Studies on rat parotid-cell actomyosin

Terence KEALEY* and Philip J. RANDLE

Nuffield Department of Clinical Biochemistry, John Radcliffe Hospital, Headington, Oxford OX3 9DU, U.K.

(Received 15 December 1983/Accepted 25 January 1984)

Actomyosin was partially purified from rat parotid cells dispersed by collagenase digestion and found to possess different solubility characteristics from that from (undispersed) rat parotid tissue. This is attributed to the decrease in vascular contamination effected by the isolation of parotid cells, yielding a non-muscle actomyosin [Adelstein, Conti, Johnson, Pastan & Pollard (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3693–3697]. Myosin light-chain kinase was partially purified from dispersed rat parotid cells by calmodulin affinity chromatography and shown to be activated by Ca^{2+} –calmodulin. The calmodulin content of dispersed rat parotid cells was shown to be 6.50 ± 0.59 ng of calmodulin/ μg of rat parotid-cell protein (mean $\pm$ S.E.M.), as determined by the activation of purified bovine brain phosphodiesterase by heat-treated extracts of dispersed rat parotid cells.

The mechanism subserving the movement of granules during exocytosis in secretory tissues is not known, but there is evidence that actomyosin may be involved. Microfilaments in secretory cells are concentrated in areas of granule–cytosolic membrane interaction (Bauduin *et al.*, 1975), and cytochalasins inhibit exocytosis (Butcher & Goldman, 1972). Actin, which is essentially ubiquitous in vertebrate cells (Korn, 1978), is associated with exocytotic granules and the cytosolic membrane in secretory tissues (Gruenstein *et al.*, 1975; Jockush *et al.*, 1977). Two separate models have been proposed to account for an actin-mediated movement of secretory granules. These are the ‘treadmill hypothesis’ (Margolis & Wilson, 1981) (unipolar depolymerization of actin filaments into dimers associated with the contrapolar polymerization of actin dimers) and the ‘sliding-filament hypothesis’ (Sheetz & Spudich, 1983), which invokes the movement of myosin along actin filaments (Huxley, 1969). So as to distinguish between the two hypotheses, it seemed useful, therefore, to attempt the isolation of actomyosin from parotid cells.

Myosin has been isolated from a range of secretory tissues, including platelets (Adelstein *et al.*, 1971), leucocytes (Stossel & Pollard, 1973),

bovine adrenal medulla (Creutz, 1977) and brain (Burrige & Bray, 1975). However, the myosins thus isolated may not necessarily be secretory in function. The blood cells are motile, and myosin has been implicated in aspects of amoeboid movement (Daniel *et al.*, 1981). Creutz (1977) has shown that the myosin isolated from sessile tissues such as the adrenal medulla may be largely of vascular origin. It was important therefore to attempt the isolation of actomyosin from the sessile parotid cells of the rat, dispersed by collagenase away from vascular material and blood cells.

Myosin of non-striated-muscle origin is regulated by light-chain phosphorylation mediated by myosin light-chain kinase (Adelstein & Conti, 1975). In the present paper we describe also the detection of myosin light-chain kinase and its partial purification from rat parotid cells. Non-striated muscle myosin light-chain kinase is regulated by Ca^{2+} –calmodulin (Klee *et al.*, 1980), and the calmodulin regulation of partially purified parotid-cell myosin light-chain kinase was determined, as was the calmodulin content of the cells.

Materials

Biochemicals, enzymes and protein electrophoresis standards were obtained from Sigma Chemical Co., Poole, Dorset, U.K., or BCL, Lewes, East Sussex, U.K. Cyclic [8- ^3H]AMP and [γ - ^{32}P]ATP were from Amersham International, Amersham, Bucks., U.K. Sephadex G-200,

Abbreviations used: DTT, dithiothreitol, PMSF, phenylmethanesulphonyl fluoride; SDS, sodium dodecyl sulphate.

* Present address: Department of Clinical Biochemistry, Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP, U.K.

