IMMUNOCYTOCHEMISTRY OF THE ACELLULAR SLIME MOULD PHYSARUM POLYCEPHALUM

I. PREPARATION, MORPHOLOGY, AND RELIABILITY OF RESULTS CONCERNING CYTOPLASMIC ACTOMYOSIN PATTERNS IN SANDWICHED PLASMODIA

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SUMMARY

Small phaneroplasmodia of Physarum polycephalum migrate, under sandwich conditions between two agar sheets and a membrane of cellophane, as thin protoplasmic sheets. This method suitably simulates the situation in the natural habitat of acellular slime moulds; i.e. the narrow clefts of the forest soil. The highly differentiated system of cytoplasmic fibrils displayed under these conditions survives both long-term extraction with glycerol and fixation with methanol, procedures that remove the strong inherent autofluorescence, thus allowing the use of immunocytochemical studies. The complicated fibrillar system of sandwiched plasmodia consists of: (1) a membrane-associated cortical filament layer in the anterior region; (2) a more or less regular polygonal fibrillar network in the intermediate region; and (3) a helically twisted fibrillar system encircling endoplasmic pathways as well as isolated strands in the posterior region. So far, three different cytoskeletal proteins have been identified immunocytochemically as constituents of the fibrillar structures: actin, myosin and AM-protein (fragmin). No positive identification of α-actinin, filamin and tropomyosin was obtained using antibodies against vertebrate proteins. Electron microscopy of glycerol-extracted specimens treated with antibodies against actin and myosin revealed that the 6 nm filaments consist of actin, whereas the electron-dense material between single actin filaments appears to be myosin. The AM-protein modulating the polymer status of actin is located in all fibrillar structures.

INTRODUCTION

Acellular slime moulds, especially Physarum polycephalum, are classical objects in cell motility research (Kamiya, 1959; Komnick, Stockem & Wohlfarth-Bottermann, 1973; Wohlfarth-Bottermann, 1979). The motive force necessary for cytoplasmic streaming and thereby for the mass transport connected with the locomotion of the plasmodia is generated by a cytoplasmic actomyosin system (Wohlfarth-Bottermann, 1962, 1964). In large plasmodia, the fibrillar arrangement of this system reaches dimensions visible at the light-microscopical level (Wohlfarth-Bottermann, 1975; von Kortzfleisch, 1976). Furthermore, the size of P. polycephalum favours biochemical analysis of its contractile proteins (Vandekerckhove & Weber, 1978; Hatano &
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Oosawa, 1966a, b; Nachmias, 1979; Zechel & Weber, 1978). These advantages explain the wealth of results obtained for this organism (Wohlfarth-Bottermann, 1979; Kamiya, 1981; Kessler, 1982). The possibility of applying immunocytochemical techniques using fluorescent antibodies (Lazarides & Weber, 1974; Weber & Gröschel-Stewart, 1974) to the plasmodial system has been limited by the strong inherent autofluorescence and the thickness of the organism. However, the autofluorescence can be removed by extraction with glycerol (Wohlfarth-Bottermann, 1964; Kamiya & Kuroda, 1965; Achterrath, 1969) and the thickness can be decreased by sandwiching (Ogihara & Kuroda, 1979; Hülsmann, Haberey & Wohlfarth-Bottermann, 1974). The aim of the present study is to describe the effects of extraction with glycerol on the morphology of thin plasmodia (Ishigami, Nagai & Kuroda, 1981; Naib-Majani et al. 1982) and on the fine structure of the cytoplasmic actomyosin system (Wohlfarth-Bottermann & Stockem, 1982). Furthermore, the immunocytochemical localization of three different proteins within the fibrillar structures is shown at the light- and electron-microscopical levels.

