J. Cell Sci. 19, 203–213 (1975)
Printed in Great Britain

CALCIUM-BINDING PROTEINS IN A VORTICELLID CONTRACTILE ORGANELLE

W. B. AMOS, L. M. ROUTLEDGE AND F. F. YEW
The Department of Zoology, Downing Street, Cambridge CB2 3EJ, England

SUMMARY

The proteins of the contractile spasmoneme of *Zoothamnium* have been examined for comparison with other motile systems. Though capable of calcium-induced contraction, glycerinated preparations of the spasmoneme contain neither actin nor tubulin at levels that can be detected in polyacrylamide gels. Sixty per cent of the protein in sodium dodecyl sulphate gels migrates in a band at a molecular weight of approximately 20000, consisting largely of 2 similar protein species which are here given the name of *spasmins*. The amino acid composition of 2 spasmin fractions has been determined by a fluorimetric method. They are rich in Asx, Glx and serine, but have few aromatic amino acids and no cystine or methionine. In calcium-buffered polyacrylamide gels, it was observed that a reduction in the electrophoretic mobility of the spasmins was induced specifically by calcium (but not magnesium) at the same low concentrations as induce contraction. This indicates that the spasmins are calcium-binding proteins which may be involved directly in the calcium-induced contraction of the spasmoneme.

INTRODUCTION

The spasmoneme, a contractile organelle which occurs in the vorticellids, a family of ciliated protozoa, differs fundamentally from other motile structures in cells. Earlier work (Hoffmann-Berling, 1958; Amos, 1971) has indicated strongly that in this system the binding of calcium brings about contraction directly rather than by triggering an ATPase. This effect presumably depends on an alteration in conformation of a protein which occurs when calcium becomes bound to it. The calcium-binding of the spasmoneme as a whole is discussed in the preceding paper (Routledge *et al.* 1975).

Little information is available about the chemical composition of the spasmoneme. Randall & Hopkins (1962) found that the spasmoneme of *Carchesium* showed cytochemical reactions typical of proteins but gave a negative result for carbohydrate with the periodic acid-Schiff method.

The present study was undertaken to discover whether a protein fraction is present which binds calcium in the normal range of intracellular concentrations.

Microanalytical methods have made it possible to study the proteins from microgramme quantities of spasmoneme material, which was obtained in this study from the giant stalk of colonies of *Zoothamnium geniculatum* by direct dissection (Weis-Fogh & Amos, 1972) or simple extraction procedures. These methods were necessary because of the small quantities of material that could be collected.

MATERIAL AND METHODS

Extraction of proteins

Colonies of *Zoothamnium geniculatum* were glycerinated for several months in the medium described by Amos (1971), and then washed in 50 % v/v glycerol to remove salts. For some experiments, where it was necessary to ensure that the spasmoneme alone was included in the analysis, the organelles were dissected individually from the main stalks of the colonies. In other cases, an extracting solution was used. The solutions for extracting the proteins contained either 2 % SDS (sodium dodecyl sulphate) with 0·06 M Tris buffer at pH 6·8 or 3 M guanidine hydrochloride. It was sufficient to cut off the branches of the colonies and immerse the main stalk in the extracting solution, which dissolved the spasmoneme and surrounding cytoplasm, leaving the stalk sheath undissolved. Almost all the material extracted in this way was from the spasmoneme.

The highest yield of spasmoneme material was obtained by a method which could be applied in bulk. The zooids were first detached by shearing the colonies gently in 0·02 % SDS by repeated pipetting. The detached zooids were then removed by washing, leaving the spasmonemes intact within the stalks of the colonies. The spasmonemal material was then extracted.

Dialysis and concentration of protein extracts

To remove the salt from small volumes of guanidine hydrochloride extract, a Colover ultramicrodialysis cell (Electrothermal Engineering Co.) was used; 0·25 ml of extract could be dialysed against distilled water or buffer in this apparatus. The dialysed protein solution was concentrated in the same apparatus by absorbing the water into 30 % polyethylene glycol (mol. wt. 20000). Both operations were performed at 4 °C.