DEAE-Sephadex A-50, QAE (quaternary amino-ethyl)-Sephadex A-25 and CNBr-activated Sepharose 4B were from Pharmacia, Hounslow, Middx., U.K. All other chemicals were from BDH Chemicals, Enfield, Middx., U.K., and of the highest purity available. Calmodulin, prepared from bovine brain to apparent homogeneity (Dedman *et al.*, 1977), was a gift from Dr. M. P. Esnouf of this department. Partially purified calmodulin-deficient phosphodiesterase prepared from bovine brain cortices by the method of Klee & Krinks (1978) was a gift from Dr. D. E. Harrison of this department. Trifluoperazine (Stelazine) was a gift from Smith, Kline and French Laboratories to Dr. S. J. H. Ashcroft of this department.

Methods

Preparation of isolated parotid cells

These were prepared by the method of Kanagasuntheram & Randle (1976) from the parotids of four 300g male Wistar rats dissected free of vascular, lymphatic, muscle and duct tissue. The yield amounted to approx. 60×10^6 cells containing approx. 35mg of protein.

Preparation of parotid-tissue (undispersed) actomyosin

Actomyosin was extracted in the classical manner, which is based on its solubility at high ionic strength and its insolubility at low ionic strength and at pH 6.4 (Needham, 1971). Tissue was homogenized by a Polytron homogenizer (10s, mark 4) into 2 vol. of 10mM- $\text{Na}_4\text{P}_2\text{O}_7$ /20mM-imidazole/0.9M-KCl/5mM- MgCl_2 /3mM-DTT/2mM-PMSF, pH 7.0, at 4°C and centrifuged (35000g for 30min at 4°C). The supernatant was removed and diluted 1:4 with 2mM- MgCl_2 , the pH was lowered to 6.4 and the precipitate was collected by centrifugation (12000g for 30min at 4°C).

Preparation of parotid-cell actomyosin

Packed rat parotid cells after centrifugation were suspended in 5ml of ice-cold 20mM-imidazole/60mM-KCl/15mM- MgCl_2 /15mM- CaCl_2 /1mM-DTT/1% (v/v) Triton X-100, pH 7.0, and ruptured by a Dawe Soniprobe (10s, mark 3). The homogenate was centrifuged (11000g for 20min at 4°C), and the precipitate was suspended (Soniprobe, 2s, mark 3) in 0.4ml of 40mM-imidazole/0.6M-KCl/20mM-EDTA/1mM-DTT, pH 7.0, at 4°C. The suspension was centrifuged (52000g for 20min at 4°C), and the supernatant was applied to a 1cm $\times$ 50cm column of Sephadex G-200 equilibrated with 40mM-imidazole/0.6M-KCl/1mM-EDTA/1mM-DTT, pH 7.0, at 4°C (flow rate 8ml/h). The eluate was monitored for ATPase activity

and for absorption at 280nm, the myosin-containing fractions were pooled, 0.25M- $\text{Na}_4\text{P}_2\text{O}_7$, pH 7.0 (at 20°C), was added to 40mM and the mixture was dialysed against two changes of 100vol. of 40mM- $\text{Na}_4\text{P}_2\text{O}_7$ /1mM-DTT, pH 7.5, at 4°C. The retained material was applied to a 1cm $\times$ 13cm DEAE-Sephadex A-50 column equilibrated in the dialysis buffer (flow rate 8ml/h). After loading of the sample, one bed volume of the buffer was applied, followed by a linear gradient of KCl (0–0.9M) in 40ml of the same buffer. The eluate was monitored for ATPase activity by measurement of the release of $^{32}\text{P}_i$ from [γ - ^{32}P]ATP (Cooper *et al.*, 1974), for absorption at 280nm and by SDS/polyacrylamide-tube-gel electrophoresis (Weber & Osborn, 1969).

Preparation of parotid-cell myosin light-chain kinase

Cells were homogenized as described above into 250mM-KCl/40mM-imidazole/1mM-DTT/1mM-PMSF, pH 7.0, at 4°C and the homogenate was centrifuged (150000g for 90min at 4°C). The supernatant was made up to 40mM-imidazole/50mM-KCl/0.2mM- CaCl_2 /3mM- MgCl_2 /0.1mM-DTT, pH 7.0, at 4°C and applied to a calmodulin-Sepharose 4B affinity column, prepared by the method of Klee & Krinks (1978), equilibrated in the same buffer. The column was washed with one bed volume of equilibration buffer, followed by the same buffer made up to 0.2M-KCl, until the absorption at 280nm of the eluate equalled that of the equilibration buffer.

