Comparison of the localization of several muscle proteins in relaxed and contracted myofibrils[1]

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Summary. The localization of several muscle proteins in relaxed and contracted myofibrils is compared. The morphology of the myofibrils and the behavior of these proteins was also investigated under extraction conditions.

A number of proteins, isolated from chicken muscle, e.g. parvalbumin-like protein, β-actinin, glycogen phosphorylase b and MM-creatine kinase have been shown by us to be located in distinct regions of the relaxed myofibrils[2-6]. In pursuing these studies, 3 major questions were raised concerning: a) the specificity of the detection method used (indirect immunofluorescence technique) under varying experimental conditions, b) the binding of these proteins also in strongly contracted myofibrils, where the interfilamentary distances are extremely reduced, and c) the extraction under conditions applied for the 'specific' removal of the M-line structure. A number of experiments has been undertaken to test our usual approach (indirect immunofluorescence) under conditions where morphological and functional state of the myofibrils are considerably changed.

Methods. Relaxed myofibrils were prepared according to Kundrat and Pepe[7], and incubations were done as described earlier[2-6]. Antisera against the protein components were obtained and characterized as reported[2-6]. Anti-MM-creatine kinase and anti-IgG fraction against chicken gizzard actin were kind gifts of M. Caravatti and Dr B. Jockusch. Antisera were diluted 1:40 except

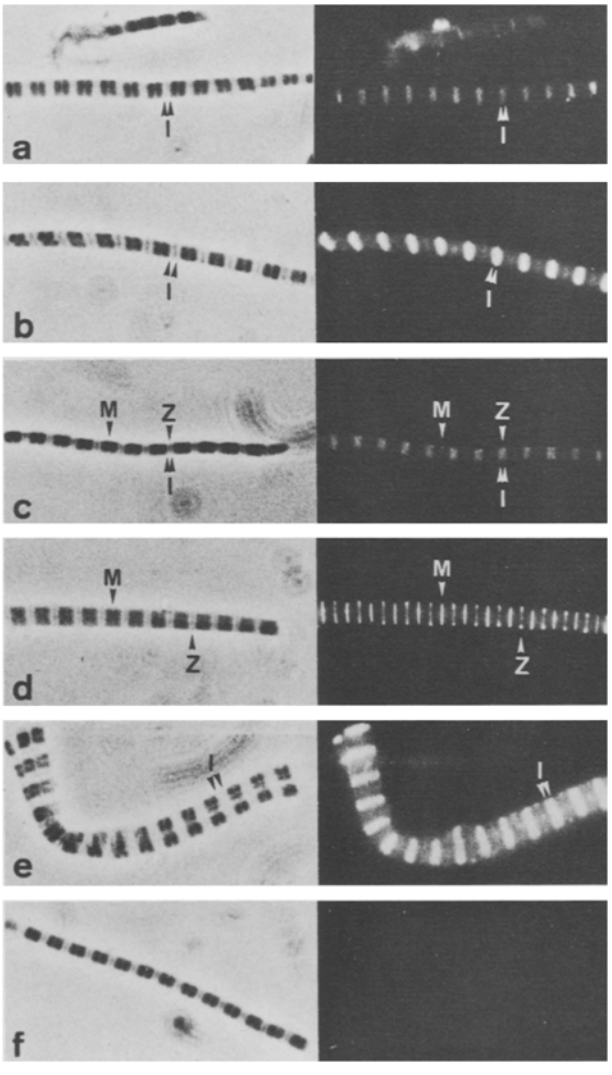

Fig. 1, A. Localization of *a* parvalbumin-like protein, *b* β-actinin, *c* phosphorylase, *d* MM-creatin kinase and *e* actin in relaxed myofibrils isolated from chicken breast muscle, *f* control, incubated with nonspecific control serum. The exposures on the left show phase contrast and on the right the fluorescence of the same myofibril. ×3500.