Electrophoresis

Polyacrylamide gels were used. A microslab gel apparatus (Amos, 1975) was constructed so that runs could be carried out with samples from as few as two *Zoothamnium* colonies, containing approximately 1·5 μg of spasmonemal protein in all. Loading of the 5-μl sample with a hand-held pipette was facilitated by adding 0·02 % fluorescein to the sample as a marker. The fluorescein also served as tracking dye. Discontinuous SDS gels were made according to the formulae of Laemmli & Favre (1973) but the electrode baths were filled with 0·1 M Tris-glycine buffer instead of 0·2 M. The samples were dissolved in a solution containing 2 % SDS, 0·06 M Tris buffer, pH 6·8 and 1 % v/v mercaptoethanol. In some experiments the sample solution was heated to 100 °C for several minutes in a sealed capillary tube to destroy metastable aggregates.

For the electrophoresis of undenatured protein, polyacrylamide gels without SDS but with the same discontinuous buffer system were used. The separating gel contained 0·38 M Tris-glycine buffer, pH 8·0 and the spacer gel 0·06 M Tris-HCl buffer, pH 6·8. In some experiments either 1 mM $CaCl_2$ or 0·5 mM EDTA was added to the gels and to the solutions in the electrode baths. In other experiments, the calcium ion concentration was controlled by the addition of EGTA (ethylene glycol bis(β-amino ethyl ether) tetraacetic acid) buffers to the gels. An EDTA (ethylene diamine tetraacetic acid) buffer was used when it was necessary to control magnesium ion levels in the range 10^{-4} to 10^{-6} M. The constants given by Sillén & Martell (1964) were used to calculate the buffer compositions appropriate to each pH.

The gels were stained with either Coomassie blue or fast green in 50 % methanol with 7 % acetic acid and destained by leaching in 7 % acetic acid, 5 % methanol. They were scanned directly with a Joyce–Loebl microdensitometer and the relative amounts of protein determined from the areas under the peaks.

Elution of protein from gels

In order to obtain proteins for amino acid analysis 15 % polyacrylamide gels were frozen and cut into sections 0·3 mm thick in a Joyce–Loebl gel slicer. The position of the proteins was determined either by staining with Coomassie blue before sectioning or by staining a parallel

gel. Microgramme quantities of protein were eluted from the gel slices with 2 changes of 1 % SDS each of 0·1 ml. The resulting solution was combined with 9 times its volume of cold acetone and the precipitate was collected.

Preparation of protein hydrolysate

The acetone-precipitated proteins were hydrolysed in 6 N HCl containing 0·001 M thioglycollic acid in sealed tubes under nitrogen for 20 h at 110 °C. For tryptophan determinations, the concentration of thioglycollic acid was increased to 2 % and the hydrolysis tubes were evacuated down to 50 μm Hg (6·65 N m^{-2}) as described by Matsubara & Sasaki (1969). Cystine was oxidized to cysteic acid by performic acid for determination.

Amino acid analysis

Amino acid analysis was performed on a Technicon Autoanalyser with a single column (75 cm), the sample being eluted in a continuous buffer gradient from pH 2·8 to 5·0 at 65 °C. Since each protein sample extracted from the SDS gel was less than 1 μg, the amounts of amino acids present in the hydrolysates were below the limit of detection of conventional ninhydrin systems. A fluorescence technique developed by Roth & Hampai (1973) was used instead. The reagent solution with which amino acids react to produce intensely fluorescent compounds contains 0·08 % w/v *o*-phthaldehyde, 0·02 % v/v mercaptoethanol and 1 % v/v ethanol in 0·1 M sodium borate buffer pH 10·5. The effluent and the reagent were mixed at equal flow rates of 0·7 ml/min and allowed to flow through mixing coils for 5 min at room temperature before entering the flow cell. The flow cell was fitted into a Zeiss spectrofluorimeter, with excitation at 360 nm. The fluorescence was measured at 470 nm. The output of the fluorimeter was fed into a pen recorder and the relative amount of fluorescence associated with each amino acid was calculated by weighing the paper under the appropriate peak of the chromatogram. Norleucine was used as internal standard. Standard chromatograms were prepared from a mixture of amino acids containing 5 nmol of each, together with a 'blank extract' of a piece of SDS-gel of the same size as the sample-gel but containing no protein. This mixture was subjected to hydrolysis and column chromatography in exactly the same way as the protein sample.

