OBSERVATIONS OF INTERACTIONS BETWEEN MYOFIBRILLAR PROTEINS BY CHEMICAL CROSS-LINKING

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1. Introduction

The lattice of striated muscle contains, and is presumed to depend for its morphology on, a series of minor protein components, besides the actin and myosin, which between them account for the bulk of the myofibril. Some of the minor proteins of the thin-filaments [1], namely tropomyosin, the three troponin components and α-actinin, have been located with reasonable certainty. However, at least five other myofibril proteins are electrophoretically observable [2], and of these only one, the so-called C-protein has been located in the thick filament lattice, where it is responsible for a set of periodic striations observed in the A-bands by electron microscopy [3,4]. This protein interacts with myosin filaments, but no evidence of interaction with myosin was obtained in the analytical ultracentrifuge at higher ionic strength, at which myosin is soluble and monomeric. The other most prominent minor component occurring in myosin preparations is the B-protein of Starr and Offer [2], which has an apparent mol. wt of about 160 000 in gel electrophoresis in the presence of SDS. This may be a component of the M-line. One M-line protein was purified by Morimoto and Harrington [5] and was subsequently identified [6] as creatine kinase. This is evidently one of the components found in M-line extracts by Eaton and Pepe [7], and has subunit a mol. wt of some 40 000. The same workers reported a further component of mol. wt 100 000 which may possibly be the E-protein in the terminology of Starr and Offer, and M-line component II of Masaki and Takaiti [8], tentatively identified as glycogen phosphorylase. The latter authors in addition report the existence of a more abundant M-line protein of apparent subunit mol. wt 165 000, further characterised in its native state by Landon and Oriol [9], which binds strongly to myosin. The possibility was raised that this could be equated with the B-protein of Starr and Offer.

The complexity of the mixture of proteins extracted from the myofibril, allied to the possibility that some of them may be artefacts of proteolysis, makes it imperative to try to reconcile the data reported by different laboratories. The approach here described involves the use of reversible cross-linking reagents, which make it possible to establish which proteins in a soluble extract are specifically associated with one another. The method has been successfully applied to studies of the interaction of proteins in complex mixtures in membranes [10] and ribosomes [11]. The first upshot of the present study is the demonstration that the B-protein is associated with myosin even at an ionic strength of 0.5, and can be almost certainly identified with the M-protein of Masaki and Takaiti. One further association of a minor protein component with myosin is also reported.

2. Experimental

Myosin from rabbit skeletal muscle was prepared by the method of Perry [12]. C-protein prepared as

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described by Offer et al. [3] was a gift from Dr G. W. Offer. The cross-linking reagents used were dimethylmalonimidate, dimethyladipimidate, dimethylsuberimidate, dimethylsebacimidate, all prepared from the corresponding dinitriles [13], and the cleavable reagents, dimethyl-3,3′-dithiobispropionimidate and dithiobissuccinimidylpropionate, were obtained from Pierce Chemical Co. For cross-linking, protein at 2 mg/ml was allowed to react with the cross-linker in 0.5 M sodium chloride, 0.05 M triethanolamine, pH 8.0, with stirring in the cold for 1.5 h. The optimal concentration of cross-linking agent was found to be about 0.2 mg/ml. It was added either as a solid, dissolved in water, or in the case of the last of the above reagents, as a concentrated solution in acetone. Reversal of cross-linking was achieved by incubation with 5% β-mercaptoethanol for 30 min. Before electrophoresis the solution was made 1% in SDS and heated for 5 min in a boiling water bath, or in the case of the dimethyldithiobispropionimidate, warmed for 1 h at 37°C. It was then applied to cylindrical gels 6 mm ID. The acrylamide concentration was variously 2.8, 3.0 and 3.5%, and the buffer was 0.1 M boric acid, 0.1 M sodium acetate, 0.1% SDS, pH 8.5. The electrophoresis was allowed to proceed for 2–5 h at 8–9 mA/tube. Staining and destaining ensued as described by Weber and Osborn [14]. The gels stained with Coomassie brilliant blue were subjected to scanning in a Gilford densitometer.

