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The Interaction of C-Protein with Heavy Meromyosin and Subfragment-2

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C-protein has previously been shown to bind to the light-meromyosin region of the myosin tail. Examination of mixtures of C-protein with heavy meromyosin or subfragment-2 or subfragment-1 in the analytical ultracentrifuge shows that there is also a binding site for C-protein in the subfragment-2 region of the tail.

The thick filaments of vertebrate skeletal muscle consist not only of myosin but also of C-protein and several other proteins (Offer, 1972; Offer *et al.*, 1973; Pepe & Drucker, 1975; Craig & Offer, 1976; Craig, 1977). The myosin molecules are packed so that the light-meromyosin region of their tails forms the backbone of the filament, whereas the rest of the molecule, the heavy-meromyosin region, consisting of the remainder of the tail and the two globular heads, emerges at the surface (Huxley, 1963; Lowey, 1971). Labelling of muscle with antibodies has shown that C-protein is located at seven axial positions about 43 nm apart in each half of the filament (Offer, 1972; Pepe & Drucker, 1975; Craig & Offer, 1976). Because C-protein was thus shown to be accessible to antibody and the antibody was bound near the backbone of the filaments (rather than in the inter-filament spaces), it was concluded that C-protein is present, at least in part, on the surface of the filament (Craig & Offer, 1976). In such a position the C-protein might interact with the light-meromyosin region of the myosin tail or with some portion of the heavy-meromyosin region of the molecule or possibly with both.

To determine the location of C-protein-binding sites in myosin, the interaction of C-protein with fragments of myosin has been studied. Previous work has shown that C-protein binds not only to myosin filaments but to aggregates (paracrystals) of light-meromyosin (Moos, 1972; Moos *et al.* 1975). We have now investigated whether C-protein can bind to heavy meromyosin and its proteolytic subfragments, subfragment-2 (the truncated tail) and subfragment-1 (a cleaved head) (see Fig. 2*b*). Because these proteins are soluble under physiological ionic conditions, binding experiments are not so straightforward as those with light meromyosin. We have therefore used the analytical ultracentrifuge to detect binding. The sedimentation profile of a mixture of two proteins that bind rapidly and reversibly is not the sum of the profiles of the individual components, but consists of a fast-spreading reaction boundary and a slower boundary of one of the components; the component producing the slower boundary depends on the initial concentration of the components and the binding constant (Gilbert & Jenkins, 1959; Nichol *et al.*, 1964).

Experimental

C-protein was prepared by the method of Offer *et al.* (1973) and heavy meromyosin by tryptic digestion of myosin for 20 min at a myosin concentration of 15 mg/ml and a trypsin concentration of 5 μg/ml (Lowey & Cohen, 1962).

Subfragment-2 was prepared by papain digestion of myosin for 60 min at 20°C in 0.5 M-KCl/10 mM-potassium phosphate, pH 6.0. The papain concentration was 0.06 mg/ml and that of myosin was 18 mg/ml. The proteins soluble at low ionic strength were precipitated with alcohol, and the material soluble in 0.5 M-KCl was reprecipitated by dialysis at pH 4.5 (Lowey *et al.*, 1969).

Sedimentation velocity runs were performed in a Beckman model E ultracentrifuge, with double-sector cells with a filled Epon centrepiece in an AnD rotor.

Results and Discussion

In Figs. 1(*a*) and 1(*b*) the sedimentation pattern of a mixture of C-protein and heavy meromyosin is compared with the patterns of the individual proteins. The schlieren peak of C-protein alone (Fig. 1*a*) is broad and skewed because C-protein associates at the low ionic strength used (Offer *et al.*, 1973). By contrast the schlieren peak of heavy meromyosin is hypersharp because the molecule is elongated (Fig. 1*b*). The sedimentation pattern of the mixture is markedly different from the sum of the individual patterns. The slow peak sedimented with an $s_{20,w}$ value of approximately 5.3 S and we presume this to represent the boundary of C-protein; the area under this peak was much smaller than that of an identical

