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Aldolase Binding to Actin-Containing Filaments

FORMATION OF PARACRYSTALS

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Electron-microscope observations show that when aldolase binds to F-actin or F-actin-tropomyosin, highly ordered paracrystalline structures are formed consisting of tightly packed filament bundles cross-banded at 36 nm intervals. Morphologically different paracrystalline arrays are formed between aldolase and F-actin-tropomyosin-troponin. The filament bundles are far more extensive and are characterized by a prominent cross-striation at 38 nm intervals. It is suggested that this reflects an interaction between troponin and aldolase.

Histochemical studies have established that the majority of glycolytic enzymes are localized within the I-band of the muscle fibre (Sigel & Pette, 1969), and biochemical studies have shown that fructose biphosphate aldolase and a number of other glycolytic enzymes interact strongly with F-actin, the major structural protein of the I-band (Arnold & Pette, 1968; Arnold *et al.*, 1971). Further studies (Clarke & Masters, 1975) have shown that enzyme binding can occur at ionic strengths and protein concentrations approximating to those found intracellularly and that the regulatory proteins tropomyosin and troponin exert an important influence on enzyme adsorption on actin-containing filaments.

Although the kinetic parameters of aldolase are known to be altered on adsorption (Arnold & Pette, 1970), the biological significance of this binding remains unclear. In the present paper we report the formation of ordered aggregates between aldolase and actin-containing filaments.

Materials and Methods

F-actin and the tropomyosin-troponin complex were prepared from an acetone-dried powder of ox skeletal muscle by the method of Spudich & Watt (1971). Tropomyosin and troponin were purified as described by Clarke *et al.* (1976). The F-actin-tropomyosin and F-actin-tropomyosin-troponin complexes were prepared as described by Ishiwata (1973). Ox muscle aldolase was prepared by the method of Penhoet *et al.* (1969). All preparations were examined by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Clarke *et al.*, 1976) to determine whether significant amounts of impurity were present.

For examinations by electron microscopy, aldolase

(0.25 mg/ml) and the filament preparations (0.5 mg/ml) were mixed in 5 mM-imidazole/10 mM-KCl/1 mM-MgCl₂/2 mM-dithiothreitol, pH 6.8. The paracrystals were formed by raising the ionic strength by adding one of the following salts: KCl to 25 mM, MgCl₂ to 5 mM, or BaCl₂ to 5 mM final concentrations. A drop of the mixture was applied to a carbon-coated grid and the excess quickly removed, then stained with 1% uranyl acetate. The grids were examined at 60 or 80 kV in a Philips EM 300 electron microscope, calibrated by the method of Dickson (1974). To test for enzyme adsorption the mixtures were centrifuged at 120 000g for 60 min, and the resulting supernatants and pellets assayed for enzyme activity (Richards & Rutter, 1961).

Results and Discussion

Plate 1(a) shows the appearance of the aggregates formed between F-actin-tropomyosin-troponin filaments and aldolase in the presence of 5 mM-BaCl₂. These complexes between F-actin-tropomyosin-troponin and aldolase consist of tightly packed bundles of filaments 0.1–0.4 μ m in width and of considerable length, often in excess of 20 μ m (Plate 1b). These structures are characterized by strong cross-striations which repeat at 38 nm along the filament bundle, and there is another less prominent cross-banding midway between the strong striations. An interfilament spacing of 11 ± 0.8 nm has been measured. Although the Plates all show the aggregates formed on addition of BaCl₂, identical results were obtained on addition of MgCl₂ or KCl.

The formation of these ordered aggregates is due to aldolase binding, as only single filaments are observed under the same conditions in the absence of the enzyme. Independent evidence of enzyme

binding was obtained from centrifugation experiments (see the Materials and Methods section), which demonstrated the complete adsorption of the aldolase on the filaments under these conditions. There are marked morphological differences from the paracrystals of F-actin-tropomyosin-troponin alone, which form at 50 mM-MgCl₂ (Gillis & O'Brien, 1975), an example of which is shown in Plate 1(c) for purposes of comparison.

Paracrystalline structures are also formed when aldolase binds to F-actin or F-actin-tropomyosin. These aldolase-F-actin (Plate 2a) and aldolase-F-actin-tropomyosin aggregates (Plate 2b) are similar to each other and consist of filaments in parallel alignment with regular cross-banding at 36 nm. In general these aggregates are less extensive than those formed with F-actin-tropomyosin-troponin and they lack the secondary banding present when troponin is associated with the filament. The inter-filament spacing is 11 ± 1 nm. Once again these structures are markedly different from the paracrystals formed with F-actin alone at high MgCl₂ concentrations (Plate 2c).

The formation of well-ordered aggregates between actin-containing filaments and aldolase provides more information on the nature of aldolase interaction with the structural proteins of the I-band of muscle. These structures appear to be formed by aldolase cross-linking adjacent filaments, suggesting that aldolase must have multiple sites of binding to structural proteins. The coincidence of the aldolase repeat banding at 38 nm on the F-actin-tropomyosin-troponin filaments with the known repeat of troponin on these filaments (Gillis & O'Brien, 1975) points to

the possibility that troponin itself may be involved in enzyme binding in this instance.

This is not to suggest that these paracrystalline arrangements have significance in the I-band of muscle, only that an interaction of aldolase with troponin warrants fuller investigation.

The formation of these ordered aggregates opens the way for a detailed analysis of filament-enzyme interactions by the image-processing techniques now available.

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EXPLANATION OF PLATE I

Aldolase-F-actin-tropomyosin-troponin paracrystals

(a) Paracrystal structure formed between aldolase and F-actin-tropomyosin-troponin in presence of 5 mM-BaCl₂. Large arrows indicate the 38 nm cross-striations, small arrows indicate the secondary striations. Bar represents 40 nm. (b) Low-magnification micrograph of aldolase/F-actin-tropomyosin-troponin mixture, illustrating extensive formation of ordered filament bundles. Bar represents 0.5 μ m. (c) Paracrystal of F-actin-tropomyosin-troponin in the presence of 50 mM-MgCl₂ in the absence of aldolase. Bar represents 40 nm.

EXPLANATION OF PLATE 2

Aldolase-F-actin and aldolase-F-actin-tropomyosin paracrystals

(a) Paracrystal structure formed between aldolase and F-actin in presence of 5 mM-BaCl₂. Note parallel alignment of filaments with regular cross-banding at 36 nm intervals. Bar represents 40 nm. (b) Paracrystal structure formed between aldolase and F-actin-tropomyosin in the presence of 5 mM-BaCl₂. Note similarity to structure in (a). Bar represents 40 nm. (c) Paracrystal of F-actin in the presence of 50 mM-MgCl₂ in the absence of aldolase. Bar represents 40 nm.