3:6 Light scattering from muscle during the onset of rigor mortis

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Introduction

The appearance of meat may be profoundly affected by the metabolic events which take place in muscle post mortem and a major influence upon its appearance is exerted by the light scattering power of the constituent muscle fibres. A particularly dramatic instance of this effect is the appearance of pale soft and exudative pork which owes its peculiar appearance very largely to its very high light scattering ability (1).

In view of its importance, we aim to study the light scattering properties of muscle to establish which structures within the muscle fibre are the main contributors to the scatter and to determine how their contribution changes with variations in the physiological state of the cell.

This preliminary study has been devoted to the light scattering change induced by the onset of rigor mortis. It has shown that a large increase in scatter results from the establishment of 'rigor bonds' between the thick and thin filaments of the contractile apparatus.

It is known that the light scattering power of a relaxed muscle is increased both during contraction (2,3,4) and with the onset of rigor mortis (5) and there are indications from previous work that both the optical changes due to contraction (4) and those due to rigor (6) are dependent upon the degree of overlap of the thick and thin filaments. The present measurements, which have been made both upon substantial pieces of muscle tissue and upon single muscle fibres, aim to pursue the rigor-induced scattering increase in a quantitative manner and to compare the behaviour of whole muscle and of single muscle fibres. In the latter case the experimental conditions can be rather precisely controlled and indeed the relaxed, contracting and rigor states can be generated at will; it is, however, highly desirable to be able to relate experimental results obtained with single fibres to the behaviour of the whole muscles which are the concern of commercial practice and this was a particular aim of the present measurements.

Methods

Three different optical set-ups were used in these experiments (see Fig. 1). A and B were employed with substantial pieces of muscle and C was used to make optical measurements on single muscle fibres; the latter were combined with simultaneous mechanical measurements of isometric force and of stiffness using exceedingly small (~0.03%) sinusoidal length changes (at 77Hz) as previously described (7).

The illumination, derived from an appropriate light emitting diode in each case, was delivered to the specimen by a suitable fibre optic light guide. In B and C a light guide was also used to convey light from the specimen to the detector - a silicon photodiode. The various light emitting diodes were pulsed at high frequency (30-50 KHz) and the resultant pulsed illumination produced a modulated signal at the photodetector. This arrangement permitted the rejection by high-pass filters, of steady or slowly changing interfering signals caused by background illumination, whilst in (C) it enabled, in addition, the use of lock-in amplifiers to extract from background noise the rather small signal received at the photodetector.

The 3 light emitting diodes (LED's) used in A provided rather narrow band illumination (25-40 nm at half height) centred upon 565, 655 and 940 nm, whilst all the measurements reported here with arrangements B and C used a near infra-red (940 nm) source alone. In arrangement A, measurements at three wavelengths were made effectively simultaneously by projecting pulses at the sample in rapid succession from three different LED's each of which was illuminated for 8 μs. The repetition rate of the pulse train was 15 KHz. Suitable decoding circuitry was used to demodulate the resultant pulsed signal from the photodiode detector. In measurements of myoglobin oxygenation a LED emitting maximally at 585 nm was substituted for the 940 nm LED. The light guides used consisted of a bundle of glass optical fibres which was subdivided by diverting the fibres at random into three or two sub-bundles in A and B respectively. The overall diameter of the combined guide was 2 mm (A) and 3 mm (B). In C, single silica optical fibres (0.25 mm core diameter) were used, with the muscle fibre mounted between a force transducer and a displacement transducer (along an axis normal to the plane of the paper).

The geometry of the arrangement was such that A measured light transmission by the specimen (path length 3mm). The light guide and detector were in close contact with 1 mm thick perspex plates which sandwiched the preparation (detector area 9mm^2). B by contrast, measured light back-scattered from the preparation. In both of these cases the muscle fibre axis was normal to the optic axis of the light guide. In C, however, such measurements of transmission and backscatter were difficult to make for technical reasons and so modulated infra- red light incident upon the specimen normal to the muscle fibre axis was collected by two optical fibres (each subtending about 0.1 steradians at the specimen), one at 90° to the incident beam and the other at approximately 135° to it.

In arrangements A and B the muscle specimen was cut to size and totally enclosed without access to the air. It rapidly became completely anaerobic as indicated by changes in the absorption spectrum of the muscle myoglobin which could be conveniently monitored by measurements with 565 nm and 585 nm LEDs (manuscript in preparation).

Sarcomere length was measured by optical diffraction using a He Ne laser at 633 nm.

Results and discussion

Fig. 2 records the changes in light transmission measured in apparatus of type A when a sample of beef sternomandibularis muscle was allowed to pass into rigor at 22°C.