Determination of proline

The proline content of the sample was determined separately, since proline does not react with the *o*-phthaldehyde-mercaptoethanol reagent. In the period when proline was being eluted from the chromatographic column, the effluent was collected before the fluorescence reagent had been added. It was lyophilized or dried in a rotary evaporator and redissolved in 0·5 ml 0·01 N HCl, 0·2 ml of 1 % sodium hypochlorite was added and after mixing vigorously the solution was allowed to stand for 5 min at room temperature to allow the oxidation of proline to proceed to completion. It was then heated in a boiling water bath for 2–3 min to destroy excess sodium hypochlorite. After cooling to room temperature 1 ml of the *o*-phthaldehyde-mercaptoethanol solution was added to the reaction mixture. Fluorescence due to the oxidation product of proline was measured after 5 min.

An additional method used for proline determination was dansylation of the protein hydrolysate. The dansylated amino acid was chromatographed on a 7·5 × 7·5 cm^2 polyamide plate by the method of Woods & Wang (1967). Since dansyl-proline migrated near the solvent front in the benzene-acetic acid system, it was very easily identified. The polyamide plate was then photographed under ultraviolet, and the amount of dansyl-proline was determined with a Vitatron flying-spot densitometer.

OBSERVATIONS

Composition of SDS extracts

Both fresh and glycerinated spasmonemes dissolved totally within 30 s in 2 % SDS buffered at pH 6·8. Glycerinated preparations isolated by dissection which contain

only the spasmoneme and a thin surrounding layer of cytoplasm left no visible residue when dissolved in this way. When subjected to electrophoresis in a 15% polyacrylamide SDS gel the solution of glycerinated spasmonemes gave the pattern shown in Fig. 2B. Adding mercaptoethanol to reduce labile disulphide linkages or preheating the sample affected the pattern only in minor details. Molecular weights were determined by comparison with standard proteins: bovine serum albumin, carbonic anhydrase and lysozyme. A prominent feature of the SDS gel pattern was a complex band corresponding to a molecular weight of 20000. The band consisted of one prominent peak, often with a shoulder, and a number of minor peaks which appear not to be gel artifacts since they occurred regularly in both tube and slab gels. The 20000 mol. wt. band was found by densitometry to contain 40–60% of the protein, the 2 stains yielding identical results in this respect. Most of the remaining 40–60% consisted of slowly migrating material with a molecular weight above 100000. When the spasmoneme sample was run in parallel with actin and tubulin in an SDS slab gel, no bands co-migrating with these proteins were found in the spasmoneme pattern (Fig. 3). This shows the fundamental dissimilarity between this contractile system and those based on actin filaments or microtubules.

Guanidine hydrochloride extracts

Guanidine hydrochloride functions as a protein solvent chiefly by destabilizing hydrophobic interactions (Mahler & Cordes, 1966), but unlike SDS it can readily be removed from proteins by dialysis. Guanidine hydrochloride extracts of the spasmoneme were made and subsequently dialysed in an attempt to obtain spasmoneme proteins in a native state.

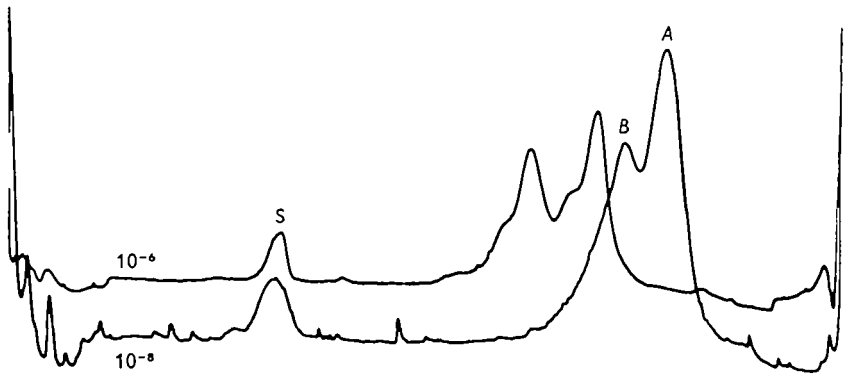


Fig. 1. Superimposed densitometer tracings of two 15% polyacrylamide gels maintained at calcium ion concentrations of 10^{-6} and 10^{-8} M by means of CaEGTA buffers. *Zoothamnium* spasmoneme proteins extracted with 3 M guanidine hydrochloride from glycerinated preparations were subjected to electrophoresis in the gels, migration being from left to right (anodal), and stained with Coomassie blue. In the low-calcium gel (10^{-8} M) the prominent double peak is due to incompletely resolved *A* and *B* components of the 20000 mol. wt. band. The rate of migration of these proteins is markedly reduced at 10^{-6} M Ca^{2+}, while the mobility of an unidentified slower-migrating protein (S) is unchanged.

