# ACTIN IN THE ADRENAL MEDULLA

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## 1. Introduction

The bovine adrenal medulla is a very convenient tissue for studies of cellular processes related to exocytosis. It contains granules with a high internal concentration of catecholamines (the 'chromaffin' granules) which are released by calcium-dependent exocytosis on depolarisation of the cell membrane [1]; in many ways it resembles noradrenergic cells of the sympathetic nervous system. While it seems likely that nerve cells contain special mechanisms for transporting secretory vesicles to synaptic release sites (axoplasmic transport), it is not known whether similar mechanisms are found in other secretory cells. However, recent findings of the association of actin-like filaments with migrating pigment granules [2], of actin with platelet granules [3], and of the association in vitro between chromaffin granule membranes and purified actin [4], together with long-standing reports of actin in secretory cells (review, see [5]), including the adrenal medulla [6], have led us to characterise the distribution of actin in the adrenal medulla in some detail.

It has recently been shown that actin is one of the major protein components of fibroblasts [7]; much of it may be present in an unpolymerised form (D. Bray, personal communication). In this communication we demonstrate the existence of actin in the adrenal medulla and investigate its subcellular distribution: it seems likely that at least 70% is unpolymerised.

**Abbreviations:* HEPES, 2-(*N*-2-hydroxyethylpiperazine-*N'*-yl)ethane-sulphonic acid; SDS, sodium dodecyl sulphate.

* The actin band of chromaffin granules is not clearly resolved from other bands; a very approximate estimate is given.

## 2. Materials and methods

### 2.1. *Tissue fractionation*

Bovine adrenal glands were obtained from a local slaughter house and used within two hours; conventional fractionation procedures [8] were used. The minced medulla was homogenised in 0.3 M sucrose buffered with 10 mM HEPES*, pH 7.0. Microsomes, crude mitochondria and crude chromaffin granules were obtained by centrifugation. Mitochondria and granules were purified on gradients of buffered sucrose (0.7 to 2.0 M and 1.0 to 2.3 M, respectively; 3 hr at 200 000 *g* at 4°C). Chromaffin granule membranes were prepared by lysing granules prepared on discontinuous sucrose gradients [9] with excess 10 mM HEPES, followed by flotation centrifugation through a continuous sucrose gradient [8]. In some experiments 0.3 M sucrose was replaced by 0.15 M KCl; in such cases sucrose gradients were supplemented with 0.15 M KCl, and granule lysis was performed by freezing and thawing a suspension of granules in 0.15 M KCl buffered with 10 mM HEPES.

### 2.2. *Polyacrylamide gels*

Protein contents of subcellular fractions were determined by the method of Hartree [10] following trichloroacetic acid precipitation and solubilisation in 3% sodium hydroxide containing 2% sodium deoxycholate [11]. From 50 to 100 μg of protein were solubilised in 2% (w/v) sodium dodecyl sulphate containing 6% (v/v) mercaptoethanol and applied to 8% SDS-polyacrylamide slab gels with 3% stocking gels [12]. The gels were stained with Coomassie brilliant blue (0.05% in 45% aqueous methanol containing 9% acetic acid). Strips of the gel slab were cut out and scanned using a Gilford 2410 linear gel scanner at 570 nm.

2.3. *Identification of actin*

Bovine actomyosin was prepared by extraction of swollen myofibrils [13] with Weber-Edsall solution; it was subjected to SDS-gel electrophoresis and the actin-containing band cut out. Bands of similar mobility were used from gels of adrenal medulla fractions. Protein was eluted from the gels and subjected to iodination with sodium [$^{125}$I]iodide, tryptic digestion and fingerprinting following the methods of Bray and Brownlee [14]. Oxidation of the digestion products was omitted.

## 3. Results

3.1. *Identification of actin*

Bovine adrenal medulla was subjected to a conventional subcellular fractionation. Each fraction was solubilised in sodium dodecyl sulphate and analysed by electrophoresis in a polyacrylamide slab gel containing SDS. Fig.1 shows a gel of several purified fractions, together with bovine muscle actin and myosin, and serum albumin. All medulla fractions contain a protein component which migrates with the same mobility as muscle actin, having a subunit mol. wt of about 45 000.

