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THE SKINNED FIBER TECHNIQUE AS A POTENTIAL METHOD FOR STUDY OF MUSCLE AS A FOOD

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Abstract

Skeletal, smooth and cardiac muscle cells can be skinned by physical means or a variety of chemical techniques. The skinned fibers have been used to study the molecular mechanisms of contraction and the regulation of contraction by Ca^{++}. Skinned fiber preparations are also useful for study of muscle as a food. For example, it is now possible to determine fiber type of skinned fibers following study of their physical properties.

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Introduction

Research on biological phenomenon often originates with an investigation of the relevant structure or biochemistry, but eventually attention becomes focused on function. Such has been the case with muscle -- enormous detail is known about structure and about the biochemical events which collectively provide a source of energy for contraction. Study of function (contraction) was done originally in either intact muscle preparation or in solution systems consisting of isolated proteins. In response to the need to have a system in which the structural contractile components were left intact and organized but, at the same time, removing the selective barrier properties of the sarcolemma the so-called skinned fiber preparation was developed.

Procedures

Two procedures have been developed, one being chemical skinning in which the permeability of the sarcolemma is altered by exposing it to various chemicals so that the sarcolemma is not actually removed nor is the cell subjected to strong physical disruption. On the other hand, mechanical skinning, as the term implies, is a procedure for actual physical removal or disruption of the sarcolemma.

Szent-Gyorgyi (1949) is credited with describing chemical skinning of muscle fibers. In his classical studies of contraction, there was a need to establish whether the interaction of ATP with the protein actomyosin was indeed responsible for contraction. This required preparation of fibers which were free of ATP, which contained the contractile system in an intact state and which were permeable to ATP so that it could be added back and any effect observed. Szent-Gyorgyi obtained satisfactory results by extracting fiber strips in 50 per cent glycerol at 0°C for two days and then storing the preparation at -20°C. When fiber bundles were removed, placed in Ringers solution containing $MgCl_2$ and then exposed to ATP, contraction resulted. Moreover the preparations could be stored in glycerol for weeks without loss of activity. This simple technique is still used extensively nearly forty

years later in both modern experimentation and in class room demonstration.

The Japanese scientist Natori (1954) is credited with developing the mechanical skinning technique. This procedure consists of physically rolling back a portion of the sarcolemma of a single fiber, either in relaxing solution or under oil. Contraction can be elicited in response to electrical stimuli or application of Ca^{++} and other divalent cations and can be observed microscopically.

Applications

Two examples of the useful application of the skinning technique to study structure and function are cited here. Constantin et al. (1965) used a Natori preparation of frog muscle as a means to directly control the composition of the solution in the vicinity of the myofibrils. When calcium solution was applied to the fibers, followed by oxalate, an electron-opaque material, calcium was precipitated in the terminal sacs of the sarcoplasmic reticulum. These regions of calcium accumulation were identified with the intracellular calcium sink that controls the relaxation phase of contraction-relaxation cycle. Wood et al. (1975) used a chemical skinning technique to irreversibly disrupt the sarcolemma of human skeletal muscle so that calcium and other diffusible solutes were allowed access to the myofilament space. The skinning solution contained EGTA, potassium propionate, magnesium acetate, ATP and imidazole. They found that single skinned fibers developed isometric tensions of about 1.5 kilograms per square centimeter when exposed to ionized calcium even after 1 or 2 weeks of storage. Thus the technique allows controlled study of the intracellular processes involved with tension development and calcium regulation.

The skinning technique was used subsequently by a number of investigators, and an excellent and extensive review of the contributions of research on skinned fibers was published recently by Stephenson (1981). She gives a clear explanation of the four main types of preparations which derive from the two original techniques. There are two variations of the Natori type preparation. The classical one, already described, is prepared by rolling back the sarcolemma. This leaves the sarcoplasmic reticulum membranes functional but the T tubules may seal over at the surface. Contractile responses are measured by microscopic observation, or if the fiber segment is skinned completely, actual mechanical measurements may be made. Endo (1977) conceived a split fiber technique. In this case, a single fiber is placed in relaxing solution containing EGTA and is divided longitudinally for a portion of the length. This yields a split fiber comprised of two protruding halves each having a portion covered with intact sarcolemma and a portion exposed directly to the bathing media. The sarcoplasmic reticulum membranes remain intact and the T tubules probably remain functional. Differing chemical treatments result in two additional preparations of skinned fibers. Treatment with non-ionic detergents such as Brij-58, Triton X-100 and Lubrol-WX yield skinned preparations with a highly permeable sarcolemma and sarcoplasmic reticulum membranes. On the other hand, skinning with glycerol or EGTA results in a preparation having a highly permeable sarcolemma but in which the sarcoplasmic reticulum is still functional. Another approach to chemical skinning is to use the plant-origin glycoside saponin (Endo and Kitazawa, 1978). They have demonstrated that saponin acts on the surface membrane without affecting the sarcoplasmic reticulum. Clearly, the skinning procedure employed must be matched to the specific aims of a particular research project.

