Actin from pig and rat uterus

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Smooth-muscle actin was isolated from pig uterus and from pregnant-rat uterus. Methods involving acetone-dried powders were unsuccessful, and a column-chromatographic procedure was developed, with proteinase inhibitors and avoiding polymerization as a purification step. The yield of pure actin was 0.8–1.5 mg/g wet wt. of uterus, which should be compared with an expected yield of actin from skeletal muscle of 2–4 mg/g wet wt. The actin was pure as judged by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, and exhibited α -, β -, and γ -forms on isoelectric focusing. It possessed a blocked *N*-terminal amino acid residue, and its amino acid analysis conformed to those of other actins. The rat uterine actin was available only in small amounts (5–10 mg) and did not polymerize. The pig uterine actin could be obtained in amounts up to 30 mg, polymerized reversibly, and activated a skeletal myosin Mg^{2+} -dependent ATPase.

It has become clear that at least six different genes for actin may operate in one mammalian organism (Vandekerckhove & Weber, 1978) and that actin has several different functions at different times and in different cell types (Clarke & Spudich, 1977; Korn, 1978). The alterations in amino acid sequence between the various actins are so slight and conservative, however, as to suggest that the different roles of actin could derive from regulatory factors bearing on the actin rather than from significant differences in the properties of the actin molecules themselves (Gordon *et al.*, 1977). Skeletal- and cardiac-muscle actins have been well studied (Mannherz & Goody, 1976), as have the non-muscle actins (Pollard & Weihing, 1974; Korn, 1978), but smooth-muscle actin has been relatively neglected (Murphy, 1976). For smooth-muscle actin, chicken gizzard has proved to be the best source (Sobieszek & Bremel, 1975; Suzuki *et al.*, 1978; Strzelecka-Golaszewska *et al.*, 1980), but a good source of mammalian smooth-muscle actin remained to be found. Carotid arteries (Rüegg *et al.*, 1965; Gosselin-Rey *et al.*, 1969), bovine aorta (Hirata *et al.*, 1977) and uterus (Carsten, 1965) have been only briefly studied. For this reason, and also in relation to work on proteinases and protein turnover in the

pregnant uterus (Afting *et al.*, 1979), we considered it worthwhile to isolate uterine actin.

Conventional procedures involving acetone-dried powders having failed, we describe here the isolation, by column-chromatographic methods adapted from those of Gordon *et al.* (1976), of rat and pig uterine actin, and its characterization by amino acid analysis, isoelectric focusing, and activation of myosin ATPase activity. The isolation procedures were designed to minimize the damage possibly caused by the high activities of proteolytic enzymes present in uterus (Afting *et al.*, 1979) and also to avoid selection of any subgroup of actin molecules by omitting polymerization. The yield of actin isolated from uterine smooth muscle was similar to that expected from skeletal muscle.

Materials and methods

Sprague–Dawley rats, with pregnancies of known date, were obtained from Canadian Breeding Farms Ltd., St. Constant, P.Q., Canada. Pig uterus was obtained from a local slaughterhouse within 30 min of the death of the animal. The pigs were 6–9 months old and the uteri varied considerably in size.

All operations were carried out at 0–4°C.

Myosin

Myosin was prepared from rabbit back and leg muscles by standard methods (Perry, 1957; Tono-

Abbreviations used: SDS, sodium dodecyl sulphate; Mg-ATPase, Mg^{2+} -dependent ATPase; dansyl, 5-dimethylaminonaphthalene-1-sulphonyl; RNAase, ribonuclease.

mura *et al.*, 1966; Offer *et al.*, 1973) in the presence of 1 mM-2-mercaptoethanol and with two low-ionic-strength-precipitation cycles. The product was stored in 0.4 M-KCl/50% (w/v) glycerol/15 mM-imidazole, pH 6.8, at -60°C and a protein concentration of 6 mg/ml.