The organelle dissolved almost completely in 3 M guanidine hydrochloride, leaving a transparent inelastic residue with very low mass, but retaining the outline of the spasmoneme. When the guanidine hydrochloride extract was dialysed against distilled water, the proteins remained in solution and could be concentrated and run in an SDS gel system. The 3 M extract was then found to contain all the main components of the spasmoneme, whereas the 'ghost' contained no detectable protein. Since the proteins remained in solution after removal of the salts by dialysis, they could be subjected to electrophoresis without SDS.

The pattern obtained on a 15% acrylamide gel at pH 8·0 was similar to that shown in Fig. 1; 60% of the protein migrated as a fast component (*A*) and a slightly slower component (*B*), the 2 being incompletely resolved. *A* also migrated slightly faster than *B* when run in a second dimension in a 15% polyacrylamide SDS gel. *A* evidently has a slightly lower molecular weight than *B*, but both are components of the 20000 mol. wt. band observed in SDS gels. *A* and *B* migrated faster in a non-SDS gel at pH 8·8 than myoglobin (pI 6·9) and α-casein (pI 4·9) which are of similar molecular weight to the spasmoneme protein. This indicates that the spasmoneme proteins *A* and *B* are quite acidic (pI < 4·9).

Amino acid analysis

Several gels prepared at different times, some stained and others unstained, were used for amino acid analyses. The averaged results from 6 separate analyses are shown in Table 1, together with the standard errors. The agreement between the

Table 1. *Amino acid composition of components A and B of the* 20000 *mol. wt. protein band from the spasmoneme of Zoothamnium*

	Fast band (*A*)	Slow band (*B*)
Aspartic acid + asparagine	12·48 ± 1·24	11·30 ± 2·03
Threonine	7·95 ± 0·17	6·20 ± 0·94
Serine	15·05 ± 4·15	12·58 ± 2·25
Glutamic acid + glutamine	8·57 ± 1·09	7·01 ± 0·50
Proline	6·33 ± 1·47	5·83 ± 1·38
Glycine	9·32 ± 2·07	8·90 ± 2·08
Alanine	6·87 ± 0·89	6·99 ± 1·11
Valine	3·98 ± 0·47	4·15 ± 0·74
Cystine	0	0
Methionine	0	0
Isoleucine	2·82 ± 0·26	4·08 ± 0·53
Leucine	4·34 ± 0·41	4·92 ± 1·11
Tyrosine	3·89 ± 0·80	3·30 ± 0·76
Phenylalanine	1·95 ± 0·37	2·20 ± 0·74
Tryptophan	1·15 ± 0·23	1·77 ± 0·36
Lysine	6·95 ± 1·10	7·85 ± 1·35
Histidine	3·74 ± 0·71	7·63 ± 1·64
Arginine	4·59 ± 0·71	6·33 ± 2·42

The results are expressed as residues per 100 residues, ± S.E. Proline was determined separately from the other amino acids. Each value is the average of 6 determinations.

separate analyses is good for such small sample quantities. Considerable similarity was found between the compositions of components *A* and *B*. The sulphur-containing amino acids cystine and methionine appear to be totally absent. There is no obvious preponderance of hydrophobic over hydrophilic residues nor of acidic over basic. Aspartic acid and glutamic acid, which were not distinguished by the method of analysis from asparagine and glutamine, were found to be abundant, as was serine. There were no significant unknown peaks in the chromatogram.