3. Results and discussion

A densitometric scan of a typical polyacrylamide gel, run in the presence of SDS, of a myosin preparation is shown in fig.1(a). In the stained gel, six components (including myosin) can be clearly discerned in the range of apparent mol. wt 78 000–225 000. (We are not concerned here with the components of lower molecular weight, amongst which the myosin light chains predominate.) The apparent mol. wts (which depend slightly on the gel concentration, and on the standards used) are 225 000, corresponding to the myosin heavy chains, 195 000 (very faint), 160 000, 135 000, 105 000 (faint) and 78 000. Apart from the myosin, the two most prominent bands are the B- and C-proteins [2]. Thus assignment is confirmed by a comparison with purified C-protein and electrophoresis of the preparation augmented with C-protein (fig.1).

All the cross-linking agents, except dimethylmalonimidate, which produces no appreciable effect, generated profound changes in the electrophoretic pattern. All or most of the myosin heavy chains were transformed into the dimeric species, migrating with a mobility in the SDS-containing gel that corresponds

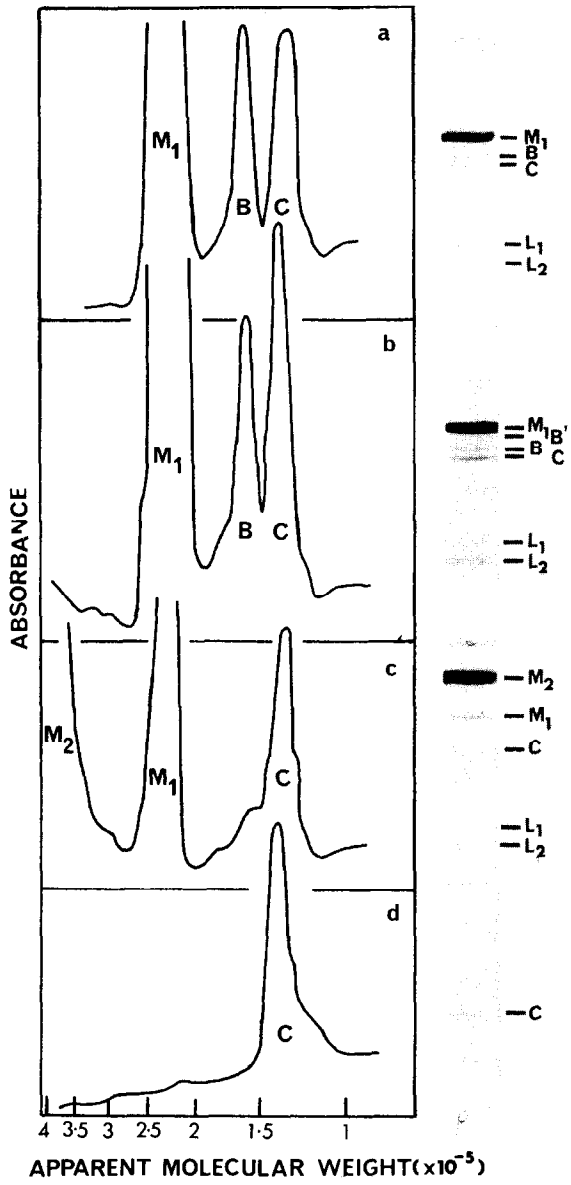

Fig.1.

to a mol. wt of 460 000. In the conditions used, only traces of higher (intermolecular) oligomers of heavy chains are generated. The shortest of the bifunctional agents to bring about substantial cross-linking, dimethyladipimidate, has a chain length between the α-carbon atoms of 6 Å, fully extended, or 4 Å, root-mean-square end-to-end, which therefore defines the upper limit of the distance of closest approach of amino groups in the associated species. The second prominent effect of cross-linking is the total disappearance of the B-protein, whereas the C-protein zone remains undiminished. When the reversible cross-linker, dithiobissuccinimidyl propionate, is used, reduction of the reaction products with β-mercaptoethanol leads to cleavage of the cross-linker and complete regeneration of the original gel pattern.