The column was then washed with the elution buffer, namely 40mM-imidazole/0.2M-KCl/1mM- MgCl_2 /2mM-EGTA/0.1mM-DTT, pH 7.0, at 4°C, and elution was monitored for absorption at 280nm and myosin light-chain kinase activity.

Myosin light-chain kinase assay

Kinase activity was measured by determining the amount of $^{32}\text{P}_i$ transferred from [γ - ^{32}P]ATP to 2 μg of isolated myosin light chains prepared as described by Perrie & Perry (1970) from gizzard smooth-muscle myosin (Sobiesek & Bremel, 1975). The assay mixture was made in 25 μl of 40mM-imidazole/60mM-KCl/1mM- MgCl_2 /1mM-DTT, pH 7.0, at 30°C containing either 0.1mM- CaCl_2 or 1mM-EGTA with or without 2 μg of light chains. The reaction was initiated by the addition of [γ - ^{32}P]ATP (3–7 d.p.m./fmol) at a final concentration of 20 μM , and at 5min intervals a 5 μl portion was removed on to a square of filter paper that was immediately immersed into ice-cold 10% (w/v) trichloroacetic acid and processed as described by Corbin & Reiman (1974). Calmodulin was assayed as described by Sugden *et al.* (1979), ^{32}P -labelled proteins were resolved by electrophoresis and auto-

radiographed as described by Gagliardino *et al.* (1980), and protein was assayed as described by Lowry *et al.* (1951) or with Coomassie Blue (Bradford, 1976), with bovine serum albumin as standard.

Results and discussion

Preparation of isolated rat parotid cells

The final pellet of cells was examined routinely by light-microscopy and was apparently free of vascular or duct material. It was noted that filamentous structures separated from parotid cells during passage of the parotid digest through nylon gauze. Red and white blood cells were discarded in the supernatants of the three 50g washes.

Preparation of actomyosin

The purification of myosin was monitored by the presence on SDS/polyacrylamide-gel electrophoretograms of a band of M_r 200000 (the M_r of the heavy chain of all vertebrate myosins; Pollard & Weihing, 1974), by the increase in the 'K/EDTA-ATPase' specific activity and the fall in the 'K/Mg-ATPase' specific activity (Seidel, 1969). These enzyme activities are studied experimentally in the presence of 0.6M-KCl and either EDTA (K/EDTA-ATPase) or Mg^{2+} (K/Mg-ATPase).

The K/EDTA-ATPase activities of homogenates of rat parotid tissue and dispersed rat parotid cells were similar (Table 1). The application of the classical techniques of actomyosin preparation to rat parotid tissue resulted in a 19-fold increase in the specific activity of the K/EDTA-ATPase activity in the low-ionic-strength precipitate, of which an SDS/polyacrylamide-gel electrophoretogram (Fig. 1a) demonstrated six prominent bands, including two with the apparent M_r values of the heavy chain of myosin and actin respectively, namely 200000 and 42000. The application of the same techniques to homogenates of dispersed rat parotid cells did not result in comparable increases in the K/EDTA-ATPase specific activity in the low-ionic-strength precipitate (Table 1), of which an SDS/polyacrylamide-gel electrophoretogram (Fig. 1b) was similar to that of the low-ionic-strength precipitate of parotid tissue with the omission of bands of apparent M_r 200000 and 42000. No significant difference was noted when $Na_4P_2O_7$, $MgCl_2$ or PMSF was omitted from the preparative buffers or when the pH 6.4 step was omitted. The failure of the classical method of actomyosin extraction from isolated parotid cells was in contrast with its success when applied to whole parotid tissue, and this is attributed to the decrease in vascular contamination effected by the isolation of parotid cells yielding a

non-muscle actomyosin of different solubility characteristics (Adelstein *et al.*, 1972).

