INHIBITION OF MITOCHONDRIAL ADENOSINE TRIPHOSPHATASE ACTIVITY BY TROPONIN COMPONENT, TN-I.

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Summary; One of the troponin components, TN-I, strongly inhibits the ATPase activity of AS-particles obtained from mitochondria, while troponin and the other components, TN-C and TN-T, do not. The inhibition of the ATPase activity by Component TN-I is effective only in the presence of Mg^{2+} and ATP. Component TN-I digested with trypsin completely loses the inhibitory action of the ATPase activity. Adding tropomyosin does not affect the inhibitory effect of Component TN-I on the ATPase activity.

During the course of the investigations of muscle contraction, it has been found by Ebashi and Endo that troponin plays an important role in muscle contraction(1). Recently, Greaser and Gergely(2) separated troponin into three components and clarified their physico-chemical properties and functions(3); Component TN-I (mw = 23,000) shows an inhibitory action to the ATPase activity of actomyosin, Component TN-T(mw = 37,000) has a binding site with tropomyosin and Component TN-C(mw = 18,000) a binding site with Ca^{2+} ion. The reconstitution of the three components leads to the restoration of the regulatory system of the ATPase activity of actomyosin with tropomyosin in the presence and in the absence of Ca^{2+} ion(2).

The mitochondrial ATPase inhibitor has been isolated in a pure form and its properties have been characterized by Horstman and Racker(4). The inhibitor seems to be a component of the coupling factor(F_1) with the molecular weight of 15,000 and its physiological function may be related to the oxidative phosphoryl-

ation or to the respiratory control(4,5).

The present paper is to prove that Component TN-I causes the inhibition of the mitochondrial ATPase activity and to clarify the role of the ATPase inhibitor in the regulation mechanism of the energy transformation process of ATP.

Experimentals

AS-particles were obtained from heavy layer beef heart mitochondria by the method of Racker and Horstman(6). AS-particles thus obtained have a high ATPase activity, probably due to the removal of the mitochondrial ATPase inhibitor. Troponin and tropomyosin were prepared from a rabbit skeletal muscle according to the procedure of Greaser and Gergely(2). The troponin preparation was further purified by DEAE-cellulose chromatography(7). The troponin components, TN-I, TN-T and TN-C, were separated by DEAE-Sephadex chromatography in 6 M urea(2). The concentrations of proteins were determined by the method of Lowry et al.(8).

Before measuring the ATPase activity, AS-particles in the medium containing 0.5 mM $MgSO_4$, 0.5 mM ATP, 25 mM KCl and 15 mM Tris-HEPES(pH 6.7) were preincubated with troponin or its components for 15 min at 30°. After the incubation, the sample solution(25 µl) was taken out and subjected to the assay of the ATPase activity(9). To this sample solution was added 0.5 ml of an assay solution containing 15 mM Tris-Sulfate buffer(pH 7.7), 5 mM $MgSO_4$, 4 mM ATP, 5 mM phosphoenol pyruvate and 17 µg pyruvate kinase. The reaction was carried out for 10 min at 30°. The amount of inorganic phosphate liberated from ATP was measured by the method of Martin-Doty(10).

Results and Discussion

Inhibitory effect of troponin and its components on the ATPase

activity of AS-particles

The ATPase activity of AS-particles was measured after the preincubation of AS-particles with troponin or its components in the presence of Mg^{2+} and ATP. Figure 1 shows the inhibition

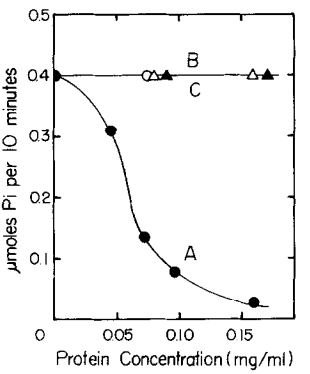
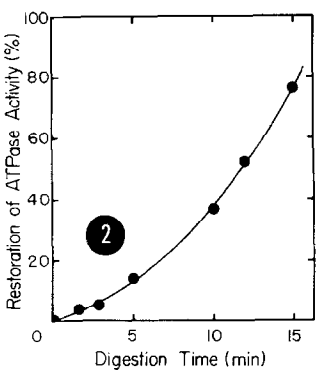


Fig. 1. Inhibition of the ATPase activity of AS-particles by troponin and its components. The ATPase activities were measured after preincubation of AS-particles(0.4 mg protein/ml) with troponin(curve B) and its component, TN-I(curve A), TN-T(curve C) and TN-C(○).

curves of the ATPase activity obtained at the various concentration of troponin and its components. The ATPase activity was strongly reduced by increasing Component TN-I concentration and was almost completely lost at 0.16 mg/ml proteins(curve A), while no inhibition of the activity was observed in the case of troponir (curve B), Component TN-T(curve C) and Component TN-C. The solubility of Component TN-C is so small that its inhibitory effect on the ATPase activity could not be tested at a higher concentration than 0.08 mg/ml. These results indicate that Component TN-I acts as a potent inhibitor of the mitochondrial ATPase activity, probably due to the interaction of AS-particles with Component TN-I. Component TN-I was digested with trypsin,

and its inhibitory effect was also tested after the preincubation of AS-particles with the digested Component TN-I. The restoration of the ATPase activity by Component TN-I digested with trypsin was plotted against the incubation time of the digestion. As is clear from Figure 2, the digestion of Component TN-I with trypsin gives rise to the complete restoration of the ATPase activity. This indicates that the higher order structure of the protein inhibitor seems to be related to the inhibition of the ATPase activity.