The identification of these bands as actin, or largely actin, was achieved by examination of tryptic digests of the protein following iodination with [$^{125}$I] iodide [14]. Bands with the mobility of actin were cut out of slab gels (such as that shown in fig.1) and also from disc gels of muscle actomyosin and adrenal medulla post-microsomal supernatant. Electrophoresis of tryptic digests at pH 3.6 and pH 6.5 showed that material in the bands gave rise to radioactive products with the same mobilities as those from muscle actin. (A band from chromaffin granule membranes, unlike bands from other fractions, contained a number of additional components.) Two-dimensional finger-prints of the digests of the bands from muscle and from adrenal medulla supernatant are shown in fig.2. Tracings were made of the autoradiographs and spots classified as major or minor. The material from muscle had 13 major and 18 minor spots, of which 12 and 8 respectively were also found in the material from the adrenal; the latter had 12 major and 11 minor spots, with 11 and 9 respectively being present in the material from muscle. Material eluted from an SDS gel is not expected to be completely pure; although ideally finger-prints of non-iodinated digests should be compared, these results, with twenty overlapping spots, do strongly suggest that the material from the adrenal medulla is indeed actin. The maps are closely similar to the two-dimensional fingerprints of tryptic digests of chick actin published by Fine and Bray [15]; in fact it has recently been suggested that there may be small differences between muscle and non-muscle actins [16].

3.2. *Subcellular distribution of actin*

Mitochondria and chromaffin granules are usually obtained from the adrenal medulla as pellets by centrifugation. Such material contains a protein comigrating with actin on gels as a fairly prominent component. Both organelles were repurified on continuous sucrose gradients (see Materials and methods); peak fractions from the gradients were analysed, and pellets were discarded. Gels of such material (shown in fig.1) contain comparatively little actin. These gels were scanned and the areas under the protein peaks determined. This was used to give a rough indication of the subcellular distribution of actin (table 1). The results suggested (a) that actin is about 3–4% of the extra-nuclear protein; and (b) that about 70% is found in the post-microsomal supernatant fraction. The remainder, found in the other fractions, or discarded during purification steps, may be in the form of filamentous actin, or associated with cell membranes [17].

Subcellular fractionations of the adrenal medulla are conventionally performed at low ionic strength. To check whether this was affecting the distribution of the actin, a fractionation was performed in which all solutions contained 0.15 M KCl, at which ionic strength muscle actin is in the filamentous form. Gels of fractions corresponding to those in fig.1 showed a number of minor differences in protein bands, but the overall distribution of the putative actin band was unaffected.

3.3. *Membrane-associated actin*

Chromaffin granule membranes can be obtained in a comparatively pure form by subjecting lysed granules to flotation centrifugation [8]. This has the advantage that membranes are well separated from proteins such as actin which tend to polymerise. Examination of gels of such membranes showed the presence of a band with the mobility of actin as a minor component: it was more prominent in the case of membranes from homogenates prepared in 0.15 M KCl than in the case of

