EFFECT OF TROPONIN COMPONENT TN-C ON THE INHIBITION OF ADENOSINE TRIPHOSPHATASE ACTIVITIES OF MITOCHONDRIA AND CHLOROPLASTS BY TROPONIN COMPONENT TN-I

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1. Introduction

Studies on the regulatory proteins in the muscle contraction system have been extensively performed. The ATPase activity of actomyosin in the presence of tropomyosin is regulated by Ca^{2+} ions and troponin [1] which is composed of three components, TN-I (mol. wt. 23 000), TN-T (37 000) and TN-C (18 000) [2].

During the course of investigations of the energy-transducing processes of oxidative phosphorylation in mitochondria and of photophosphorylation in chloroplasts, it was found that inhibitors exist [3,4], which inhibit the ATPase activities of the mitochondrial coupling factor 1(F_1) and the chloroplast coupling factor 1 (CF_1) and these may play an important role in regulation of the energy transducing system.

In previous reports, it was demonstrated that one of the troponin components, TN-I, strongly inhibits the ATPase activity of AS-particles from mitochondria in a non-competitive mode [5,6] and also inhibits the Ca^{2+}-dependent ATPase actity of chloroplast coupling factor 1(CF_1) [7].

As reported in the present paper, it was found that troponin component TN-I inhibits the Mg^{2+}-dependent ATPase activity as well as the Ca^{2+}-dependent ATPase activity of chloroplast coupling factor 1(CF_1), and that the inhibited activities of the Ca^{2+}- and Mg^{2+}-dependent ATPase and of the mitochondrial ATPase were restored by adding troponin component TN-C. These results may clarify the regulatory mechanism in energy-transducing processes.

2. Experimental

Troponin was prepared from a rabbit skeletal muscle according to the procedure of Greaser and Gergely [2]. Troponin components, TN-I, TN-T and TN-C, were isolated by DEAE-Sephadex chromatography in 6 M urea [2].

AS-particles were obtained from heavy layer beef heart mitochondria by the method of Racker and Horstman [8]. The chloroplast coupling factor 1(CF_1) was prepared by the method of Lien and Racker [9] and was activated by heat at 60°C for 4 min or by trypsin digestion at 25°C for 6 min. Protein concentrations were determined by the Lowry method [10], using bovine serum albumin as a protein standard. The measurement of the mitochondrial ATPase activity was performed as follows: before measuring the ATPase activity, AS-particles suspended in 0.5 mM $MgSO_4$, 0.5 mM ATP, 0.25 M sucrose and 15 mM Tris–HEPES (pH 6.7), were incubated with Components TN-I and TN-C for 15 min at 30°C. After the incubation, an aliquot (25 μl) was assayed for ATPase activity [5]. The Ca^{2+}-dependent ATPase activity of chloroplast coupling factor 1(CF_1) was measured by the method described previously [7]. In order to induce the inhibition of the Mg^{2+}-dependent ATPase activity of the activated CF_1 by Component TN-I, 50 μl of the activated CF_1 (8 μg) in 8 mM Tricine–NaOH (pH 8.0) containing 0.4 mM EDTA, 4 mM ATP and 2 mM dithiothreitol was added to 50 μl of each troponin component (14 μg) in 2 mM Tris-HCl (pH 7.5) containing 1 mM $CaCl_2$. The mixture was incu-

bated at 30°C for 20 min. For measuring the ATPase activity, the mixed solution (100 μl) was added to the assay solution (0.5 ml) containing 4 mM ATP, 1 mM EGTA, 2 mM $MgCl_2$, 5 mM phosphoenol pyruvate and 17.5 μg pyruvate kinase in 40 mM Tris-HCl buffer (pH 8.0). The reaction mixture was incubated for 10 min at 37°C, and stopped by adding 2.0 ml of 3% trichloroacetic acid. The amount of inorganic phosphate liberated from ATP was measured by the method of Martin and Doty [11].