In order to extract actomyosin from dispersed parotid cells, they were disrupted into low-ionic-strength buffer containing 15mM- $CaCl_2$ and 15mM- $MgCl_2$, which promote smooth-muscle actomyosin precipitation (Sobiesek & Bremel, 1975), and 1% Triton X-100, which solubilizes cell membranes and their associated proteins. The homogenate was centrifuged (11000g for 20 min), and actomyosin was collected in the precipitate. Triton X-100 was chosen because cardiac- and smooth-muscle myofibrils have been reported to be unaffected by it (Sobiesek & Bremel, 1975; Solaro *et al.*, 1971). Triton X-100 caused a 50% decrease in the K/EDTA-ATPase and a 90% decrease in the K/Mg-ATPase specific activities of cell and tissue homogenates (Table 1), which may be accounted for by the Triton X-100-induced inhibition of mitochondrial and microsomal ATPases (Ferdman *et al.*, 1970).

The low-ionic-strength-bivalent-cation precipitate of cell homogenates recovered 60% of the 1%-Triton X-100-homogenate K/EDTA-ATPase activity (Table 1), of which the high-ionic-strength buffer solubilized 76%, which was applied to the Sephadex G-200 column. Essentially all the K/EDTA-ATPase activity was recovered in the void volume. An SDS/7.5%-polyacrylamide-gel electrophoretogram of a portion of the void volume (Fig. 2a) shows two peaks, of apparent M_r 200000 and 43000, and these may correspond to the heavy chains of myosin and actin respectively, which indicates that at high ionic strength in the absence of bivalent cations parotid myosin is associated with actin. The two small- M_r peaks are similar to those found to contaminate actomyosin from other non-muscle sources, and are ascribed to histones (Adelstein & Conti, 1972). The actomyosin was subjected to ion-exchange chromatography on a DEAE-Sephadex A-50 column. The recovery of K/EDTA-ATPase activity was approx. 30% (Table 1), which was eluted in a single peak at 0.25M-KCl (Fig. 3). The recovery of K/Mg-ATPase was 1.5%, and this residual activity can be ascribed to myosin, as it is co-eluted with the K/EDTA-ATPase, and myosins extracted from a range of tissues exhibit a small K/Mg-ATPase activity (Johnson *et al.*, 1977).

An SDS/7.5%-polyacrylamide-gel-electrophoretogram scan of a sample of the peak K/EDTA-ATPase activity shows a band of apparent M_r 200000, which corresponds to the M_r of the heavy chains of myosin (Fig. 2). However, there are other bands, including one of apparent M_r 160000, which may be heavy meromyosin, a proteolytic product of myosin, and two of M_r 98000 and 71000, which may represent further proteolytic

Table 1. Recovery of actomyosin from rat parotid cells

Three preparations of cells and tissue respectively from the parotids of four rats were homogenized by sonication and Polytron homogenizer as described in the text. ATPase assays were performed in 100 µl of 40 mM-imidazole/0.6 M-KCl/1 mM-DTT, pH 7.0, at 37°C containing 20 mM-EDTA or 1 mM-EDTA for homogenates and column eluates respectively (K/EDTA-ATPase) or 10 mM-MgCl₂ (K/Mg-ATPase). The reactions were initiated by the addition of 2 mM-[γ-³²P]ATP and monitored by the production of ³²P_i by the removal of 15 µl into 0.5 ml of 1 M-HCl/Norit charcoal GSX (5 mg/ml) at 4 min intervals (20 s intervals for homogenate K/Mg-ATPase). All observations were made in duplicate. (Values for whole tissue are given in parentheses.)

	ATPase (nmol/min per mg of protein at 37°C)		Total K/EDTA-ATPase (nmol/min at 37°C)	Ratio of K/Mg-ATPase to K/EDTA-ATPase	Total protein (mg)	Percentage recovery of protein	Percentage recovery of K/EDTA-ATPase	Percentage of specific activity of cell-homogenate K/EDTA-ATPase
	K/EDTA-ATPase	K/Mg-ATPase						
Cell homogenate	1.436 ± 0.085 (1.423 ± 0.082)	148.0 ± 16.4 (220.0 ± 54.0)	48.97	103:1 (154:1)	34.25 ± 13.7	100		
Low-ionic-strength precipitate (classical actomyosin)	1.67 ± 0.27 (27.1 ± 3.4)	110.0 ± 22.3 (189.4 ± 27.6)		65.8:1 6.9:1		5.0 ± 1.7 (3.1 ± 1.2)	5.8 (57.3)	116 (1904)
Triton X-100 homogenate	0.772 ± 0.046 (0.74 ± 0.08)	15.7 ± 1.5 (28.9 ± 8.6)	26.37	20.4:1	34.25 ± 13.7		53.8 (52.1)	-46.2
Low-ionic-strength-bivalent-cation precipitate	1.75 ± 0.05	8.05 ± 1.4	15.8	4.3:1	9.0 ± 2.7		32.2	122
High-ionic-strength supernatant	1.49 ± 1.1		12.1		8.1 ± 3.1		24.7	104
Sephadex G-200 void volume	4.47 ± 0.35	6.25 ± 0.55	11.9	1.4:1	2.66 ± 0.94		24.3	312
DEAE-Sephadex A-50 (pooled ATPase fractions)	17.6 ± 2.1	1.21 ± 0.05	3.7	0.068:1	0.212 ± 0.083		7.5	1228.4
DEAE-Sephadex A-50 0.25 M-KCl (peak K/EDTA-ATPase fraction)	26.0 ± 2.6	4.68 ± 0.18	1.43	0.18:1	0.055 ± 0.019		2.8	1814.7