Total actin content

Acetone-dried powders were prepared by homogenization of minced tissue in 3×10 vol. of acetone cooled to -20°C . The actin content of the acetone-dried powders was determined by means of the double-isotope technique of Anderson (1976, 1979).

Actin

Acetone-dried powders were prepared as described by Carsten & Mommaerts (1963), which involves a series of washes of the minced tissue in 0.1 M-KCl, 0.05 M- NaHCO_3 , 0.1% Triton X-100, water and acetone. In addition, the wash solutions contained 0.2% 2-mercaptoethanol.

Skeletal-muscle actin was extracted from acetone-dried powders of rabbit back muscle, pig heart and rat hindleg muscle, and polymerized and depolymerized as described by Spudich & Watt (1971).

Rat uteri weighing 2.5–3.0 g each were taken from rats on day 20 of pregnancy, and the endometrium was removed by gentle scraping (Afting *et al.*, 1979). Pig uterus was not scraped. The uteri were cut into small pieces and usually processed immediately, although some tissue was frozen in liquid N_2 and stored at -60°C .

The uteri were minced in a meat grinder with 3 mm-diameter holes, and the mince was homogenized in 5 vol. (ml/g wet wt.) of 10 mM-EDTA/0.5% (w/v) Triton X-100/10 mM-2-mercaptoethanol/0.5 mM-phenylmethanesulphonyl fluoride/0.2% (v/v) dioxan/3 mM-imidazole, pH 7.5, for 3×30 s in a Sorvall Omnimixer at maximum speed. The homogenate was centrifuged for 10 min at $12000 g_{av.}$, and the supernatant was discarded. The sedimented material was homogenized for 3×30 s at maximum speed and then for 7 min at minimum speed in the Omnimixer in 5 vol. of 0.2 mM-ATP/0.2 mM- CaCl_2 /10 mM-2-mercaptoethanol/0.5 mM-phenylmethanesulphonyl fluoride/0.2% (v/v) dioxan/3 mM-imidazole, pH 7.5 (Ca/ATP buffer). After centrifugation of the mixture as described above, the supernatant was applied immediately to a column (2.5 cm $\times$ 15 cm) of DEAE-cellulose prepared and run in ATP-containing buffers as described by Gordon *et al.* (1976). This column is one-third of the volume of that described by Gordon *et al.* (1976). After the extract had been applied, it was followed first by 50–100 ml of 0.1 M-KCl and then by a linear gradient of 0.1–0.5 M-KCl in a total volume of 700 ml of ATP-containing buffer. The actin was located by an SDS/polyacrylamide-slab-

gel scan of the fractions, which was run and stained as rapidly as possible, since it was important to minimize the time elapsed between elution of actin from the DEAE-cellulose column and its application to the Sephadex G-100 column. The fractions containing actin were pooled (40–60 ml) and the solution was applied directly to a column (5 cm $\times$ 87 cm) of Sephadex G-100, which was eluted with 0.1 mM- CaCl_2 /2 mM-ATP/10 mM-mercaptoethanol/4 mM-imidazole, pH 7.5. In later work, 1 mM-*p*-aminobenzamidine was also present in all the wash, extraction and column buffers.

Electrophoresis

SDS/polyacrylamide-gel electrophoresis was performed on 10% (w/v)-acrylamide running gels with 5% (w/v) stacking gels in the Tris/glycine system described by Laemmli (1970), with a Bio-Rad vertical-slab-gel apparatus. The gels were stained in 0.25% Coomassie Brilliant Blue R in methanol/acetic acid/water (22:5:23, by vol.) and destained in the same solvent mixture. Alternatively, proteins were located within 45 min by immersion of the gel in 4 M-sodium acetate (Higgins & Dahmus, 1979); the gels were subsequently washed overnight in 7% (v/v) acetic acid and stained for permanent record.

