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Final Report

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Henry R. Henney, Jr.

Abstract

A method was established for the isolation and purification of nuclei in high yield from the microplasmodia of Physarum flavicomum. Purified nuclei were resistant to breakage by methods commonly employed for isolated plant and animal nuclei. Incubation of nuclei with 5 mM Dithiothreitol at pH 9.2 was found to be the simplest and most effective method for breaking the nuclei. Several methods for the extraction of nuclear protein were compared. Incubation of nuclear lysates with either 2 M NaCl, with or without 5 M urea, or 1 M $CaCl_2$ resulted in the extraction of nuclear actin together with histones. The histones were chemically fractionated into the 5 basic groups common to other eucaryotic tissue. Amino acid analyses of the total histone were also performed. Nuclear actin was found to have a molecular weight of 41,000 ± 4,000 daltons as determined by SDS polyacrylamide gel electrophoresis. The amino acid composition of the nuclear actin was established.

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Introduction

The myxomycete *Physarum flavicomum* is a eucaryotic organism with a life cycle exhibiting alteration of generations. The diploid phase consists of a motile syncytial plasmodium, characterized by a natural synchronous nuclear division (Cheung *et al.*, 1974; Gray and Alexopoulos, 1968). Large quantities of the plasmodia can be obtained by growth in pure culture in synthetic media (Henney and Henney, 1968). Thus, the organism provides a unique opportunity for the analysis of the replication and expression of the eucaryotic genome.

As the initial step for such studies, we have established a method for the preparation of purified nuclei in high yield from the plasmodia. In addition, nuclear proteins have been extracted and fractionated and some properties of the major protein classes established.

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Materials and Methods

Organism

Diploid microplasmodia of Physarum flavicomum variety 1 were grown in our semidefined medium (Henney and Henney, 1968) in 9 liter glass bottles as previously described (Teague and Henney, 1973). Microplasmodia were subsequently allowed to settle from the growth medium for 10 to 15 min at 4°C, most of the medium was poured off and the cells collected by centrifugation at 2,000 x g for 5 min at 2°C. The cells were washed by suspension and centrifugation in two volumes of 0.01 M Tris-HCl, pH 7.2 containing 0.25 M sucrose and 0.01 M EDTA.

Isolation of nuclei

The washed packed cells were immediately suspended in 0.01 M Tris-HCl, pH 7.2 containing 0.25 M sucrose, 0.01% Triton X-100 and 0.005 M $CaCl_2$ and homogenized for 2 min at high speed in a Waring blender. All procedures were performed at 4°C. The homogenate was filtered through two thicknesses of milk filters (Rapid Flow, single gauze-faced, Johnson and Johnson Co.) previously saturated with homogenization medium. The filtrate was diluted to 2½ times the original packed cell volume and refiltered. The filtrate in 100 ml quantities was dispensed into round bottom polycarbonate bottles (250 ml) and underlaid with 50 ml quantities of 0.01 M Tris-HCl, pH 7.2 containing 1 M sucrose, 0.01 Triton X-100 and 0.005 $CaCl_2$. The bottles were centrifuged at 1,000 x g for 10 min in a refrigerated swinging bucket rotor. The yellowish sediment was resuspended in 1/2 volume of homogenization medium and blended for 1 min. The nuclear suspension was underlaid with 1/2 volume of the 1 M sucrose solution and the

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centrifugation repeated.

The sedimented nuclei were suspended in twice the original packed cell volume of wash medium containing 0.25 M sucrose, 0.02 M EDTA, 0.5 M KCl and 0.01 M Tris-HCl with a final pH of 6.3. The nuclear suspension was blended at low speed for 1 min and centrifuged at 2,000 x g for 5 min. The washing was repeated about twice more until microscopic examination, after staining with 0.1% Azure C in 0.25 M sucrose, revealed a purity of at least 95%. The concentration of nuclei was determined using a hemacytometer.

Breakage of nuclei

Purified nuclei were virtually unaffected by the following treatments: repeated freezing and thawing; suspension in distilled water or 0.2 mM EDTA, pH 7.2; homogenization in a blender or Dounce homogenizer in hypotonic solutions; or grinding with glass powder or alumina for 30 min. Sonication for 20 min, repeated grinding with dry ice, or passage through a French press at 10,000 psi produced about 70% breakage. A Parr Cell Disruption Bomb at 2,000 psi or incubation with pancreatic lipase for 30 min at pH 8.0 produced about 50% breakage. However, incubation of the nucleic with 5 mM Dithiothreitol (DTT) at pH 9.2 at room temperature for 30 min produced 100% breakage.

Chromatin preparation

The viscous nuclear solution after DTT incubation was dialyzed against 50 volumes of 0.2 mM EDTA, pH 7.2 at 4°C with two additional changes over a period of 24 h. This crude chromatin was centrifuged at 54,000 x g for 15 min at 4°C. The clear viscous material on top was designated as "free chromatin" and the gelatinous opaque material at the bottom was referred to as the "chromatin pellet".

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Extraction of chromatin proteins

Three methods of extraction of proteins from chromatin were used. The first method was the extraction of true chromatin with 2 M NaCl or 2 M NaCl and 5 M urea (recrystallized from 95% ethanol). The solution was stirred in the cold for 30 min, centrifuged at 30,000 x g for 18 h at 4°C and the supernatant containing the soluble proteins was dialyzed for 24 h against distilled water and lyophilized.

The second method extracted the chromatin pellet or true chromatin with 1 M $CaCl_2$ for 24 h at 4°C. After clarification by centrifugation at 50,000 x g for 15 min, a solution of trichloroacetic acid (TCA) was added to produce a 25% concentration, followed by incubation at 4°C for 2 h. After centrifugation at 50,000 x g for 15 min, the precipitate was suspended in cold 0.04 N H_2SO_4 by use of a glass homogenizer. After dialysis against 100 volumes of 0.04 N H_2SO_4 for 24 h at 4°C, the suspension was centrifuged at 50,000 x g for 15 min. The supernatant was dialyzed in the cold against 100 volumes of distilled water and lyophilized.