Fig. 1. SDS/7.5%-polyacrylamide-gel-electrophoretogram scans of the tissue and cell low-ionic-strength precipitates. Portions of the low-ionic-strength precipitates of the high-ionic-strength supernatants of rat parotid tissue (a) and isolated rat parotid cells (b) were applied to tube gels as described in the Methods section. The apparent M_r values of the prominent peaks are indicated above them.

Fig. 2. SDS/7.5%-polyacrylamide-gel-electrophoretogram scans of a Sephadex G-200 void-volume eluate (a) and of a DEAE-Sephadex A-50 eluate demonstrating maximum K/EDTA-ATPase specific activity (b). Portions were applied to the tube gels as described in the Methods section. The apparent M_r values of the prominent peaks are indicated above them.

products, despite the inclusion of 1 mM-PMSF in the 40 mM-pyrophosphate buffer. To confirm the M_r values of the two slowest-migrating bands, samples were electrophoresed on SDS/5%-polyacrylamide gels (results not shown) against standards of cross-linked haemocyanin (M_r values in the range 280 000–70 000), and this showed that the M_r values were $210\,000 \pm 7\,000$ and $160\,000 \pm 9\,000$.

The recovery of initial parotid-cell K/EDTA-ATPase activity in the pooled fractions from the DEAE-Sephadex A-50 column that demonstrated K/EDTA-ATPase activity was 7.5%, which com-

pares well with those reported for other non-muscle tissues, including platelets (Adelstein *et al.*, 1971), leucocytes (Stossel & Pollard, 1973) and adrenal medulla (Creutz, 1977). However, the initial quantities of those tissues from which actomyosin was recovered were at least an order of magnitude greater than that yielded by the collagenase digestion of four rats' parotids. Thus the pooled K/EDTA-ATPase-containing fractions from the DEAE-Sephadex A-50 column contained only 212 μ g of protein, which precluded further purification of the myosin. The study of actin activation

Fig. 3. Elution of actomyosin from a DEAE-Sephadex A-50 column

The void of the Sephadex G-200 column was adjusted to 40 mM- $\text{Na}_4\text{P}_2\text{O}_7$ /1 mM-DTT, pH 7.5, at 4°C by dialysis as described in the Methods section and applied to a 1 cm $\times$ 13 cm DEAE-Sephadex G-200 column equilibrated in the same buffer. After loading of the sample one bed volume of buffer was applied under gravity flow at 8 ml/h, followed by a linear gradient of KCl (0–0.9 M) in 40 ml of the same buffer. The eluate was monitored for protein by absorption at 280 nm (○) and for K/EDTA-ATPase (●) and K/Mg-ATPase (□) enzyme activities as described in the Methods section.

of myosin and a sufficiently heavy loading of gels so as to make the myosin light chains detectable (Stossel & Pollard, 1973) would require a several-fold scaling-up of the preparation, and this was precluded by the method of collagenase digestion.

Preparation of myosin light-chain kinase

The detection of myosin light-chain kinase activity in homogenates of isolated parotid cells was made by autoradiography, which showed no detectable phosphorylation, Ca^{2+} -sensitive or otherwise, at M_r 20000, the expected M_r of endogenous myosin light chains (lanes c and d in Fig. 4b). However, the addition of 2 μg of light chains produced a significant Ca^{2+} -sensitive phosphorylation of the 20000- M_r light chain (lane b in Fig. 4b). The lack of obvious myosin light-chain phosphorylation in the absence of added light chains reflects the small myosin content of rat parotid cells.

