IMMUNOMORPHOLOGICAL CHARACTERISTICS OF ANTISERA AGAINST SKELETAL AND SMOOTH MUSCLE MYOSIN

B. V. Shekhonin, A. S. Alkadarskii, V. S. Rukosuev, and G. G. Ivanov

UDC 612.734.14+612.744.14].017.4

KEY WORDS: myosin of striated muscles; myosin of smooth muscles; antisera; immunomorphology.

To study the localization of contractile proteins, including myosin, in muscle cells and cells of nonmuscular tissues, immunomorphological methods are widely used [3, 5, 7]. Preparations of myosin used for immunization and the quality of the immune sera against myosin in investigations of this sort must satisfy a number of demands. It must be remembered that even minimal impurities in myosin preparations may be sufficient to give an immune response and to cause the appearance of ballast antibodies. That is why not only maximal purification of the myosin but also subsequent careful monitoring of the specificity of the antisera by various methods, including immunomorphological methods, are essential. It is best to use native myosin for immunization, i.e., myosin whose biochemical and antigenic properties are best preserved. In that case the preparations used for immunization will correspond most closely to the myosin in the cell studied. Antigenic differences between myosin of different species of animals [2] must also be borne in mind, and for that reason it is desirable to use species-specific antisera.

As a rule the demands listed above are not completely satisfied, especially in immunomorphological studies of the structural organization of myosin in different cells.

With the above considerations in mind, native myosin was isolated from skeletal and smooth muscles and purified in order to obtain immune sera.

This paper gives a brief account of the biochemical characteristics of the myosin preparations, the results of immunologic and immunomorphological analysis of antisera against the myosin, and an account of methods of increasing their specificity.

EXPERIMENTAL METHOD

Skeletal muscle myosin was extracted from the quadriceps femoris and sartorius muscles taken at autopsy 4 h after death. Smooth muscle myosin was isolated from a uterus removed at operation. Skeletal muscle myosin was obtained by the method suggested for isolation of myosin from the heart [10], with some modification. After salting out with 37.5-42.5% $(NH_4)_2SO_4$ the precipitate was dissolved in 0.05 M Tris-HCl buffer, pH 7.5, containing 0.5 M KCl and 0.001 M dithiothreitol. Dialysis was then carried out against the same buffer for 48 h at 4°C and this was followed by centrifugation for 1.5 h at 150,000g. The supernatant was used as the final myosin preparation.

Smooth muscle mysoin was obtained by the method described for isolating myosin from platelets [8], with modifications. Gel chromatography of myosin was carried out on a column filled with sepharose 4B, using a two-component KCl–ATP and KI–ATP buffer system. The myosin fraction was concentrated to 2 mg/ml with polyethylene glycol with molecular weight of 40,000. Subsequent dialysis and centrifugation were as described for obtaining skeletal muscle myosin.

The ATPase activity of the myosin was determined in 0.1 M Tris-HCl buffer, pH 7.5, containing 1 mM ATP and 10 mM $CaCl_2$ at 25°C [4].

All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR. Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 5, pp. 618-620, May, 1981. Original article submitted October 8, 1980.