MATERIALS AND METHODS

Preparation of translucent plasmodia

Small and thinly spread phaneroplasmodia (P. polycephalum, ATCC no. 44912) were prepared according to Kamiya & Kuroda (1965) using slight modifications (Naib-Majani et al. 1982). Square pieces (2 mm x 2 mm) were cut from the advancing front of migrating plasmodia and placed on a glass slide covered with a thin film (0.5 mm) of 1% Bacto-Difco agar in a moist chamber. During the initiation of locomotory activity, the protoplasmic mass spreads on the agar surface (Beylina et al. 1982). Subsequently, the small plasmodia were covered with a cellophane membrane and finally sandwiched with another sheet of agar (thickness 2 mm). After 6 h the specimens had reached a diameter of 1–2 cm (vertical thickness, only 10 μm) and were rapidly transferred to a cold 50% (v/v) glycerol solution containing 50 mM-KCl, 10 mM-EDTA and 10 mM-PIPES-buffer (pH 7.0) at 4°C. Plasmodia were extracted in this solution for at least 10 days at 4°C.

Immunocytochemical procedures

After 10–14 days extracted plasmodia were removed from the glycerol solution and immediately fixed in 100% methanol (at −10°C) for 15 min. After thorough washing in phosphate-buffered saline (PBS) without calcium and magnesium, the specimens were covered with 30 μl of a solution containing purified antibodies. The antibodies were raised in rabbits against: (1) dodecyl sulphate-denatured actin purified from chicken gizzard (Webster, Osborn & Weber, 1978); (2) dodecyl sulphate-denatured myosin heavy chain purified from chicken gizzard (Webster, Osborn & Weber, 1978) or myosin purified from P. polycephalum; (3) actin-modulating (AM) protein (fragmin; Hasegawa, Takahashi, Hayashi & Hatano 1980; Hinssen, 1981a, b) purified from P. polycephalum; (4) α-actinin purified from chicken gizzard (Webster et al. 1978); (5) filamin purified from chicken gizzard (Webster et al. 1978); and (6) tropomyosin purified from bovine brain (Webster et al. 1978). All antibodies were affinity-purified on the corresponding antigen isolated from the same source and coupled to Sepharose 4 B (Weber, Rathke, Osborn & Franke, 1976). They were used at a final concentration of 50 μg/ml. The fluorescein-labelled second antibody, goat anti-rabbit immunoglobulin G (IgG), was obtained from Miles-Yeda and used at a final concentration of 0.5 mg/ml. After incubation with the first antibody for 30 min (at 37°C) the specimens were washed thoroughly in PBS and treated with the second antibody as described. They were then mounted and examined under a Zeiss microscope equipped with an Olympus OM-10 miniature camera. Photographs were taken using Ilford film (FP-4 at a setting for 27 DIN).
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Control experiments were carried out with the second antibody, fluorescein-labelled goat anti-rabbit IgGs, without prior treatment with a specific antibody.

Preparation for light and electron microscopy

All essential steps during the immunocytochemical preparation, i.e., the status of the plasmodium under sandwich conditions, the effects of cooling and extraction with glycerol, as well as the effects of fixation with methanol and incubation with antibody, were checked individually by normal light and electron microscopy. Semithin and ultrathin sections from specimens fixed with 5% glutaraldehyde in 0.5 M cacodylate buffer (pH 6.8) or with a mixture of 1% OsO4 and 0.5% HgCl2 (pH 6.4) in distilled water and embedded in styrol methacrylate were cut on LKB ultrotomes. Light and electron microscopy of sections was performed with a Leitz-Ortholux-Orthomat on Ilford Pan F (15 DIN) and a Philips EM 200 on Agfa ortho 25 professional, respectively. Macrophotographs of total plasmodia were made with a Zeiss-Tessovar on Ilford Pan F (15 DIN).

Results

Influence of pretreatment

In surface-cultured plasmodia with diameters comparable to those of the sandwiched plasmodia, corresponding fibrillar patterns were not demonstrated because of the greater thickness of the specimens. Therefore, the question arises as to whether the patterns visualized by decoration with antibodies in extremely thin sandwiched plasmodia represent a system of cytoplasmic actomyosin comparable to that found in semi- and ultrathin sections of larger, surface-cultured plasmodia (Wohlfarth-Bottermann, 1975; von Kortzfleisch, 1976).