The first step in the purification of myosin light-chain kinase was centrifugation of the homogenate; myosin light-chain kinase activity of 58.8 ± 17.2 pmol of ^{32}P /min per mg of protein at 30°C was recovered in the supernatant (mean $\pm$ S.E.M. for three preparations). The final stage in the partial purification of myosin light-chain kinase was calmodulin-Sepharose 4B affinity chromatography. All of the parotid-cell supernatant myosin light-chain kinase activity was recovered in the column elution, 80% unbound, which may be accounted for in part by the binding of myosin light-chain kinase to endogenous calmodulin (Miyamoto *et al.*, 1981; Penn *et al.*, 1982), and 20% with the EGTA eluate.

It was noted that the myosin light-chain kinase activity in the EGTA eluate was lost on storage at 4°C with a half-life of 12 h. This could not be prevented by concentration of the eluate, by storage in 50% (v/v) glycerol at –20°C or by the addition of 3 mM- CaCl_2 , and so all eluates were assayed at once. The mean $\pm$ S.E.M. myosin light-chain kinase activity of three preparations was 156.4 ± 10.7 pmol of ^{32}P /min per mg of protein at 30°C. This declined to 54.2 ± 6.2 pmol of ^{32}P /min per mg of protein and 18.6 ± 4.5 pmol of ^{32}P /min per mg of protein at 30°C in the presence of 1 mM-EGTA and 0.1 mM-trifluoperazine respectively, and both these values were significantly different ($P < 0.001$) from that obtained with Ca^{2+} , as determined by Student's *t* test. Fig. 4 shows that the only significant phosphorylation coincided with the 20000- M_r light chain. The lack of complete inhibition of myosin light-chain kinase under these circumstances can be accounted for by the loss of Ca^{2+} -calmodulin-sensitivity that occurs with partially proteolysed myosin light-chain kinase (Hathaway & Adelstein, 1979).

Calmodulin assay

Calmodulin was assayed by an adaptation (Sugden *et al.*, 1979) of the method of Thompson *et al.* (1974), which is based on calmodulin activation of brain phosphodiesterase. The specific activity of the phosphodiesterase in the presence of 0.05 mM- CaCl_2 but in the absence of added calmodulin was shown to be 9.93 ± 0.93 pmol/min per μg of protein at 30°C. This was taken as a control value of 100%. This activity could be increased progressively by the addition of extracts of parotid cells up to 25 μg

Fig. 4. SDS/12.5%-polyacrylamide-gel electrophoretograms and autoradiographs of a rat parotid-cell homogenate and an EGTA-induced eluate of the calmodulin affinity column.

Portions (20 μ l) of parotid-cell homogenates were incubated in 40 mM-imidazole/60 mM-KCl/1 mM-MgCl₂/1 mM-DTT, pH 7.0, at 30°C as described in the text, with the following additions: lane (a) 2 μ g of light chains/1 mM-EGTA; lane (b) 2 μ g of light chains/0.1 mM-CaCl₂; lane (c) 1 mM-EGTA; lane (d) 0.1 mM-CaCl₂. At 15 s after being made up to 20 μ M-[γ -³²P]ATP the samples were removed into SDS, heated to 100°C and subjected to electrophoresis (a) and autoradiography (b). Lane (s) contains the protein *M_r* standards. A 25 μ l portion of the EGTA-induced eluate of the calmodulin affinity column containing 795 ng of protein was incubated as described above with 0.1 mM-CaCl₂, 2 μ g of light chains and 1 μ g of calmodulin and made up to 20 μ M-[γ -³²P]ATP. After 20 min the whole was removed into SDS and subjected to electrophoresis (c) and autoradiography (d). → indicates the 20 000-*M_r* light chain on the gel and its position on the autoradiograph.

of protein, whereupon the phosphodiesterase demonstrated a specific activity of 72.23 ± 1.07 pmol/min per μ g of protein at 30°C (Fig. 5). The phosphodiesterase was not entirely calmodulin-free, as shown by the fall in specific activity when it was assayed in the presence of 1 mM-EGTA or 0.05 mM-CaCl₂ and 0.1 mM-trifluoperazine. The addition of amounts of calmodulin up to 100 ng gave an analogous activation of phosphodiesterase, and a comparison of the two activation curves yielded a calmodulin content of 6.50 ± 0.59 ng of

calmodulin/ μ g of rat parotid-cell protein (mean $\pm$ S.E.M. for three preparations).