At each of the three measuring wavelengths a decrease in light transmission, with similar kinetics, is associated with rigor onset. The muscle tissue is more opaque at shorter wavelengths and this is attributable partly to increased light scatter at the shorter wavelength (6). At 565 nm, however, a substantial part of the attenuation is due to absorption by haem pigments - mainly myoglobin (the myoglobin concentration in this sample is approximately 2.8 mM). At 655 nm, however, myoglobin absorption is small (7% of the value at 565 nm) and at 940 nm it is even less significant (less than 2% of the 565 nm value). The weak wavelength-dependence of the scattering compared with the strong wavelength-dependence of the pigment absorption enables one, by using such multi-wavelength measurements, to separate these two contributions to the overall attenuation. In this particular experiment, comparison between the 655 and 940 nm measurements yields, a $\lambda^{-1.6}$ dependence of scattering intensity upon wavelength, for the relaxed pre-rigor muscle (6h post mortem), and a $\lambda^{-1.7}$ relationship for the muscle when fully in rigor (24 h post mortem). The extent, relative to the relaxed state, of the rigor-induced decrease in the light transmission is similar; at all 3 wavelengths transmission is approximately halved by rigor onset.

Figure 3 records an experiment in which geometry B was used to follow the onset of rigor at 22°C in beef sternomandibularis muscle. It is evident that the intensity of the light back-scattered from the surface of an optically almost infinitely thick preparation rises as rigor sets in by a factor (about twofold) similar to the decrease in transmission which rigor produces under similar conditions. It therefore appears that the scattered light intercepted by this geometry (B) is quite representative of that light which is deflected away from the transmitted beam by the light scattering process.

The above measurements concern muscle fibres going into rigor anaerobically as a result of metabolic depletion. In order to investigate the rigor-induced change under more controlled conditions, measurements were made on single glycerol-extracted beef sternomandibularis muscle fibres using geometry C. The relaxed state was achieved by bathing the fibre in a MgATP-containing solution lacking Ca^{2+} ions and the rigor state was produced by substituting Ca^{2+}-free solution lacking MgATP. The preparation passed into rigor (see Fig 4) as indicated by an increase in isometric force and in stiffness.

The light scattered at both 90° and 135° to the incident 940 nm beam increased with kinetics similar to the onset of rigor force and stiffness. When relaxation was produced by replacing the rigor-inducing solution with relaxing solution once more, the force, stiffness and light scattering signals all dropped to their relaxed values; both optical and mechanical phenomena are reversible. It is noteworthy that light scatter measured at 135° and at 90° shows a similar amplitude change when rigor is induced; the increase is about twofold. (This experiment was conducted at pH 6.8; as the pH of the bathing medium is lowered towards 6.0 the scatter produced by relaxed muscle rises somewhat and the rigor-induced change diminishes slightly in amplitude - results not shown).

A particular advantage of the single fibre preparation is the opportunity it offers to vary the sarcomere length in a controlled manner. Measurements like those of Fig. 4, but conducted at different sarcomere lengths, show that the rigor-induced scattering increase is very sarcomere-length dependent - see Fig. 5. An almost linear relationship exists between sarcomere length and the ratio of rigor scatter to relaxed scatter. This relationship extrapolates to 1.0 at a sarcomere length close to that (3.8 μm) at which no overlap exists between the thick and thin myofilaments. It therefore appears that the increase in scatter associated with rigor is proportional to the degree of filament overlap over a wide range, implying a close connection between the number of rigor bonds (8) which develop between the interdigitating thick and thin filaments and the magnitude of the associated optical change.

Apart from the contractile apparatus, cellular membranes, particularly the extensively developed sarcoplasmic reticulum membrane system (9) might be expected to contribute to light scattered by muscle fibres. In order to estimate this contribution, intact single muscle fibres which had not been glycerol extracted were observed in apparatus C in the relaxed state and when treated with the non-ionic detergent Brij 58 (0.5% w/v) to disrupt all membranes under conditions where the fibre remained relaxed. A small decrease in light scatter (~10%) was produced by this treatment, indicating that the membranes contribute rather little to the overall light scatter of the relaxed cell.

Conclusion

The results described above indicate that there is a very substantial increase in light scattering when relaxed beef muscle enters the rigor state. These results also demonstrate that the light scattering behaviour of single muscle fibres undergoing this transition is very similar to the response of a block of muscle tissue. It is evident that this light scattering increase induced by rigor is closely related to the extent of establishment of rigor bonds between the thick and thin myofilaments.

References

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Figure legends

Figure 1 Schematic diagrams of the optical set-ups used.