The following possibilities were considered in a series of control experiments, in which we analysed whether: (a) the polygonal fibrillar pattern is a result of sandwich conditions; (b) the pattern is a result of the cold treatment, before extraction with glycerol is accomplished and, finally; (c) whether the pattern forms during the initial phase of the extraction with glycerol.

We analysed step-by-step the influence of cold treatment and extraction with glycerol with the aid of normal light- and electron-microscopical techniques (semithin and ultrathin sections). The results demonstrate that the treatment of sandwiched specimens with ice-cooled water or 5–20 min does not lead to a recognizable increase in the number of fibrils, and that on the other hand, extraction with glycerol rather decreases the number of fibrils. As the fibrils are also present in sandwiched plasmodia that were released from the agar sheet and cellophane membrane for 1–5 h, the formation of the polygonal pattern does not seem to be a response of the plasmodium to the sandwich conditions (unpublished observations). Further investigations with the aim of proving the existence of a polygonal fibrillar pattern in surface-cultured specimens of higher thickness should be carried out with improved appropriate investigation techniques (compare Hülsmann et al. 1974).

The frequency of shuttle-streaming reversal is considerably decreased in sandwiched plasmodia as compared with uncovered specimens (average periods: uncovered = 1.3 min (Wohlfarth-Bottermann, 1977), sandwiched = 2.4 min). At present, we cannot conclude whether the higher mechanical pressure exerted by the agar sheet or a decreased oxygen supply (compare Sachsenmaier & Hansen, 1973), or
both effects, may be responsible for this decrease in frequency. We have to consider that both increased mechanical pressure and decreased oxygen tension prevail in the natural habitat compared to standard surface-culture conditions in the laboratory.

General morphology of sandwiched plasmodia

Thin plasmodia of *P. polycephalum* show a morphological organization characteristic of the nutritive stage of acellular slime moulds. Three different regions can be distinguished during locomotion (Figs 1, 3): (a) an anterior region represented by the leading edge; (b) an intermediate region characterized by a continuous protoplasmic sheet containing primarily differentiated endoplasmic pathways; and finally (c) a posterior region composed of single, i.e. isolated, protoplasmic strands (compare Naib-Majani et al. 1982). Extraction with glycerol over a time range of 10–14 days has no visible influence on the macroscopic morphology. A comparison of the same specimen before (Figs 1, 3) and after extraction with glycerol (Figs 2, 4) demonstrates the preservation of both the general shape and the plasmodial network of strands. The essential difference between the corresponding photographs taken at different stages of preparation is the increased transparency of the extracted specimens. The results from semithin sections (Figs 5–8) show that the increased transparency can be explained by the extraction of soluble cytoplasmic material during the course of treatment with glycerol. It is important to note that, in spite of this loss of soluble material, the general morphological organization, e.g. the topography of the plasmalemma invagination system (Fig. 5, asterisks), is not affected by treatment with glycerol (Fig. 6). The same is valid for the cytoplasmic actomyosin system (Figs 7, 8), which is partly in close connection with the plasmalemma invagination system (Wohlfarth-Bottermann, 1974, 1975). The subsequent fixation and dehydration in 100% methanol at −10°C for 15 min has no further damaging influence on the external and

Fig. 1. Survey picture of a multidirectionally migrating, living plasmodium grown between two sheets of agar and a cellophane membrane. Bar, 1 cm.

Fig. 2. The same plasmodium as shown in Fig. 1 after long-term extraction with glycerol (10 days; for details see Materials and Methods).

Fig. 3. Higher magnification of Fig. 1 showing a frontal plasmodial region. Bar, 0.1 cm.