General discussion and conclusions

The purpose of the present work was to isolate actomyosin from sessile secretory cells free of vascular, ductal and blood material and to investigate some of its properties. The difference in the solubility characteristics of cellular actomyosin from whole-tissue actomyosin, which is assumed to re-

Fig. 5. Activation of phosphodiesterase by extracts of parotid cells

Mixtures (100 μ l) containing 2.71 μ g of phosphodiesterase and 10 nmol of cyclic $[8\text{-}^3\text{H}]\text{AMP}$ in 50 mM-Tris/HCl/3 mM-MgCl₂/bovine serum albumin (10 μ g), pH 8.0, at 30°C were incubated with heat-treated extracts of parotid cells in the range 0–25 μ g and either 50 μ M-CaCl₂ (○) or 1 mM-EGTA (Δ) or 50 μ M-CaCl₂/100 μ M-trifluoperazine (□). Results are expressed as percentages of the phosphodiesterase activity in controls with 50 μ M-CaCl₂ and no added calmodulin (9.93 pmol/min per μ g of protein). Each point is the mean of three observations, performed in duplicate.

flect the different solubility characteristics of non-muscle actomyosin from muscle actomyosin, suggests that the separation of rat parotid secretory cells from vascular and ductal contamination was successful. However, the purity of the final cell pellet was not confirmed quantitatively by other means. It should be noted that no attempt was made at excluding myoepithelial cells from the final cell pellet, although the non-muscle nature of the extracted actomyosin suggests that the contribution of these muscle-like cells was small.

The extraction of actomyosin from isolated parotid cells may reflect a role for it in secretion, for the only other aspect of cell motility that these sessile cells might undertake would be cell division, and the rate of cell proliferation in the rat parotid gland is very low (Barka, 1965). Although the mechanism of an actomyosin-mediated exocytosis would be dependent on the regulation of actin polymerization effected by the actin-binding proteins (Margolis & Wilson, 1981), the coexistence of myosin may indicate a role for this protein in providing motive power analogous to that seen in muscle, as discussed by Sheetz & Spudich (1983). The partial purification of a calmodulin-dependent myosin light-chain kinase from isolated rat parotid cells indicates that rat parotid-cell myosin may be regulated by Ca²⁺-dependent phosphorylation.

The major secretagogues of protein secretion in the parotid are β -adrenergic agonists, which stimu-

late the formation of cyclic AMP (Schramm & Selinger, 1975). The incubation of parotid tissue with adrenaline causes a release of Ca²⁺ from the mitochondrial and microsomal fractions (Dormer & Ashcroft, 1974), and this effect can be mimicked in isolated rat parotid cells by the administration of dibutyryl cyclic AMP (Kanagasuntheram & Randle, 1976). Those findings, and the present one of a calmodulin content of rat parotid cells comparable with those found in other secretory tissues (Sugden *et al.*, 1979), are compatible with a role for cyclic AMP of releasing Ca²⁺ into the cytosol, there to bind to calmodulin and so couple stimulation with secretion.

We thank Dr. B. Bullard and Dr. D. Mercola for very helpful advice. T. K. was a Medical Research Council Training Fellow during the course of this work.