Effect of calcium and magnesium ions on the electrophoretic mobility of the 20000 *mol. wt. proteins*

Because of the small quantities of material available, a direct assay of calcium binding could not be made. In order to test whether the 20000 mol. wt. proteins bind calcium, their electrophoretic mobilities were compared in low-calcium gels containing 1 mM EDTA and in gels to which 0·5 mM $CaCl_2$ had been added. It was convenient to have the separation gel at pH 8·0 in order to be able to use the discontinuous buffer system (see Ornstein, 1964), but this pH was not so high that it blocked the calcium-induced contraction of glycerinated preparations. The proteins were extracted in 3 M guanidine hydrochloride and dialysed and concentrated as before. Then further dialyses were carried out against 20 mM EDTA/Tris at pH 8·0 and distilled water, in order to reduce or eliminate bound calcium. After electrophoresis at pH 8 in a 15% acrylamide gel containing EDTA, a prominent leading band containing the 20000 mol. wt. material was evident. By comparison, the 20000 mol. wt. material in gels containing 0·5 mM $CaCl_2$ migrated much more slowly, while the relative mobility of other spasmonemal proteins remained the same. In order to define the range of calcium concentrations over which the change in electrophoretic mobility occurred, a series of experiments was conducted in which EGTA buffers were included in the gels. A uniform concentration of free calcium ions throughout the discontinuous gel system was required, so the calcium buffer composition was adjusted to compensate for the pH difference between the spacer gel (pH 6·8) and the separation gel (pH 8·0). By this method, gel systems were prepared with a uniform free calcium ion level of either 10^{-6} or 10^{-8} M. As in the experiments described in the previous paper, the calcium buffer concentration was varied so that the total concentration of calcium ([CaEGTA] + [Ca^{2+}]) was the same (1 mM) in the gel systems with high and low [Ca^{2+}]. During electrophoresis at pCa 8 the 20000 mol. wt. material formed a complex leading band consisting of a prominent peak with a trailing shoulder (see Fig. 1). In another calcium-buffered gel, also containing 1 mM total calcium, but with the free Ca^{2+} level increased to 10^{-6} M, the leading band was markedly retarded relative to other spasmoneme proteins which did not change their mobility. Also, the leading band became divided into 2 principal components at the higher Ca^{2+} level (Fig. 1).

To determine whether the observed decrease in electrophoretic mobility could be produced by magnesium ions also, similar experiments were conducted with magnesium-buffered gels. MgEDTA was used as the buffering species, since the dissociation constant of MgEGTA is too high (see Sillén & Martell, 1964). No reduction

in mobility occurred with up to 10^{-5} M Mg^{2+}. A decrease was, however, observed with Mg^{2+} added to the gel as a simple solution of magnesium chloride at 5×10^{-4} M.

DISCUSSION

Absence of actin and tubulin

The fact that actin could not be detected in the spasmoneme is clear evidence that the mechanism of contraction is different from that of muscle. During contraction, the spasmoneme exceeds striated muscle in power output (Amos, 1971) so it is unlikely that trace amounts of actin, or of any other protein, are responsible for the contraction. It is not surprising that tubulin also was absent from the electrophoretic pattern, since few microtubules have been found in the stalks of peritrichs. Most of them are in axoneme-like bodies distributed sparsely in the cytoplasm outside the spasmoneme (Amos, 1972; Allen, 1973), and probably lost when spasmonemes are isolated by dissection. Though it cannot be concluded that actin and tubulin are totally absent, neither protein is sufficiently abundant in the spasmoneme to be responsible for contraction. Our attention has therefore been directed to the major protein components, and in particular to the 20000 mol. wt. band.

Calcium binding

The 2 components *A* and *B* of the 20000 mol. wt. band both undergo a large reduction in electrophoretic mobility as the calcium ion level is raised from 10^{-8} to 10^{-6} M. This shows that they bind calcium with high affinity and that the binding occurs over the same range of calcium ion concentrations as contraction of glycerinated spasmonemes. The binding does not occur with magnesium ions at the same concentration, but some magnesium is bound at concentrations 500 times higher. This specificity suggests that the high-affinity binding site is the same as that involved in contraction of glycerinated preparations, which is neither induced nor blocked by magnesium, but is induced by calcium ions at 10^{-7} M. It is not known at present whether the reduction in electrophoretic mobility is due to an alteration in charge or in conformation, but it is a large effect, and seems likely to have some bearing on the primary transduction event in contraction.

Stoichiometry

Evidence was presented in the preceding paper (Routledge *et al.* 1975) that 1·7 g of calcium per kg of dry mass is taken up by the glycerinated spasmoneme during contraction. If it is assumed that all this calcium is bound to a protein with a molecular weight of 20000, which constitutes 40–60% of the total dry mass, the number of calcium ions bound per molecule is 1·4–2·1.