Analytical isoelectric focusing was performed on an LKB 2117 Multiphor flat-bed apparatus with water cooling. The gels (11 cm $\times$ 25 cm $\times$ 0.2 cm) contained 10 M-urea, 4% (w/v) acrylamide, 0.2% bisacrylamide, 2% (w/v) Nonidet P40, 1.6% (w/v) Ampholines pH range 5–7 and 0.4% Ampholines pH range 3–10, and were prepared as described by O'Farrell (1975), except that twice the amount of *NNN'*-tetramethylethylenediamine and three times the amount of ammonium persulphate described by him were used to polymerize the gel. After prefocusing for 1 h at 500 V, actin samples in 10 M-urea/20% (w/v) glycerol/2% (w/v) Nonidet P40/2% (w/v) Ampholines pH range 5–7 were loaded into wells cut into the gel remote from the final position of the actin, and focusing was continued for 5 h at 500 V. The gels were washed overnight in methanol/acetic acid/water (40:7:53, by vol.) to remove the Ampholines and stained with Coomassie Brilliant Blue R.

Polymerization of actin

The actin eluted from the Sephadex G-100 column was polymerized by the addition of fresh 2-mercaptoethanol (5 mM), fresh ATP (2 mM), MgCl_2 (2 mM) and KCl (75 mM) and leaving the solution at 0°C overnight, followed by addition of solid KCl to 0.6 M and centrifugation for 3 h at $80000 g_{av.}$. The pelleted material was dispersed and was dialysed against 0.2 mM- CaCl_2 /0.2 mM-ATP/5 mM-2-mercaptoethanol/2 mM-Tris/HCl, pH 8.0 (Spudich & Watt, 1971). The yield of reversibly

polymerizable actin was taken as the amount of protein that remained in the supernatant after clarification of the dialysed actin solution by centrifugation at 80000 g_{av} .

For small-scale experiments, polymerization was monitored in 1.5 ml cuvettes by observation of changes in absorption of actin solutions at 232 nm with a Beckman M IV recording spectrophotometer (Spudich & Cooke, 1975; Kuczmarski & Rosenbaum, 1979).

Amino acid analysis

Samples containing approx. 1 mg of protein were dialysed against water and freeze-dried. When required, the residues were oxidized with performic acid (Moore, 1963). The residues were then hydrolysed for 24 or 48 h in sealed tubes at 110°C in 1 ml of 6 M-HCl. After evaporation to dryness, the residues were redissolved in 1 ml of 0.2 M-citrate, pH 2.2, and analysed on a Beckman model 120C single-column amino acid analyser. The values for serine and threonine were obtained by extrapolation to zero time.

ATPase assay

The activation of myosin Mg-ATPase by actin was measured at 25°C in a total volume of 0.2 ml of 3 mM-MgCl₂/0.5 mM-EGTA/30 mM-KCl/0.5 mM-[γ -³²P]ATP (50 μ Ci/mmol)/20 mM-Tris/HCl, pH 8.0, containing 38 μ g of rabbit skeletal myosin and 0–20 μ g of F-actin (Mockrin & Spudich, 1976). After preincubating the myosin and actin together for 10 min, the reaction was initiated by addition of the [³²P]ATP, and terminated after 2–20 min by addition of 0.3 ml of ice-cold 0.8 M-HClO₄/0.5 mM-H₃PO₄. A 20 mg/ml suspension of Norit A in water (0.5 ml) was then added to each assay mixture, the mixtures were centrifuged, and the radioactivity in 0.5 ml of the supernatant was determined by liquid-scintillation spectrophotometry (Shimizu, 1978). The reactions were linear with respect to time for the given incubation period.

Protein determination

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Trichloroacetic acid/deoxycholate precipitation of the protein was used when necessary to remove interfering substances (Peterson, 1977, 1979).

Results

Isolation of actin

For ease of comparison, throughout the Results and Discussion sections, all yields of actin have been expressed in terms of mg of actin per g wet wt. of

tissue, without regard to the yield of acetone-dried powder which was involved in some procedures. In fact, the yield of acetone-dried powder from skeletal, cardiac and uterine tissue, prepared as described by Carsten & Mommaerts (1963), was in the range 10–14 g/100 g wet wt. of tissue. When prepared by direct treatment of minced heart or uterus in acetone, the yield of acetone-dried powder varied more widely, from 25 to 40 g/100 g wet wt. of tissue.

