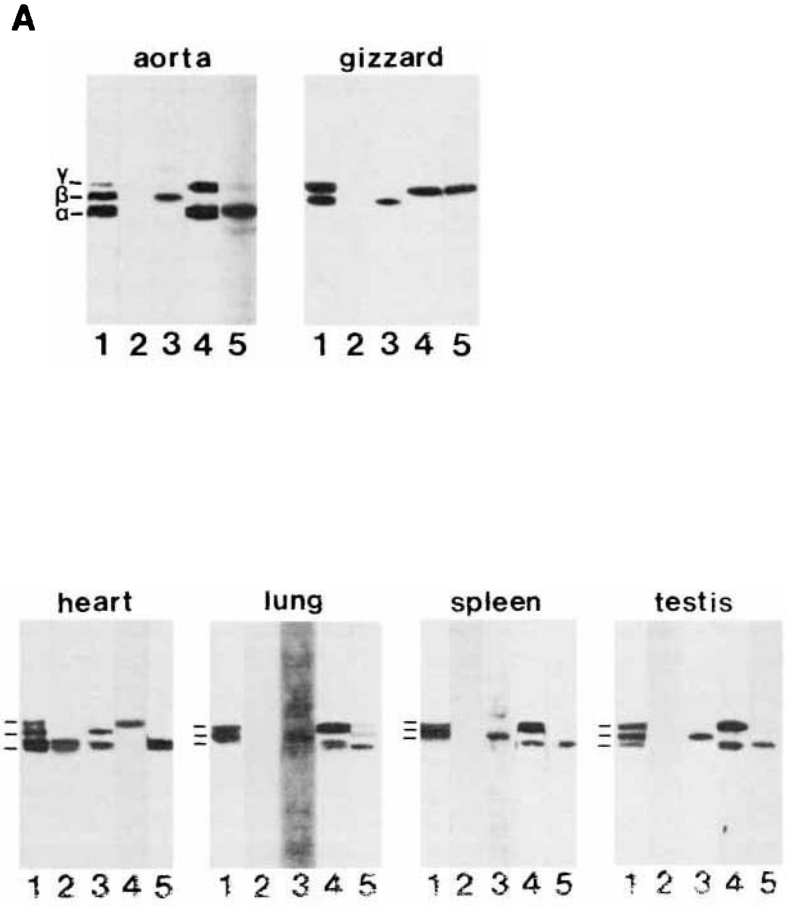

Fig. 3. Characterization of actin isoforms that displayed an α-like IEF position. Five identical samples of the following tissue extracts were electrofocused and transferred to nitrocellulose: **A**) 30 μg rabbit aorta and 30 μg chicken gizzard. **B**) 200 μg rat diaphragm and 100 μg each of rat heart; rat lung, rat spleen, and rat testis. Lane 1 of each set in A and B shows an IEF blot stained with C4 general actin monoclonal antibody; lane 2, IEF blot stained with Sα peptide antibody; lane 3, IEF blot stained with affinity-purified β peptide antibody; lane 4, IEF blot stained with γ peptide antibody; and lane 5, IEF blot stained with B4 muscle-selective monoclonal antibody. α, β, and γ represent the IEF positions of α, β, and γ actin isoforms.

γ actin present in gizzard tissue is nonmuscle γ. However only the smooth muscle γ and nonmuscle α actin forms were detected previously [23].

The antibody reactivities of each of the actin isoforms are summarized in Table I. The profile of antibody reactivity coupled with the IEF position is unique for each of the six isoforms except the sarcomeric α actins, thus allowing the identification of isoforms on IEF Western blots. The smooth muscle α isoform, for example, is the only isoform with an α electrophoretic position that reacts with the B4 and the γ peptide antibodies, but not with the Sα peptide antibody. We have made use of this information to probe further the identity of the α-like bands in each of the rat tissues. As shown in Figure 3B, the α band, which stained with the γ peptide antibody in extracts of lung, spleen, and testis, did not react with the Sα peptide antibody, but did react with the B4 antibody. Thus, these α bands, previously not detected in these

TABLE I. Reactivity of a Panel of N-Terminal Peptide and Monoclonal Actin Antibodies With the Actin Isoforms of Warm-Blooded Vertebrates

	Peptide antibodies			Monoclonal antibodies	
	Sα	β	γ	B4	C4
Skeletal α	+	+		+	+
Cardiac α	+	+		+	+
Smooth muscle α			+	+	+
Smooth muscle γ			+	+	+
Nonmuscle β		+			+
Nonmuscle γ			+		+

+ indicates detectable reactivity in IEF immunoblot assays of either purified actins or tissue extracts. Assays were performed as described in Materials and Methods.

tissues, can be unequivocally identified as smooth muscle α actin. The α species that stained with the β peptide antibody in extracts of diphragm and heart tissues were also reactive with the α peptide antibody but were not stained with the γ peptide antibody. This confirms our supposition and the previous results of others [23] that the sarcomeric α actins are the only α isoforms expressed in these tissues. In three tissues (lung, spleen, and testis), the B4 muscle-selective actin antibody also reacted faintly with a β and a γ band. Although this is probably the result of cross-reactivity of the muscle-selective antibody in the presence of an excess of the nonmuscle actins, the possibility that these three tissues contain small amounts of smooth muscle γ actin cannot be ruled out.

