THE PRESENCE OF AN INHIBITOR OF ACTIN-TROPOMYOSIN COMBINATION IN CRUDE ACTIN PREPARATIONS AND THE EFFECT OF PHOSPHATE ON THE ACTIN-TROPOMYOSIN INTERACTION

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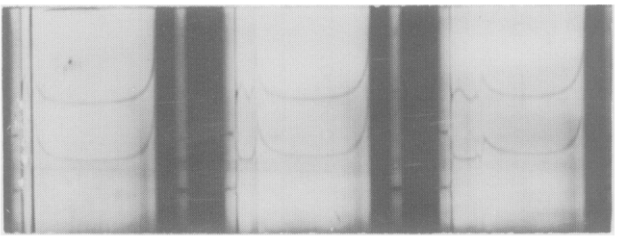
Received April 25, 1963

A preparation of actin with a capacity for combination with myosin greater than the preparation obtained by Mommaerts (1) was described by one of us (2). It was assumed that the actin prepared according to Mommaerts was combined with another protein through an unknown mechanism.

It is now known from the work of Laki and co-workers (3)(4)(5) and Martonosi (6) that not only crude actin preparations but also purified ones - made according to Mommaerts (1) - contain varying amounts of tropomyosin. In order to gain insight of what controls the amount of tropomyosin in actin preparations we dialyzed purified actin preparations (made according to the method of Mommaerts) against crude preparations made according to Straub

(°) This work was supported by grants from "l'Institut National d'Hygiène", "la Caisse Nationale de Sécurité Sociale", "le Centre National de la Recherche Scientifique", "la Délégation Générale à la Recherche Scientifique", France, "The Muscular Dystrophy Associations of America, Inc.", New York, and by a research grant N° GM-06016 from the Division of General Medical Sciences, Public Health Service, U.S.A.

and co-workers (7). We found that after 24 hours of dialysis the polymerizability of the purified actin preparations decreased, and a new component appeared in ultracentrifuge experiments (figure 1 and table I). The new component had sedimentation properties very similar to those found by Martonosi (6) and identified to be a complex of tropomyosin and actin. The viscosity of the F actin formed from the dialyzed G actin under these conditions was also less than that of the undialyzed sample. It appears then that we are confronted with a dialyzable (through collodion membranes) inhibitor present in crude actin preparations, that prevents the polymerization of actin from going to completion, probably by interfering with the actin-tropomyosin combination.



Ultracentrifugal pattern of the purified F actin before and after dialysis against crude actin (see legend to table I).
Lower half of figure : F actin before dialysis 2,5 mg/protein/ml
Upper half of figure : F actin after dialysis (of the G actin) 3,1 mg/protein/ml
The pictures were taken at 4 min. intervals, RPM. 37 020 at 19°C.
Solution represented by the lower pattern was placed into a regular cell, the solution represented by the upper pattern was placed into a cell equipped with a wedge.

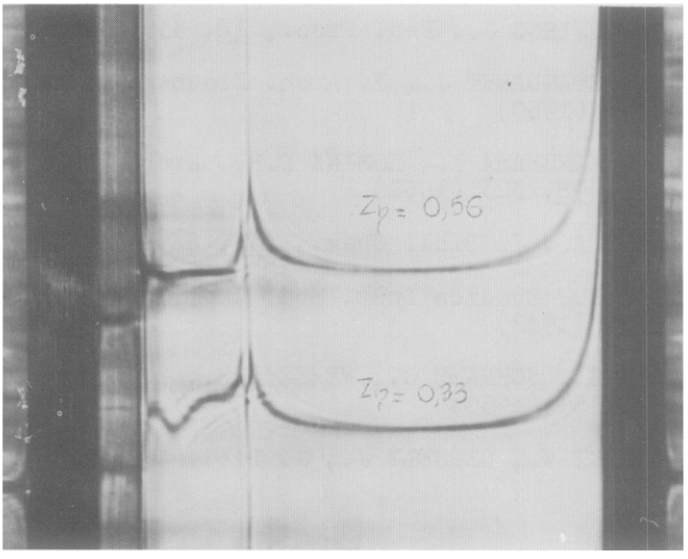
In our search of the inhibitory substance we noticed that inorganic phosphate in small concentrations had a promoting effect on actin polymerization by reducing the amount of a non polymerizing portion of the actin preparation. In order to examine this effect we purified G actin free of tropomyosin (9) and added

