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PHOSPHOLIPIDS in MYOFIBRILS

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The myofibrils were shown to be lipoprotein structures. Lipoprotein nature of these structures was confirmed by their lipid phosphorus to protein ratio in a completely dispersed myofibril preparation of the skeletal muscle cells. The ratio was found to be 49.65 ± 3.95 microgram P / mg protein. The phospholipids present in the myofibril preparation were determined to be phosphatidylcholine and phosphatidylethanolamine.

INTRODUCTION

It is known that the myofibrils, filamentous contractile structures of the muscle cell, are composed of proteins. Although small amounts of lipids, carbohydrates and nucleic acids could be found in these structures (1, 2), they were generally accepted as contractile filamentous structures containing unconjugated proteins.

In the last two decades, it has been acknowledged that filamentous structures like myofibrils are found in every kind of eukaryotic cell. These non-muscle filamentous structures contain proteins similar to those of myofibrils. Several reports in the literature indicated the presence of lipid and carbohydrate components in these structures (3-5). More recently, filamentous structure of neuron was suspected to be of lipoprotein nature (6).

Considering results indicated above, we intended to test whether the myofibrils are composed of lipoproteins, also.

To decide if myofibrils are lipoprotein structures we determined their lipid phosphorus to protein ratio, since it is a

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characteristic of each lipoprotein. Moreover we showed the kinds of phospholipids present in these structures.

METHODS

Isolation of Myofibrils: A specimen of the back muscle was obtained from a dog. The myofibrils were isolated and purified as described by Etlinger et al. (7).

Characterization of Myofibrils: The myofibrillar pellet was suspended in two volumes of water. The suspended myofibrils were sonicated in ice bath until they were completely dispersed. The dispersed myofibrils were extracted with 15 volumes of chloroform-methanol (1:1, v/v). The organic phase was separated and evaporated on a water bath at 60° C. To the dried extracts 10 N H_2SO_4 was added, and heated at 160° C for 3.5 hours. All charred material was removed by adding 30 % H_2O_2 followed by further heating at 160° C for 2.5 hours more, to complete the combustion and to decompose all the peroxide.

In the digest inorganic phosphate determination was made as described by Bartlett (8). Protein in the dispersed myofibril preparation was determined by the method of Lowry et al. (9) with bovine serum albumin as the standard.

