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Pressure-induced Changes in Myofibrillar Proteins

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Abstract

Natural actomyosin, synthetic actomyosin, myosin and F-actin were subjected to lactoperoxidase-catalysed iodination with ^{125}I before and after exposure to a pressure of 150 MNm^{-2}. After iodination myosin was split into its subfragments, light and heavy meromyosin, and the actomyosins were separated into their component proteins.

The effect of the pressure treatment on the extent of iodine incorporation into both the light and heavy chains of myosin and into actin depended on whether the myosin and actin were present as components of natural or synthetic actomyosin or in the purified state. The results suggest that conformational changes altering the exposure of tyrosine groups are induced in isolated myosin and actin by pressure treatment and are partly prevented when these proteins are combined in synthetic actomyosin and more completely prevented when they are combined in natural actomyosin.

Introduction

Physicochemical observations have shown that exposure of the major myofibrillar proteins of muscle to pressure induces changes in molecular weights and enzymatic activities (Ivanov *et al.* 1960; Ikkai and Ooi 1966). Actomyosin undergoes changes that can be accounted for in terms of depolymerization to actomyosin monomers (Ikkai and Ooi 1969) and F-actin is believed to be depolymerized into G-actin monomers (Ikkai and Ooi 1966). Since pressures up to 3000 atm (304 MNm^{-2}) favour the formation of hydrogen bonds and the disruption of hydrophobic bonds (Suzuki *et al.* 1968), it was of interest to characterize the changes taking place on the surface of the proteins using a specific probe for a particular hydrophobic group. The tyrosyl residue is considered to be one of the more hydrophobic groups in proteins at pH values below its pK_a (Tanford 1973), and it is present in all of the myofibrillar proteins discussed in this work (Bodwell 1971; Laki 1971; Maruyama 1971). It is also readily iodinated. In the present study the lactoperoxidase-catalysed iodination of tyrosyl residues was used since this procedure only labels those residues exposed on the peripheral protein surface (Morrison *et al.* 1957; Morrison and Bayse 1970).

F-actin, natural actomyosin, synthetic actomyosin, and myosin were iodinated before and after pressure treatment. The pressures employed for this work were within the range known to disrupt hydrophobic bonds. Further, such pressures were shown to be effective in bringing about substantial effects in pre- and post-rigor muscle and in the solubilization of myofibrillar proteins (MacFarlane 1973, 1974; MacFarlane and McKenzie 1976; Bouton *et al.* 1977).

Materials and Methods

All proteins were prepared from sheep semimembranosus muscle. Natural actomyosin was prepared by the method of Briskey and Fukazawa (1971), myosin by the method of Offer *et al.* (1973), light and heavy meromyosin by the method of Lowey *et al.* (1969) and F-actin by the method of Spudich and Watt (1971). Synthetic actomyosin was prepared by mixing F-actin and myosin in the proportion 1:5 (w/w) and was used without subsequent washing. Sodium dodecyl sulfate (SDS) polyacrylamide gels of actin, myosin and natural actomyosin are shown in Fig. 1.

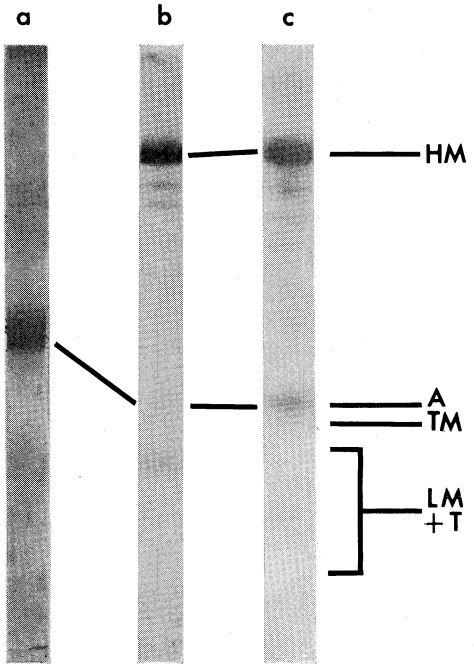

Fig. 1. SDS polyacrylamide gel electrophoresis of myofibrillar proteins actin (*a*), myosin (*b*) and natural actomyosin (*c*). Approximately 50 μg of each protein was applied to each gel. HM, heavy chains of myosin; A, actin; TM, tropomyosin; LM, light chains of myosin; T, troponin.

