The Structure and Function of MUSCLE

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Volume II

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The

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MUSCLE

Volume II

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Volume II: Biochemistry and Physiology

Volume III: Pharmacology and Disease

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PREFACE

In this volume we deal mainly with the functional rather than the structural aspects of muscle. However these two aspects are indissoluble. Those who have perused Volume I will have noted a constant referral of structure to function and in this volume a referral of function to structure will be noted. Dr. Andrew Szent-Györgyi's initial chapter in fact deals with the nature of the actual structural units of muscle—the proteins. In the other chapters the detailed biochemical activity of structures particularized in Volume I is given. Physiological aspects of muscle action and functioning of muscle as part of an integrated organism mediated by the central nervous system and the endocrines and affected by temperature, fatigue, and training all find a place within the pages of Volume II.

This particular volume will be of special interest to biochemists and physiologists and also to neurologists, pathologists, cardiologists, and others concerned with neuromuscular disease in humans and animals, since it provides the norms from which muscular action deviates in pathological conditions.

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CHAPTER I

Proteins of the Myofibril

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I. INTRODUCTION

A. PROTEINS OF MUSCLE

The proteins of muscle may be conveniently grouped into four major fractions. These fractions are: the sarcoplasmic proteins, the proteins of the granules, the proteins of the myofibril, and the stroma proteins. Such a grouping is convenient, since the members of the different fractions are localized in different entities of the muscle cell and contribute in a different fashion to its various activities. The differences in the extractability of these major fractions is one of the bases of such a classification.

1. *The Sarcoplasmic Proteins*

The sarcoplasmic proteins are extracted with the greatest of ease and are frequently mentioned as the "soluble proteins" of muscle. These proteins occupy mostly the space between the myofibrils and can be brought into solution readily with water or with neutral salt solutions of low ionic strength ($\Gamma/2<0.2$). The solution thus obtained has a low viscosity. The proteins extracted are myoglobin and enzymes, including

all the components of the glycolytic system and the various phosphokinases. The sarcoplasmic proteins do not contribute significantly to the filamentous organization of muscle, and after their removal, the characteristic morphological features of the different types of muscles remain apparently unaltered. In fact, these features can be best resolved in muscle preparations from which the soluble proteins have been previously extracted. The sarcoplasmic proteins do not seem to be directly involved in the structural reorganization which results in contraction. Their function is directed to other, mainly metabolic, activities of the cell. The contribution of sarcoplasmic proteins to the total proteins of muscle varies from species to species and depends on the embryonic development of the cell. The variation in the soluble proteins is considerably greater than the variation in fibrillar proteins. In adult rabbit or chick striated muscles, about 30% of the total protein is of sarcoplasmic origin (Hasselbach and Schneider, 1951; Robinson, 1952a, b). In the early stages of development (14 days), the sarcoplasmic proteins may comprise nearly 70% of the total proteins of embryonic chick muscle.

2. *Granules*

Most of the granules are removed from a well-homogenized muscle together with the sarcoplasmic proteins, by solvents of low ionic strength. Differential centrifugation is a convenient way to separate them from the soluble proteins. The important components of this fraction are the nuclei, the sarcosomes or mitochondria, and the microsomes. Several important activities are associated with these particles. The sarcosomes carry the enzyme systems of the oxidative cycle. The amount present in different muscles varies widely, and there is a good correlation between oxidative activity and sarcosomal content of various muscles (Paul and Sperling, 1952; Chappell and Perry, 1953). The important "relaxing factor" (Marsh, 1951) is closely associated with the microsomal particles (Kumagai *et al.*, 1955; Portzehl, 1957). The magnesium-activated ATPase of muscle which is not myosin appears to originate also from the microsomal fraction (Kielley and Meyerhof, 1950). The granules are localized between the myofibrils and in some instances appear to be localized in register with the Z membranes (Perry, 1956). Their presence is not a necessary requirement of contraction, although some of their components or products may significantly modify the behavior of the proteins participating in con-

traction, and may thus exert a controlling function. Elucidation of their role will certainly help to bridge the gap between the *in vitro* contraction of extracted fibers and that of the intact muscle fiber.