The total actin content of two samples of different pig uteri was estimated by the method of Anderson (1979), and found to be 9.4 and 10.9 mg/g. The total actin content of skeletal and cardiac muscle, estimated by the same procedure, was 18–20 mg/g.

Actin was isolated from an acetone-dried powder of rat hindleg muscle in an average yield of 2 mg/g. From pig heart treated in the same way, the yield of actin was 0.85 mg/g.

When an acetone-dried powder was prepared from pig uterus by the method of Carsten & Mommaerts (1963), the successive wash solutions contained much protein with a mobility on SDS-polyacrylamide gels corresponding to that of actin. The Ca/ATP-buffer extract of the resultant acetone-dried powder also appeared to contain some actin, but neither the actin in the washes nor in the extract could be polymerized. An acetone-dried powder of pig uterus was therefore prepared by direct homogenization of minced uterus in 3 $\times$ 4 vol. of acetone at –20°C (Rüegg *et al.*, 1965), and also by homogenization in acetone of minced uterus that had been washed once only in 0.5% (w/v) Triton X-100. The acetone-dried powders were extracted with Ca/ATP buffer in the presence of 1 mM-*p*-aminobenzamidine, but no polymerizable actin could be obtained.

Attempts were therefore made to purify actin from uterine extracts by the DEAE-cellulose column procedure of Gordon *et al.* (1976).

When washing of the uterine mince was omitted entirely, almost 2 g of protein was extracted on homogenization of 25 g of the mince in Ca/ATP buffer, the actin peak in the DEAE-cellulose column eluate contained many contaminants, and a precipitate appeared rapidly in the actin-containing fractions. The precipitate did not redissolve on dialysis against Ca/ATP buffer, and on an SDS/polyacrylamide-gel electrophoresis was shown to consist of only two proteins, one of mobility corresponding to actin, and the other having a molecular weight of the order of 200 000, but not further characterized.

A single wash of the minced uterus in buffer containing 0.5% Triton X-100 and proteinase inhibitors was therefore carried out, and the Ca/ATP extract (540 mg of protein) was applied to the DEAE-cellulose column (Figs. 1 and 2). Efforts to polymerize the actin eluted from the DEAE-cellu-

Fig. 1. *DEAE-cellulose chromatography of a pig uterus extract*

An extract of 25 g of pig uterine mince containing 540 mg of protein was applied to a column (2.5 cm $\times$ 15 cm) of DEAE-cellulose, run at a flow rate of 60 ml/h. Fractions 51–56 were pooled for application to the Sephadex G-100 column.

Fig. 2. *SDS/polyacrylamide-gel electrophoresis*

The protein in the samples was precipitated with trichloroacetic acid/deoxycholate, and redissolved by boiling in SDS/2-mercaptoethanol-containing solubilizer solution. The results shown are a composite of several slab gels; from the DEAE-cellulose column (Fig. 1): (a) fraction 40; (b) fraction 48; (c) fraction 52; (d) fraction 56; (e) fraction 60; (f) fraction 66; (g) the standard proteins were: phosphorylase *b* (mol.wt. 94 000); bovine serum albumin (68 000); ovalbumin (43 000); carbonic anhydrase (29 000); soya-bean trypsin inhibitor (21 000); lysozyme (14 300); (h) fraction 86 from the Sephadex G-100 column (Fig. 3).

lose column gave rise to a precipitate that could not be redissolved in Ca/ATP buffer. The fractions containing actin were pooled within 12 h of their elution and applied immediately to the Sephadex

Fig. 3. *Sephadex G-100 column chromatography*

The pool of actin-containing fractions eluted from the DEAE-cellulose column (Fig. 1) was applied to a column (5 cm $\times$ 87 cm) of Sephadex G-100 run at a flow rate of 60 ml/h. Fractions 80–90 were pooled for ultrafiltration and polymerization.