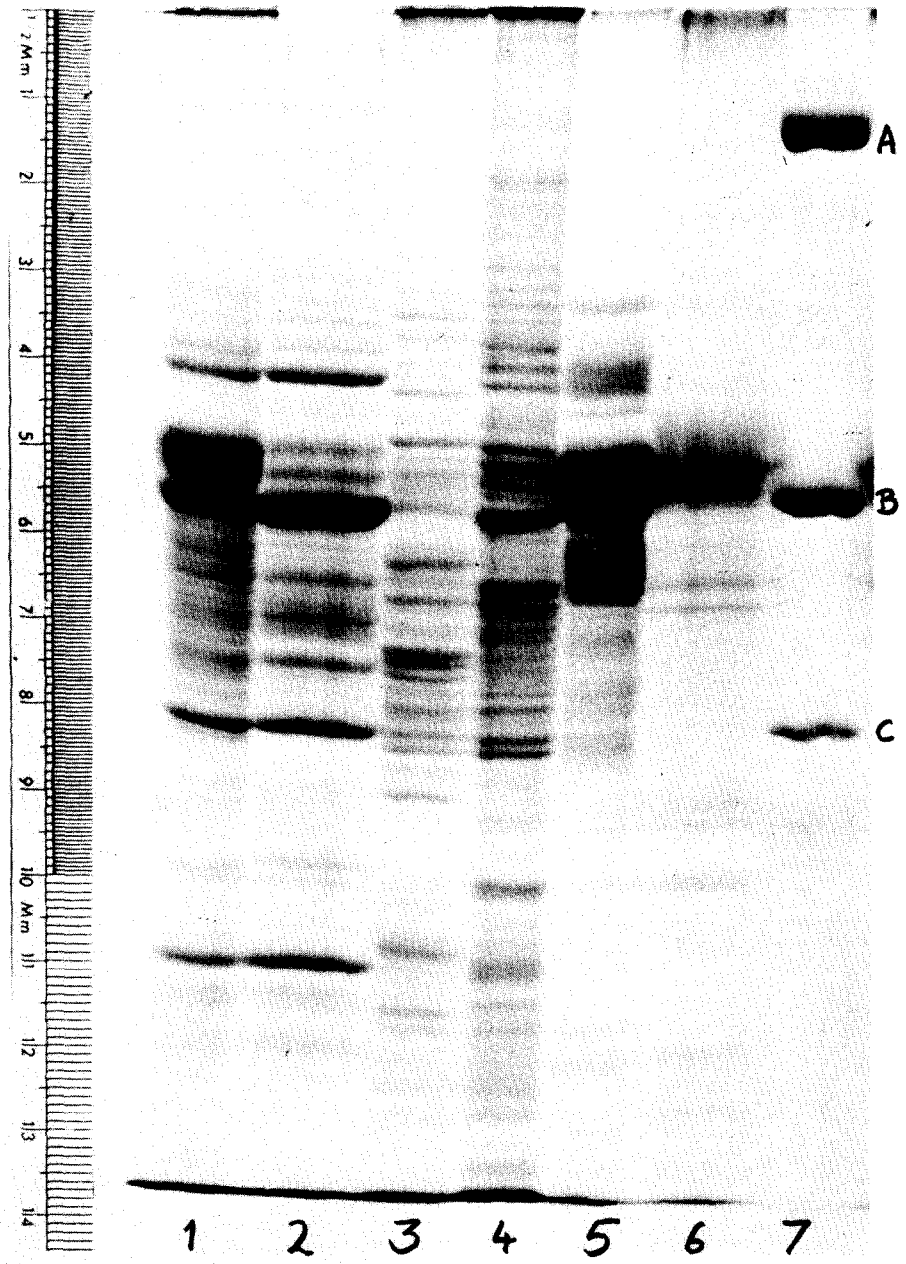


Fig.1. SDS-polyacrylamide gel electrophoresis of subcellular fractions from bovine adrenal medulla: (1) tissue homogenate, following removal of nuclei and cell debris; (2) post-microsomal supernatant; (3) purified mitochondria; (4) microsomes; (5) purified chromaffin granules; (6) purified chromaffin granule membranes; (7) bovine myosin (A), serum albumin (B) and muscle actin (C). Migration was from top to bottom.

those prepared at low ionic strength. It was impossible to estimate the band quantitatively owing to the presence of chromogranin, the major internal protein of the chromaffin granule, in the membrane preparation; it was probably of the order of 2% of the total membrane protein. The band is not sufficiently well separated from other proteins to enable us to identify it unequivocally as actin by iodination.

Prolonged dialysis of the membranes at low ionic strength in the presence of 0.2 mM ATP, 0.2 mM $CaCl_2$ and 0.2 mM dithiothreitol, or solution in 0.6 M KI, appeared to reduce the intensity of the putative actin band somewhat; these conditions disaggregate F-actin. Gels of such membranes resembled those of membranes prepared at low ionic strength in buffered sucrose.

## 4. Discussion

Examination of homogenates and fractions from the adrenal medulla by electron microscopy following 'decoration' with myosin subfragment 1 and negative staining fails to reveal actin filaments (J. H. P., unpublished data). On the other hand, isolated chromaffin granules associate in vitro with purified F-actin [4] and G-actin (K. Burridge and J. H. P., unpublished data). These observations seem to be consistent with our present finding that, while the adrenal medulla contains plenty of actin, this is mainly in the final supernatant and therefore, presumably, non-filamentous, although a small amount may be associated with the granule and other membranes. The putative actin that is associated in this way is far less in amount than can be attached to the