0007-4888/81/9105-0688$07.50

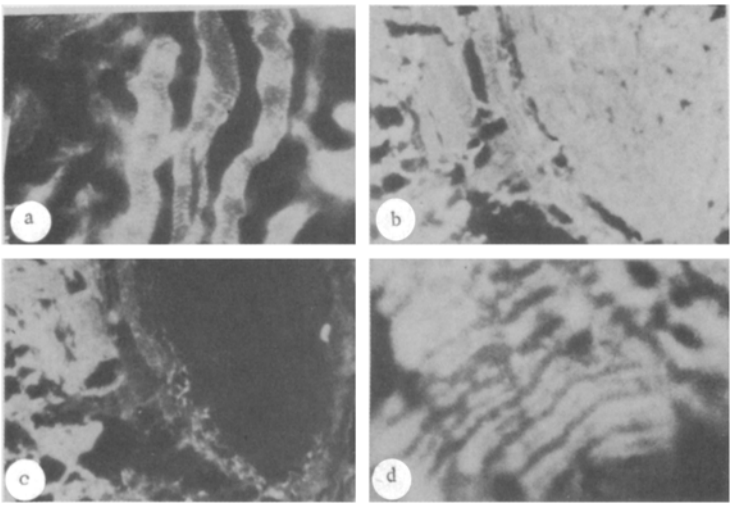

Fig. 1. Wall of upper third of esophagus. Treated with antiserum against skeletal muscle myosin: a, b) before; c, d) after absorption by immunosorbent from extract of myometrium. a) Striated muscles in wall of esophagus, 120×; b) specific fluorescence of striated fibers and positive reaction with smooth muscle cells (right), 60×; c) serial section, same place. Specific fluorescence of striated muscles only, 60×; d) specific reaction with myosin in A disks of striated muscle fiber, 270×.

The purity of the preparations was verified by electrophoresis in 10% polyacrylamide gel (PAG) with sodium dodecylsulfate [9]. Myosin preparations were applied to the electrophoresis tubes in doses of 20 and 150 mg. Proteins with mol. wt. between 10,000 and 300,000 daltons were used as markers.

Antisera were obtained by immunization of rabbits in accordance with the following scheme: first injection – antigen 0.3 mg/kg into the footpads together with Freund's complete adjuvant, second injection – 1 month later, the same dose of antigen subcutaneously, without adjuvant. Blood was taken the first time 7 days after the second injection. Later the animals were reimmunized subcutaneously without adjuvant at intervals of 1 month for 3 months. Throughout this period blood was taken each week. The resulting antisera were analyzed immunologically by the precipitation test in agarose made up in 0.6 M KCl in physiological saline, buffered with 0.01 M phosphate buffer, pH 7.2 (PBS). The indirect fluorescent antibodies method with pure goat antibodies against rabbit IgG, labeled with fluorescein isothiocyanate, was used for the immunomorphological investigation. Frozen sections 4 μm thick from various organs of a rat were fixed for 5 min in 95% ethanol at 18°C. The technique of incubation with immune sera and conjugated antibodies and the manner of setting up the controls were the same as those generally adopted [6]. The antisera against myosin were diluted with BPS in the ratios of 1:10 and 1:20 before addition to the sections.

For crossed immunosorption of antisera extracts with 0.6 M KCl were obtained from skeletal and smooth muscles, and immunosorbents were then prepared by cross-linkage with glutaraldehyde [1]. The antisera were incubated in a volume of 10 ml with 3 g of the sorbent for 24 h at 18°C.

EXPERIMENTAL RESULTS

Electrophoresis in PAG showed that both myosin preparations contained heavy chains of myosin with mol. wt. of 200,000 daltons and light chains. Three types of light chains with mol. wt. of 14,000, 16,000, and 25,000 daltons were found in preparations from skeletal muscles and two types with mol. wt. of 16,000 and 19,000 in preparations from smooth muscles. On application of 150 μg protein to the tube, contaminating fractions were found with mol. wt. of over 100,000 (about 8% for skeletal muscles and about 50% for smooth muscles) and under 100,000 (1% in the preparations). To judge from the molecular weights of these fractions, the high-molecular-weight impurities were the rod-shaped part of myosin, C-protein, and in the smooth muscle preparation, Ca^{++}-activated protein kinase also. The low-molecular-weight fractions perhaps included tropomyosin, fragments of the heads of the mysoin molecules, light chains of protein kinase, and hydrolysis products. Preparations of both types of myosin were free from actin, troponin, and creatine phosphokinase.