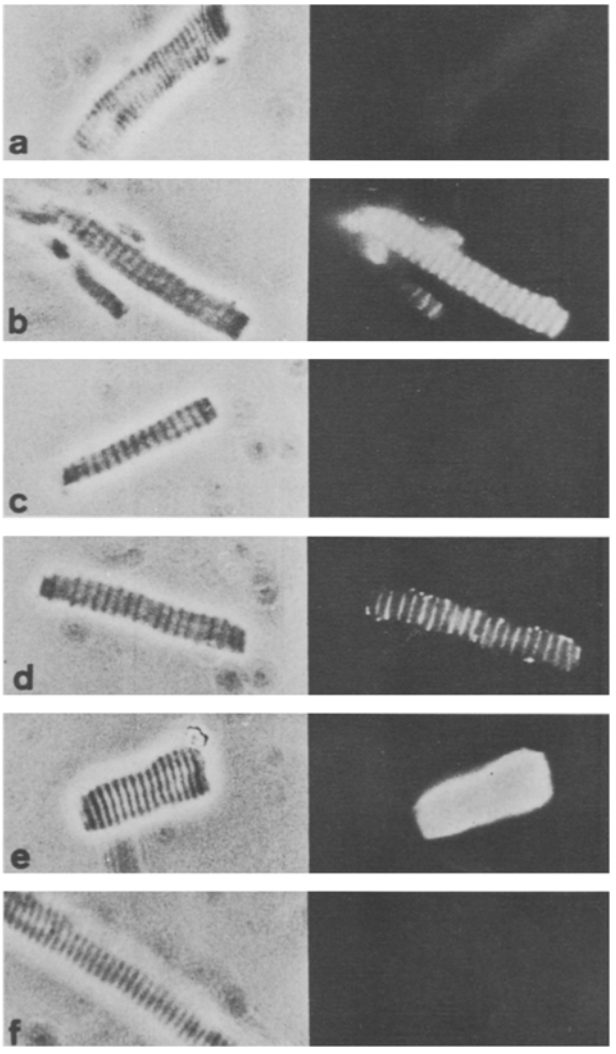

Fig. 1, B. Localization of *a* parvalbumin-like protein, *b* β-actinin, *c* phosphorylase, *d* MM-creatin kinase and *e* actin in strongly contracted fibrils, *f* control.

for parvalbumin-like protein and anti-actin (IgG) which were diluted 1:25. Controls (pre-immune sera) were appropriately diluted. Goat anti-rabbit globuline conjugate (FITC, fluorescein isothiocyanate) was obtained from the Grand Island Biological Company and diluted 1:200.

The following buffers were used:

Relaxing buffer: 0.1 M KCl, 1 mM EGTA, 5 mM EDTA, 1 mM dithiothreitol pH 7.

Contraction buffer: 0.1 M KCl, 5 mM $MgCl_2$, 0.2 mM $CaCl_2$, 1 mM dithiothreitol, 0.5 mM ATP pH 7.

Extraction buffers: a) low ionic strength, 5 mM Tris-HCl, 1 mM dithiothreitol pH 7.7; b) high ionic strength, 0.6 M KCl, 1 mM $MgCl_2$, 10 mM sodium pyrophosphate, 0.1 M potassium phosphate pH 7.

Results and discussion. 1. Properties of isolated myofibrils. When myofibrils were kept in relaxing buffer either at 4 °C or in relaxing buffer containing 50% glycerol at −20 °C for longer than 3 days or several weeks respectively, an increased FITC mediated fluorescence in the I-band region could be observed upon incubation with unspecific control (pre-immune) serum. However, no or only little unspecific binding was seen when fresh myofibrils, prepared from muscle tissue which was put into relaxing buffer immediately after excision, were used. Under these conditions approximately 90% of the fibrils, isolated from leg or breast muscles, were found to be in the relaxed state. This is in contrast to heart muscle where up to 80% of the myofibrils were contracted. Prolonged storage of the excised (leg or breast) muscles in ice or in the frozen state resulted also in a high percentage of contracted myofibrils. Contracted myofibrils were normally obtained by addition of contraction buffer to the pellet of relaxed myofibrils. They were considerably more unstable than in the relaxed state. Raising of the ATP concentration up to 1 mM resulted in a splitting of the fibrils into smaller fragments. Incubation with immune-sera and successive washing steps had to be carried out in relaxing buffer in order to avoid further fragmentation of the fibrils. A reversion of the contraction by resuspension in relaxing buffer (even for 24 h) was never observed. The state of contraction corresponded to stage D as defined by Tunik and Holtzer[8] with closely spaced contraction bands of equal optical density.