The third method was the direct extraction of true chromatin or the chromatin pellet with 0.4 N H_2SO_4 for 30 min at 4°C. The sample was centrifuged at 35,000 x g for 15 min, the supernatant saved and the sediment re-extracted. The combined supernatants were dialyzed against distilled water and lyophilized.

Column chromatography

Sephadex G-100 (fine) and G-75 (medium) columns (9 mm X 220 cm) were used at room temperature in the presence of 0.02% sodium azide. Proteins were eluted with 0.1 N HCl at a constant flow rate of 6 ml/h. Fractions were monitored for protein at 230 nm using a spectrophotometer.

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Electrophoresis

Polyacrylamide gel electrophoresis according to Bonner, et al., (1968) was used to monitor basic nuclear proteins. The separating gels contained 7.5% acrylamide and 6.25 M urea. The buffer was 3.2% ß-alanine and 0.08% acetic acid, pH 4.4 and a charge of 4 ma/gel was applied for 1.5 h. Basic proteins were separated from actin by preparative electrophoresis in 2.2 x 3 cm gel tubes for 5 h - the basic proteins were collected in a dialysis bag. SDS polyacrylamide gel electrophoresis with 10% acrylamide was used to determine the molecular weight of actin (Weber and Osborn, 1969).

Gels were stained for protein in 0.1% Amido Black in 7% acetic acid for 30 min, and destained in a solution of 7% acetic acid - 10% ethanol. Densitometric tracings were prepared with a Gilford linear transport apparatus at 575 nm.

Chemical analyses

Analyses for protein and RNA were as described previously (Henney and Jungkind, 1969). DNA was determined with diphenylamine (Burton, 1968).

Amino acid analyses

Proteins were hydrolysed and amino acid analyses performed as previously described (Henney and Jungkind, 1969). In addition, spectrophotometric analyses for tyrosine and tryptophan were performed according to Bencze and Schmid (1957).

Chemical fractionation of whole histone

The procedure of Oliver et al. (1972) was used to fractionate [illegible] fraction I (very lysine rich), fraction II (slightly lysine rich), III (very arginine rich), IIb1 (slightly lysine rich), and IV (slightly arginine rich).

Electron microscopy

Nuclei were fixed with 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.4, followed by 1% osmium tetroxide in the phosphate buffer. Other procedures were according to Choung et al. (1974).

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Results

Purified nuclei

The purified nuclei were about 5 μ in diameter (4 to 6 μ range) and the nucleoli occupied about half the nuclear volume. About 1% of the nuclei had two nucleoli. The nuclear membrane was quite elastic since many methods attempted to produce breakage were unsuccessful but did produce volume changes and distortions of the nuclei. The yield of purified nuclei was about 4×10^8 nuclei/gram wet weight of microplasmodia. The composition of the nuclei was (picogram/nucleus ± standard deviation): protein 10.50 ± 0.50, RNA 1.59 ± 0.21 and DNA 0.84 ± 0.05.

Figures 1 and 2 present photomicrographs and electron micrographs, respectively, of the nuclear preparations, illustrating the purity and integrity of the organelles.

Chromatin preparation

Regardless of the method employed to break the purified nuclei, the resultant true chromatin (the clear gelatinous supernatant after the 54,000 x g centrifugation) had a typical $\frac{A260}{A280}$ of about 1.72 with negligible turbidity (i.e., A320 of about 0.04). In addition, the true chromatin represented about 75% of the total nuclear protein.

Nuclear protein extraction

A nuclear protein preparation should have an $\frac{A260}{A280}$ of less than 1 indicating negligible contamination with nucleic acid. Extraction of true chromatin or the chromatin pellet with the following

M NaCl; 2 M NaCl + 5 M urea; 1 M $CaCl_2$; and 0.4 N H_2SO_4. However, extraction with 1 M $CaCl_2$ followed by TCA precipitation and re-extraction with

0.04 N H_2SO_4 eliminated the nucleic acid contaminations and resulted in an $\frac{A260}{A280}$ of about 0.85. Direct extraction of nuclei with 0.04 N H_2SO_4 also produced a clear basic nuclear protein preparation but the protein yield was less than 1%. Table 1 summarizes the nuclear protein yields using various extraction reagents. It is clear that the reagents which extracted the most nuclear protein(NaCl + or - urea) also extracted the most non-histone protein. The latter protein (which we later identified as actin) created a problem in that it collected at the top of the gel during pH 4.4 disc gel electrophoresis. We could eliminate this protein from the histone preparation by preparative electrophoresis and by gel filtration chromatography. Using the latter procedure the actin protein eluted before the histones with a high degree of purity. However, the gel filtration chromatography did not sufficiently separate the histones into their fractions without cross contamination.

Figure 3 presents a tracing of the total histone fraction separated by gel electrophoresis at pH 4.4. The actin protein fraction was eliminated prior to electrophoresis. Table 2 presents the amino acid compositions of the total histone and non-histone (actin) proteins. Tryptophan was not detected in either protein. SDS gel electrophoresis of the nuclear actin revealed it to have a molecular weight of 41,000 ± 4,000 daltons (Figure 4).

The gel electrophoresis scans presented in Figure 5 finely illustrate the separation of the total histone fraction into the individual fractions by the procedure.