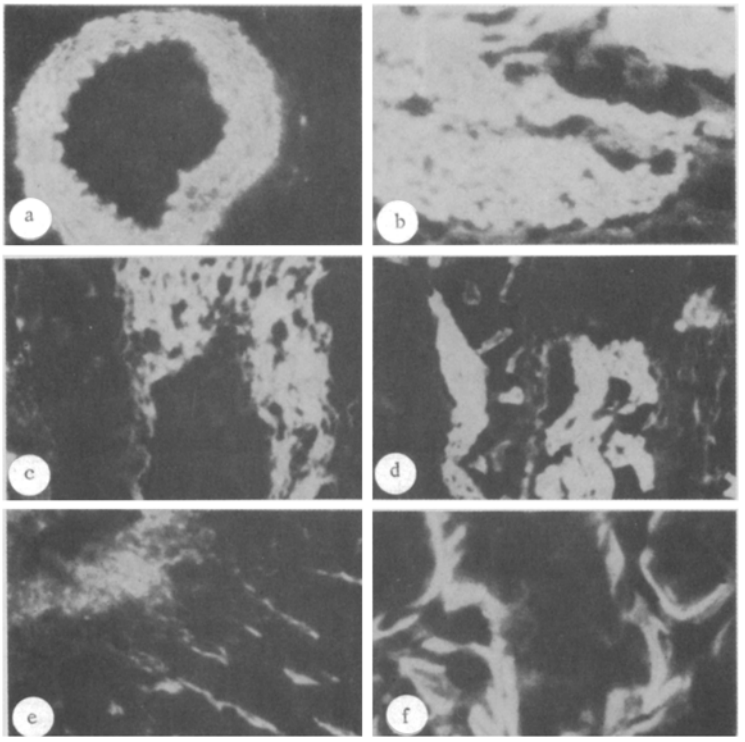

Fig. 2. Rat tissues, treated with antiserum against smooth muscle myosin: a, b) before, c, e, f) after absorption with immunosorbent from skeletal muscle extract. a) Smooth-muscle cells of blood vessel wall in rat kidney, 120×; b) smooth muscle cells in wall of esophagus, 120×; c) specific fluorescence of smooth muscle cells in wall of upper third of esophagus, no fluorescence of striated muscle fibers, 30×; d) serial section, same place, control. Identification of previously nonfluorescent striated muscle fibers after treatment with absorbed antiserum against skeletal muscle myosin, 30×; e) smooth muscle cells in gastric mucosa, 60×; f) myoepithelial cells in salivary gland, 270×.

The high ATPase activity (0.2 and 1.8 μg/mg·min respectively for striated and smooth muscle myosin respectively) indicated that the native state of the myosin was preserved to the highest degree.

For investigation of the specificity of the immune sera by the precipitation test in agarose extracts of skeletal muscles and of the myometrium in 0.6 M KCl in BPS and preparations of purified myosin were used as antigens. The antisera reacted only with the corresponding extracts and with purified myosin.

On treatment of sections of skeletal muscles, the upper third of the esophagus, and also of the heart with antiserum against skeletal muscle myosin by the immunofluorescence method bright specific fluorescence of the striated muscle fibers, located in the A disks, was observed (Fig. 1a-d). However, examination of preparations from various organs showed that despite the distinct specific reaction, this immune serum also reacted with smooth muscle cells (Fig. 1b). To increase the specificity of the antiserum crossed immunosorption was carried out by means of an immunosorbent prepared from extract of myometrium. The absorbed antiserum did not react with smooth muscle cells or with any other cells than striated muscle cells (Fig. 1c, d).

Antiserum against smooth muscle myosin reacted specifically with smooth muscle cells of the uterus, esophagus, stomach, intestine, and blood vessels in various organs (Fig. 2a, c-f). During reimmunization ballast antibodies could be removed. The antisera kept their titer of 1:20 and their specificity for smooth muscle myosin (Fig. 2c-e). Myosin could be detected very clearly in myoepithelial cells of the salivary and sweat glands, mammary gland, testis, and ovary (Fig. 2f).