LG - fibre optic light guide
M - muscle orientated with fibre axis perpendicular to optical axis
D - detector (silicon photodiode, area 9mm^2 operated under reverse bias, response time 100 ns).
OF - Single (250 μm core diameter) optical fibre
m - muscle fibre
$\lambda_1\lambda_2\lambda_3$ light emitting diodes emitting maximally at 565, 655 and 940 nm respectively
dimension l = 3 mm (A) and 15 mm (B).

Figure 2 Time course of light transmission at 3 different wavelengths in beef sternomandibularis muscle as it enters rigor mortis. Measurements were made in apparatus A, temperature 22^{0}C.

Figure 3 Time course of light intensity back scattered from beef sternomandibularis muscle as it enters rigor mortis. Measurements were made in apparatus B, temperature 22^{0}c.

Figure 4 Mechanical and optical changes in a single glycerinated beef sternomandibularis fibre. Measurements were made in apparatus C, temperature 21^{0}C, sarcomere length 2.4 μm. Initially the fibre was bathed in 100 mM KCl, 6 mM $MgCl_2$, 5 mM ATP, 3 mM, EGTA, 3 mM imidazole pH 6.9; at arrow (a) this was replaced by a similar medium but lacking ATP and $MgCl_2$; at arrow (b) the original solution was readmitted, fibre diameter 75 μm.

Figure 5 Sarcomere length dependence of the fractional light scattering increment caused by rigor onset. Experiments were performed as described for Fig. 4 (measurements at 90^0) but the sarcomere length was varied. Open and closed symbols describe results from two different fibres, diameters (at 2.4 μm sarcomere length) 68 μm ○ and 74 μm ● respectively.

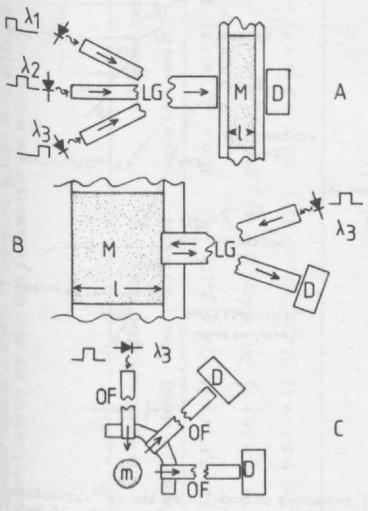

Figure 1

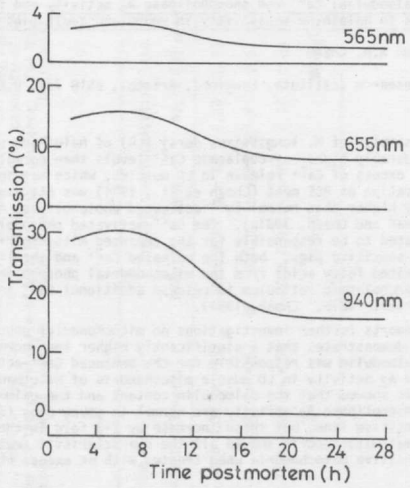

Figure 2

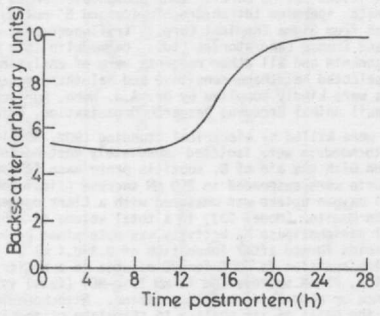

Figure 3

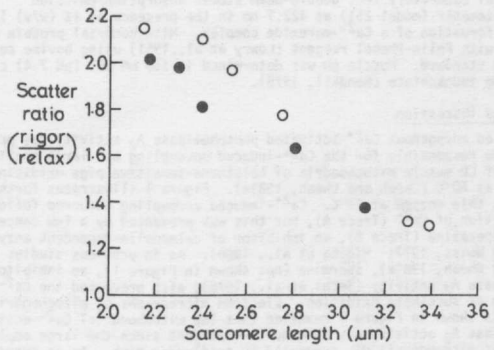

Figure 4

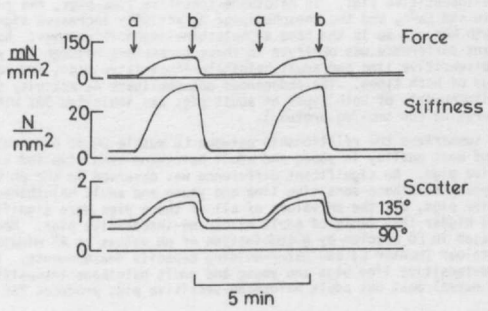

Figure 5