3. Results and discussion

3.1. *Inhibition of the Mg^{2+}-dependent chloroplast ATPase activity by Component TN-I*

The inhibitory action of Component TN-I on the Mg^{2+}-dependent ATPase activity is strongly evident when the mixture of Component TN-I and the activated CF_1 in 4 mM Tricine–NaOH buffer (pH 8.0) was incubated in the presence of 0.5 mM $CaCl_2$ at 30°C. The results are shown in fig.1, in which curves A and B represent the Mg^{2+}-dependent ATPase activity of the heat-activated CF_1 in the presence and the absence of Component TN-I, respectively. The inhibition of the ATPase activity by Component TN-I

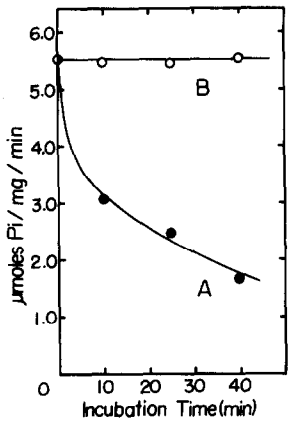

Fig.1. Generation of the inhibitory action of the Mg^{2+}-dependent ATPase activity of the heat-activated CF_1 by Component TN-I, after Component TN-I (14 μg) and the activated CF_1 (8 μg) in 4 mM Tricine-NaOH (pH 8.0) containing 0.2 mM EDTA, 2 mM ATP and 1 mM dithiothreitol were incubated at 30°C in the presence of 0.5 mM $CaCl_2$. Curve A; with Component TN-I. Curve B; without Component TN-I.

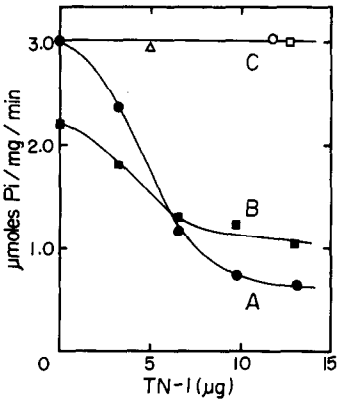

Fig.2. Inhibition of the Mg^{2+}-dependent ATPase activity of the activated CF_1 by troponin and its components. Curve A; ATPase activity of the heat-activated CF_1 (7.5 μg) with Component TN-I. Curve B; ATPase activity of the trypsin-activated CF_1 (7.7 μg) with Component TN-I; Curve C; ATPase activity of the heat-activated CF_1 (7.5 μg) with troponin (○), Component TN-T (△) and Component TN-C (□).

increases with the time of incubation (curve A). No inhibition was observed in the absence of Component TN-I (curve B). The inhibition of the ATPase activity by Component TN-I did not change when the Ca^{2+} ions added to the incubation mixture were replaced by Mg^{2+} ions.

Fig.2 shows a plot of the Mg^{2+}-dependent ATPase activity of CF_1 activated by heat (curve A) and by trypsin digestion (curve B) against concentration of Component TN-I. The Mg^{2+}-dependent ATPase activity of the heat-activated CF_1 decreases with increasing Component TN-I concentration and remains at a constant level of 23% of the original activity at more than 10 μg Component TN-I. The inhibition of the ATPase activity of the trypsin-digested CF_1 by Component TN-I was to approx. 45% of the original activity. Deters et al. reported [12] that the trypsin-activated CF_1 is lacking in the γ and δ subunits of the CF_1 molecule and that these subunits may play an important role in binding CF_1 to the chloroplast ATPase inhibitor. This may be reflected in the difference in inhibition by Component TN-I between the heat-activated CF_1 and the trypsin-activated CF_1. Troponin components, TN-C and TN-T, and troponin do not inhibit the Mg^{2+}-dependent ATPase activity of the heat-activated CF_1, as shown by curve C in fig.2.

Component TN-I was digested by trypsin (1/100 w/w) at 30°C for 5 min. No inhibition of the Mg^{2+}-

dependent ATPase activity by the digested Component TN-I occurred. This result suggests that the higher order structure of Component TN-I is closely associated with the inhibition of the Mg^{2+}-dependent ATPase activity.