2. Localization of the muscle proteins in relaxed and contracted myofibrils. As can be seen from figure 1, A, parvalbumin-like protein, β-actinin and phosphorylase b

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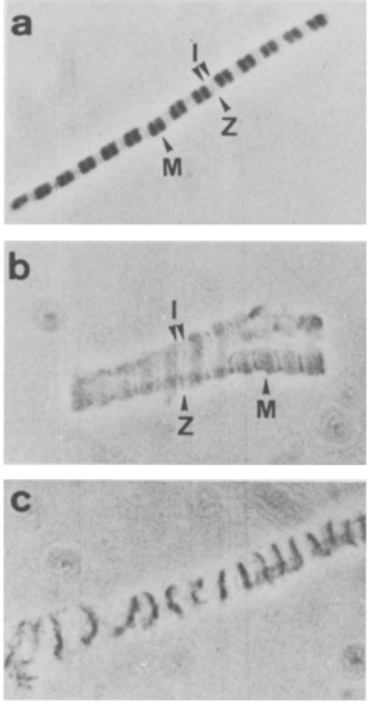

Fig. 2. Myofibrils, *a* prior to extraction in relaxing buffer, *b* in extraction buffer (5 mM Tris-HCl pH 7.7), and *c* in high salt medium (0.6 M KCl pH 7).

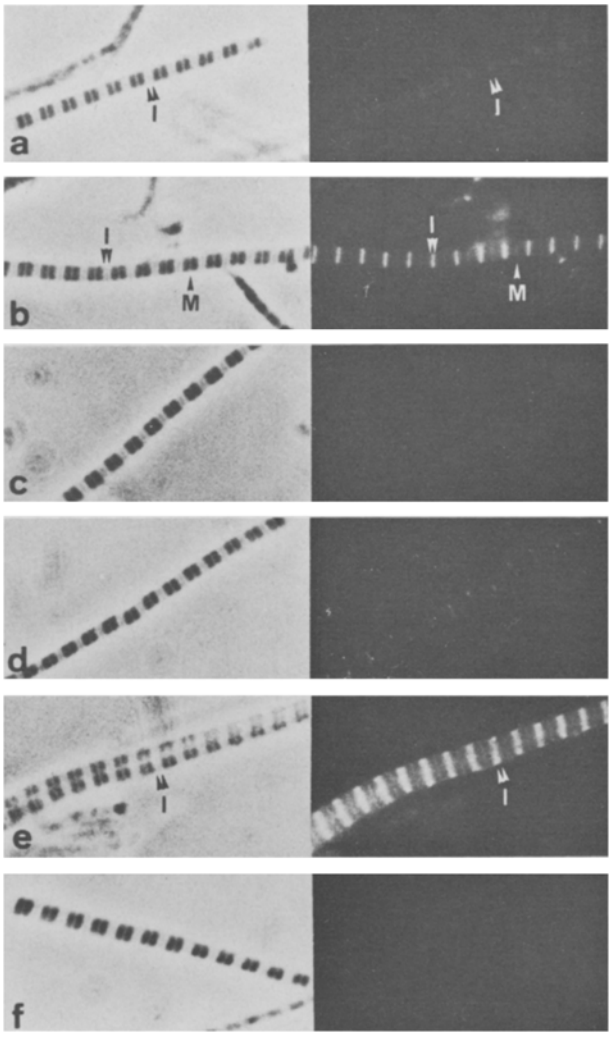

Fig. 3, A. Localization of *a* parvalbumin-like protein, *b* β-actinin, *c* phosphorylase, *d* MM-creatin kinase, and *e* actin in relaxed fibrils after extraction with 5 mM Tris-HCl pH 7.7; *f* control.