Fig. 4. Identical region to that shown in Fig. 3, but after 10 days extraction with glycerol. Note that the general morphology of the plasmodium has not changed by the extraction, except for an increase in transparency.

Fig. 5. Longitudinal semithin section through a plasmodial control strand showing the organization of the plasmalemma invagination system (asterisks). Phase-contrast, glutaraldehyde fixation. Bar, 50 μm.

Fig. 6. A plasmodial strand of corresponding size to that shown in Fig. 5 but after 10 days of extraction with glycerol. The general morphology, as judged from the preservation of the plasmalemma invaginations, is not obviously affected by the extraction. Phase-contrast, glutaraldehyde fixation.

Fig. 7. Cytoplasmic actomyosin fibrils (arrows) in a control strand. Phase-contrast, glutaraldehyde fixation. Bar, 25 μm.

Fig. 8. Cytoplasmic actomyosin fibrils in a strand after 10 days extraction with glycerol. Phase-contrast, glutaraldehyde fixation.
internal morphology of the glycerol-extracted plasmodia (results not shown; for fine structural results see Figs 21–24).

**Differentiation of the fibrillar cytoplasmic actomyosin system in sandwiched plasmodia**

Sandwich conditions, i.e. plasmodia migrating between an agar sheet and a cellophane membrane, simulate the environmental situation of the natural biotope of this organism much better than the conventional laboratory method of keeping the plasmodia on agar or filter paper, i.e. uncovered and with direct contact to the atmosphere.

Immunofluorescence microscopy using actin antibodies revealed a well-developed fibrillar actin pattern. The anterior and intermediate plasmodial regions, especially, displayed a highly differentiated fibrillar arrangement (Figs 9–11). A cortical actin filament layer (Fig. 11, cc) in close contact with the anterior plasma membrane is continuously connected, more posteriorly, with a more or less regular polygonal fibrillar network, which is constructed of nodes interconnected by struts (Figs 9–11). Short fibrillar segments located, predominantly, within or adjacent to the nodes are not decorated by the actin antibody (Fig. 9, arrow) and reveal electron-dense material intimately intermingled with actin filaments (Fig. 11, arrow; compare Götz von Olenhusen, Jücker & Wohlfarth-Bottermann, 1979). The chemical nature of this electron-dense material is not known.

**Decoration of different cytoskeletal proteins by antibodies to actin, myosin and AM-protein (fragmin)**

A total of seven rabbit antibodies were used in antigen affinity-purified form. Five were directed against vertebrate cytoskeletal proteins (actin, myosin, tropomyosin, α-actinin, filamin) and two against *Physarum* proteins (myosin, AM-protein). In addition, a rabbit serum prepared against *Physarum* actin was used. Positive specific reaction was found for actin (Fig. 12), myosin (Fig. 13) and AM-protein (Fig. 14). These proteins are identified in the fibrillar system of all plasmodial regions including endoplasmic pathways. Especially in single (isolated) strands of the posterior region, actin, myosin and fragmin (Figs 12–14) are constituents of the fibrils organized as a helically twisted system. This system is composed of diagonal fibrils, surrounding the endoplasmic pathways, and single strands. The diagonally running fibrils originate from longitudinal fibrils running parallel to the outer plasma membrane in the ectoplasm.
Figs 9–11
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The other antibodies, i.e. anti-α-actinin (Fig. 15), anti-filamin (Fig. 17) and antitropomyosin (not shown), gave no fibrillar staining but resulted in a weak, more or less uniform, background fluorescence over the entire plasmodium indicating that the reaction was non-specific. The subsequent additional staining with anti-actin (Figs 16, 18) demonstrated that these antibodies did not react specifically with the fibrillar system. Control experiments staining the frontal region (Fig. 19) or single plasmodial strands (Fig. 20) of sandwiched plasmodia with the second antibody, fluorescein-labelled goat anti-rabbit IgGs, without prior treatment with a specific antibody to the different fibrillar proteins revealed no distinct fluorescence.