Fig. 4. *Isoelectric focusing of rat leg and uterine actins*

The actin sources and weights in each track were: (a) leg (10 μ g); (b) leg (10 μ g) + uterus (30 μ g); (c) uterus (30 μ g).

G-100 column (Fig. 3). The purification of actin from 25 g of uterine mince is summarized in Table 1. At a scale of 10–25 g, this procedure has been repeated about 10 times, giving yields of actin in the range 0.8–1.5 mg/g, and the yield of actin was not affected by freezing pieces of uterus in liquid N₂ and storing them at –60°C. On a scale of 100 g, however, the actin in the DEAE-cellulose column eluate tended to precipitate; efforts to dialyse this actin pool and to decrease its volume by ultrafiltration before the Sephadex G-100 column led to further losses, and the final yield was only 0.4 mg/g.

Table 1. *Purification of actin from 25 g of pig uterus*

	Volume (ml)	Protein (mg)
Ca/ATP buffer extract	125	540
Actin peak from DEAE-cellulose column	63	60.5
Actin peak from Sephadex G-100 column	115	31.7
After ultrafiltration	38	22.6
Polymerized–depolymerized	6.5	12.6

The actin finally eluted from the Sephadex G-100 column was close to 100% pure (Fig. 2), but dilute (0.3 mg/ml or less), and when polymerization was required, it was necessary to concentrate the actin solution by ultrafiltration on an Amicon PM 10 membrane, in the presence of fresh ATP and 2-mercaptoethanol. Some actin was lost during ultrafiltration, since it formed a gel that adhered loosely to the membrane.

Characterization of actin from rat and pig uterus

Electrophoresis. The uterine actin isolated by this procedure had the same mobility as skeletal-muscle actin on SDS/polyacrylamide gels, when run separately or as a mixture.

On isoelectric focusing, rat leg-muscle actin showed a single band of the α -form, and rat uterine actin contained apparently equal amounts of β - and γ -forms, with a lesser amount of the α -form (Fig. 4).

N-Terminal amino acid. All actins so far characterized have been shown to possess an N-terminal acetyl group. By the technique of dansylation (Narita *et al.*, 1975), and by using RNAase (N-terminal lysine) as a positive control and oval-

Table 2. *Amino acid analysis of rat leg and rat uterus actins*

Cysteine was determined as cysteic acid after performic acid oxidation. In the analyser column system used, 3-methylhistidine was not resolved from histidine. Values are given as the mean number of residues per molecule of actin $\pm$ S.D. for four analyses of rat leg actin and ten analyses of rat uterus actin. Some published analyses of other actins have been included for ease of comparison. Abbreviation: N.D., not determined.

Content (residues/molecule)

Amino acid	Source of actin ...	Rat leg	Rat uterus	Human uterus*	Chicken gizzard†	Rabbit skeletal muscle‡
Lys		19.2 $\pm$ 1.6	19.3 $\pm$ 1.5	19.8	20.1	19
His		11.2	8.2 $\pm$ 1.0	7.4	7.6	8
Arg		18.5 $\pm$ 1.8	16.8 $\pm$ 2.0	18.4	17.4	18
Asp		34.3 $\pm$ 3.5	33.2 $\pm$ 2.6	34.4	32.3	33
Thr		27.6 $\pm$ 3.7	25.1 $\pm$ 3.0	27.4	25.2	27
Ser		23.1 $\pm$ 2.4	23.1 $\pm$ 3.2	26.5	22.2	23
Glu		41.1 $\pm$ 1.0	41.3 $\pm$ 3.1	41.4	40.1	38
Pro		19.9 $\pm$ 1.2	18.7 $\pm$ 1.9	19.0	21.1	20
Gly		26.9 $\pm$ 3.9	35.5 $\pm$ 5.5	28.7	28.8	29
Ala		31.4 $\pm$ 2.8	30.0 $\pm$ 1.3	31.0	29.2	29
Cys		6.0 $\pm$ 0.5	6.5 $\pm$ 0.3	5.2	6.1	5
Val		19.0	19.7	18.7	19.5	21
Met		14.8	15.2 $\pm$ 1.1	11.9	14.1	16
Ile		26.1 $\pm$ 2.8	25.3 $\pm$ 1.8	26.7	24.8	30
Leu		26.6 $\pm$ 1.8	28.5 $\pm$ 1.4	28.2	26.8	25
Tyr		14.3	13.4 $\pm$ 2.4	13.9	15.7	15
Phe		11.7 $\pm$ 0.8	11.9 $\pm$ 1.0	12.2	14.3	12
Trp		N.D.	N.D.	3.3	N.D.	5
Total		371.7	371.7	374.1	365.3	374