Spasmin

The *A* and *B* components both have molecular weights close to 20000, and similar though not identical amino acid compositions. How different their amino acid compositions are is not at present clear, since *A* and *B* were not completely separated

in the gels. We propose the name spasmin for this class of calcium-binding proteins, recognizing that the two species are not identical, and intending the name to be generic, like tubulin. The spasmins are acidic proteins of low molecular weight which bind a small number of calcium ions with high affinity. In these respects they resemble certain other calcium-binding proteins, such as parvalbumin (Kretsinger & Nockolds, 1973) and troponin C (Hartshorne & Pyun, 1971). However, the amino-acid composition of the spasmins is different from that of known muscle proteins, particularly in the lack of sulphur-containing residues.

The location of the spasmins in the ultrastructure of the spasmoneme is uncertain. However, assuming a protein density of 810 Daltons/nm^3 (Lake & Leonard, 1974) the diameter of a spasmin molecule would be 3·6 nm, which is consistent with a longitudinal spacing of 3·5 nm observed in negatively stained spasmoneme filaments (Amos, 1974). This, together with the abundance of spasmin in the organelle, suggests that the longitudinal filaments in the spasmoneme may consist of spasmin molecules joined together in chains. This arrangement would make possible a series summation of contractile events occurring independently in each molecule, such as might give rise to the high intrinsic shortening velocity of the spasmoneme. It is possible that the contraction is due entirely to the spasmin molecule, which may either change shape or alter its manner of bonding in the filament when calcium becomes bound to it. In the ciliate *Stentor* the shortening of the myonemes is apparently associated with a helical coiling of microfilaments (Huang & Pitelka, 1973). These filaments may well consist of chains of spasmin molecules and the coiling may be due to a change in the angle between adjacent molecules. Coiling of the microfilaments in vorticellid myonemes has not been observed, but cannot be eliminated at present.

It seems likely that spasmin will be found in the myonemes of heterotrich ciliates such as *Stentor* and *Spirostomum* which contract as rapidly as vorticellids (Jones, Jahn & Fonseca, 1970). The question of its occurrence in other cells must await better characterization of spasmin.

We thank Dr B. M. F. Pearse for a sample of purified pig brain tubulin and Dr J. Kendrick-Jones for rabbit skeletal muscle actin. We also thank the Science Research Council for supporting this work through a grant to Professor T. Weis-Fogh.

REFERENCES

ALLEN, RICHARD D. (1973). Structures linking the myonemes, endoplasmic reticulum and surface membranes in the contractile ciliate *Vorticella*. *J. Cell Biol.* **56**, 559–579.

AMOS, W. B. (1971). A reversible mechanochemical cycle in the contraction of *Vorticella*. *Nature, Lond.* **229**, 127–128.

AMOS, W. B. (1972). Structure and coiling of the stalk in the peritrich ciliates *Vorticella* and *Carchesium*. *J. Cell Sci.* **10**, 95–122.

AMOS, W. B. (1974). Contraction and calcium binding in the vorticellid ciliates. In *Molecules and Cell Movement*, Symp. at 28th A. Meet. Soc. gen. Physiol., Pacific Grove, California, New York: Raven Press (in Press).

AMOS, W. B. (1975). An apparatus for microelectrophoresis in polyacrylamide slab gels. *Analytic. Biochem.* (Submitted.)

HARTSHORNE, D. J. & PYUN, H. Y. (1971). Calcium binding by the troponin complex and the purification and properties of troponin A. *Biochim. biophys. Acta* **229**, 698–711.

HOFFMAN-BERLING, H. (1958). Der Mechanismus eines neuen, von der Muskelkontraktion verschiedenen Kontraktionszyklus. *Biochim. biophys. Acta* **27**, 247–258.

HUANG, B. & PITELKA, D. R. (1973). The contractile process in the ciliate *Stentor coeruleus*. I. The role of microtubules and filaments. *J. Cell Biol.* **57**, 704–728.

JONES, A. R., JAHN, T. L. & FONSECA, J. R. (1970). Contraction of protoplasm. III. Cinematographic analysis of the contraction of some heterotrichs. *J. cell. Physiol.* **75**, 1–7.

