Short Communications

ral' Tropomyosin and the Factor Sensitizing Actomyosin Adenosine Triphosphatase to Ethylenedioxybis(ethyleneamino)tetra-acetic acid

By S. V. Perry, Valerie Davies and Dorothy Hayter
Department of Biochemistry, University of Birmingham

(*Received* 17 *January* 1966)

s originally reported by Perry & Grey) that the Mg^{2+}-activated ATPase* of ls and actomyosin extracted directly as nplex from muscle, so-called 'natural' sin, is strongly inhibited by low concentra- EGTA and EDTA, whereas the enzymic of the complex formed from independently actin and myosin is not. In view of the helating properties of EGTA and the fact concentrations required to produce marked n were only a few per cent of that of the the system, the observation suggested that amount of Ca^{2+} is required for the Mg^{2+} n of the ATPase of natural actomyosin. ture of the actomyosin ATPase is of physio- ignificance, for the present view is that the pump of the relaxing-factor system exerts tory action on the myofibrillar ATPase by g the low concentration of Ca^{2+} required for rolysis of ATP by the myofibrils in the of Mg^{2+}.

i (1963) has demonstrated that the super- tion behaviour of 'natural' and 'synthetic' sin preparations in the presence of chelators the effects obtained with the enzymic and has produced evidence suggesting that , the E.S.F., which confers sensitivity to on the ATPase and superprecipitation es, is present in addition to actin and myosin ral' actomyosin preparations.

i (1963) found E.S.F. activity in tropo- prepared by a method that did not involve nt with an organic solvent at any stage, and he has named 'natural' tropomyosin, preparations made by the original Bailey ethod were inactive. All the preparations ported with E.S.F. activity have contained ' tropomyosin, which suggests that this may have a special significance in the (Ebashi & Ebashi, 1964; Katz, 1965; cf. & Kodama, 1965).

extra protein (Szent-Gyorgyi, Mazia & eviations: ATPase, adenosine triphosphatase; thylenedioxybis(ethyleneamino)tetra-acetic acid GTA-sensitizing factor.

Szent-Gyorgyi, 1955; Perry & Zydowo, 1959) and the soluble protein fraction extracted at low ionic strength (Perry, 1953; Perry & Corsi, 1958) from well-washed myofibrils both contain appreciable amounts of tropomyosin, which, as organic solvents are not used in the preparation, would be expected to be in the 'natural' form. Assay of both of these myofibrillar protein fractions showed the presence of E.S.F. activity that was destroyed by treatment with trypsin, as has been reported for other E.S.F. preparations (Ebashi & Ebashi, 1964; Katz, 1965).

The work of assaying E.S.F. activity (see Fig. 1) has been facilitated by the development of a simple method for the preparation of actomyosin, the Mg^{2+}-activated ATPase of which is not sensitive to EGTA and which is therefore presumably free of

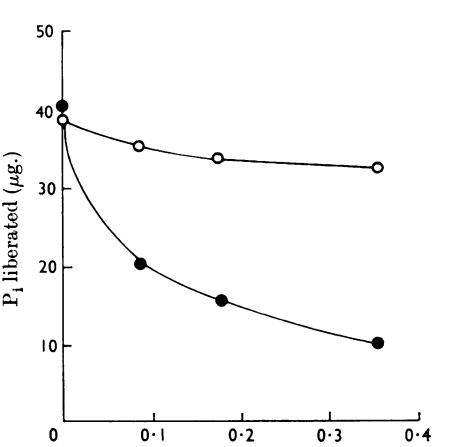

Fig. 1. Effect of EGTA on the Mg^{2+}-activated ATPase activity of desensitized actomyosin in the presence of the fraction of extra protein eluted from DEAE-cellulose by 0·1 M-KCl in 25 mM-tris–HCl buffer, pH 7·6, namely fraction I. The ATPase assay medium (2 ml.) contained $MgCl_2$ (2·5 mM), tris–ATP (2·5 mM), tris–HCl buffer, pH 7·6 (25 mM), actomyosin (1·24 mg.) and fraction I as indicated; incubation was for 5 min. at 25°. ○, No further addition; ●, EGTA (1 mM) added.

E.S.F. 'Natural' actomyosin prepared from myofibrils as described by Perry & Corsi (1958) was washed three times at 0° with water by centrifugation for 30min. at 1200*g*, dispersed in 3vol. of 5mM tris–HCl buffer, pH 8·6, and allowed to stand for 48hr. at 0°. It was then centrifuged for 15min. at 33000*g*, and the precipitate was resuspended in 3vol. of water, washed twice with water by centrifugation under the same conditions and stored as a suspension in water at pH 7·0. These preparations usually possessed higher Mg^{2+}-activated ATPase activity than comparable preparations made from separately purified actin and myosin and have obvious advantages over the actomyosin preparations desensitized to EGTA by trypsin treatment as described by Ebashi (1963).