* Carsten (1965).

† Suzuki *et al.* (1978).

‡ Nakamura *et al.* (1979).

Table 3. *Activation of rabbit skeletal myosin Mg-ATPase by pig cardiac and uterine actin*

The incubation mixtures contained 38 µg of rabbit skeletal myosin in a total volume of 0.2 ml of 3 mM-MgCl₂/0.5 mM-EGTA/30 mM-KCl/0.5 mM-[γ-³²P]ATP (50 µCi/mmol)/20 mM-Tris/HCl, pH 8.0. The values are the means of duplicate determinations.

Actin source	Actin/myosin weight ratio	ATPase activity (nmol of P _i /min per mg of myosin)	Activation (fold)
None		29	
Heart	1:8	330	11.4
Heart	1:2	845	29
Uterus	1:8	275	9.5
Uterus	1:2	394	13.6

bumin (*N*-terminal *N*-acetylglycine) as a negative control, no *N*-terminal amino acid could be identified in the rat leg and rat uterus actin samples.

Amino acid analysis. Amino acid analyses for rat leg and rat uterus actins are shown in Table 2, which includes, for ease of comparison, some relevant values from the literature. The values obtained for pig uterus actin did not differ significantly from those for the rat actins.

Polymerization. Rat leg actin (0.33 mg/ml) polymerized when exposed to 2 mM-MgCl₂ and 75 mM-KCl, as shown by an increase in *A*₂₃₂ to 0.05 unit within 10 min (Kuczmarski & Rosenbaum, 1979), but rat uterus actin (0.4 mg/ml) showed no increase in *A*₂₃₂, either alone or mixed in equal proportion with rat leg actin. Pig uterus actin, however, polymerized reversibly in the Spudich & Watt (1971) procedure. When 22.6 mg of pig uterus actin (0.58 mg/ml) was treated with MgCl₂ and KCl, 12.6 mg polymerized reversibly.

Actin activation of myosin Mg-ATPase. Initial experiments were performed with pig heart myosin, with a view to species homology of myosin and actin sources, but the pig heart myosin was less stable than rabbit skeletal-muscle myosin, and its Mg-ATPase activity was lower, so that the rabbit enzyme was used for subsequent work. As Table 3 shows, at an actin/myosin weight ratio of 1:8 the pig heart and pig uterus actins gave very similar activation. The activation by uterus actin increased very little on addition of further actin up to a ratio of 1:2, but cardiac actin gave increased activation up to a maximum at a ratio of 1:2.

Discussion

The aim of the present work was to find ways to improve the yield and quality of actin extracted from mammalian smooth muscle. As a basis for comparison, it is useful first to summarize the known actin values for skeletal muscle. Both Bray & Thomas (1975) and Anderson (1976), by different methods, estimated the total actin content of skeletal muscle to be 18–20 mg/g. The amount that can be

isolated in a biologically active form is, however, only 15–20% of this, since preparation of an acetone-dried powder followed by extraction with Ca²⁺ and ATP (Spudich & Watt, 1971) gives rise to a yield of 2–4 mg/g.