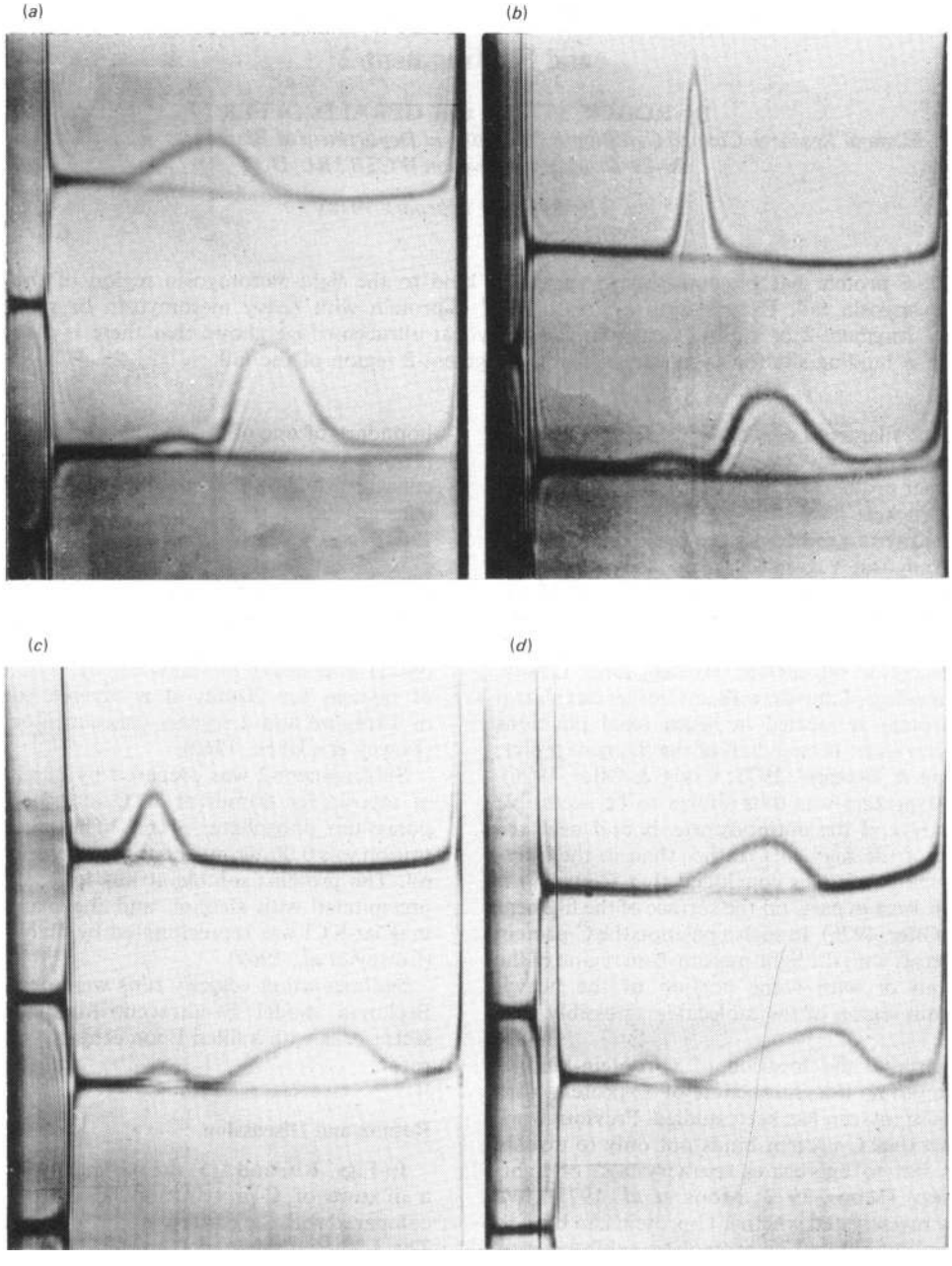

Fig. 1. *Sedimentation patterns of mixtures of C-protein with heavy meromyosin or subfragment-2*
(*a*) Upper trace, 2.5mg of C-protein/ml; lower trace, 2.5mg of C-protein/ml + 3.9mg of heavy meromyosin/ml. (*b*) Upper trace, 3.5mg of heavy meromyosin/ml; lower trace, 2.5mg of C-protein/ml + 3.5mg of heavy meromyosin/ml. Photographs (*a*) and (*b*) were taken 60min after reaching the final speed of 52640rev./min with a bar angle of 50°. (*c*) Upper trace, 1.9mg of subfragment-2/ml; lower trace, 3.6mg of C-protein/ml + 1.9mg of subfragment-2/ml. (*d*) Upper trace, 3.6mg of C-protein/ml; lower trace, 3.6mg of C-protein/ml + 1.9mg of subfragment-2/ml. Photographs (*c*) and (*d*) were taken 90min after reaching the final speed of 60000rev./min with a bar angle of 55°. The solvent in all cases was 0.07M-KCl/10mM-imidazole chloride, pH 7.0 (measured at 5°C), and the rotor temperature was 20°C.

concentration of C-protein. The broad leading peak, corresponding to the reaction boundary, sedimented faster ($s_{20,w} = 8.6$S) than either C-protein ($s_{20,w} = 6.2$S) or heavy meromyosin ($s_{20,w} = 6.6$S) alone. (In other experiments the velocity of this leading peak increased with increasing concentrations of C-protein, as theory predicts.) Ahead of the leading peak there was some very-fast-sedimenting material, suggesting that material of high molecular weight was formed. The experiment clearly shows that C-protein binds to heavy meromyosin under these ionic conditions. In 0.1 M-KCl binding was less strong, as was the case at the lower ionic strength at pH 7.5.

Figs. 1(*c*) and 1(*d*) show a similar experiment with C-protein and subfragment-2. As with the above result the sedimentation profile of the mixture was not the sum of the individual components. The area of the slower-moving peak was much smaller than that of an equal concentration of subfragment-2, whereas the leading peak was faster and broader than that of an equal concentration of C-protein. We conclude that C-protein binds to subfragment-2. However, C-protein does not bind to subfragment-1, since the sedimentation profile of a mixture of C-protein and subfragment-1 can be accounted for by the contribution of the components.