We also wished to determine if we could quantify actin isoform composition using antibody reactivity. To determine whether a densitometric scan of a Western blot stained with the C4 general actin antibody could be used to determine the ratio of actin isoforms present in complex mixtures of proteins, we tested extracts of HeLa cells, as we wished to compare our results with those that had previously been published for this cell line by Vandekerckhove and Weber [26]. HeLa cell extracts and DNase-purified HeLa actin were resolved on IEF gels, and the gels were either protein stained or blotted onto nitrocellulose and immunostained with the C4 antibody. As shown in Figure 4, the electropherogram of HeLa extract contained two bands at the same electrophoretic positon at which β and γ actins focused. Two bands were also immunostained with the C4 antibody in both the HeLa extract (Fig. 4b) and the purified HeLa actin (Fig. 4d). Densitometric scanning of the protein-stained gels and the immunostained blots was performed, the actin peaks were integrated and the β:γ ratio was calculated. As shown in Table II, the β:γ ratio calculated from the protein-stained gel of purified actin was identical to the ratio obtained by immunostaining blots with the C4 antibody. The result obtained from the immunostained HeLa extract blot was also similar (it differed by $< 5\%$) to the value obtained for the electropherogram of the purified actins, and an identical ratio was obtained over a wide range (100-fold) of protein amounts electrophoresed (data not shown). All three results were in good agreement with the values of 65:35 previously reported by Vandekerckhove and Weber [26]. The β:γ ratio obtained from protein-stained electropherograms of HeLa extracts ($45:55 \pm 0.7$), however, was markedly different from the value obtained in the other three determinations (Table II). This indicated that a protein other than actin focused at the same position as γ actin, and this result was confirmed by electrophoresis in the second (SDS) dimension. Thus, although densitometry of protein-stained gels of extracts gave an incorrect determination of isoform composi-

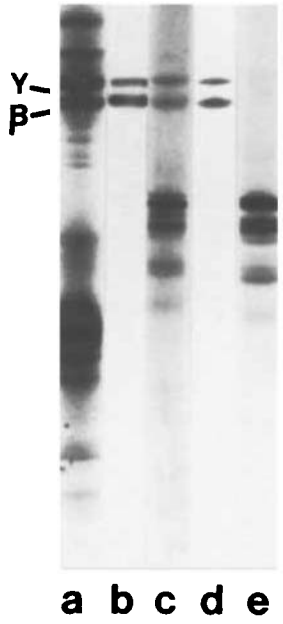

Fig. 4. Quantification of β and γ isoforms in cell extracts. HeLa cell extract (100 μg) was electrophoresed on IEF gels and **a**) stained with Coomassie Brilliant Blue; **b**) transferred to nitrocellulose and stained with C4 general actin monoclonal antibody. DNase-purified actin (10 μg) from HeLa extract was electrophoresed on IEF gels and **c**) stained with Coomassie Brilliant Blue or **d**) transferred to nitrocellulose and stained with C4 antibody. **e**) 12 μg DNase I was focused and stained with Coomassie Brilliant Blue. β and γ denote isoelectric positions of the β and γ actin isoforms.

TABLE II. Quantitative Analysis of the Actin Isoforms of HeLa Extracts and DNase-Purified HeLa Actins

	β:γ	
	Protein stained	Immunostained
Extract	45:55 ± 0.7[a]	62:38 ± 2.6
Purified actin	59:41 ± 2.0	59:41 ± 3.3

Ratio of β:γ actins were obtained from densitometry of stained IEF gels and immunoblots.
[a] ± Standard deviation.

tion, an analysis of an IEF Western blot provided a simple and accurate measure of the isoform composition of the extract.

Quantitative information of the isoform composition of the nine rat tissues was obtained from densitometric scans of immunoblots. These data were also used to calculate the β:γ ratio. As shown in Table III, the composition of β and γ isoforms ranged from 1:1 in testis to 6.1:1 in heart. For two representative tissue extracts (lung and spleen), these ratios were determined for a range of protein loads (25–2,000 μg per IEF gel); within this range, the actin isoform composition was independent of the amount of protein electrophoresed.