References

- Adelstein, R. S. & Conti, M. A. (1972) *Cold Spring Harbor Symp. Quant. Biol.* **37**, 599–605
- Adelstein, R. S. & Conti, M. A. (1975) *Nature (London)* **256**, 597–598
- Adelstein, R. S., Pollard, T. D. & Kuehl, W. M. (1971) *Proc. Natl. Acad. Sci. U.S.A.* **68**, 2703–2707
- Adelstein, R. S., Conti, M. A., Johnson, G. A., Pastan, I. & Pollard, T. D. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3693–3697
- Barka, T. (1965) *Exp. Cell Res.* **39**, 355–364
- Bauduin, H., Stock, C., Vincent, D. & Grenier, J. F. (1975) *J. Cell Biol.* **66**, 165–181
- Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
- Burridge, K. & Bray, D. (1975) *J. Mol. Biol.* **99**, 1–14
- Butcher, F. R. & Goldman, R. H. (1972) *Biochem. Biophys. Res. Commun.* **48**, 23–29
- Cooper, R. H., Randle, P. J. & Denton, R. M. (1974) *Biochem. J.* **143**, 625–641
- Corbin, J. D. & Reiman, E. M. (1974) *Methods Enzymol.* **38**, 287–290
- Creutz, C. E. (1977) *Cell Tissue Res.* **178**, 17–38
- Daniel, J. L., Molish, I. R. & Holmsen, H. (1981) *J. Biol. Chem.* **256**, 7510–7514
- Dedman, J. R., Potter, J. D., Jackson, R. L., Johnson, J. D. & Means, A. R. (1977) *J. Biol. Chem.* **252**, 8415–8422
- Dormer, R. L. & Ashcroft, S. J. H. (1974) *Biochem. J.* **144**, 533–550
- Ferdman, D. L., Dyadyusha, G. P. & Himmelreich, N. G. (1970) *Ukr. Biokhim. Zh.* **42**, 411–416
- Gagliardino, J. J., Harrison, D. E., Christie, M. R., Gagliardino, E. E. & Ashcroft, S. J. H. (1980) *Biochem. J.* **192**, 919–927
- Gruenstein, E., Rich, A. & Weihing, R. R. (1975) *J. Cell Biol.* **64**, 223–234
- Hathaway, D. R. & Adelstein, R. S. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1653–1657
- Huxley, H. E. (1969) *Science* **164**, 1356–1366
- Jockush, B. M., Burger, M. M., DaPrada, M., Richards, J. G., Chapponier, C. & Gabbiani, G. (1977) *Nature (London)* **270**, 628–629

- Johnson, D. H., McCubbin, W. D. & Kay, C. M. (1977) *FEBS Lett.* **77**, 69–74
- Kanagasuntheram, P. & Randle, P. J. (1976) *Biochem. J.* **160**, 547–564
- Klee, C. B. & Krinks, M. H. (1978) *Biochemistry* **17**, 120–126
- Klee, C. B., Crouch, T. H. & Richman, P. G. (1980) *Annu. Rev. Biochem.* **49**, 489–515
- Korn, E. D. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 588–599
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Margolis, R. L. & Wilson, L. (1981) *Nature (London)* **293**, 705–711
- Miyamoto, E., Matsui, K., Fukunaga, K., Nishume, S. & Iwasa, Y. (1981) *Biomed. Res.* **2**, 341–346
- Needham, D. M. (1971) *Machina Carnis*, Cambridge University Press, London
- Penn, E. J., Brocklehurst, K. W., Sopwith, A. M., Hales, C. N. & Hutton, J. C. (1982) *FEBS Lett.* **139**, 4–8
- Perrie, W. T. & Perry, S. V. (1970) *Biochem. J.* **119**, 31–38
- Pollard, T. D. & Weihing, R. R. (1974) *CRC Crit. Rev. Biochem.* **2**, 1–65
- Schramm, M. & Selinger, Z. (1975) *J. Cyclic Nucleotide Res.* **1**, 181–192
- Seidel, J. C. (1969) *Biochim. Biophys. Acta* **189**, 162–170
- Sheetz, M. P. & Spudich, J. A. (1983) *Nature (London)* **303**, 31–35
- Sobiesek, A. & Bremel, R. D. (1975) *Eur. J. Biochem.* **55**, 49–60
- Solaro, R. J., Pang, D. C. & Briggs, N. F. (1971) *Biochim. Biophys. Acta* **245**, 259–262
- Stossel, T. P. & Pollard, T. D. (1973) *J. Biol. Chem.* **248**, 8288–8294
- Sugden, M. C., Christie, M. R. & Ashcroft, S. J. H. (1979) *FEBS Lett.* **105**, 95–100
- Thompson, W. J., Brooker, G. & Appleman, M. M. (1974) *Methods Enzymol.* **38**, 205–212
- Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406–4412