3. *Myofibrillar Proteins*

These are the proteins which are responsible for the filamentous organization of muscle and which directly participate in contraction. Their removal is accompanied by the disorganization and disappearance of the myofilaments (Hasselbach, 1953; Hanson and Huxley, 1953), and by a complete loss of birefringence. The myofibrillar proteins are frequently denoted as the "structure proteins" or "insoluble proteins" of muscle. For their extraction, neutral salt solutions of high ionic strength ($\Gamma/2 > 0.5$) are required, even though, after extraction, some of them are soluble at lower ionic strength. The resistance to extraction is partly a result of the intimate associations and interactions between these proteins within the myofilaments. The high viscosity of the extract indicates the fibrous nature of the proteins brought into solution. From myofibrils of striated muscles and from most smooth muscles, three well identified components can be isolated: myosin, actin, and tropomyosin. In the "catch" muscles of Molluscs and Annelids, there is an additional major component—paramyosin. Actin, myosin, and tropomyosin comprise about 80% of the proteins of the myofibril of rabbit skeletal muscle (Perry, 1956). Since these structural proteins can be extracted under conditions which are sufficiently mild that they retain their biological activities, and since the solubilized proteins do not show obvious signs of denaturation, they are uniquely suited for study of some of the general properties of fibrous proteins, apart from the specific question of their participation in contraction.

4. *Stroma Proteins*

These are the proteins retained in the residue after prolonged extraction of a well-homogenized muscle with strong salt solutions. The residue contains some material of a collagenous nature contributing to the structure of the sarcolemma and possibly to the Z membrane. Because of their poor extractability, our knowledge of the components of stroma proteins is rather limited and their characterization has hardly begun.

B. Terminology and Reviews

This chapter will describe some of the properties of the structure proteins of muscle. Since their role in contraction and their contribution to the structure of the muscle cell is discussed in other chapters, emphasis will be laid upon their isolation, characterization, and properties. Actin, myosin, tropomyosin, and paramyosin will be discussed in some detail. Apart from these, a number of other proteins, presumably of myofibrillar origin, have been reported. These are: delta protein (Amberson *et al.*, 1957), metamyosin (Raeber *et al.*, 1955), contractin or Y protein (Dubuisson, 1948; Schapira *et al.*, 1957), and X protein (A. G. Szent-Györgyi *et al.*, 1955). The identification and characterization of these proteins and their possible relationship to the major group of myofibrillar proteins is still in an initial stage, and they will be dealt with only cursorily.

The following nomenclature will be used:

Myosin denotes preparations free of actin or poor in actin. It includes the "crystalline" myosin of Szent-Györgyi (1943), the L-myosin of Weber (Schramm and Weber, 1942), and myosin A, a relatively actin poor preparation (Banga and Szent-Györgyi, 1941). The myosin β electrophoretic component of Dubuisson (1946a, b) corresponds to myosin.

Actomyosin denotes the myosin B of Szent-Györgyi (Banga and Szent-Györgyi, 1941) obtained by direct extraction from muscle, and the "artificial" or "synthetic" actomyosin prepared by mixing actin and myosin solutions. Those preparations described in the literature as "myosin" which contain considerable amounts of actomyosin, as deduced from the way of preparation, will be designated as actomyosin (e.g. the "myosin" of the era preceding the discovery of actin). The myosin α electrophoretic component of Dubuisson (1946a, b) corresponds to actomyosin.

Tropomyosin denotes tropomyosin, the soluble tropomyosin of Bailey (1946, 1957), and the tropomyosin B of Kominz *et al.* (1957c).

Paramyosin denotes paramyosin (Hall *et al.*, 1945), the insoluble tropomyosin of Bailey (1957c), and the tropomyosin A of Kominz *et al.* (1957c).

Since 1950, a number of books (Mommaerts, 1950; Szent-Györgyi, 1951, 1953; Dubuisson, 1954) and reviews (Weber and Portzehl, 1952, 1954; Bailey, 1954; Mommaerts, 1954; Hamoir, 1955a; Hanson and