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Discussion

P. flavicomum nuclei contain histones which can be fractionated into the same 5 basic groups as are found in all plant and animal tissues (DeLange and Smith, 1971). Amino acid analyses also reveals a similarity in composition to the histones of Tetrahymena pyriformis (Iwai et al., 1965) and P. polycephalum (Bradbury et al., 1973). This is not surprising since histones have been found to be conserved during the course of evolution (DeLange and Smith, 1971, 1972). Some differences in properties and functions of histones can be accounted for by modification of amino acid side chains by acetylation, methylation, oxidation-reduction and phosphorylation. Other differences in histones which can occur between tissues is the relative quantity of each histone fraction. Histones are involved in regulating the conformation as well as the activity of DNA (DeLange and Smith, 1971; Skidmore and Walker, 1973). As organisms become more complex in structure and function, the relative quantities of the various fractions apparently varied rather than their chemical composition since the latter would be less tolerated.

Another essential protein found in all living cells which is also highly conserved is actin (Clarke and Spudich, 1977; Korn, 1978). This study has demonstrated the occurrence of actin as a major protein in the nuclei of P. flavicomum. The molecular weight and amino acid analysis of the nuclear actin are similar to that occurring in the cytoplasm of P. polycephalum plasmodia as well as other cells (Adelman and Taylor, 1969; Adelman, 1977; Hatano and Oosawa, 1966b; Hatano and Onnuma, 1970; Goldman et al., 1976). Early studies on cytoplasmic actin of P. polycephalum

(Hatano and Oosawa, 1966; Hatano and Ohnuma, 1970) showed that the protein was easily extracted with a neutral salt solution. In this study we found that nuclear actin is also readily extracted together with histones by $NaCl_2$ (with and without urea) or $CaCl_2$.

The presence of actin in the nucleus may explain the plasticity, stability and resistance to breakage of isolated diploid nuclei. The contractile protein may be associated with the nuclear membrane, thus giving the membrane a flexible nature to accomodate stress. It is also noteworthy that mitosis in plasmodia is intranuclear in that the nuclear membrane remains intact instead of disintegrating (Gray and Alexopoulos, 1968). Only incubation at pH 9.2 and room temperature with DTT, which has been used to disrupt membranes of other organisms (Epel et al., 1970), was found to achieve complete nuclear and nucleolar breakage.

Actin probably plays other roles in the cell nucleus. Actin may be necessary for the constriction of the nuclear membrane during telophase in intranuclear mitosis. There is also some evidence for the involvement of actin in the movement of chromosomes during mitosis and meiosis (Goldman et al., 1976). In addition, nuclear actin may be associated with chromatin condensation (Goldstein et al., 1977). An understanding of these complex nuclear events may well depend on further characterization of this universally occurring contractile protein.

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References

ADELMAN M. R. 1977. Physarum actin. Observations on its presence, stability, and assembly in plasmodial extracts and development of an improved purification procedure. Biochemistry 16 4862-4871.

ADELMAN M.R. and Taylor, E.W. 1969. Further purification and characterization of slime mold myosin and slime mold actin. Biochemistry 8 4976-4987.

BENCZE W.L. and Schmid K. 1957. Determination of tyrosine and tryptophan. Analyt. Chem. 29 1193-1196.

BONNER J., Chalkley G.R., Dahmus M., Fambrough D., Fujimura F., Huang R.C., Huberman J., Jensen R., Marushige K., Ohlenbusch H., Olivera B. and Widholm J. 1968. Isolation and characterization of chromosomal nucleoproteins. In Methods in Enzymology. 12B. Edited by L. Grossman and K. Moldave. pp. 1-65. Academic Press.

BRADBURY E.M., Inglis R.J. Matthews H.R. and Sarner N. 1973. Phosphorylation of very lysine-rich histone in Physarum polycephalum. Eur. J. Biochem. 33 131-139.

DeLANGE R. J. and Smith E.L. 1972. The structures of histones. Acc. Chem. Res. 5 368-373.

EPEL D., Weaver A.M. and Mazia D. 1970. Methods for removal of the vitelline membrane of sea urchin eggs. 1. Use of Dithiothreitol. Exp. Cell Res. 61 64-67.

GOLDMAN R., Pollard T. and Rosenbaum J. 1976. Cell motility. Cold Spring Harbor Conference on Cell Proliferation 3 1-1373.

GOLDSTEIN L., Ko C. and Errick J. 1977. Nuclear actin: an apparent association with condensed chromatin. Cell Biol. Intern. Reports 1 511-515.

GRAY W.D. and Alexopoulos C.J. 1968. Biology of the myxomycetes. The Ronald Press Co., N.Y.

HATANO S. and Ohnuma J. 1970. Purification and characterization of myosin A from the myxomycete plasmodium. Biochim. Biophys. Acta 205 110-120.

HATANO S. and Oosawa F. 1966. Isolation and characterization of plasmodium actin. Biochim. Biophys. Acta 127 488-498.

HENNEY H.R. JR. and Henney, M. R. 1968. Nutritional requirements for the growth in pure culture of the myxomycete Physarum rigidum and related species. J. Gen. Microbiol. 53 333-339.

HENNEY H.R. JR. and Jungkind D. 1969. Characterization of ribosomes from the myxomycete Physarum rigidum grown in pure culture. J. Bacteriol. 98 249-255.

IWAI K., Shiomi H., Ando T. and Mita T. 1965. The isolation of histone from Tetrahymena. J. Biochem. 58 312-314.

KORN E.D. 1978. Biochemistry of actomyosin-dependent cell motility. Proc. Natl. Acad. Sci. USA 75 588-599.

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OLIVER D., Sommer K. R., Panyim S., Spiker S., Chalkley R. 1972. A modified procedure for fractionating histones. Biochem. J. 129 349-353.

SKIDMORE C. and Walker I.O. 1973. Structure of partially histone depleted nucleohistone. FEBS Lett. 32 175-178.