KRETSINGER, R. H. & NOCKOLDS, C. E. (1973). Carp muscle calcium-binding protein. II. Structure determination and general description. *J. biol. Chem.* **248**, 3313–3326.

LAEMMLI, U. K. & FAVRE, M. (1973). Maturation of the head of bacteriophage T4. I. DNA packaging events. *J. molec. Biol.* **80**, 575–599.

LAKE, J. A. & LEONARD, K. R. (1974). Structure and protein distribution for the capsid of *Caulobacter crescentus* bacteriophage ϕCbK. *J. molec. Biol.* **86**, 499–518.

MAHLER, H. R. & CORDES, E. H. (1966). *Biological Chemistry*, p. 118. New York and London: Harper & Row.

MATSUBARA, H. & SASAKI, P. B. (1969). High recovery of tryptophan from acid hydrolysates of protein. *Biochem. biophys. Res. Commun.* **35**, 175–181.

ORNSTEIN, L. (1964). Disc electrophoresis. I. Background and theory. *Ann. N.Y. Acad. Sci.* **121**, 321–342.

RANDALL, J. T. & HOPKINS, J. M. (1962). On the stalks of certain peritrichs. *Phil. Trans. R. Soc. Ser. B* **245**, 59–79.

ROTH, M. & HAMPAI, A. (1973). Column chromatography of amino acids with fluorescence detection. *J. Chromat.* **83**, 353–356.

ROUTLEDGE, L. M., AMOS, W. B., GUPTA, B. L., HALL, T. A. & WEIS-FOGH, T. (1975). Microprobe measurements of calcium binding in the contractile spasmoneme of a vorticellid. *J. Cell Sci.* **19**, 195–201.

SILLÉN, L. G. & MARTELL, A. E. (1964). *Stability Constants of Metal Ion Complexes*, special publication no. 17, pp. 634 and 697. London: The Chemical Society.

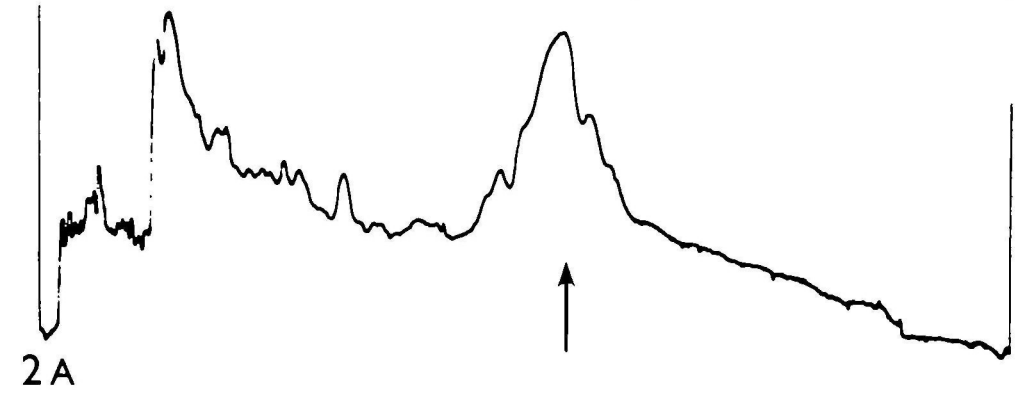
WEIS-FOGH, T. & AMOS, W. B. (1972). Evidence for a new mechanism of cell motility. *Nature, Lond.* **236**, 301–304.

WOODS, K. R. & WANG, K. T. (1967). Separation of dansyl-amino acids by polyamide layer chromatography. *Biochim. biophys. Acta* **133**, 369–370.

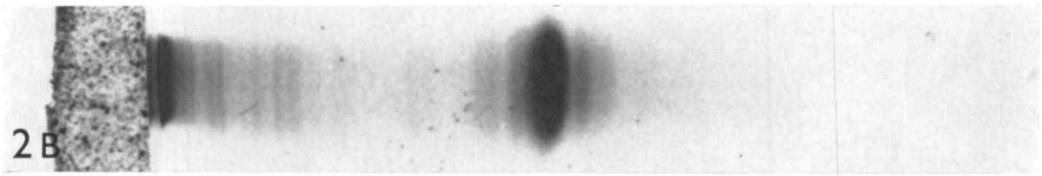
(*Received* 4 *March* 1975)

Fig. 2. A, densitometer tracing and B, photograph of a 15% polyacrylamide SDS microgel. The sample was a solution in SDS of 15 spasmonemes. The peak corresponding in position to a molecular weight of 20000 is indicated by an arrow. The granular material to the left of the separation gel is the remains of the stacking gel.