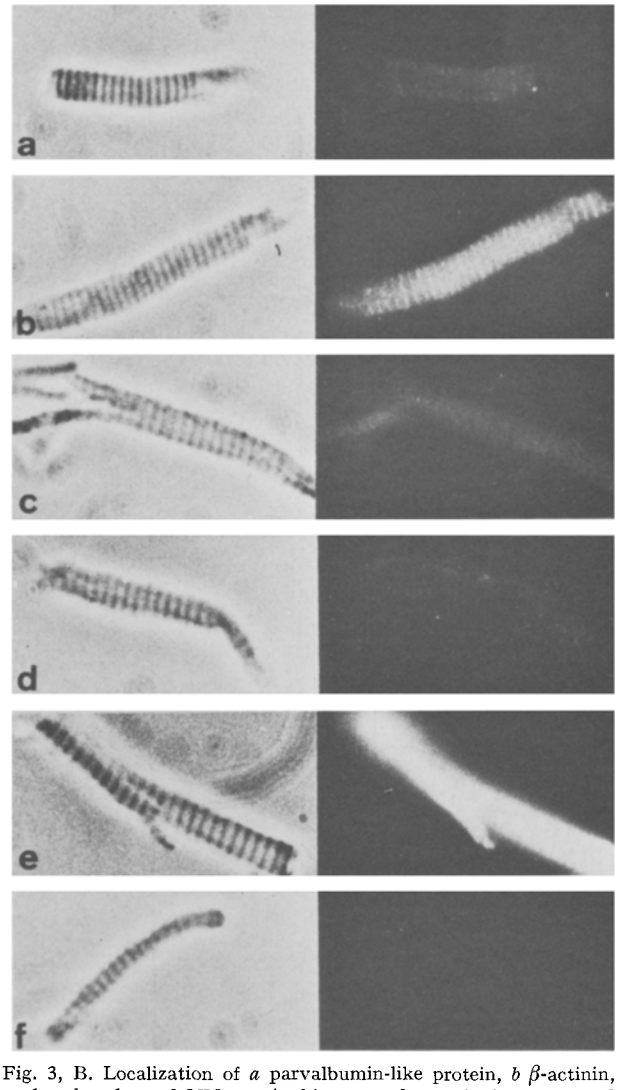

Fig. 3, B. Localization of *a* parvalbumin-like protein, *b* β-actinin, *c* phosphorylase, *d* MM-creatin kinase, and *e* actin in contracted myofibrils after extraction in 5 mM Tris-HCl pH 7.7; *f* control.

are found in the I-band (like actin, the major protein of the thin filament which was chosen for a direct comparison) in the relaxed state, whereas phosphorylase also shows some additional binding in the H-zone[4], MM-creatine kinase was localized mainly in the M-line.

The question was raised whether these components are also bound in strongly contracted myofibrils where the interfilamentary distances are extremely reduced. In this state, the A- and I-bands had disappeared, not allowing a clear localization of the fluorescence, in defined structural regions.

Figure 1, B, shows the localization of the components in contracted myofibrils. Cross-striation is still observed upon incubation with anti-β-actinin and anti-MM-creatin kinase but not with parvalbumin-like protein and phosphorylase.

3. Extraction of the muscle components from relaxed and contracted myofibrils. Mainly 2 procedures for the extraction and isolation of M-line proteins (from myofibrils) are described in the literature: a) a more 'specific' procedure in low salt buffer[9] and b) one under high salt conditions (0.6 M KCl)[10] after extensive washing of the fibrils in relaxing buffer.

The influence of these conditions on the morphology of single fibril is shown in figure 2. It demonstrates a considerable swelling of the still relaxed myofibril in hypotonic, low salt buffer (up to double size) which is immediately reversed by reincubation in relaxing buffer. Extraction (for longer than 2 min) in high salt buffer resulted not only in the extraction of the M-line and A-band, but almost complete destruction of the fibril (figure 2, c). Therefore only low salt conditions were used for extraction and localization. Figure 3, A, shows that creatine kinase and phosphorylase are extracted in 5 mM Tris-HCl pH 7.7 (for 15 min) from relaxed fibrils but not β-actinin or actin. Extraction, however, of contracted fibrils (under conditions used for relaxed myofibrils) resulted in a disappearance of MM-creatine kinase while β-actinin and actin are still visible. Due to the weak fluorescence after incubation with anti-phosphorylase and anti-parvalbumin-like protein sera in contracted fibrils (prior to extraction), no clear result was obtained.

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A differential effect of L-serine on the incorporation of ^{3}H-deoxycytidine and ^{3}H-thymidine into DNA of rat thymocytes[1]

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Summary. The addition of L-serine to short-term cultures of rat thymocytes stimulated the incorporation of ^{3}H-deoxycytidine into DNA, but simultaneously depressed the incorporation of ^{3}H-thymidine into DNA.

Although L-serine is not considered to be an 'essential' amino acid, previous studies have shown that proliferation of certain mammalian cell types can be stimulated by L-serine supplementation[2]. In addition it has been found that the full lymphocytic response to phytohemagglutinin requires an adequate supply of L-serine[3]. Part of the reason for this requirement may be due to an inadequate synthesis of L-serine from glycolytic intermediates[4].

In the process of testing the effect of amino acids on the proliferation of rat thymocytes in vitro, we found that while the addition of L-serine stimulated the incorporation of ^{3}H-deoxycytidine into DNA, the incorporation of ^{3}H-thymidine into nucleic acid was depressed.

Materials and methods. Sprague-Dawley derived male rats, 35–40 days old, weighing 125–150 g, were purchased from Sasco, Omaha, NE, and were maintained on water and