TEAGUE M. and Henney H.R. Jr. 1973. Purification and properties of cytoplasmic and mitochondrial malate dehydrogenases of Physarum polycephalum. J. Bacteriol. 116 673-684.

WEBER K. and Osborn M. 1969. The reliability of molecular weight determinations by dodecyl sulfate polyacrylamide gel electrophoresis. J. Biol. Chem. 244 4406-4412.

TABLE 1. Comparison of reagents for extraction of nuclear protein.

Extraction reagent	Source	Method of nuclear breakage	% of total nuclear protein extracted	% non-histone protein (actin)
0.4 N H_2SO_4	True chromatin	CO_2	0.58	0
0.4 N H_2SO_4	Chromatin pellet	CO_2	0.50	0
0.4 N H_2SO_4	True chromatin	DTT	0.25	0
0.4 N H_2SO_4	Chromatin pellet	DTT	0.50	0
0.4 N H_2SO_4	True chromatin	Sonication	0.30	0
0.4 N H_2SO_4	Chromatin pellet	Sonication	0.78	0
2 M NaCl	True chromatin	DTT	16.00	50
2 M NaCl + 5 M urea	True chromatin	DTT	30.00	75
1 M $CaCl_2$; TCA precipitation; 0.04 N H_2SO_4	True chromatin	DTT	5.00	10
1 M $CaCl_2$; TCA precipitation; 0.04 N H_2SO_4	Chromatin pellet	DTT	5.00	10
1 M $CaCl_2$; TCA precipitation; 0.04 N H_2SO_4	Chromatin pellet	N_2	5.00	10

TABLE 2. Amino acid composition of nuclear actin and total histone.

Amino Acid	Histone (mole %)	Actin (mole %)
Aspartic acid (A)	6.1	10.0
Threonine	5.4	5.8
Serine	6.9	5.5
Glutamic acid (A)	10.1	11.6
Proline	3.8	5.5
Glycine	13.3	12.4
Alanine	7.5	8.5
Valine	6.6	3.6
Cystine/2*	trace	trace
Methionine	0.7	1.2
Isoleucine	5.0	5.0
Leucine	7.3	9.0
Tyrosine	3.3	2.6
Phenylalanine	1.9	4.4
Lysine (B)	12.8	7.6
Histidine (B)	3.7	2.3
Arginine (B)	5.6	4.8
B/A	1.4	0.7

*Determined as cysteic acid.

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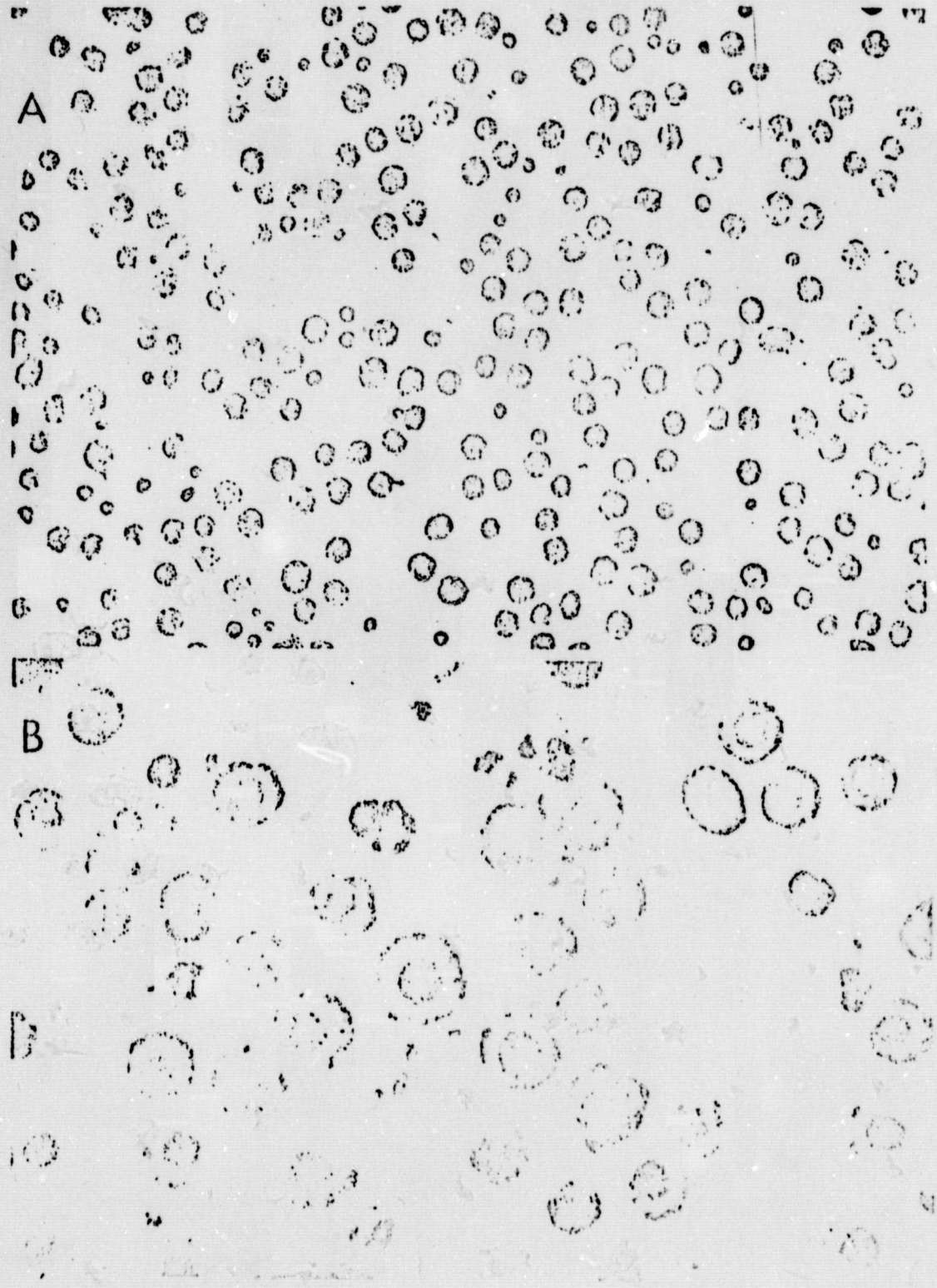

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Figure 2. Electron micrographs of purified nuclei fixed with 2.5% glutaraldehyde followed by 1% osmium tetroxide in 0.1 M sodium phosphate, pH. 7.4. (A) 9,000 X, (B) 15,000 X.