Sobieszek & Bremel (1975) reported for chicken gizzard a total actin content of 20–30 mg/g, based on densitometry of stained gels and therefore possibly an overestimate (Anderson, 1976, 1979). Suzuki *et al.* (1978) obtained 2 mg of actin/g of chicken gizzard by a conventional acetone-dried-powder procedure. In pig uterus, we have shown that the total actin content is 10 mg/g, but could isolate only 10–15% of this in pure form. The total actin content of uterine smooth muscle is therefore about half that of skeletal muscle, reflecting the much higher proportion of connective tissue in the myometrium.

The difficulty of obtaining good actin samples from smooth muscle, which has been often noted, may be due to its greater ease of extraction by low-ionic-strength media (Hamoir, 1973), to differences in polymerization, or to the effects of proteinases, whose activities are high in smooth muscle (Rüegg *et al.*, 1965; Sobieszek & Bremel, 1975; Adelman, 1977; Uyemura *et al.*, 1978). We have therefore avoided extensive washes of the tissue before extraction of the actin, have used proteinase inhibitors, and have not included polymerization as a purification step. In fact, it is highly probable, regardless of the cell type from which they are isolated, that all actins when pure and undenatured have very similar polymerization properties (Gordon *et al.*, 1977).

Carsten (1965) obtained actin from acetone-dried powders in yields of 0.35 mg/g (human uterus) and 0.14 mg/g (pregnant-sheep uterus). The amino acid analysis of this actin was close to values obtained more recently, and the actin polymerized and activated a myosin Mg-ATPase. This work has, however, proved difficult to reproduce with various types of smooth muscle. Rüegg *et al.* (1965) made an acetone-dried powder by direct homogenization of bovine carotid arteries in aq. 50% (v/v) acetone;

actin was obtained from the powder in a yield of 0.6–1.0 mg/g. In contrast, Hirata *et al.* (1977) could isolate actin from bovine aorta only by prior isolation of actomyosin followed by chymotrypsin digestion, but the actin did not activate a myosin Mg-ATPase.

In our laboratory all attempts to obtain a polymerizable actin from acetone-dried powders of pig uterus were unsuccessful, whether the powders were prepared with or without extensive tissue washes. It appeared therefore that, although smooth-muscle actin can be obtained from chicken gizzard by the method of Suzuki *et al.* (1978), further developed by Strzelecka-Golaszewska *et al.* (1980), no reliable preparation of a mammalian smooth-muscle actin existed. Our attention was therefore directed to methods not involving acetone-dried powders that had been developed for non-muscle actins (Gordon *et al.*, 1976; Adelman, 1977; Uyemura *et al.*, 1978). A rapid procedure was essential, since Adelman (1977) found that G-actin lost its ability to polymerize with a half-life of 4–6 h, even in the presence of proteinase inhibitors, possibly as a result of loss of bound ATP, which is followed by an irreversible denaturation (Engel *et al.*, 1977).

Various precautions were taken to minimize proteolysis. The pH of 7.5 used throughout inhibited lysosomal proteinases, and phenylmethanesulphonyl fluoride and *p*-aminobenzamidine were added to inhibit serine proteinases. EDTA was used in the initial wash to inhibit the uterine Ca^{2+} -dependent proteinase (Puca *et al.*, 1977), and since this proteinase is readily soluble in EDTA buffers, it is probably largely removed in the first wash. The requirement of actin for Ca^{2+} (Suzuki *et al.*, 1978) prevented the use of EDTA in subsequent steps, although some Ca^{2+} -dependent proteinase may still be present.

It was found necessary to accept the losses of actin associated with a single wash of the uterine mince in 0.5% Triton X-100, since the actin extracted with Ca/ATP buffer was then eluted from the DEAE-cellulose column with lower amounts of contaminants, particularly of the high-molecular-weight contaminant (Fig. 1), and seemed to be more stable. The factors that lead to rapid precipitation in the actin-containing fractions derived from unwashed tissue are not understood. Even from Triton X-100-washed tissue, the actin, which is eluted in 0.25 M-KCl and is therefore prone to polymerization, tended to precipitate at higher protein concentrations or on standing, and the precipitates did not redissolve in Ca/ATP buffer. It was therefore not feasible to work with more than about 25 g of uterine mince at one time, leading to about 30 mg of purified actin. More actin could be obtained by using a larger column and a larger gradient volume

(Gordon *et al.*, 1976), but the actin peak is then of larger volume, and the solution must be concentrated before application to the Sephadex G-100 column. It was found impossible to polymerize the smooth-muscle actin reversibly at this point, or to concentrate the actin solution, even after dialysis at low ionic strength, without considerable loss.