Electron-microscopical localization

The fibrillar system developed in the different plasmodial regions is ultrastructurally characterized by parallelly oriented 6 nm filaments. Under isometric conditions (see Fleischer & Wohlfarth-Bottermann, 1975; Wohlfarth-Bottermann, 1979, and references therein) the filaments in unextracted cells are in parallel arrangement and interconnected by an electron-dense material, often arranged at distances of approximately 14 nm (Fig. 21, arrowheads; Wohlfarth-Bottermann & Stockem, 1982). A very similar ultrastructural appearance of the fibrils is observed after long-term glycerol extraction (Fig. 22). Characteristic changes as a consequence of extraction with glycerol are, however: (1) a loss of soluble cytoplasmic components; and (2) the appearance of fibrillar regions containing small filamentous aggregates (Fig. 22, arrows), probably due to an aggregation of actin filaments (compare Figs 22 and 23, arrows) during extraction with glycerol. The decoration of glycerol-extracted fibrils with actin antibody provides clear ultrastructural evidence for the actin nature of the 6 nm filaments (Fig. 23). The actin antibody is deposited circumferentially from all sides along the filaments. Due to the approximate size of the diameter of an antibody

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Fig. 12. Immunocytochemical demonstration of actin in the diagonally running fibrillar system of a single plasmodial strand in the posterior region after staining with actin antibody. Bar, 100 μm.

Fig. 13. Immunocytochemical demonstration of myosin in the fibrillar system of a plasmodial strand in the posterior region after staining with myosin antibody. Bar, as Fig. 12.

Fig. 14. Immunocytochemical demonstration of AM-protein (fragmin) in the fibrillar system of a plasmodial strand in the posterior region after staining with AM-protein antibody. Bar, as Fig. 12.

Fig. 15. Immunocytochemical demonstration of α-actinin in a plasmodial strand after staining with α-actinin antibody. Note the absence of typical fibrillar staining and a general weak fluorescence, probably indicating non-specific background fluorescence. Bar, as Fig. 12.

Fig. 16. Same region as that shown in Fig. 15 after staining with α-actinin antibody followed by staining with actin antibody. Note the localization of actin in a fibrillar pattern, in contrast to α-actinin. Bar, as Fig. 12.

Fig. 17. Immunocytochemical demonstration of filamin in a plasmodial strand after staining with filamin antibody. Bar, as Fig. 12.

Fig. 18. Same region as that shown in Fig. 17 after staining with filamin antibody followed by staining with actin antibody. Note the localization of actin in a fibrillar pattern, in contrast to filamin. Bar, as Fig. 12.
Fig. 19. Control experiment using the second antibody, fluorescein-labelled goat anti-rabbit IgGs, without prior treatment with a specific antibody. The polygonal fibrillar network in the front region is not stained. Bar, as Fig. 20.

Fig. 20. Control experiment using the second antibody, fluorescein-labelled goat anti-rabbit IgGs, without prior treatment with a specific antibody. The diagonally running fibrillar system in a single plasmodial strand is not stained. Bar, 100 μm.

Fig. 21. Detail of the fine-structural organization of a cytoplasmic actomyosin fibril after conventional glutaraldehyde fixation. Arrowheads point to cross-bridges between neighbouring actin filaments. Bar, as Fig. 24.

Fig. 22. Detail of the fine-structural organization of a cytoplasmic fibril after long-term extraction with glycerol and fixation in glutaraldehyde. Arrows point to filamentous aggregates. Bar, as Fig. 24.

Fig. 23. Detail of the fine-structural organization of a cytoplasmic fibril after long-term extraction with glycerol, fixation in glutaraldehyde, and subsequent treatment first with rabbit actin antibody and then with goat anti-rabbit IgGs. Note the increased thickness of filaments. Arrowheads point to short filament segments not decorated by the antibody sandwich. Arrows point to filamentous aggregates (compare with Fig. 22). Bar, as Fig. 24.