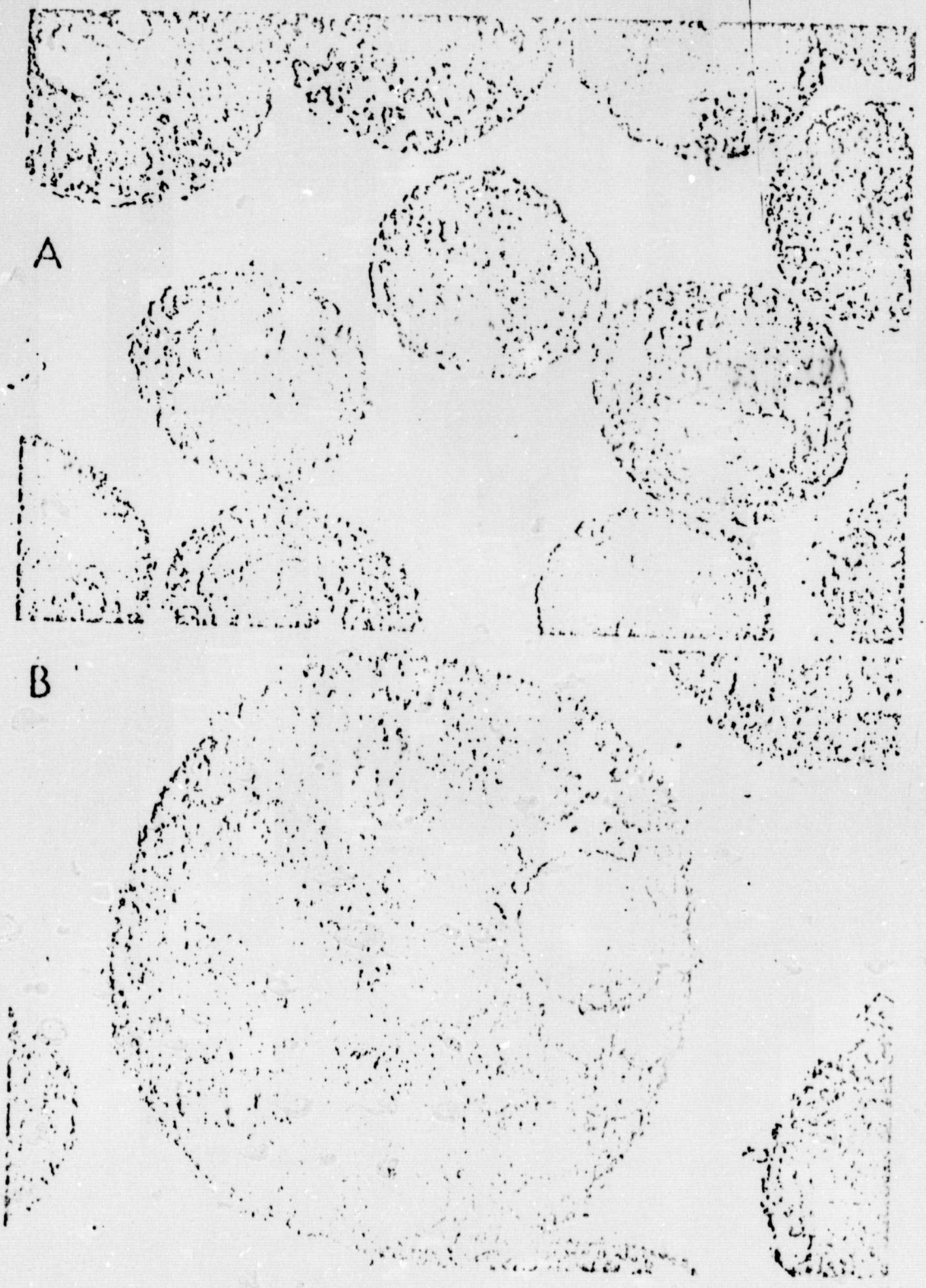

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Figure 3. Densitometric tracing of histones separated by polyacrylamide gel electrophoresis at pH 4.4. The gels were stained with Amido Black and scanned at 575 nm. The arrow indicates the direction of movement of the proteins during electrophoresis.