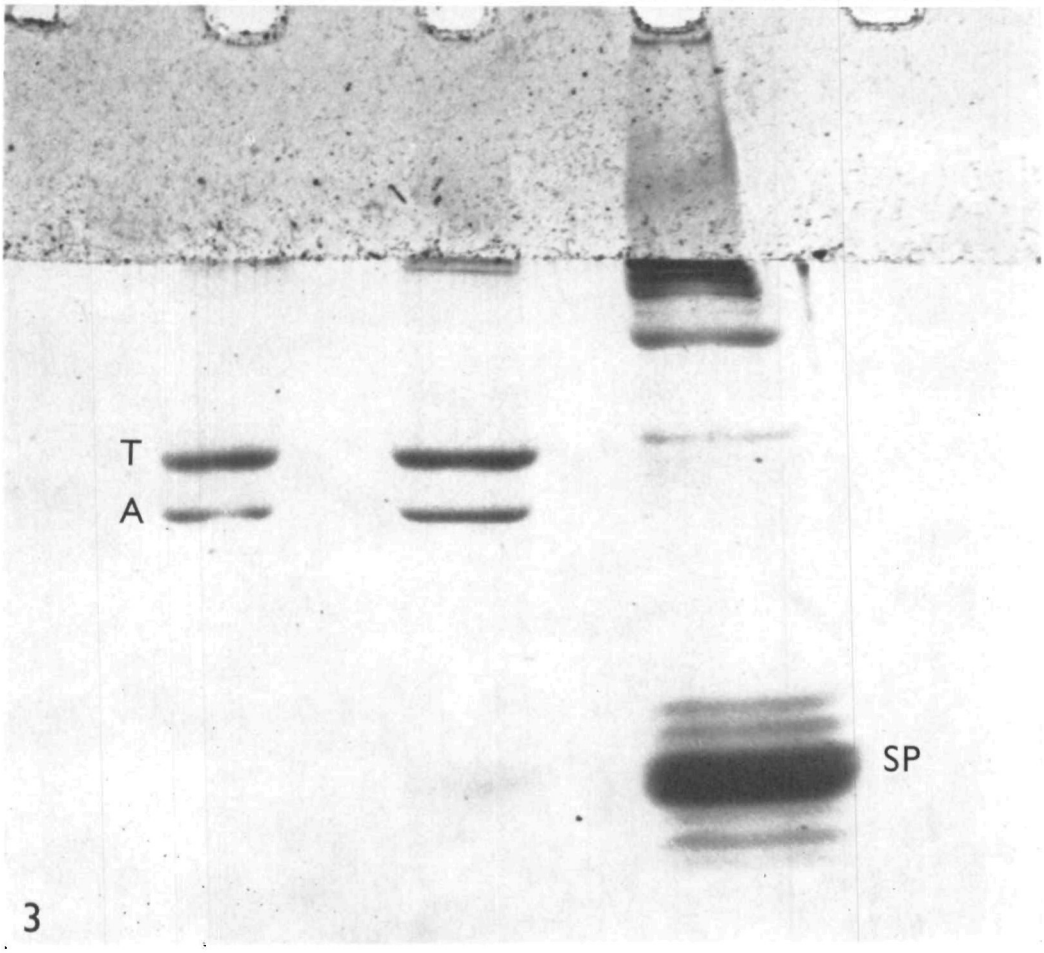
Fig. 3. Electrophoresis in a 15% polyacrylamide SDS microslab gel stained in Coomassie blue. Three samples were loaded: the right-hand one was an SDS extract of 10 *Zoothamnium* spasmonemes, the left-hand contained 0·2 μg each of tubulin (T) and actin (A), and the sample in the centre contained actin, tubulin and a small quantity of spasmoneme extract. No band in the electrophoretic pattern of the spasmoneme corresponds precisely to either tubulin or actin. The 20000 mol. wt. band (SP) is prominent.



2 A



2 B



3