Both extra protein and the soluble protein fractions can be fractionated on DEAE-cellulose, but there are difficulties with the latter protein fraction owing to the unstable nature of the inactive form of actin present in this preparation. Attention has therefore been directed particularly to the extra protein because of its lower tropomyosin content and satisfactory resolution by DEAE-cellulose into three fractions by elution with 0·1M-KCl, 0·22M-KCl and 0·4M-KCl, all in 25mM-tris–HCl buffer, pH 7·6, namely fractions I, II and III respectively (Perry & Zydowo, 1959). When assayed at constant protein concentration, measured by the biuret method of Lane (1957), comparable E.S.F. activity was usually found in all three fractions although occasionally activity was higher in fraction III.

The presence of appreciable amounts of tropomyosin only in fraction III was confirmed by measuring the marked fall in viscosity obtained on increasing the ionic strength, a property that is characteristic of tropomyosin (Bailey, 1948). Addition of 0·5M-KCl produced very slight falls in relative viscosity (0·01–0·16 with protein concentrations in the range 1–4mg./ml.) when added to fraction I in 25mM-tris–HCl, pH 7·6. Under similar conditions large falls in relative viscosity (3·0–18·0 with protein concentrations in the range 1–4mg./ml.) were obtained with fraction III. These results confirm the earlier findings that fraction III consists largely of tropomyosin, which is eluted as a sharp peak at 0·29M-KCl in 25mM-tris–HCl buffer, pH 7·6 (Perry & Zydowo, 1959). In contrast, for reasons that are not yet apparent, the E.S.F. is not sharply fractionated on DEAE-cellulose, considerable amounts being eluted at much lower ionic strength than is required for tropomyosin.

In some circumstances E.S.F. preparations obtained that inhibited the Mg^{2+}-activated AT of actomyosin in the absence of EGTA. S inhibitory action, which was enhanced at h protein concentrations, is apparent in the fract assayed in Fig. 1. This inhibitory effect in absence of EGTA is described by Hartshorne, P & Davies (1966).

Morphological fractionation of rabbit p muscle homogenized in 25mM-KCl in 39mM-b buffer, pH 7·1 (Perry, 1953), into a washed r fibrillar fraction sedimented by centrifugatio 15min. at 600*g*, a heterogeneous fraction consi of mitochondria and sarcoplasmic reticulum mented after 60min. at 100000*g*, and the so sarcoplasm, indicated that significant E activity could only be detected in the myofib fraction (cf. Mueller, 1965). Assays were carrie as indicated in Fig. 1 on whole sarcoplasm an soluble protein fractions obtained by extractin granular fraction and the myofibrils for 3 days 5mM-tris–HCl buffer, pH 8·6. These extracts used in the case of the granules and the myofi as the whole fractions have considerable AT activity, which would make assay of the f difficult, if not impossible. Extraction with 5 tris–HCl buffer, pH 8·6, has been shown to solub the E.S.F., and the protein extracted in this way no ATPase activity.

These investigations suggest that the E.S. localized in the myofibrils of skeletal muscle, w it is present as a minor protein component identical with tropomyosin.

Bailey, K. (1948). *Biochem. J.* **43**, 271.
Ebashi, S. (1963). *Nature, Lond.*, **200**, 1010.
Ebashi, S. & Ebashi, F. (1964). *J. Biochem., Toky* 604.
Ebashi, S. & Kodama, A. (1965). *J. Biochem., Toky* 107.
Hartshorne, D. J., Perry, S. V. & Davies, V. (1966). *N Lond.* (in the Press).
Katz, A. M. (1965). *Fed. Proc.* **24**, 208.
Lane, E. (1957). In *Methods in Enzymology*, vol. 3, p. Ed. by Colowick, S. P. & Kaplan, N. O. New Y Academic Press Inc.
Mueller, H. (1965). *Fed. Proc.* **24**, 599.
Perry, S. V. (1953). *Biochem. J.* **55**, 114.
Perry, S. V. & Corsi, A. (1958). *Biochem. J.* **68**, 5.
Perry, S. V. & Grey, T. C. (1956*a*). *Biochem. J.* **64**, 5
Perry, S. V. & Grey, T. C. (1956*b*). *Biochem. J.* **64**, 18
Perry, S. V. & Zydowo, M. (1959). *Biochem. J.* **71**, 22
Szent-Gyorgyi, A. G., Mazia, D. & Szent-Gyorgyi, A. (1 *Biochim. biophys. Acta*, **16**, 339.