Pressure treatment was performed on proteins dissolved in 0·6 M KCl, 0·05 M tris-HCl, pH 6·8, at a concentration of 2 mg protein/ml. In order to prevent a reversal of the pressure effects upon release of pressure 5 mM EDTA was included in the solutions of actomyosin and F-actin (Ikkai and Ooi 1966). The samples were placed in glass vials with neoprene plungers and then subjected to a pressure of 150 MNm^{-2} for 1 h at 0°C.

Iodination was performed at 23°C on aliquots of pressure-treated and untreated protein samples essentially as described by Phillips and Morrison (1971). The reaction mixture (2 ml) contained 0·6 M KCl, 0·05 M tris-HCl, pH 6·8, 0·05 mg lactoperoxidase preparation, 0·01–0·1 mCi $Na^{125}I$ (high specific activity) and 4 mg control or pressure-treated muscle protein preparation. The reaction was catalysed by the addition of thirty-six 10-μl aliquots of 1·56 mM H_2O_2 in 0·6 M KCl and 0·05 M tris–HCl, pH 6·8, at 15-s intervals. These conditions have been found to give adequate iodination by other workers (Phillips and Morrison 1971). In some experiments the iodination reaction mixture contained ^{125}I which had been mixed with 50 μl of 10 μM sodium sulfite in order to ensure that the iodination was vectorial (Morrison 1974). The addition of sodium sulfite made no difference to the results and it can be assumed therefore that vectorial iodination occurred in all experiments.

The iodination reaction was terminated by the addition of 2 ml of 10% (w/v) trichloroacetic acid (TCA) solution. The resulting precipitate was washed three times with 10% (w/v) TCA, dissolved in 1 ml of 1% (w/v) SDS in 8 M urea and then dialysed against successive changes of 0·1% (w/v) SDS until the ^{125}I in a 1·0 ml aliquot of the dialysate at equilibrium was below 200 cpm.

The SDS-solubilized proteins (approximately 50 μg) were separated by SDS polyacrylamide gel electrophoresis (Weber and Osborn 1969), using 5% (w/v) gels. After electrophoresis the proteins in the gels were stained with Coomassie brilliant blue R and the gels scanned in a Kipp and Zonen

recording densitometer model DD2 and photographed. The gels were then cut into 1·5 mm slices and ^{125}I determined on each slice using a Packard liquid scintillation spectrometer model 3385 with a manual gamma counting attachment. Each result reported is the average of duplicate incubations.

Protein concentrations were determined using the method of Lowry *et al.* (1951). Lactoperoxidase was obtained from Calbiochem (San Diego, California) as a lypophilized powder which contained approximately 15% (w/w) protein in polyethylene glycol. Carrier-free ^{125}I was obtained from New England Nuclear, Boston, Massachusetts as a solution in 0·1 M NaOH.

Table 1. Effect of pressure on the iodination of myofibrillar proteins

Results expressed as thousands of counts per minute per milligram protein

Protein	Control	Pressure-treated	Protein	Control	Pressure-treated
Natural actomyosin			Myosin		
Prepn 1	127	171	Prepn 1	1950	9510
2	130	170	2	477	1490
Synthetic actomyosin			3	1158	3058
Prepn 1	149	186	Fragmented myosin[A]		
2	179	229	Heavy meromyosin	730	2410
F-actin			Light meromyosin	2430	4470
Prepn 1	880	360			
2	58	23			

[A] Myosin digested with papain after iodination.

