MEAT - AN ENVIRONMENTAL SCANNING MICROSCOPICAL VIEW

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S-IVB.48

SUMMARY

An Environmental Scanning Electron Microscope (ESEM) is a recently developed scanning electron microscope used for a number of reasons, viz, to avoid sample preparation of fresh and wet biological specimens. Biological samples may be imaged at low voltage in order to obtain fine surface structures. Samples of meat from near the achilles tendon from a chilled beef carcass were obtained by dissection. Environmental scanning microscopy was carried out on the specimens using the material unprocessed and fresh or stained with 'Protogold', a colloidal gold preparation developed for staining proteins and gels. Imaging was done using an EST secondary electron detector. The images of unstained fresh muscle show clear relief features of individual muscle fibres prepared longitudinally highlighting the endomysial structures in detail. Transverse sections show the ends of the muscle fibres visualising the slight emergent myofibrils. Staining with 'Protogold' (British Biocell International, Cardiff, UK) enhanced the contrast of these features and particularly endomysial fibres. Skeins of connective tissue between slightly tensed muscle fibres were seen containing perforations. In some preparations subsurface structure could be discerned particularly that of the sarcomeres. The methodology described is being extended to a back scattered electron detector which should improve the signal to noise ratio in gold stain material in comparison to unstained.

Introduction

In traditional structural view of muscle eg, by Lawrie, (1985) a connective tissue matrix of different hierarchial order consisting of intercellular material containing different aggregated states of collagen and elastin holds together striated muscle fibres and fat cells. Purslow, (1991) summarises the appearance of muscle both before and after mastication models and used scanning electron microscopy to present a structural view of the inter-relationships of muscle fibres in meat. However, due to the preparation artifacts encountered for normal scanning electron microscopy distortions due to processing have been asserted (Peters, 1992). The introduction of a new type of scanning electron microscope capable of working with fully hydrated specimens and offering the high resolution of the scanning electron microscope particularly at low kilovoltage offers a new way of examining the structure of meat. Peters, (1992) also suggested the use of surface contrasting agents to augment contrast and improve the signal to noise in this new mode of observation. This study reports the first examples of meat observed by this new methodology and incorporates the use of 'Protogold', a colloidal gold preparation to enhance contrast.

Materials and Methods

Meat from a one year old aged carcass of yearling beef was obtained from a commercial butchery. Muscle was removed from the region continuous with the Achilles tendon. Sharp razors were used to excise the muscle and prepare it for observation. Thin strips approximately 3-4 mm thick were placed on a stub in the environmental scanning microscope (ESEM) and observed using an EST secondary electron detector. A direct observation was carried out on a cool stage operated at 4°C. Two methods were utilised for observation. In one the meat was left untreated and in the other 'Protogold' (a colloidal gold solution from British Biocell International, Cardiff, UK) was employed as a staining solution. The staining procedure was modified from that supplied by Biocell Research Laboratories. Phosphate buffered saline (PBS) pH 7.4 was used to wash the surface of the meat and blotted off. The meat was then washed with distilled water for 1 min and then incubated in the 'Protogold' solution for 3 hours. It was then rinsed with saline and blotted off and then examined.

Results and Discussion

Meat samples which were observed unstained were examined in several orientations. Transverse cut ends show slightly emergent myofibrils protuding from the cut ends (figure 1). Longitudinal views show the cylindrical muscle fibres (figure 2). Occasionally it is possible to discern subsurface structure of the muscle fibres indicating sarcomeres (figure 3).

In muscle stained with 'Protogold' an enhanced visibility of surfaces can be noted versus, the unstained (figure 4). It is possible to observe clearly the investment of elastin and connective tissues fibres of the endomysial fibres. In places where muscle has been slightly tensed serving to separate the muscle fibres slightly skeins of connective tissue may be observed (figure 5). The high resolution which this technique affords can be seen in figure 6 which shows the fine detail of the connective tissue. Specimens removed after observation in the ESEM retained its wetness.

Conclusions

The environmental scanning electron microscope is shown here as a powerful new tool for the investigation of meat structure. It is able to reveal the surface features of muscle fibres and connective tissue in the meat in a fully wet state. Concomitantly the use of contrast agents in investigating wet proteinaceous materials is demonstrated. The extension of these techniques to gold labelled antibodies used to specifically identify surface muscle components is projected. The use of back scattered electron detectors and silver enhancement (Biocell Research Laboratories) should further improve identification, contrast and signal to noise in imaging.

References

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Figure Legends

Figure 1 Transverse raw beef muscle section from near Achilles tendon. Myofibrils slightly emergent from cut ends may be seen (arrow). Magnification 360x

Figure 2 Longitudinal raw beef muscle section from near Achilles tendon. Condensed water droplets on surface may be seen (arrow). Subsurface evidence of sarcomeres may be discerned. Magnification 270x

Figure 3 Longitudinal raw beef muscle section from near Achilles tendon. Sarcomeres clearly evident (arrow). Endomysial connective tissue is discernible (double arrow). Magnification 655x

Figure 4 'Protogold' stained longitudinal beef muscle from near Achilles tendon. Slight tension between muscle fibres reveals connective tissue skeins (arrow). Magnification 575x

Figure 5 'Protogold' stained oblique section through beef muscle from near Achilles tendon. Connective tissue of endomysium is seen (arrowed). Magnification 335x

Figure 6 'Protogold' stained connective tissue of endomysium showing fibrils and sheets. Magnification 288x