INHIBITION OF ADENOSINE TRIPHOSPHATASE ACTIVITY OF CHLOROPLAST COUPLING FACTOR (CF_1) BY TROPONIN COMPONENT, TN-I

Shojiro YAMAZAKI, Haruhiko TAKISAWA, Yutaka TAMAURA, Shigehisa HIROSE and Yuji INADA

Laboratory of Biological Chemistry, Tokyo Institute of Technology, Ookayama, Meguroku, Tokyo 152, Japan

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1. Introduction

In order to clarify the regulation mechanism of the energy transformation processes of photophosphorylation in chloroplasts and of oxidative phosphorylation in mitochondria, the chloroplast coupling factor 1 (CF_1) [1] and the mitochondrial coupling factor (F_1) [2] and their inhibitors [3,4] have been isolated and studied extensively regarding their structures and functions [5]. The investigation on the molecular basis of muscle contraction led to the isolation of troponin, which consists of three components [6], TN-I (Mol. wt = 23 000), TN-T (37 000) and TN-C (18 000) and plays an important role in the regulation of the ATPase activity of actomyosin with tropomyosin in the presence of Ca^{2+} ions [7].

In previous studies [8,9], it was found that one of the troponin components, TN-I, strongly inhibits the ATPase activity of AS-particles from beef heart mitochondria and component TN-I acts as a non-competitive inhibitor of the mitochondrial ATPase activity. The present paper deals with the inhibition of the Ca^{2+}-dependent ATPase activity of the chloroplast coupling factor 1 (CF_1) by the troponin component, TN-1.

2. Materials and methods

The chloroplast coupling factor 1 (CF_1) was prepared by the method of Lien and Racker [10] and was activated by heat at 60°C for 4 min or by trypsin digestion at 20°C for 6 min. The ATPase activities of the activated CF_1 were 30–25 μmol P_i/mg of protein/min in the presence of Ca^{2+} ion and 12–10 μmol P_i/mg of protein/min in the presence of Mg^{2+} ion. The concentration of protein was determined by the method of Lowry et al. [11] Measurement of the ATPase activity of the activated CF_1 was performed by the method of Lien and Racker [10]. Troponin and tropomyosin were prepared from a rabbit skeletal muscle according to the procedure of Greaser and Gergely [7]. The troponin components, TN-I, TN-T and TN-C, were separated by DEAE-Sephadex chromatography in 6 M urea [7].

A mixture of the activated CF_1 (1.5 μg) and component TN-I (less than 8 μg) in 50 μl of 20 mM Tricine-NaOH buffer (pH 8.0) containing 1 mM EDTA and 0.1 mM dithiothreitol was prepared. The mixed solution (50 μl) was added to 0.5 ml solution containing 8 mM ATP, 10 mM $CaCl_2$ in 40 mM Tricine-NaOH buffer (pH 8.0) for the assay of the Ca^{2+}-ATPase activity or to 0.5 ml solution containing 8 mM ATP, 2 mM $MgCl_2$, 60 mM sodium maleate in the same buffer as above for the assay of Mg^{2+}-ATPase activity. After the reaction mixture had been incubated for 10 min at 37°C, the reaction was 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-Doty [12].

3. Results and discussion

Inhibitory effect of troponin and its components on the Ca^{2+}-ATPase activity of CF_1 activated by trypsin or by heat was tested, and the results are

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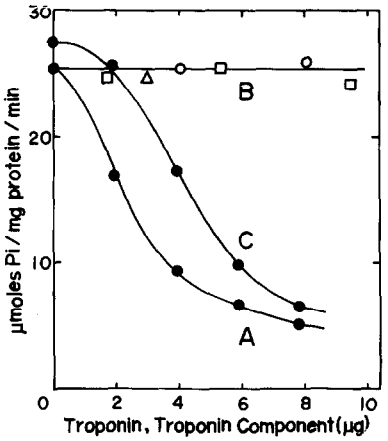

Fig.1. Inhibition of the Ca^{2+}-ATPase activity of the activated CF_1 by troponin and its component. The reaction mixture (0.55 ml) contains 8 mM ATP, 10 mM $CaCl_2$, 40 mM Tricine-NaOH (pH 8.0) and 1.3 μg activated CF_1 with various amounts of troponin, or its component. Curve A; the ATPase activity of the trypsin-activated CF_1 with component TN-I. Curve B; the ATPase activity of the trypsin-activated CF_1 with troponin (○–○), component TN-T (△–△) or component TN-C (□–□). Curve C; the ATPase activity of the heat-activated CF_1 with component TN-I.

shown in fig.1. The Ca^{2+}-ATPase activity of CF_1 activated by trypsin decreases by increasing component TN-I concentration and is almost completely inhibited with (80%) 8 μg of component TN-I, as is seen in curve A. On the other hand, no inhibitory effect on the ATPase activity of CF_1 is observed, when troponin, component TN-C or component TN-T (curve B) was used instead of component TN-I. The solubility of component TN-T is so small that its inhibitory effect on the ATPase activity could not be tested beyond 3.0 μg. The Ca^{2+}-ATPase activity of CF_1 activated by heat is also inhibited by component TN-I, which is shown by curve C. However, the Mg^{2+}-ATPase activity of CF_1 activated by trypsin was not inhibited by component TN-I. These results indicate that component TN-I acts as a potent inhibitor of the Ca^{2+}-ATPase activity of CF_1, probably due to the interaction of CF_1 with component TN-I. The concentration of component TN-I, at which 50% inhibition of the Ca^{2+}-ATPase activity occurs was 100 pmol per 1 μg CF_1 and this value was twice the concentration of CF_1 inhibitor, 50 pmol per 1 μg CF_1, obtained for its inhibition of the ATPase activity of CF_1 [3].