Fig. 24. Detail of the fine-structural organization of a cytoplasmic fibril after long-term extraction with glycerol, fixation in glutaraldehyde, and subsequent treatment first with rabbit myosin antibody and then with goat anti-rabbit IgGs. The antibody is localized between neighbouring actin filaments (arrowheads, compare with Fig. 21). Bar, 50 nm.
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Figs 21–24
molecule of close to 9 nm, the thickness of the decorated 6 nm actin filaments increases to nearly 42 nm (6 nm + (4 × 9) nm; see Fig. 23). This is especially obvious in short filamentous segments not decorated with actin antibody (Fig. 23, arrowheads).

A quite different pattern of ultrastructure and distribution was obtained when glycerol-extracted fibrils were decorated with myosin antibody (Fig. 24). The myosin antibody is located between the parallel arrays of the 6 nm filaments at the same distance (Fig. 24, arrowheads) as the electron-dense material in undecorated specimens (compare Fig. 21, arrowheads). This indicates that the cross-bridges already described in another study (Wohlfarth-Bottermann & Stockem, 1982) represent myosin. Because of their short length (25 nm) they represent an oligomeric aggregation stage.

**DISCUSSION**

**Reliability of the applied method**

There is no doubt, that the cytoplasmic actomyosin fibrils present in semithin sections of large plasmodial strands show a weak birefringence in polarized light, even after osmium fixation (Wohlfarth-Bottermann, 1964). Weak birefringent phenomena, sometimes also observable in living sandwiched plasmodia, are very similar to the pattern of immunofluorescence of the same probes. However, when interpreting birefringence patterns in plasmodia (Nakajima, 1964; Cieslawska & Grebecki, 1978; Hülsmann et al. 1974; Ishigami et al. 1981; Kamiya, 1981), one has to consider that the actomyosin fibrils regularly follow the plasmalemma invagination system, which is filled with excreted slime (Wohlfarth-Bottermann, 1975). This slime is birefringent, as can be seen on the slime traces left behind a locomoting plasmodium. Because, within the plasmalemma invaginations, slime filaments and actomyosin sheets run in the same direction (von Kortzfleisch, 1976) and are geometrically related and in close neighbourhood, it is very difficult to discriminate between birefringent phenomena resulting from F-actin bundles, on the one hand, and from slime filaments, on the other hand. Both probably contribute to the observed optical phenomena. Appearance and disappearance of birefringence in temporal coincidence with the contraction—relaxation cycle (Nakajima, 1964; Kamiya, 1981) does not allow us to conclude that the birefringence results solely from the actin dynamics (Nagai, Yoshimoto & Kamiya, 1978; Götz von Olenhusen & Wohlfarth-Bottermann, 1979; Wohlfarth-Bottermann & Isenberg, 1976; Wohlfarth-Bottermann & Fleischer, 1976), because the plasmalemma invaginations (as insertion points of the actomyosin system) show temporally corresponding dynamics (Gawlitta, Wolf, Hoffmann & Stockem, 1980). This important fact means that the slime organization of the invaginations also follows (passively) these movements, thus potentially producing or adding to changes in birefringent aspects, depending on the contraction cycle of the plasmodial strands. In our opinion, it is difficult to differentiate between slime-filament birefringence and actin-filament birefringence, irrespective of the thickness of the specimens, if abundant plasmalemma invaginations are present in the corresponding plasmodial regions. Thus the immunofluorescence method not only offers
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a high cytochemical specificity for the cytoplasmic fibrillar system, but also provides a higher topographical resolution as well as higher reliability than birefringence optics.

A close correlation of certain birefringence phenomena and fibrillar structures was only gained by Ishigami et al. (1981), by combining polarized light- and electron-microscopical analysis of thin-spread plasmodia. The lack of abundant plasmalemma invaginations in thin-spread protoplasmic sheets excludes the objections discussed above. Thus, under sandwich conditions, polarized light can be used for the study of the dynamic behaviour of the fibrils (Ishigami et al. 1981). Combined birefringence and immunofluorescence studies should contribute to the functional analysis of different cytoplasmic actomyosin patterns in thin-spread plasmodia in areas without plasmalemma invaginations.