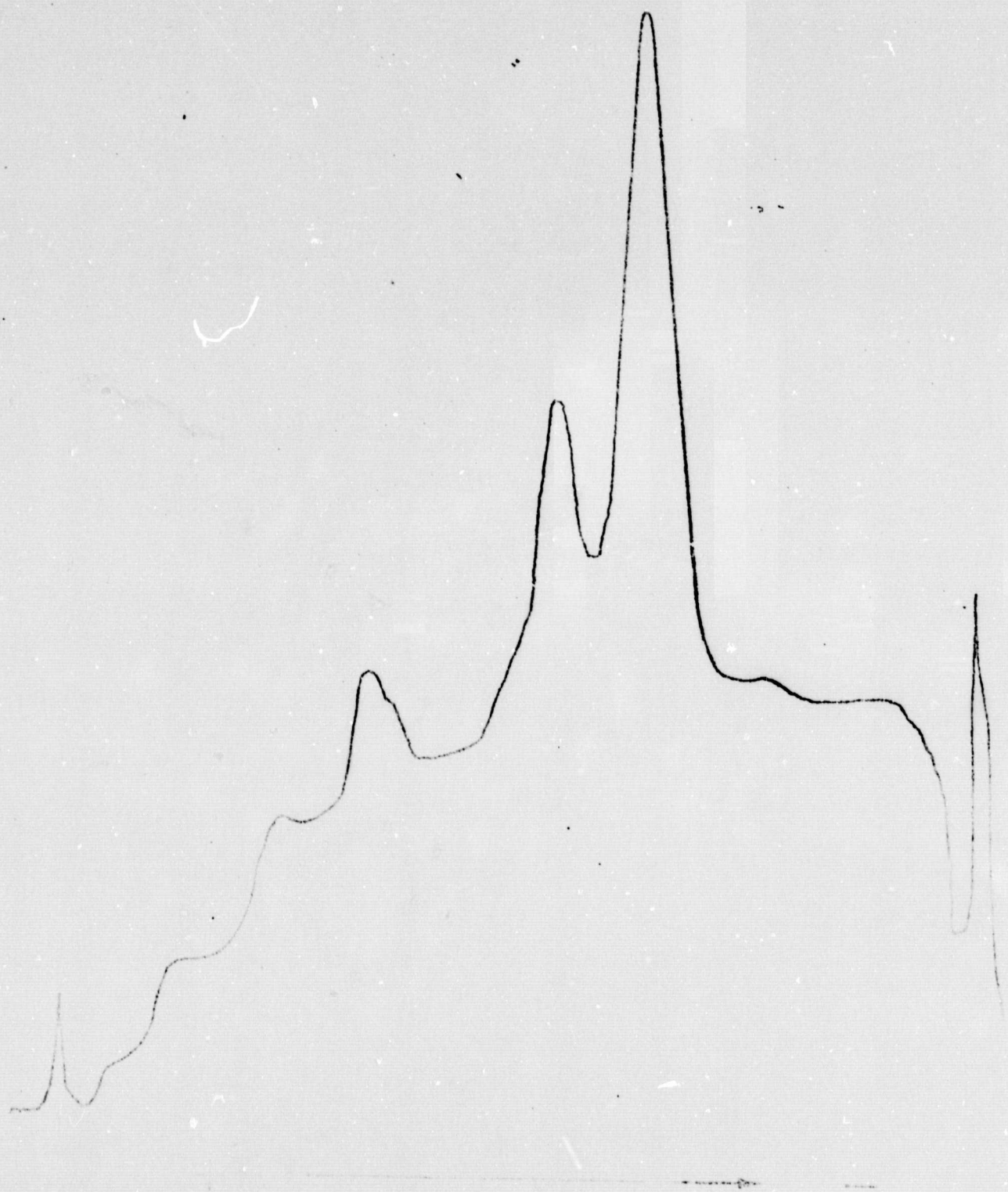


Figure 4. Molecular weight determination of nuclear actin by SDS gel electrophoresis. The actin was extracted with 2 M NaCl, isolated by gel filtration chromatography, and electrophoresed on 10% acrylamide gels containing 0.1% SDS (Weber and Osborn, 1969). Standards used were:

(a) Bovine serum albumin 68,000 daltons

(b) Ovalbumin 43,000 daltons

(c) Cytochrome C 12,000 daltons

The actin sample(d) had a molecular weight of 41,000 ± 4,000 daltons. Insert (D) is a densitometric tracing of the actin sample gel.