Immune sera against striated and smooth muscle myosin satisfying the necessary requirements, i.e., monospecific and in sufficiently high titer, were thus obtained. Considering their species specificity, these

immune sera can be regarded as most suitable for the study of myosin. They have the great advantage over antisera against animal myosin usually used. The necessity for and value of thorough and extensive immunomorphological analysis of the specificity of the antisera which was undertaken must be particularly emphasized. The highly sensitive immunofluorescence method enabled ballast antibodies to be found in the immune sera despite their apparent purity as shown by precipitation in agarose. The crossed absorption of the antisera proved to be a sufficiently effective method of increasing their specificity. The impurities causing the formation of these ballast antibodies can be interpreted differently. In our view, preparations of skeletal muscle myosin contain C-protein. It may have been this which caused the formation of ballast antibodies and the crossed reaction with smooth muscles. The possibility likewise cannot be ruled out that myosin of vascular smooth muscles may have been responsible for this crossed reaction, for this is invariably present, even if in small quantities, in preparations of skeletal muscle myosin. As regards the antisera against smooth muscle myosin, C-protein or protein kinase were evidently the impurities against which ballast antibodies were formed. The problem of a true crossed reaction with myosin as a result of common antigenic determinants in striated and smooth muscle myosin likewise must be regarded as still unsolved.

LITERATURE CITED

1. S. Avrameas and T. Ternynck, Immunochemistry, 6, 53 (1969).
2. B. Burke, C. Mahlmeister, and U. Groschel-Stewart, Histochemistry, 60, 135 (1979).
3. J. Chamley-Campbell, G. R. Campbell, and R. Ross, Physiol. Rev., 59, 1 (1979).
4. K. Itaya and M. Vi, Clin. Chim. Acta, 14, 361 (1966).
5. F. Miller, E. Iazarides, and J. Elias, Clin. Immunol. Immunopathol., 5, 416 (1976).
6. R. C. Nairn, Fluorescent Protein Tracing, Edinburgh (1976).
7. F. A. Pepe, J. Histochem. Cytochem., 32, 543 (1975).
8. T. D. Pollard, S. M. Thomas, and R. Niederman, Anal. Biochem., 60, 258 (1974).
9. M. A. Porzio and A. M. Pearson, Biochim. Biophys. Acta, 490, 27 (1977).
10. J. Wikman-Coffelt, R. Zelis, C. Feuner, et al., Biochem. Biophys. Res. Commun., 51, 1097 (1973).

HEPATOCYTE ULTRASTRUCTURE DURING ACCUMULATION AND SECRETION OF BILE PRODUCTS

M. M. Kalashnikova

UDC 612.357.3:612.35.014.2

KEY WORDS: hepatocyte ultrastructure; bile secretion; Golgi complex; clasmatosis.

The fine morphology of the hepatocyte during synthesis and secretion of bile products has not been adequately studied [1, 2, 13-15]. The object of this investigation was to study the dynamics of changes in the ultrastructure of hepatocyte organelles during the accumulation and secretion of bile products. The liver of the chick embryos is a convenient object for this purpose, for its mode of nutrition changes in the course of its development: until the 8th day of incubation an extraintestinal mode of nutrition with protein and fat of the yolk, and from the 9th through the 13th days of incubation, in addition to extraintestinal assimilation of yolk, an intestinal mode of nutrition connected with swallowing of amniotic fluid by the prefetus [4, 5]. It must be expected that the process of bile formation and bile secretion in the liver of the chick embryo begins early, and that the ultrastructure of hepatocytes can thus be studied during the period of formation of these functions.

EXPERIMENTAL METHOD

Hepatocyte ultrastructure was studied in chick embryos from the 6th through the 13th days of incubation. Pieces of liver were fixed daily in Palade's fixing solution at pH 7.2-7.4 and embedded in Araldite. Ultrathin sections were stained by Reynolds' method and studied in the EM-7A electron microscope.

Laboratory of Evolutionary Histology, A. N. Severtsov Institute of Evolutionary Morphology and Ecology of Animals, Academy of Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Sciences of the USSR V. E. Sokolov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 5, pp. 620-623, May, 1981. Original article submitted November 11, 1980.