The immunocytochemical results prove that the fibrils forming the elaborate filament pattern seen by light- and electron-microscopy in sandwiched plasmodia contain the two main contractile proteins, actin and myosin. This means, that the fibrillar patterns found in small sandwiched plasmodia are identical in nature and probably also in function to the actomyosin fibrils known from larger plasmodial strands. Electron-microscopical studies performed by Ishigami et al. (1981) led these authors to the same conclusion. Because extraction with glycerol does not destroy the main light-microscopical cellular architecture (Figs 3-4, 5-6, 7-8), the preparation is reliable and the resulting fibrillar pattern can be used as a model to study the localization of proteins in this system and in other plasmodial structures of Physarum. Because it is the aim of this paper to consider the technical problems of and future possibilities for immunocytochemistry in this system, a more detailed functional discussion, especially of the polygonal patterns (compare Gordon & Bushnell, 1979; Ireland & Voon, 1981; Rathke, Osborn & Weber, 1979; Lazarides, 1976; Hämmerli, Strub, Jockusch & Sträuli, 1982), will be given elsewhere.

Ultrastructural localization of antibodies

Previous studies have shown that extraction with glycerol, in principle, allows a preservation of cytoplasmic actomyosin fibrils in Physarum (Alléa, Beck & Wohlfarth-Bottermann, 1971), and that the glycerol-extracted fibrils are capable of contracting under appropriate conditions (Achterrath, 1969; Komnick, Stockem & Wohlfarth-Bottermann, 1970; Alléa & Wohlfarth-Bottermann, 1972). Figs 21 and 22 demonstrate that most of the parallel arrangement of F-actin filaments within the fibrils is maintained after long-term extraction with glycerol. The ultrastructurally different antibody decoration pattern observed after incubation with antibodies to actin (Fig. 23) and myosin (Fig. 24) are in agreement with the current view that most of the filaments represent actin, and that myosin is arranged at distances of approximately 14 nm between the parallel F-actin filaments (Wohlfarth-Bottermann & Stockem, 1982). Obviously, the myosin is present only in an oligomeric aggregated stage (Alléa & Wohlfarth-Bottermann, 1972). The ultrastructural distribution of myosin antibodies within a fibril (Fig. 24) indicates the absence of thick myosin filaments and contributes to the mechanism of interaction of the two proteins.
Advantages, possibilities and shortcomings

Our results show the possibility of analysing in detail the essential components of cytoplasmic actomyosin fibrils of Physarum in situ using immunocytochemical techniques. Such investigations should provide results complementary to biochemical techniques and contribute to an improved interpretation of the function of the cytoplasmic actomyosin system. At least under the preparative conditions used, it is possible to obtain a complete overview of the system ranging from the macroscopic (Figs 1, 2) to the supramolecular level (Figs 21-24). Thorough and stepwise control of the applied preparation procedures, i.e. from cold treatment to extraction with glycerol, fixation, incubation with antibody and the ultrastructural localization of the antibodies, revealed that Physarum plasmodia are now accessible as a reliable model system for immunocytochemical studies. The following remaining shortcomings must be mentioned:

(1) Without release of the sandwich, small plasmodia are spatially less accessible for experimental assays (e.g. amputation, tensiometry etc.) as compared with large uncovered plasmodia.

(2) It should also be kept in mind that the structures stained in the glycerol-extracted models reflect only some of the respective proteins in the living plasmodia, since a large proportion of these proteins (especially actin and fragmin) seems to exist in soluble form in the cytoplasm and are extracted during the preparation. Hence, the model system used can be regarded only as a cytoskeleton or even as reflecting only the most stable part of the general cytoskeleton, that is able to resist the glycerol-extraction procedure.

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