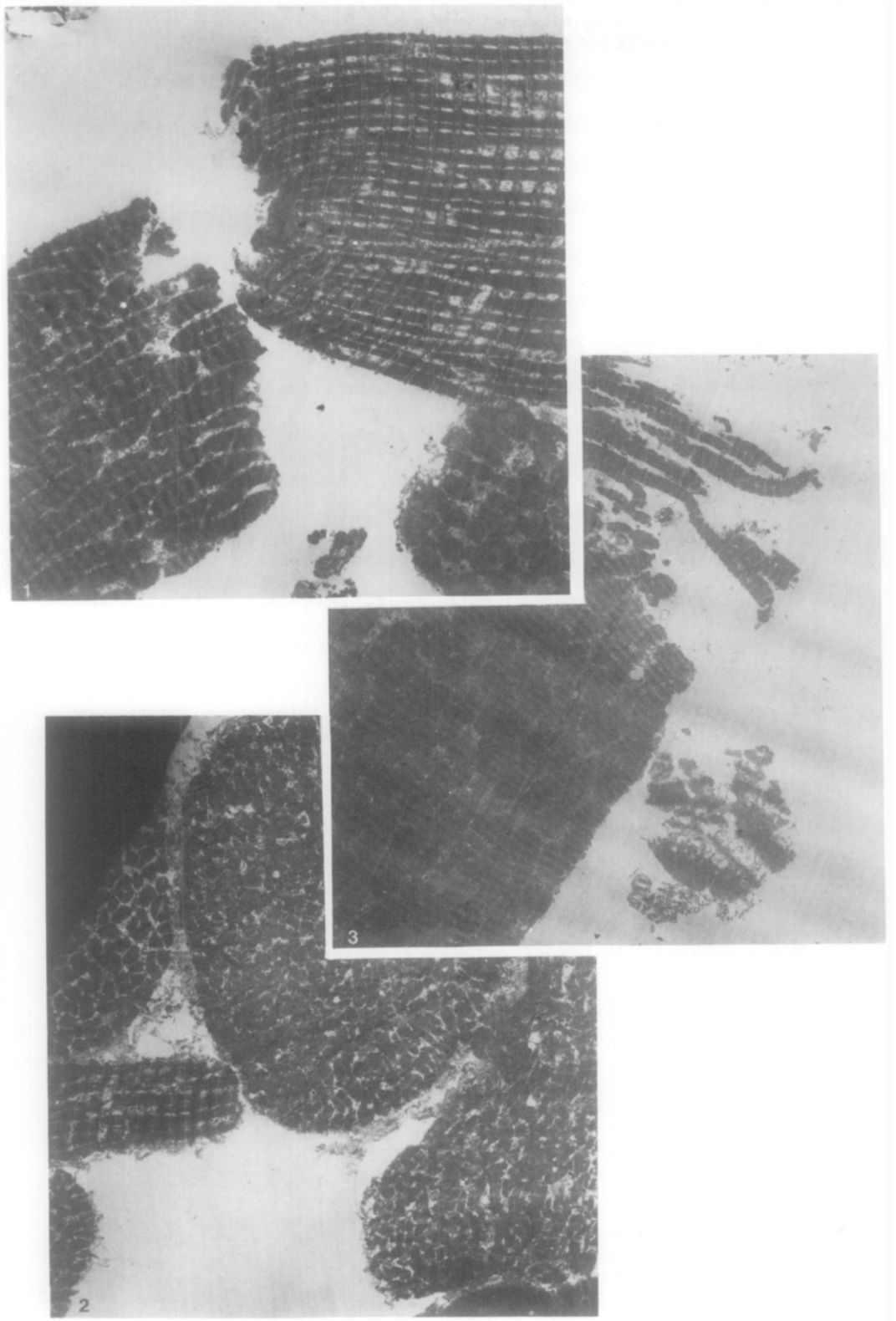
The kinds of phospholipids present in the myofibril preparation were determined by thin-layer chromatography of the chloroform-methanol extracts of dispersed myofibrils. Chromatography was done by using silica gel G plates. The solvent system used was chloroform-methanol-water(65:25:4, v/v) (10). Spots were observed by carbonisation with 50 % H_2SO_4 and by reaction with ninhydrin (10, 11). Phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine, lysolecithin, and sphingomyelin were used as chromatographic standards.

Electron Microscopy of Isolated Myofibrils: Samples obtained from two different regions of the myofibrillar pellet were fixed with 1 % OsO_4 and dehydrated in a graded series of ethanol. Dehydrated myofibrils were taken into propylene oxide before embedding in Araldite. Sections of blocks were stained with uranyl acetate and lead citrate and examined in electron microscope.

RESULTS and DISCUSSION

Determinations of lipid phosphorus and protein in the purified and dispersed myofibril preparation gave a ratio of 49.65±3.95 microgram P/mg protein (result of five determinations). The chloroform-methanol extract of this preparation was applied to silica gel G plates for one dimensional thin-layer chromatography (five applications). The presence of phosphatidylcholine and phosphatidyl-ethanolamine was seen after carbonisation with 50 % H_2SO_4. The presence of phosphatidylethanolamine was confirmed also by reaction with ninhydrin.

As can be seen from figures 1-3, our myofibril preparation was not completely pure and probably contaminated with membranous struc-



Figs. I-3 Electronmicrographs of thin sections of myofibrillar pellet. Myofibrils were obtained and prepared to electron microscopy as described in Methods. x 4750

tures of the muscle cell. However, these contaminations does not change our ratio considerably, since the corresponding ratio of membranous parts of muscle cell is much lower except that of transverse tubules (I2). Moreover, lipid content of our preparate must not be much affected by transverse tubules since in spite of the high sphingomyelin content of transverse tubules (I2), we could not find any sphingomyelin in our lipid extract. Looking to the matter from another standpoint, these contaminations do not affect our results considerably because membranous contaminations bring to the preparation both lipid and protein components. If the myofibrils have been formed from simple or unconjugated proteins we would have obtained a very small lipid phosphorus to protein ratio.

Then in summary, our results indicate that the myofibrils are lipoprotein structures. Whether all the filamentous structures are lipoproteins or conjugated proteins is yet unknown but the decision is waiting for future studies.

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