3.2. *Effect of Component TN-C on the inhibition of the mitochondrial and the chloroplast ATPase activities by Component TN-I*

It is well known that in the muscle contraction system troponin component TN-C reserves the inhibitory action of the actomyosin ATPase activity by Component TN-I [13]. In order to clarify whether Component TN-C serves as a regulatory protein on the energy-transducing systems of mitochondria and chloroplasts, the effect of Component TN-C on the inhibition of the mitochondrial and chloroplast ATPase activities by Component TN-I was examined.

Table 1
Effect of Component TN-C on the inhibitory action of the chloroplast and mitochondrial ATPase activities by Component TN-I

	Ca^{2+}-ATPase (μmoles P_i/mg protein/min (%))	Mg^{2+}-ATPase
Heat-activated CF_1	25.8 (100)	3.00 (100)
+ Component TN-C	27.6 (107)	3.22 (107)
+ Component TN-I	6.1 (24)	1.20 (40)
+ Components TN-C and TN-I	29.2 (113)	3.04 (101)

	ATPase activity (μmoles P_i/10 min (%))
AS-particles	0.34 (100)
+ Component TN-C	0.35 (103)
+ Component TN-I	0.07 (21)
+ Components TN-C and TN-I	0.29 (86)

The Upper section; chloroplast ATPase, amount of the heat-activated CF_1 and troponin components: 1.4 μg CF_1, 6.6 μg Component TN-I and 6.3 μg Component TN-C for Ca^{2+}-ATPase and 7.7 μg CF_1, 9.8 μg Component TN-I and 8.0 μg Component TN-C for Mg^{2+}-ATPase. The lower section; mitochondrial ATPase, amount of AS-particles and troponin components: 40 μg AS-particles, 15.5 μg Component TN-I and 10 μg Component TN-C.

The results are shown in table 1. The enzymic activities of the Ca^{2+}- and Mg^{2+}- dependent ATPase of the heat-activated CF_1 are markedly decreased by the addition of Component TN-I but they do not decrease with the addition of Component TN-C instead of Component TN-I. Addition of the mixture of Components TN-I and TN-C to the heat-activated CF_1 does not cause the inhibitory action of Component TN-I on the Ca^{2+}- and Mg^{2+}-dependent chloroplast ATPase activities. A simialr result was obtained for the mitochondrial ATPase activity. This indicates that the inhibitory action of Component TN-I on the chloroplast or the mitochondrial ATPase activity is restored by adding Component TN-C. Plotting of the enzymic activity of the mitochondrial ATPase in the presence of Component TN-I (15.5 μg) against Component TN-C concentration is shown in fig.3. The ATPase activity of the mitochondrial ATPase with Component TN-I increases with increasing TN-C concentration and at more than 12 μg Component TN-C the activity is completely restored, as seen by curve A in the figure. In the absence of Component TN-I, the full activity of the mitochondrial ATPase was retained upon addition of various amounts of Component TN-C (curve B). From the analysis of curve A, it was found that full restoration of the enzymic activity is caused by a stoichiometric coexistence (1 mole:1 mole) of Components TN-I and TN-C. Hartshorne and Pyun

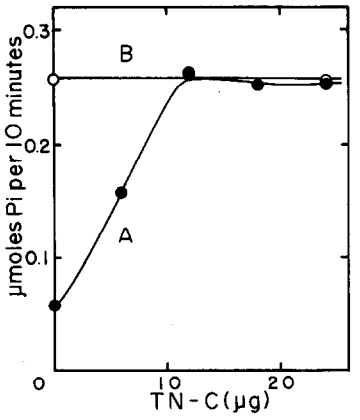

Fig.3. Restoration of the TN-I-induced inhibition of the mitochondrial ATPase activity by Component TN-C. Plotting the ATPase activity in the presence (curve A) and the absence (curve B) of Component TN-I (15.5 μg) against Component TN-C concentration.

[14] reported that an anionic polymer such as polyglutamate restores the TN-I-induced inhibition of the actomyosin ATPase activity. Component TN-C is an acidic protein and the ionic charges of Component TN-C may be responsible for the binding of Components TN-I and TN-C, which may induce the restoration of the TN-I-induced inhibition of the mitochondrial and the chloroplast ATPase activities.

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