The results permit the conclusion that the B-protein is strongly and specifically associated with myosin in solution, in that the interaction persists at ionic strength higher than 0.5. The failure of the C-protein to enter into cross-linking interactions bears out the inference of Offer et al. [3] that it does not interact to a significant degree with dispersed monomeric myosin. On the basis of its apparent molecular weight, and its strong interaction with myosin, the B-protein may with some confidence be equated with the M-protein of Masaki and Takaiti [8]. The results disprove the suggestion [15] that the B-protein is an electrophoretic artefact, associated with phosphate buffer systems in SDS-containing gels. Not only do we find that it occurs in our borate system but it also vanishes after cross-linking, reappearing when the cross-links are broken. Thus not only is the B-protein a genuine myofibrillar component, but also, together with the enzyme creatine kinase [5,6], it is evidently the major structural constituent of the M-line, serving presumably the role of a mechanical rigidity element, in which the ends of the thick filaments are rooted. It should be noted that the electrophoretic distribution of total myofibril protein, dissolved in SDS [16,17], shows no component of greater abundance than myosin, actin, the known thin-filament constituents and the B- and C-proteins. The identification of the B-protein thus completes the enumeration of all but the trace constituents of the myofibril.

Between myosin and the B-protein a faint band of apparent mol. wt 195 000 is consistently observed. We may term this B′. This zone also disappears on exposure to cross-linking reagents, and reappears when the cross-links are ruptured. This species is thus also not an electrophoretic artefact, and it appears to be associated with the myosin.

Reversible cross-linking represents an approach to the detection and study of intermolecular interactions between myofibrillar proteins which has the advantages of versatility and convenience. Its wide potential as a probe of thick filament structure is being further explored.

Fig.1. Densitometer scans of relevant sections of stained 3% polyacrylamide gels containing SDS. (a) Typical myosin preparation showing B-protein, C-protein and dissociated myosin heavy chains (M_1). The B′ component, referred to in the text, lies between M_1 and B, but is too faint to be well resolved in the scan. (b) Same preparation augmented with purified C-protein. (c) Same preparation after treatment with the cross-linking reagent, dithiobissuccinimidyl propionate, showing unchanged C-protein zone, residual monomeric heavy chains and dimers of heavy chains (M_2). (d) Purified C-protein. The mol. wt scale is based on a calibration mixture of covalently cross-linked oligomers of bovine serum albumin run in a companion gel. The corresponding gels are shown beside each trace, and show in addition to the above components the faint B′ zone, and two light chains (L_1 the major 'alkali' light chain, and L_2 the 'DTNB-light chain').

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References

[1] Ebashi, S. (1974) Essays in Biochemistry, 10, 1–36
[2] Starr, R. L. and Offer, G. W. (1971) FEBS Lett., 15, 40–44.
[3] Offer, G. W., Moos, C. and Starr, R. L. (1973) J. Mol. Biol. 74, 653–676.
[4] Offer, G. W. (1972) Cold Spring Harbor Symp. Quant. Biol. 37, 87–96.
[5] Morimoto, K. and Harrington, W. F. (1972) J. Biol. Chem. 247, 3052–3061.
[6] Turner, D. C., Wallimann, T. and Eppenberger, H. M. (1973) Proc. Nat. Acad. Sci. USA 70, 702–705.
[7] Eaton, B. L. and Pepe, F. (1972) J. Cell. Biol., 55, 681–695.
[8] Masaki, T. and Takaiti, O. (1974) J. Biochem. (Tokyo), 75, 367–380.

[9] Landon, M. F. and Oriol, C. (1975) Biochem. Biophys. Res. Commun. 62, 241–245.
[10] Wang, K. and Richards, F. M. (1974) J. Biol. Chem., 249, 8005–8018.
[11] Traut, R. R. Bollen, A., Sun, T-T., Hershey, J. W. B., Sundberg, J. and Pierce, L. R. (1973) Biochemistry, 12, 3266–3273.
[12] Perry, S. V. (1955) in: Methods in Enzymology (Colowick, S. P. and Kaplan, N. O., eds) Vol. 2, pp. 582–588, Academic Press, New York.
[13] Davies, G. E. and Stark, G. R. (1970) Proc. Nat. Acad. Sci. USA 66, 651–656.
[14] Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412.
[15] Morimoto, K. and Harrington, W. F. (1973) J. Mol. Biol. 77, 165–175.
[16] Sender, P. M. (1971) FEBS Lett. 17, 106–110.
[17] Scopes, R. K. and Penny, I. F. (1971) Biochim. Biophys. Acta, 236, 409–415.