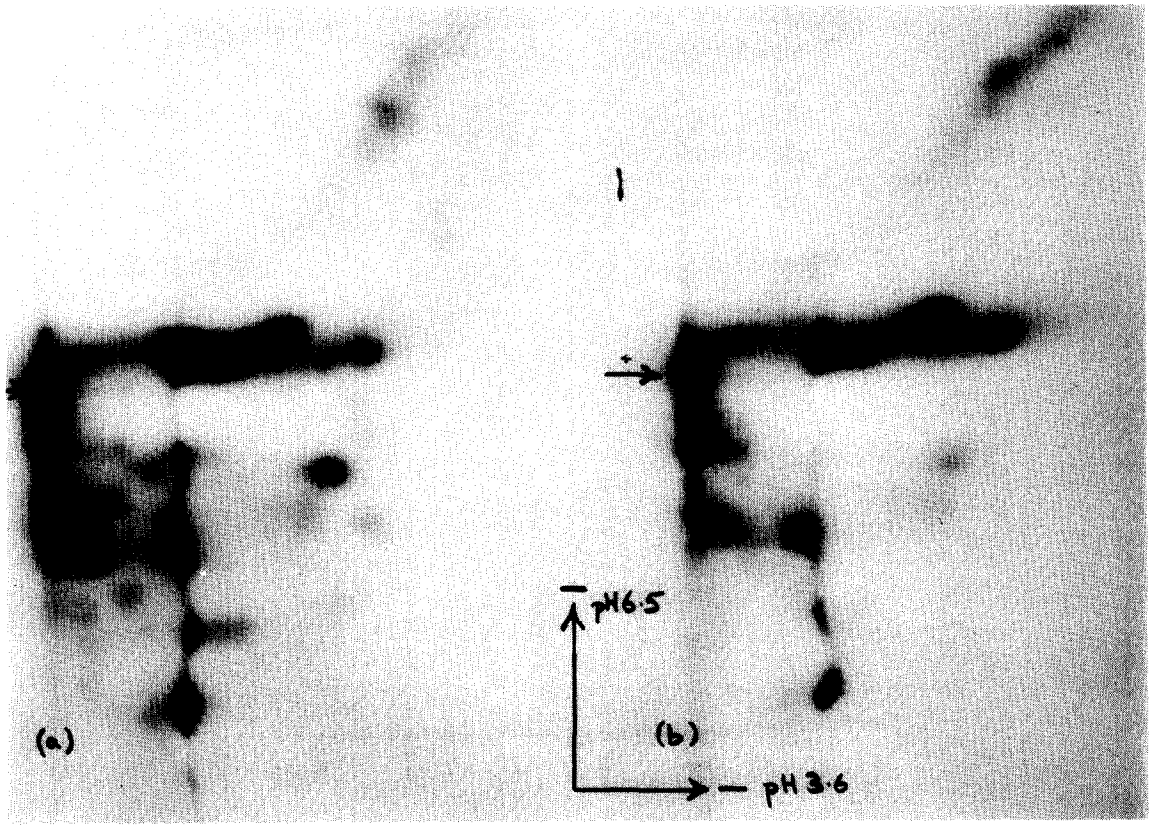


Fig.2. Radioautograph of a two-dimensional electrophoresis of tryptic digestion products of (a) muscle actin (b) putative adrenal medullary supernatant actin, iodinated with [$^{125}$I]iodide. Electrophoresis was at pH 6.5 (first dimension) and pH 3.6 (second dimension); origins are marked by arrows.

Table 1
Subcellular distribution of putative actin in the adrenal medulla

| | Actin content (% ± S.D. of total protein in fraction) | Actin content (% ± S.D. of total actin found in homogenate) |
|---|---|---|
| Homogenate | 3.4 ± 0.2 | 100 |
| Post-microsomal supernatant | 6.7 ± 0.7 | 72.9 ± 3.7 |
| Microsomes | 2.5 ± 0.6 | 4.4 ± 1.2 |
| Mitochondria | 2.6 ± 0.4 | 5.3 ± 2.5 |
| Chromaffin granules | approx. 1* | approx. 4* |

The results are taken from three separate preparations. The term 'homogenate' refers to the supernatant obtained after removel of cell debris and nuclei. Figures in the first column are taken from scans of gels; those in the second column are based on the total protein content of each subcellular fraction.

* The actin band of chromaffin granules is not clearly resolved from other bands; a very approximate estimate is given.

membranes in vitro using purified filamentous actin, when long filaments become associated with the membranes [4].

We do not suggest that our methods of estimation of the subcellular distribution of the actin are very accurate; the difficulties of estimating protein proportions from gels stained with Coomassie brilliant blue have been discussed by several authors, and elaborate experiments are needed in order to achieve any real accuracy [7]. As actin binds rather less stain than, for example, albumin, our percentages are presumably under-estimates. The major protein of the adrenal medulla appears (fig.1) to be chromogranin A (subunit mol. wt. about 70 000: about 45% of the granule protein and 17% of the extranuclear protein of the tissue); gels like that in fig.1 show clearly, however, that actin is quantitatively among the more important protein constituents. Although the medulla is dissected away from the cortex, our preparations are presumably contaminated to some extent with material of cortical origin, as well as with small numbers of smooth muscle cells; it seems unlikely that actin from these sources would make a substantial contribution in our experiments.

Any filamentous actin in the cells which is not associated with membranes would be expected to sediment during one of the centrifugation steps. Indeed, more putative actin is found on gels of crude mitochondria and granules than is found associated with these fractions after further purification on sucrose gradients (fig.1). Our experiments, however, put an upper limit to this of about 30% of the actin present in the homogenate.

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