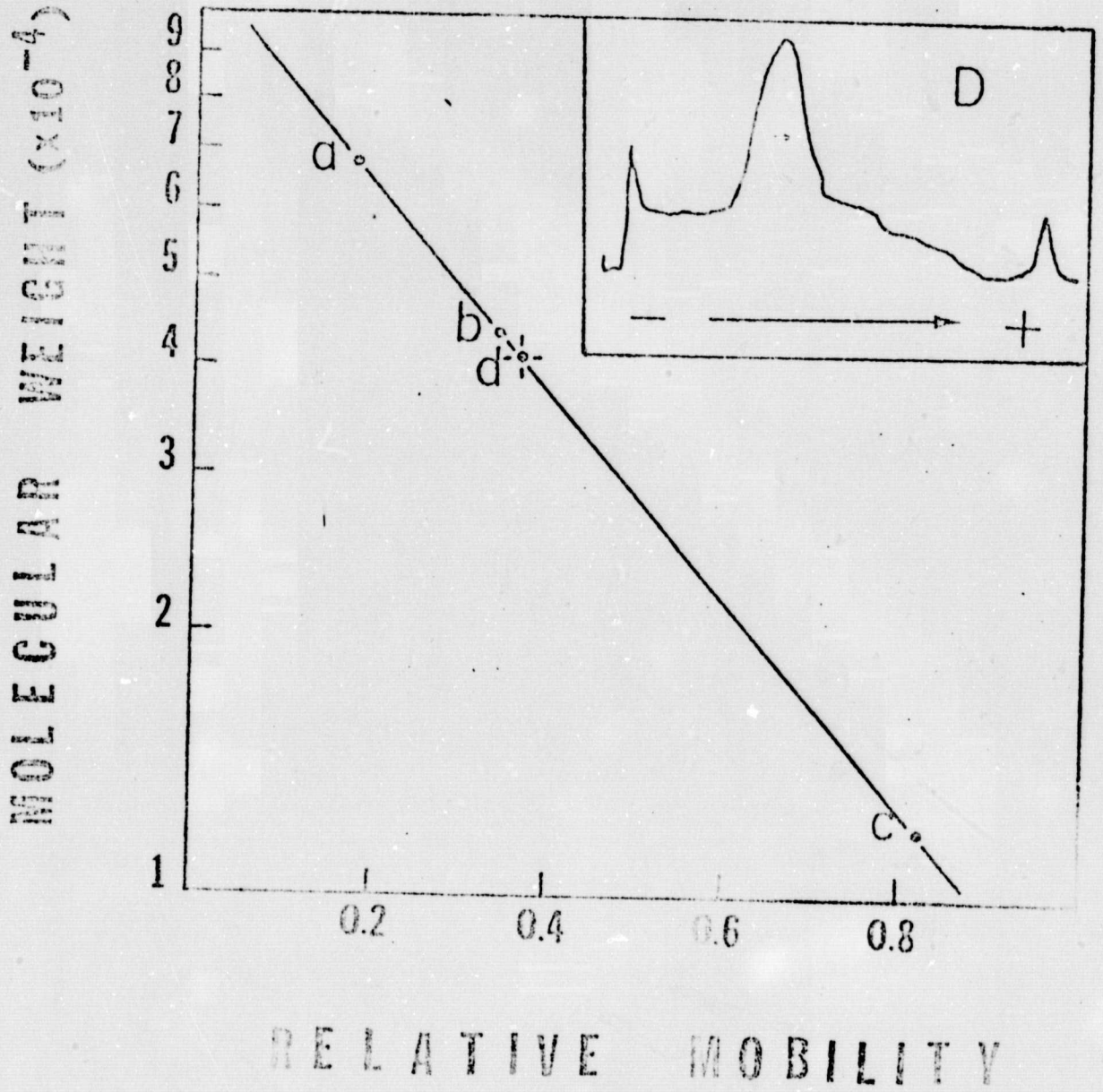
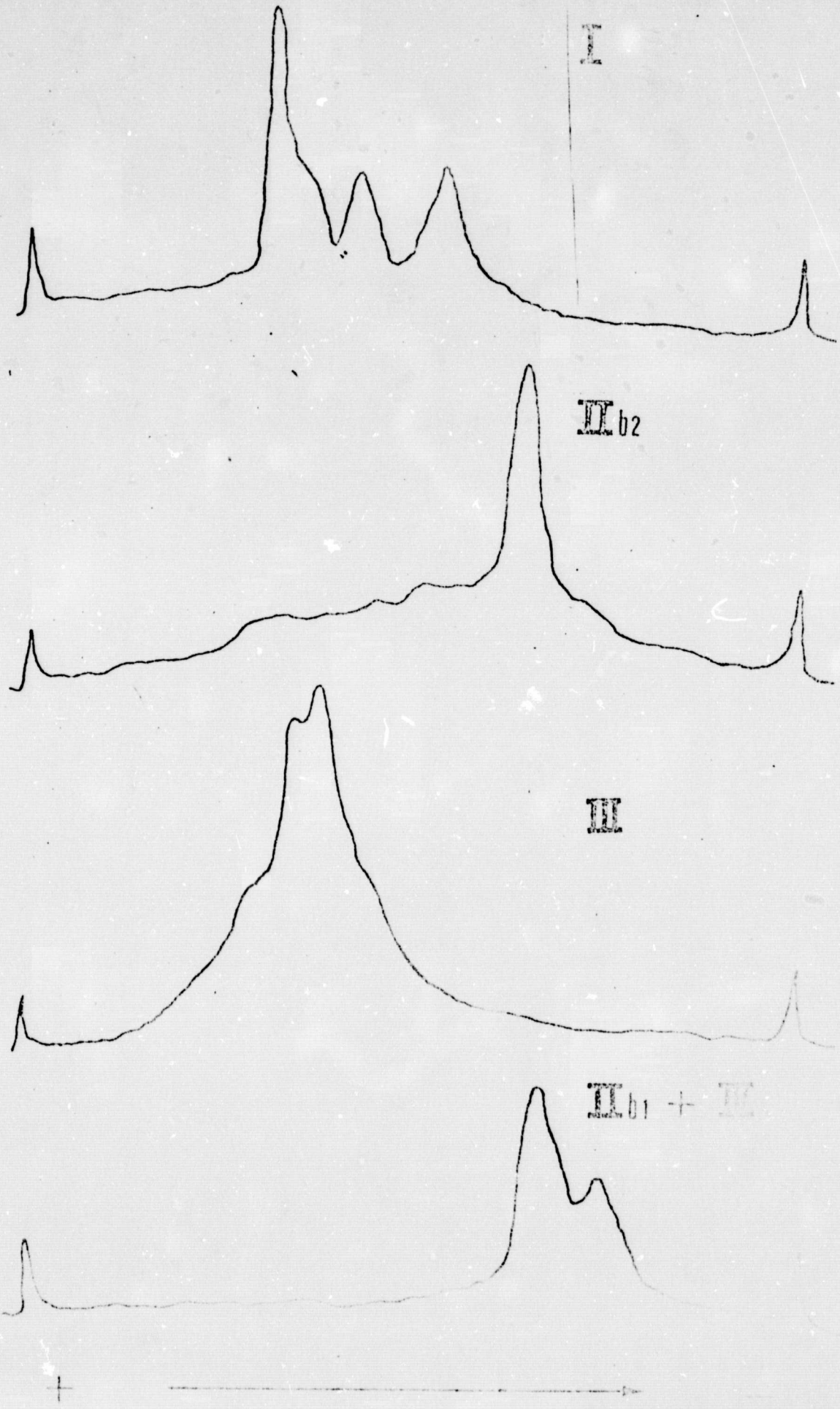

MOLECULAR WEIGHT (×10⁻⁴)
9
8
7
6
5
4
3
2
1
a
b
d
c
D
−
+
0.2
0.4
0.6
0.8
RELATIVE MOBILITY

Figure 5. Densitometric tracings of isolated histones fractionated chemically by the method of Oliver et al. (1972). (I) very lysine rich; (IIb2) slightly lysine rich; (III) very arginine rich; (IIb1) slightly lysine rich; and (IV) slightly arginine rich.