The ATPase activity of AS-particles in the presence of Component TN-I was measured as a function of pH(Figure 3). The



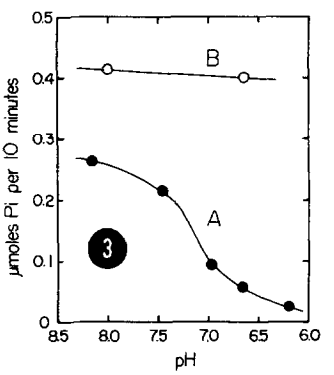


Fig. 2. Restoration of the ATPase activity of AS-particles during the course of the digestion of Component TN-I with trypsin. Component TN-I(0.6 mg/ml) in the medium containing 80 mM KCl and 6 mM Tris-HEPES(pH 6.7) was digested with trypsin(2 μg/ml) at 30° and at a given time the digestion was stopped by adding trypsin inhibitor (4 μg/ml). The ATPase activities were measured after preincubation of AS-particles(0.4 mg protein/ml) and the digested Component TN-I(0.12 mg/ml).

Fig. 3. pH-Dependence of the inhibition of the ATPase activity of AS-particles(0.4 mg protein/ml) by Component TN-I (0.16 mg/ml). The ATPase activities were measured after AS-particles was preincubated at various pH's in the presence of Component TN-I(curve A) and in the absence of Component TN-I(curve B).

pH-activity curve obtained from the ATPase activity of AS-particles with Component TN-I is shown by curve A, which forms a sigmoidal shape with pK value of 7.2(pH value at one-half of the maximal ATPase activity) in the acidic side. This pK value is in good agreement with that obtained from the inhibition of the mitochondrial ATPase activity by the mitochondrial ATPase inhibitor(4). The ATPase activity of AS-particles without Component TN-I was not reduced at the pH range between pH 6.6 and 8.0 (curve B).

Effects of Mg^{2+}, ATP and tropomyosin on the inhibition of the ATPase activity by Component TN-I

It was demonstrated by Horstman and Racker(4) that the mitochondrial ATPase activity is inhibited by the mitochondrial inhibitor in the presence of Mg^{2+} and ATP. Both of Mg^{2+} and ATP seem to be necessary for the interaction of mitochondrial ATPase with its inhibitor. In the present study, it was examined as to whether Mg^{2+}, ATP or both of them play an important role in the inhibition of the ATPase activity of AS-particles by Component TN-I. The incubation of AS-particles with Component TN-I was carried out in the presence of EDTA with and without ATP or in the presence of $MgSO_4$ with and without ATP, and the ATPase activity of AS-particles was measured. In the addition of both $MgSO_4$ and ATP to the reaction system, the inhibition of the ATPase activity of AS-particles by Component TN-I was almost complete(93%), compared with the minute inhibitory effect obtained in the presence of EDTA with and without ATP or in the presence of $MgSO_4$ without ATP(Table I). This indicates that the inhibition of the ATPase activity of AS-particles by Component TN-I was caused in the presence of Mg^{2+} and ATP.

Table I. Effect of Mg^{2+} or ATP on the inhibition of the ATPase activity of AS-particles(0.4 mg protein/ml) by Component TN-I(0.16 mg/ml). Concentrations; 5 mM EDTA, 0.5 mM $MgSO_4$ and 0.5 mM ATP.

	ATPase activity(μmoles Pi/10 min)		
	AS-particles	AS-particles + TN-I	Inhibition (%)
EDTA	0.392	0.256	35
EDTA + ATP	0.376	0.306	19
$MgSO_4$	0.355	0.242	32
$MgSO_4$ + ATP	0.364	0.037	93

Tropomyosin plays an important role in the inhibition of actomyosin ATPase activity by Component TN-I(2,3,11). To test the effect of tropomyosin on the inhibition of the mitochondrial ATPase activity by Component TN-I, AS-particles, Component TN-I, $MgSO_4$ and ATP were incubated with and without tropomyosin, and the ATPase activity was measured. As is clear from Table II, no significant difference was observed between the inhibitory effect on the ATPase activity in adding Component TN-I alone and that in adding Component TN-I together with tropomyosin.

Throughout this experiment, the presence or the absence of Ca^{2+} did not show any difference in the inhibitory effect by Component TN-I on the ATPase activity of AS-particles.

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Table II. Effect of tropomyosin on the inhibition of the ATPase activity of AS-particles by Component TN-I. Concentrations; AS-particles(0.4 mg protein/ml), Component TN-I(0.16 mg/ml) and tropomyosin(0.6 mg/ml).

	ATPase activity(μmoles Pi/10 min)	
	AS-particles	Relative activity
None	0.392	100
Tropomyosin	0.402	102
Component TN-I	0.037	9
Component TN-I + tropomyosin	0.050	13

References

1. S. Ebashi and M. Endo, Progr. Biophys. Mol. Biol., 18, 123 (1968)
2. M. L. Greaser and J. Gergely, J. Biol. Chem., 246, 4226(1971)
3. Muscle Regulatory System in Cold Spring Harbour Symposia on Quantitative Biology. 37, 1973. Cold Spring Harbour Lab., p.215
4. L. L. Horstman and E. Racker, J. Biol. Chem., 245, 1336(1970)
5. M. E. Pullman and G.C. Monroy, J. Biol. Chem., 238, 3762(1963)
6. E. Racker and L. L. Horstman, J. Biol. Chem., 242, 2547(1967)
7. I. Staprans and S. Watanabe, J. Biol. Chem., 245, 5962(1970)
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, J. Biol. Chem., 193,265(1951)
9. M. E. Pullman, H. S. Penefsky, A. Datta and E. Racker, J. Biol. Chem., 235, 3322(1960)
10. J. B. Martin and D. M. Doty, Anal. Chem., 21, 965(1949)
11. J. M. Wilkinson, S. V. Perry, H. A. Cole and I. P. Trayer, Biochem. J., 127, 215(1972)