TABLE III. Quantitative Analysis of the Actin Isoform Composition of Nine Rat Tissues

	α	β	γ	β:γ
Brain	ND	65.8 ± 1.8[a]	34.2 ± 1.8	1.9
Diaphragm	70.6 ± 0.3	22.8 ± 1.1	6.6 ± 1.1	3.4
Heart	81.1 ± 2.0	17.4 ± 2.6	2.7 ± 0.5	6.1
Kidney	ND	69.1 ± 5.9	30.9 ± 5.9	2.2
Liver	ND	77.5 ± 2.7	22.5 ± 2.7	3.4
Lung	4.4 ± 1.7	67.1 ± 1.7	29.9 ± 3.3	1.8
Spleen	3.8 ± 1.9	61.8 ± 1.5	34.3 ± 2.1	1.8
Testis	27.6 ± 3.4	35.3 ± 2.2	37.0 ± 1.7	1.0
Thymus	ND	76.3 ± 1.8	23.7 ± 1.8	3.2

[a]Values listed, in arbitrary units ± SD, were obtained from densitometry of immunoblots as described in Materials and Methods.
ND, not detected.

DISCUSSION

We have used a panel of five actin antibodies of known isoform specificity to identify and quantify the actin isoforms present in nine tissues from adult rats. By comparing the immunoreactivities of one isoform-selective monoclonal and three site-directed polyclonal antibodies, we detected a previously unreported third actin species in three nonmuscle tissues (lung, spleen, and testis), and we have identified this species as the smooth muscle α isoform of actin. Although smooth muscle α actin has been detected in cultured cells of fibroblastic origin [3,6,14], its presence in nonmuscle tissues has not been previously described. The expression of smooth muscle α actin in rat testis is of particular interest, since it may be the product of the unidentified actin mRNA that has previously been detected in rodent testis [14] (H. Erba and P. Gunning, personal communication).

The immunoassay technique we used is well-suited to the identification of actin isoforms, since it is extremely sensitive and, because the immunological reactivities of the antibodies we used are known, it results in unambiguous identification of isoforms in most cases. The peptide antibodies used in this study have previously been used to detect a single actin isoform in the presence of an excess of the other isoforms. The Sα peptide antibody could detect α actin in mouse myogenic cultures that contained a 50–100-fold excess of the β and γ isoforms [32], and the γ peptide antibody permitted the identification of the nonmuscle γ isoform in mouse L cells, which contain a 10–20-fold excess of the β isoform [20]. This isoform had been undetectable by conventional two-dimensional electrophoresis [19]. We have verified that the immunoassay technique is quantitative, and we find that our values for isoform composition are comparable, within the range of experimental error, to values reported by Vandekerckhove and Weber [26]. The immunoassay we used was advantageous because it required neither fractionation of the tissue or cell extracts nor purification of the actins; in addition, our protocol is considerably simpler than the "fingerprinting" technique that Vanderkerckhove and coworkers employ. However, we are currently unable to distinguish between the cardiac α and skeletal α isoforms with our method, and we cannot detect minor amounts of either of the γ isoforms (nonmuscle or smooth muscle) when the other γ isoform is present in excess. Although recent modifications of the technique of Vanderkerckhove and

coworkers allow for the sensitive measurement of the two sarcomeric α actins [35], these improvements have not yet been applied to the measurement of the γ isoforms.

We were interested in determining whether the novel chicken actin isoform, named Type 5 nonmuscle actin [15,27], would be detected in any of the nine rat tissues we examined. This isoform has an N-terminal sequence (Ac-Asp-Glu-Glu-Ile—) that is similar to that of striated and cardiac α actins (Ac-Asp-Glu-Asp-Glu- and Ac-Asp-Asp-Glu-Glu-, respectively) and therefore might be expected to react with the α peptide antibody, which reacts with both of these. From examining the N-terminal sequence of the Type 5 isoform, we would predict that the IEF position of Type 5 would be in between the positions of β and γ. Although Northern blot analysis has shown that the Type 5 message is expressed at a high level in chicken brain and gizzard and to a lesser extent in heart, we have not detected a band immunoreactive with Sα peptide antibody at the position predicted for Type 5 actin in any of the nine rat tissues or in chicken gizzard. Our results suggest that either the level of Type 5 message is not paralleled by the protein level in these tissues or that the Type 5 isoform does not display detectable immunoreactivity with the predicted reagent. Our results are in agreement with those of Vandekerckhove (personal communication), who with the peptide fingerprinting assay has found no evidence for accumulation of Type 5 actin in extracts of a variety of chicken tissues.

Identification of accumulated actin isoforms by immunoassay provides a useful correlate to data obtained from Northern blots probed for actin mRNAs. Such a comparison is important to determine whether each actin message is translated and whether actin isoforms are accumulated in the ratios predicted from mRNA levels. Apparently, this is not always the case, as indicated by the results of Vandekerckhove et al [35], Caravatti et al [36], and in our study. We have found that the isoform composition of some tissues differs dramatically from the mRNA ratios. In liver, for example, the β mRNA:γ mRNA ratio is 80:1 (H. Erba and P. Gunning, personal communication), but the β:γ ratio measured at the level of accumulated protein is only 3:1. This result implies that translation of the actin messages may be regulated in an isoform-selective manner. Complete information concerning both the level of each actin isoform and the level of its message in a particular tissue or cell type will give a composite picture of the expression of the actin multigene family.

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