Third Component Participating in the Superprecipitation of 'Natural Actomyosin'

It has been shown that the superprecipitation of 'natural actomyosin' is subtly controlled by a minute amount of calcium ions[1-3]; the superprecipitation is promoted or depressed by the increase or the decrease of calcium ions in the reaction medium respectively. This unique action of calcium ions not only affords the basis for the relaxing mechanism of chelating agents[3] as well as physiological relaxing factor[3], but also appears to be closely associated with the link between excitation and contraction[4].

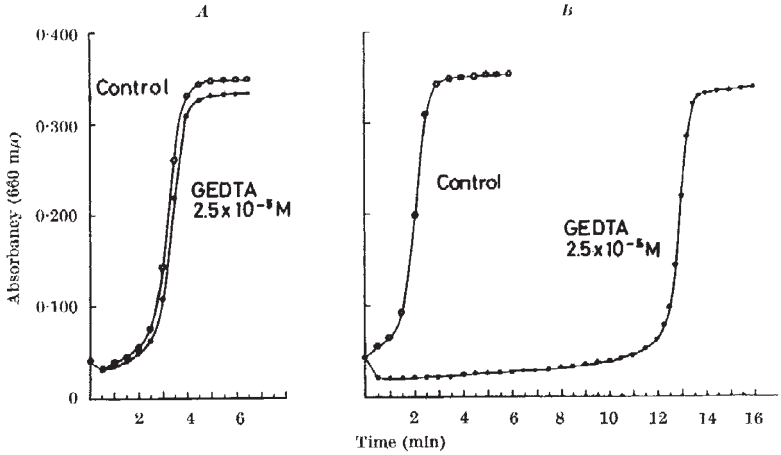

Fig. 1. Effect of tropomyosin-like protein on the response of trypsin-treated 'natural actomyosin' to a chelating agent. The suspension of 'natural actomyosin', 2 mg/ml., was incubated with trypsin, 10 μg/ml., for 20 min at 20° C. After stopping the reaction by trypsin-inhibitor the actomyosin was centrifuged and resuspended for use. *A*, The effect of glycoletherdiaminetetraacetic acid (GEDTA) on the trypsin-treated 'natural actomyosin', 0·78 mg/ml.; *B*, the effect of GEDTA on the trypsin-treated 'natural actomyosin' plus purified tropomyosin-like protein, 0·08 mg./ml. The method for measuring the superprecipitation followed those described in a previous paper (ref. 3). For response of untreated 'natural actomyosin' to GEDTA see ref. 8

In view of these findings and considerations it is interesting to enquire into a well-known fact[5-8], first noticed by Perry and Gray, that the reconstituted actomyosin is insensitive to the relaxing action of the chelating reagents; in other words, the reconstituted actomyosin lacks an important property of 'natural actomyosin' responsive to calcium ions.

Muscle mince was first extracted with Guba-Straub solution according to the routine procedures of actin preparation, then the residue of filtration was exhaustively washed several times at 0° C and finally extracted by water containing 1 mM sodium bicarbonate at about 20° C for 4 h. By filtration we obtained a very viscous solution, which was free of myosin and native actin, though it might contain some denatured actin. If a fraction of the solution is added to the reaction mixture, the reconstituted actomyosin now behaves like 'natural actomyosin' in its response to calcium ions and chelating agents.

Further purification of the active principle was made as follows: solid ammonium sulphate was added to the original solution up to 40 per cent saturation. Then the supernatant was dialysed against 0·2 mM sodium bicarbonate to eliminate the ammonium sulphate, and the volume of the solution was reduced by evaporating water under vacuum. Then the concentrated solution was dialysed against 0·2 mM sodium bicarbonate to reduce its ionic strength below 0·02. Further dialysis against water acidified slightly by carbon dioxide precipitated the essential component. After centrifugation the residue was dissolved again by adding a small amount of water by the aid of sodium bicarbonate. The protein solution thus obtained has all the properties of tropomyosin found by Bailey[9,10] in every respect: (*a*) the amino-acid composition[9,11]; (*b*) the relationship between viscosity and ionic strength[9]; (*c*) the sensitivity to trypsin digestion[12]; (*d*) the binding to *F*-actin measured by flow birefringence[13]; (*e*) the pattern of ultracentrifuge[10]; the preparation is not yet completely homogeneous but only one peak is appreciable which undoubtedly corresponds to that of tropomyosin. In spite of these evidences, usual tropomyosin preparation according to the original method of Bailey[9] is not effective. So far, it is not yet decided whether the active principle is the 'native' tropomyosin itself or not.

The foregoing facts suggest that the calcium-sensitive nature of 'natural actomyosin' is due to the presence of the active principle in it. As noted here, the factor is very sensitive to trypsin digestion. Therefore, it is interesting to know whether the nature of trypsin-treated 'natural actomyosin' would become similar to that of reconstituted actomyosin and the addition of the active principle would recover the previous properties of 'natural actomyosin'. Fig. 1 shows one of the results along this line. They are in good accordance with the expectation, indicating that 'natural actomyosin' functionally consists of three protein components—actin, myosin and the factor described here. Essentially the same results were obtained with myofibrils.

The interaction between actin and myosin in the presence of adenosine triphosphate undoubtedly represents the basic mechanism of muscular contraction. However, if we are interested in the physiological contraction of living muscle, where calcium-sensitive property of contractile elements would be of primary importance, the role of the protein factor mentioned here, which resembles tropomyosin in many respects, would be highly evaluated.

Details of this communication will be published elsewhere[14].

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Cellulase in Tomato Fruits

A cellulolytic enzyme that reduces the viscosity of sodium carboxymethylcellulose (CMC) solution was found in sodium chloride extracts of ripe tomato fruits using the method of Bell *et al.*[1] for determining activity. This is a viscometric method devised for detecting low enzyme-levels by measuring the change in drain time of solutions in Ostwald-Fenske pipettes during a 20-h period.

Further extractions were made to compare the activity in the pericarp (wall tissue) and in the locular material