The skinning technique has also been used on preparations of smooth and cardiac muscles. Such work with smooth muscle has been hampered due to the small size of the cell and the large amount of connective tissue surrounding it. However, Gordon (1978) reported Ca^{2+}-dependent tension development by single smooth muscle cells that were chemically skinned by brief exposure to the non-ionic detergent Triton X-100. Previous attempts using the usual procedures for skeletal muscle were not successful in that glycerol skinning of the smooth muscle cells appeared to modify the sensitivity of the contractile proteins to Ca^{++} and short-term exposure to EDTA resulted in maximal tension only 5-10% of that developed by intact muscle. However, with the Triton X-100 treatment, tension could be induced by increasing the $[Ca^{++}]$ in the micro-molar range. In the presence of a saturating $[Ca^{++}]$, the preparations developed nearly 100% of the control tension recorded during electrical stimulation of the intact muscle prior to treatment with the detergent. Cardiac muscle cells may be skinned with EGTA or EDTA, although there has recently been considerable discussion both with regard to the definition of skinning and the efficacy of this skinning procedure in heart muscle (Reuben et al., 1979). A reasonable working definition advanced in the aforementioned references is that a muscle cell is skinned when normally impermeable solutes such as Mg ATP, EGTA, EDTA and other high molecular weight moieties gain free access to the myofilament space.

The conclusion may then be drawn that skeletal, smooth and cardiac muscle cells can be readily skinned by a variety of techniques, some of which require a high degree of technical expertise. The resulting systems offer excellent models for the study of the detailed molecular mechanisms of contraction and the regulation of contraction by Ca^{2+}. Much of the current knowledge of the molecular mechanism of contraction in heart and skeletal muscles has been derived from *in vitro*, biochemical experiments involving the isolated contractile proteins. On the other hand, the physiological properties of contracting muscle are the result of cyclic interactions of actin and myosin which are formed into overlapping lattices of thick and thin filaments. Tension generation and shortening are accompanied

by mechanical strain, or loading, of the individual myosin cross-bridges, a condition which has yet to be duplicated in the in vitro experiments. A major problem is to relate biochemical results, in which cross-bridges are mechanically unloaded, to physiological results reflecting the behavior of cross-bridges that are both loaded and constrained to occupy positions within the filament lattice. An approach to this problem is to study the physiological properties of muscle using preparations from which the surface membranes have been mechanically or chemically removed. In this way, controlled chemical manipulations in the fluid bathing the myofilaments can be made, and the resultant physiological effects can be measured.

Potential for Study of Food

A remaining question is whether such preparations are useful for the study of muscle as a food. We believe they are because they provide an excellent tool for fundamental studies. Real advances in understanding and improving the use of muscle as a food are always based on scientific understanding of the basis of the problem. An example can be given. It has been recognized that the distribution of fiber types in a muscle plays some role in determining ultimate quality of the meat.

Recent work (Eddinger et al., 1985) has shown that chemically skinned fibers may be fiber typed using Myosin-ATPase histochemistry. Examples of chemically skinned fibers are shown in Figure 1. The skinned fiber preparation allows measurement of physical properties of single cells, which for technical reasons (primarily small size) cannot be done on living fibers. Whole muscle work in meat animals is generally impractical due to the large size of the muscles in these animals, and where possible, analysis is complicated by the heterogeneous fiber type composition of these muscles. These combined methodologies allow a new avenue of investigation into the physical properties of postmortem muscle between the various fiber types. Measurements of active and passive tension, yield stress, and sarcomere uniformity can be made and related to fiber type.

In the area of animal development, skinned fibers can be used to measure the mechanic properties of developing muscles starting with the fetus. After the mechanical measurements, the fibers can be fiber typed using myosin-ATPase histochemistry while contiguous sections of the fiber can be run on SDS polyacrylamide gels to determine their exact protein composition. Such information would add to our understanding of protein turnover and exchange and its relation to mechanical properties with development and aging. These techniques will also allow the study of various experimental manipulations, both hormonal and neuronal, in terms of their effects on the various fiber types with regard to their mechanical properties and protein composition. This information may prove useful not only for better understanding of the in vivo situation but also with regards to final meat quality.

Thus, a new avenue has been opened that will allow us to relate fiber type directly to function -- and information can be produced which is pertinent to understanding meat quality.

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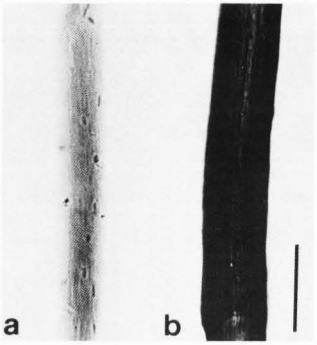

Figure 1. M-ATPase fiber typing of chemically skinned rat fibers. EDL & SOL fiber following acid preincubation (pH 4.35). a) Type IIb fiber from EDL muscle shows intermediate staining intensity. b) Type I fiber from SOL muscle shows dark staining intensity. Bar = 0.1 mm.

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