Table I A solution of G actin (purified according to Mommaerts) containing 10^{-4}M ATP (pH 8) was dialyzed through a collodion membrane against an equal volume of crude actin solution for 24 hours at 0°. After dialysis the purified G actin was centrifuged at 75 000 g for 3 hours. The solution was then made to polymerize by the addition of KCl (0,1 M KCl + 0,001 M Mg SO_4). This solution was then compared to the undialyzed purified actin solution polymerized in a similar manner. It is seen that the viscosity of the dialyzed solution is lower, that a new slow sedimenting component appeared and that the quantity of non sedimenting proteins is larger.

	viscosity of the 3 different preparations (x)	amount of non sedimenting proteins in per cent	Analysis of the components seen in fig. 1		
			number of components	sedimentation constant S 20°	amount of the components expressed as per cent of the total
before dialysis	n°1 0,48 n°2 0,50 n°3 0,58	5 to 8	2	S_2 = 38,6 S_3 = 59,7	5 95
after dialysis	n°1 0,38 n°2 0,40 n°3 0,43	20 to 27	3	S_1 = 9,4 S_2 = 38,8 S_3 = 59,4	22,5 5, 72,5

(x) The numbers refer to $Z\gamma = \frac{2{,}3 \log. \eta \text{ relat.}}{\text{mg/proteins/ml}}$ (8)

a known amount of purified tropomyosin to it in the presence and absence of inorganic phosphate (x). These mixtures were made to polymerize (in 0,1 M KCl + 0,001 M Mg SO_4) then examined in the ultracentrifuge and their viscosities compared. Figure 2 shows the result of the ultracentrifuge experiments. It is seen that in the presence of phosphate all the tropomyosin added to actin is incorporated into the heavy component. By contrast, in the absence of phosphate, a portion of tropomyosin remained as a discrete light component. Viscometric measurements also support the polymerization promoting effect of phosphate : F actin formed when 0,1 M KCl and 0,001 M Mg SO_4 are added to a mixture of G



Ultracentrifugal pattern of the purified F actin + tropomyosin
Lower half : F actin (tris 0,002 M pH 8) + tropomyosin 7 mg protein/ml
Upper half : F actin (phosphate 0,002 M pH 8) + tropomyosin 7 mg protein/ml, RPM 37 020 at 19°C.
Solution represented by the lower pattern was placed into a regular cell, the solution represented by the upper pattern was placed into a cell equipped with a wedge.

(x) We thank Dr. K. Laki for his interest in this work and for the preparation of tropomyosin.

actin and tropomyosin in the presence of phosphate has a much greater viscosity than the mixture containing no phosphate (see figure 2).

These experiments suggest that small amounts of phosphate are needed for the actin-tropomyosin formation to go to completion. The inhibitor present in crude actin preparations may interfere with the promoting effect of inorganic phosphate.

REFERENCES

1. MOMMAERTS W.F.H.M., J. Biol. Chem., 198, 445 (1952)

2. DEMOS J., DREYFUS J.C., SCHAPIRA G., C.R. Acad. Sci., 247, 1254 (1958)

3. LAKI K., CAIRNS J., Fed. Proc., 18, 335 (1959)

4. LAKI K., STANDAERT J., J. Arch. Biochem. Biophys., 86, 16 (1960)

5. LAKI K., MARUYAMA K., KOMINZ D.R., Arch. Biochem. Biophys., 98, 323 (1962)

6. MARTONOSI A., J. Biol. Chem., 237, 2795 (1962)

7. STRAUB F.B., Studies Inst. Med. Chem. Univ. Szeged, 2, 3, (1942)

8. PORTZEHL H., SCHRAMM G., WEBER H.H., Z. Natur. Forsch., 56, 61 (1950)

9. DRABIKOWSKI W., GERGELY J., J. Biol. Chem., 237, 3412, (1962)