I
IIb2
III
IIb1 + IV

Abstract

Antibodies were produced against filamentous proteins from haploid and diploid cells of Physarum flavicomum, as well as rabbit skeletal muscle actin. The optimum conditions were determined for "Ring tests" and immunodiffusion tests and for photography of immunodiffusion test plates. The data reveal that the proteins exhibit common antigenicity.

Introduction

The purpose of these experiments was to produce antibodies against the filamentous proteins from haploid and diploid cells of Physarum flavicomum and rabbit skeletal muscle actin and to determine the antigenic relatedness of these proteins.

Materials and Methods

Young adult New Zealand white rabbits were obtained for the immunization experiments. The following immunization scheme (a modification of the one for the preparation of actin antibody by Lazarides, 1976) was adopted for the production of antibody against actin: days 1 and 8 (0.25 mg administered subcutaneously in Freund's complete adjuvant); days 15, 17 and 19 (0.1 mg administered intravenously); days 22, 24 and 26 (0.2 mg administered intravenously); day 29 (0.3 mg) and day 33 (0.5 mg administered intravenously).

Actin was prepared from diploid microplasmodia and haploid amoebae-swarm cells of Physarum flavicomum as well as rabbit skeletal muscle. Rabbits were bled at day 7, boosted on day 14 with 0.2 mg protein and bled subsequently every 5 - 7 days. They were boosted every 3 weeks.

Sera was precipitated at 50% ammonium sulfate saturation, dialyzed against 0.15 M NaCl, 0.02 M Tris-HCl, pH 7.5 and stored at -20°C. The antibody content of the sera was assayed by immunodiffusion tests (Harvey et al., 1967).

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Gel diffusion plates were prepared in sterile 60 x 50 mm plastic petri dishes containing 1% agarose and 0.03% sodium azide in phosphate buffered saline (PBS), pH 7.2-7.4. A center hole and 4 circumferential holes were punched into the agar, the plugs removed and the bottom of the hole sealed with a drop of the above agarose solution. The center well was filled with serum and the 4 peripheral wells with antigen solutions; alternately, antigen was placed in the center well and sera in the peripheral wells. Incubation was at 25°C.

Immunodiffusion assays also were conducted under other conditions including: agarose-merthiolate-borate buffered plates; RM-actin in PBS; Rm-actin in Tris-ATP-mercaptoethanol (TAM); Rm-actin in 0.01 % sodium dodecyl sulfate (SDS); Rm-actin in urea; Rm-actin in TAM + 0.5 M KCl + 1% Nonidet P-40 + urea.

Additional precipitin tests were conducted in 6 X 50 mm test tubes. These "Ring tests" were conducted by placing antiserum in the bottom of the tube and layering antigen or buffer (control) on top.

Procedures for the photography of immunodiffusion plates were developed. Darkfield type lighting conditions accentuated the precipitin lines. Plus-X film was used to record the results.

Results and Discussion

The immunodiffusion assays demonstrated that antibodies were produced against the filamentous proteins from haploid and diploid cells as well as against Rm-actin. The "Ring tests" also confirmed the results of the immunodiffusion assays.

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RM-actin was best solubilized in 0.5 M KCl + 1% Nonidet P-40.

Figure 1 presents a photograph of an immunodiffusion plate containing RM-actin in the center well and antisera against RM-actin, haploid cell and diploid cell proteins. The presence of precipitin lines indicate common antigenicity of the proteins from the three different sources. These data confirm the concept that these proteins are highly conserved (Ogievetskaya, 1977).

References

Garvey, J., Cremer, N., and Sussdorf, D. (1977). Method. Immunol., W. A. Benjamin, Inc. Reading, Mass., p. 315.

Lazarides, E. (1976). J. Cell Biol. 68, 202.

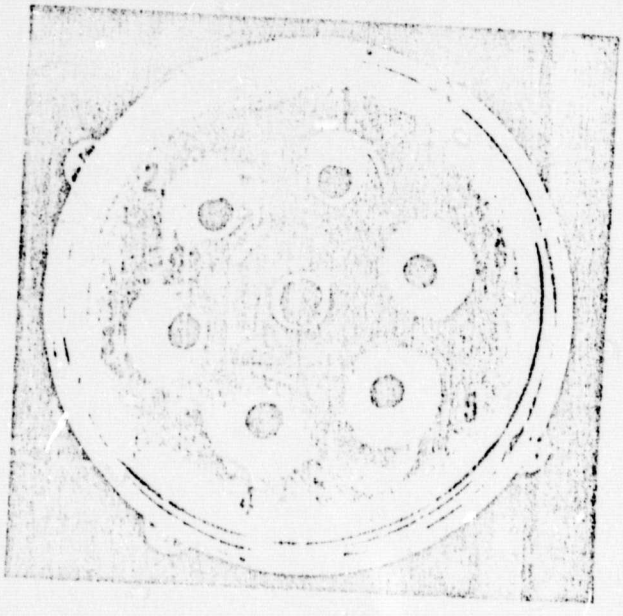
Ogievetskaya, M. (1977). Origins of Life 8, 145.

Figure 1. Immunodiffusion test.

The center well contained rabbit skeletal muscle actin in TAM + 0.5 m KCl + 1% Nonidet P-40.

The peripheral wells contained antisera against the following proteins:

1. haploid cell
2. skeletal muscle
3. diploid cell
4. skeletal muscle
5. haploid cell
6. diploid cell



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