Table 1
Effect of tropomyosin on the inhibition of the ATPase activity of the trypsin-activated CF_1 by Component TN-I

	Ca^{2+}-ATPase activity (μmol P_i/mg/min)	Relative activity (%)
CF_1 activated with trypsin	25.4	100
+ tropomyosin	26.5	104
+ Component TN-I	11.4	45
+ tropomyosin + Component TN-I	13.0	51

To the reaction mixture (0.55 ml) containing 1.1 μg CF_1 and 4.1 μg component TN-I 5 μg tropomyosin was added.

Component TN-I was digested by trypsin and the effect of the digested Component TN-I on the Ca^{2+}-ATPase activity of CF_1 was examined. In fig.2 the Ca^{2+}-ATPase activity of CF_1 is plotted against the incubation-time of the digestion. The digestion of component TN-I with trypsin gives rise to a complete

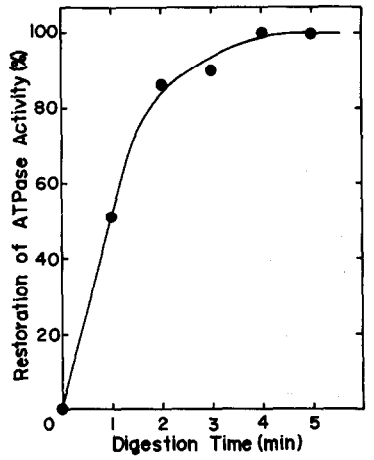

Fig.2. Restoration of the Ca^{2+}-ATPase activity of the trypsin-activated CF_1 during the course of the digestion of component TN-I with trypsin. Component TN-I (180 μg/ml) in a medium containing 10 mM Tricine-NaOH (pH 8.0) was digested with trypsin (2.1 μg/ml) at 37°C and at a given time the digestion was stopped by adding trypsin inhibitor (6.3 μg/ml). The mixed solution (50 μl) containing the activated CF_1 (1.1 μg) and component TN-I digested with trypsin (3.5 μg) was subjected to the measurement of the Ca^{2+}-ATPase activity. On this experimental condition, the degree of inhibition of the ATPase activity by non-digested component TN-I was 60%.

Table 2
Inhibitory and non-inhibitory effect of troponin component TN-I, the mitochondrial ATPase inhibitor (F_1 inhibitor), the chloroplast ATPase inhibitor (CF_1 inhibitor) and the inhibitor of elongation factor G-dependent GTPase reaction (C factor) on the ATPase and GTPase activities of various biological energy transducing systems

	TN-I	F_1 inhibitor	CF_1 inhibitor	C factor
mol. wt.	23 000 [6]	11 500 [5]	13 000 [3]	23 000 [14]
Actomyosin ATPase (Mg^{2+})	+ [6]			
Mitochondrial ATPase (Mg^{2+})	+ [8]	+ [4]	− [3]	
Chloroplast ATPase (Ca^{2+}, Mg^{2+})	+ [a]	− [b][3]	+ [c][3]	
Elongation factor G-dependent GTPase (Mg^{2+})	− [15]			+ [14]

+; inhibition, −; non-inhibition.

[a] Ca^{2+}-ATPase activity
[b] Mg^{2+}-ATPase activity
[c] Ca^{2+}- and Mg^{2+}-ATPase activity
The numbers in square brackets are references.

restoration of the chloroplast ATPase activity. This indicates that the higher order structure of component TN-I seems to be closely associated with the inhibition of the ATPase activity.

Tropomyosin plays an important role in the inhibition of actomyosin ATPase activity in the presence of component TN-I. To test the effect of tropomyosin on the inhibition of the chloroplast ATPase activity by component TN-I, the Ca^{2+}-ATPase activity of CF_1 with component TN-I was measured in the presence and the absence of tropomyosin. The results are shown in table 1. No significant difference was observed between the inhibitory effect on the ATPase activity on adding component TN-I alone and on adding component TN-I together with tropomyosin.

The ATPase inhibitors isolated from biological energy transducing systems are the muscle ATPase inhibitor (troponin component TN-I), the chloroplast ATPase inhibitor (CF_1 inhibitor) and the mitochondrial ATPase inhibitor (F_1 inhibitor), and they may play an important role in the regulation of energy transformation processes. An inhibitor of elongation factor G-dependent guanosine triphosphatase (GTPase) reaction was also isolated from ribosome wash of *Escherichia coli* Q13, and may serve as a regulator of translocation of peptidyl-tRNA on the ribosome [14]. Inhibitory effects of each inhibitor on the ATPase or GTPase activity of various biological energy transducing systems are summarized in table 2. It was demonstrated by Nelson et al. [3] that the CF_1 inhibitor inhibits only the Ca^{2+}- and Mg^{2+}-ATPase activity of CF_1 and not the mitochondrial ATPase activity of F_1 and that the F_1 inhibitor inhibits only the mitochondrial ATPase activity of F_1 and not the Mg^{2+}-ATPase activity of CF_1. However, troponin component, TN-I, inhibits the actomyosin ATPase activity and the mitochondrial ATPase activity [8] and also inhibits the Ca^{2+}-ATPase activity of CF_1, as described in the present study. It is noteworthy that the affinity of component TN-I to proteins having ATPase activity is fairly high and consequently inhibition of the ATPase activity takes place. No inhibition of the GTPase activity generated by binding of elongation factor G to ribosomes was observed on adding component TN-I [15].

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