The Sephadex G-100 column may inhibit polymerization (Adelstein *et al.*, 1963), and it separated the actin both from the KCl and from the protein impurities, which were mainly of much higher molecular weight (Fig. 2). The actin finally eluted from the Sephadex G-100 column in the monomeric form was 100% pure as judged by SDS/polyacrylamide-gel electrophoresis (Fig. 2), showing no trace particularly of tropomyosin, which is a common contaminant of actin.

The uterine actin possessed a blocked *N*-terminal amino acid, and the amino acid analyses of pig and rat uterus actins were very close both to the rat leg actin analysis and also to the published values for actins of other tissues and species (Table 2). Isoelectric focusing showed that whereas the rat leg actin consisted entirely of the α -form, the rat uterus actin contained α -, β - and γ -forms roughly in the proportions 1:5:5. This distribution of forms in uterine smooth muscle differs from that in chicken gizzard, where the γ -form predominates (Zechel & Weber, 1978), from that in human platelets, where the β -form predominates (Saborio *et al.*, 1979), and from that in bovine aorta, where the major form is closest in mobility to the skeletal actin α -form (Vandekerckhove & Weber, 1978). Expression of the actin genes is a function of species, tissue and age (Garrels & Gibson, 1976; Storti *et al.*, 1976; Saborio *et al.*, 1979). It is possible that the different forms of actin recovered from rat uterus derive to some extent from different cell types within the tissue.

The rat uterus actin did not polymerize, and seemed to inhibit the polymerization of rat leg actin. Rats with timed pregnancies being very expensive, this actin was available only in small amounts, and its failure to polymerize was not extensively studied. Neither proteolysis nor the influence of other protein factors were apparently to blame, since the actin appeared to be pure and undegraded on an SDS/polyacrylamide gel, and the failure can be attributed only partially to low concentration, since the critical concentration of actin in MgCl_2/KCl is of the order of 90 $\mu\text{g}/\text{ml}$ (Gordon *et al.*, 1977). The rat actin was not concentrated by ultrafiltration. It is possible that in spite of great care a significant proportion of this actin was denatured. The pig uterus actin, however, sedimented in the normal way on exposure to MgCl_2/KCl . From a solution containing 0.38 mg/ml, only 20% showed reversible polymerization, and at 0.58 mg/ml, 55% polymerized reversibly. For

comparison, Adelman (1977) showed that at 4.9 mg/ml about 50% of *Physarum* (slime mould) actin could be sedimented, and at 0.55 mg/ml only 20%.

The maximum activation of skeletal myosin Mg-ATPase by the pig uterus actin (13-fold) was about half of the maximum shown by pig heart actin (20-fold). These values are in agreement with the results of Spudich (1974), Suzuki *et al.* (1978) and Kuczmarski & Rosenbaum (1978), who used actin from several cell types.

Conclusion

Actin has been isolated from pig and rat uterus by column-chromatographic procedures in yields approaching those expected from skeletal muscle. Carsten (1965) obtained actin from uterus by conventional acetone-dried-powder procedures, but subsequent workers, including ourselves, have been unable to reproduce this. The actin that has been obtained is of high purity, as judged by electrophoresis, and its amino acid analysis conforms to those of other actins. Isoelectric focusing showed that the expression of actin genes in the uterus is slightly different from that in other tissues. The rat uterus actin did not polymerize, but the pig uterus actin could be polymerized in the normal way, and it activated a skeletal-muscle myosin Mg-ATPase.

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