Unlike ATP aminohydrolase, which binds specifically to subfragment-2 (Ashby & Frieden, 1977),

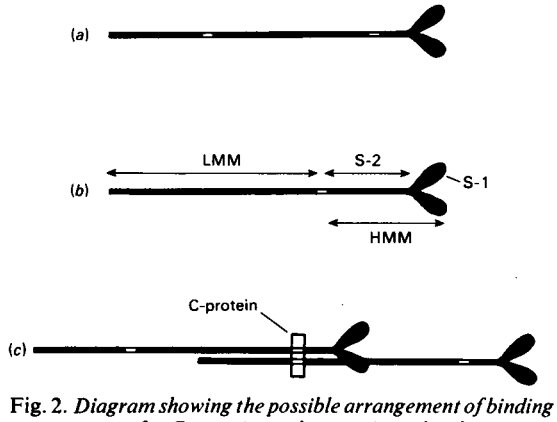

Fig. 2. *Diagram showing the possible arrangement of binding sites for C-protein on the myosin molecule*

The binding sites are indicated by the white areas in the tails. (*a*) Separate binding sites in the light-meromyosin and subfragment-2 regions; (*b*) binding site shared by the light-meromyosin (LMM) and subfragment-2 (S-2) regions; (*c*) diagram showing how in the thick filament one C-protein molecule could interact with the heavy-meromyosin (HMM) region of one myosin molecule and the light-meromyosin region of another. Abbreviation: S-1, subfragment-1. The shapes used for the myosin molecules are taken from Offer & Elliott (1978).

C-protein thus binds both to the subfragment-2 region and to the light-meromycsin region of the myosin tail. Although the binding of C-protein to heavy meromyosin and to subfragment-2 is not as strong as to myosin filaments and light-meromyosin paracrystals (Moos *et al.*, 1975), the systems are not directly comparable. In myosin filaments and light-meromyosin paracrystals each C-protein molecule may be able to bind to several tails and the binding may be correspondingly strong. The relative strengths of binding of C-protein to subfragment-2 and light meromyosin cannot therefore be determined.

There are two simple possibilities for the distribution of C-protein-binding sites along the myosin tail. In Fig. 2(*a*) the myosin tail has separate binding sites for C-protein in the light-meromyosin and subfragment-2 regions. In Fig. 2(*b*) the myosin tail has only a single binding site for C-protein that is located at the junction between the light-meromyosin and subfragment-2 regions. We have previously concluded that in the thick filament the axial extent of C-protein at each site is only about 7 nm and that the long axes of the C-protein molecules must be arranged perpendicular to the myosin tails in the backbones of the thick filament (Craig & Offer, 1976). If the C-protein molecules bind to the proteolytic fragments in a similar way (Fig. 2*c*) the length of the binding sites along the tail cannot be greater than 7 nm. However, the region of the myosin tail that is susceptible to digestion by proteolytic enzymes in the preparation of the meromyosins is longer than this (Mihalyi & Harrington, 1959; Lowey & Cohen, 1962; Huxley, 1963; Segal *et al.*, 1967; Lowey, 1971; Burke *et al.*, 1973). It is therefore unlikely that in the second case sufficient of the binding site on the myosin molecule could survive the digestion to account for the observed binding of C-protein to light meromyosin and subfragment-2. Thus it appears that the myosin tail has at least two binding sites for C-protein, with a minimum of one in the light-meromyosin region and one in the subfragment-2 region (Fig. 2*a*).

Do the interactions that have been demonstrated between C-protein and light meromyosin or subfragment-2 in solution occur in the thick filaments of muscle? If, in the thick filaments of resting muscle, the subfragment-2 regions of the myosin tails were not bent outwards but were closely attached to the light-meromyosin regions, then the surface of the shaft would be expected to consist largely of the subfragment-2 regions and partly of the light-meromyosin regions of the myosin tails (Squire, 1973). The result in the present paper that C-protein can bind to the subfragment-2 regions as well as to the light-meromyosin regions is thus consistent with previous suggestions that C-protein molecules are attached to the surface of the shaft (Offer, 1972; Craig & Offer, 1976). If the long axes of the C-protein molecules were arranged circumferentially around

the shaft each C-protein molecule might then interact with the tails of several myosin molecules, some of them in the light-meromyosin region and some in the subfragment-2 region (Fig. 2*c*).

If, in resting muscle, the subfragment-2 regions of the myosin molecules making up the thick filaments were attached to the light-meromyosin part of the filament shaft by C-protein, the outward movement of the cross-bridges of heavy meromyosin thought to occur during contraction (Huxley, 1969) would be prevented. It is an intriguing possibility that, if the binding of C-protein to the subfragment-2 region or to light meromyosin were regulated, this could form the basis of a mechanism to regulate the interaction of myosin heads with actin.

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