Results

The effect of pressure treatment on the iodination of myofibrillar proteins is shown in Table 1. Although the direction and magnitude of the change was the same for any particular myofibrillar protein, it will be noted that for some proteins there is a large difference in specific activity of labelling between the different preparations. The values shown are the extremes obtained from a large number of preparations. It is likely that these differences resulted from the use of carrier-free $Na^{125}I$ which was necessary to obtain adequate radioactivity in the SDS polyacrylamide gels. Natural actomyosin showed greater iodination after pressure treatment (Table 1). Fractionation of the natural actomyosin components by SDS gel electrophoresis (Fig. 2*a*) revealed that there was an increase in the iodination of all components. However, the increase in the actin component was greater than for any other component (4 to 1 for actin compared with approximately 2 to 1 for the other major components). The increase in labelling of these major components was much greater than that observed for the natural actomyosin preparations (approximately 30% increase) since in the latter, many of the minor constituents did not show much change after treatment.

With synthetic actomyosin, as with natural actomyosin, pressure treatment caused an increase in iodine uptake, from which it is inferred that the number of accessible tyrosyl residues on the surface of the proteins was increased (Table 1). The increase was greatest in the actin component and somewhat less in the contaminating tropomyosin. SDS gel electrophoresis revealed there to be only a very small amount of tropomyosin present; however, what was there was relatively heavily labelled. There was little or no change in the myosin light chain components while the myosin heavy chains actually showed a decrease (Fig. 2*b*).

In contrast to the above findings for the actin of actomyosin, when isolated F-actin was exposed to pressure there was a decrease in iodination (Table 1).

When isolated myosin was subjected to pressure there was a large increase in iodination (Table 1) of both the heavy and the light chains (Fig. 2*c*). This contrasts with the findings for the myosin present as a component of natural or synthetic actomyosin.

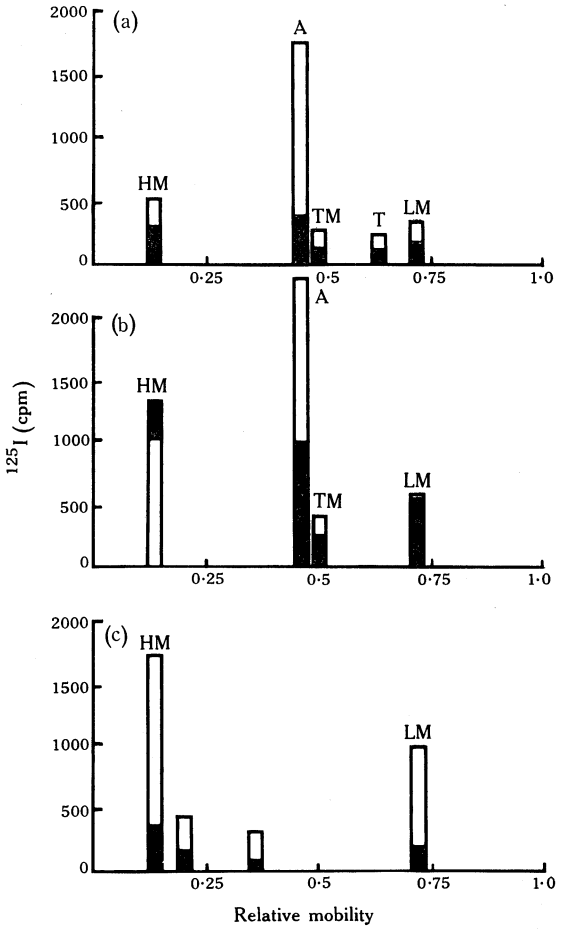

Fig. 2. Effect of pressure on the iodination of natural actomyosin (*a*), synthetic actomyosin (*b*) and myosin (*c*). The individual components were separated by SDS polyacrylamide gel electrophoresis and are shown at their approximate mobilities relative to pyronin Y. Black bars, control; white bars, pressure-treated. The three light chain components of myosin were summed for presentation. In (*a*) troponin components were also summed. Lettering as in Fig. 1. The identities of the two minor components in (*c*) are unknown.

Disruption of iodinated control samples of myosin into heavy meromyosin and light meromyosin fragments by papain digestion showed that the heavy meromyosin took up less label than did the light meromyosin (Table 1). The same was true for the pressure-treated samples even though in these the subfragments both incorporated more label than they did in the control samples.

Discussion

The object of this study was to obtain information on association–dissociation or conformational changes of a number of muscle proteins following pressure treatment. Lactoperoxidase-catalysed iodination was used to specifically label the exposed tyrosyl residues of the proteins thus allowing alterations in exposure of these groups to be measured. Whether these changes are due to conformation changes or association–dissociation will be discussed. Marchalonis (1969), using this technique, noted that the monomeric forms of flagella and of mouse immunoglobins were more readily iodinated than their polymeric forms, and suggested that the reactive tyrosyl residues might be obstructed in the polymer.

In the present work pressure-treated natural actomyosin, synthetic actomyosin, myosin and the papain subfragments prepared from pressure-treated myosin all showed an increase in iodination relative to the control samples. Since previous work has suggested that pressure treatment depolymerizes these proteins (Ikkai and Ooi 1969; O'Shea *et al.* 1976) our present findings are in keeping with those of Marchalonis (1969).

On the other hand, pressure treatment of F-actin resulted in a decrease in iodination. A possible explanation for this stems from the fact that the samples were maintained in EDTA solution to prevent reversal of the pressure effects. Under these conditions, G-actin formed as a result of pressure treatment would be irreversibly denatured by EDTA (Maruyama and Gergely 1961) and the studies of Lehrer and Kerwar (1972) indicate that EDTA-denatured G-actin has fewer exposed tyrosyl residues than has F-actin. When control G-actin was iodinated the incorporation of iodine was intermediate between F-actin and F-actin pressure treated in the presence of EDTA (T. Walsh, J. M. O'Shea and R. K. Tume, unpublished observations).

The actin component of natural actomyosin showed a large increase in labelling after pressure treatment even though EDTA was present. It appears therefore that when the other components, myosin, tropomyosin and troponin are present, actin is not denatured in the presence of EDTA. With synthetic actomyosin where myosin was essentially the only other protein present, the increase in iodination of the actin component was not as great as with natural actomyosin, suggesting that there may have been some G-actin denaturation.

Myosin (heavy and light chains), when essentially free from other muscle proteins, showed a large increase in iodination following pressure treatment. When present in natural actomyosin only a slight increase was observed and myosin heavy chains in synthetic actomyosin actually gave a reduction in labelling. It appears that when actin is present, myosin is restricted to some extent from altering its conformation on pressure treatment, just as myosin (and other components) appeared to prevent the formation of EDTA-denatured G-actin. It is therefore proposed that, in the system described here, some sort of an association between myosin and actin remains after pressure treatment.

Separation of myosin into heavy and light chains and also into heavy and light meromyosin revealed that iodination took place in all of the fractions. Despite the overall abundance of tyrosyl residues in heavy meromyosin (Lowey 1971), the light meromyosin showed the higher level of iodination indicating that more tyrosyl residues are exposed in this region. The comparatively larger increase in iodination in the heavy meromyosin following pressure treatment suggest that this region is more affected by pressure.

Although there was more iodine taken up by the myosin heavy chains than by the light chains, on a weight basis the light chains incorporated more label. Thus a greater proportion of tyrosyl residues are exposed in the light chains than in the heavy chains. Despite this difference both heavy and light chains of myosin were affected to a similar extent by pressure treatment and it is proposed that pressure has an effect over the entire surface of the myosin molecule, resulting in exposure of hydrophobic tyrosyl residues. On release of pressure the system cannot return to its original state and the tyrosyl residues remain exposed. In the presence of associated actin (where the increase for myosin on pressure treatment was considerably less) it is likely that the actin imposed certain restrictions upon the myosin molecule, reducing the exposure of hydrophobic tyrosine sites due to pressure treatment.

Acknowledgment

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