Inhibition of *E. coli* ATPase activity by a troponin component, TN-I, and by mitochondrial ATPase inhibitor

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Summary. The enzymic activity of Mg^{2+}- or Ca^{2+}-stimulated ATPase from *Escherichia coli* was inhibited by one of the troponin components, TN-I, and by mitochondrial ATPase inhibitor (F_1-inhibitor). The inhibitory ability of component TN-I against Mg^{2+}-stimulated ATPase activity was lost after digestion of component TN-I with trypsin. The Mg^{2+}-stimulated ATPase activity inhibited by component TN-I was completely restored by the addition of another troponin component, TN-C.

During the course of the study on energy transducing system in living cells, ATPases and their inhibitors have been isolated in a homogeneous state from the different sources; mitochondrial coupling factor 1 (F_1) and its inhibitor (F_1-inhibitor) from beef heart[1,2], chloroplast coupling factor 1 (CF_1) and its inhibitor (CF_1-inhibitor) from spinach[3,4], and actomyosin ATPase and a troponin component, TN-I, from rabbit skeletal muscle[5-7]. The physicochemical properties of these ATPases and their inhibitors have been studied to clarify the regulatory mechanism for ATPase activity. In previous studies[8-11], the authors reported the interactions between ATPases and their inhibitors obtained from different sources; mitochondrial or chloroplast ATPase activity was inhibited non-competitively by the troponin component TN-I, and actomyosin and chloroplast ATPase activity were also strongly inhibited by F_1-inhibitor. Bacterial ATPase was isolated from the membrane of *Escherichia coli*[12] and its physicochemical properties were similar in mol wt, 296,000, and subunit structure to those of ATPase from different sources such as chloroplasts and mitochondria. The present paper deals with the inhibition of the enzymic activity of the partially purified *E. coli* ATPase by a troponin component, TN-I, and by mitochondrial ATPase inhibitor (F_1-inhibitor).

Materials and methods. The partially purified bacterial ATPase (EC 3.6.1.3) was prepared from *Escherichia coli* K12 by the method of Futai et al.[12] except the final purification step. The partially purified ATPase was very stable during the measurement of ATPase activity under these experimental conditions and gave a constant enzymic activity, 10-13 μmoles Pi/mg of protein/min. The enzymic activity of the ATPase thus prepared was completely inhibited by the bacterial inhibitor prepared from the membrane of *E. coli* by the method of Nieuwenhuis and Bakkenist[13]. From the measurement of the band intensities in the disc-electrophoretic pattern, the purity of the preparation was estimated as approximately 30%. Troponin was prepared from a rabbit skeletal muscle by the method of Greaser and Gergely[7], and the troponin components TN-I and TN-C were purified by chromatography with DEAE-Sephadex using 6 M urea[7]. The component TN-I thus prepared showed a single band in the disc-electrophoretic pattern. The actomyosin ATPase activity was inhibited by component TN-I; it retained 5% activity when the molar ratio of actomyosin ATPase/component TN-I was 1.8. Mitochondrial ATPase inhibitor (F_1-inhibitor) was purified from beef heart mitochondria by the method of Horstman and Racker[2]. The disc-electrophoretic pattern of the mitochondrial ATPase inhibitor prepared gave a single band. The concentration of protein was determined by the method of Lowry, using bovine serum albumin as a standard[14]. The measurement of the ATPase activity was carried out as follows; a mixture (225 μl) of the partially purified *E. coli* ATPase (2.3 μg) and component TN-I (less than 68 μg) was preincubated for 10 min at 37 °C. After the preincubation, the sample solution was added to 1.0 ml of the substrate solution containing 4 mM ATP, 2 mM Mg^{2+} (or 4 mM Ca^{2+}) and 20 mM Tricine-NaOH buffer (pH 8.0). After incubating the reaction mixture for 10 min at 37 °C, the reaction was stopped by adding 1.5 ml of 7.5% trichloroacetic acid. A similar experiment was carried out for F_1-inhibitor; a mixture (75 μl) of ATPase (2.3 μg) and F_1-inhibitor (less than 26 μg) was added to 1.0 ml of the substrate solution. The amount of inorganic phosphate liberated from ATP was measured by the method of Martin-Doty[15].

Results and discussion. Figure 1 shows the inhibition of the enzymic activity of the partially purified *E. coli* ATPase by the troponin component TN-I, (the left-hand panel) and by the mitochondrial ATPase inhibitor, F_1-inhibitor (the right-hand panel). Increasing the concentration of component TN-I in the reaction mixture caused a decrease in the

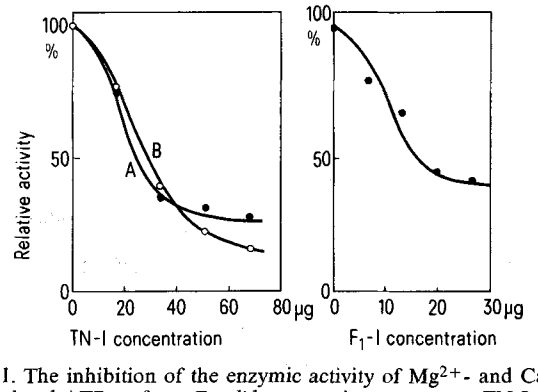

Fig. 1. The inhibition of the enzymic activity of Mg^{2+}- and Ca^{2+}-stimulated ATPase from *E. coli* by troponin component, TN-I, (the left side panel), and the F_1-inhibitor (the right side panel). Curves A and B; Mg^{2+}-ATPase and Ca^{2+}-ATPase (2.3 μg), respectively. The enzymic activity (13 μmoles Pi/mg/min) was taken as 100%.

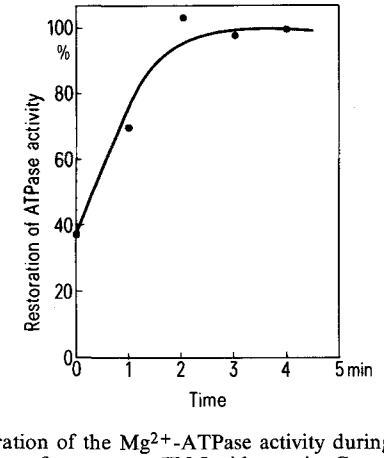

Fig. 2. Restoration of the Mg^{2+}-ATPase activity during the course of the digestion of component TN-I with trypsin. Component TN-I (31.7 μg) in a medium containing 10 mM Tris-HCl (pH 8.0) was digested with trypsin (0.5 μg) at 37 °C and at a given time the digestion was stopped by adding trypsin inhibitor (1.33 μg).

enzymic activity of ATPase stimulated by Mg^{2+} or Ca^{2+}. As seen in the right-hand panel in figure 1, mitochondrial ATPase inhibitor, (F_1-inhibitor) also inhibited the enzymic activity of Mg^{2+}-stimulated ATPase. The inhibitory action was enhanced with increasing concentrations of F_1-inhibitor and remained constant (40%) at a concentration of more than 20 µg F_1-inhibitor. No complete loss of the ATPase activity with F_1-inhibitor occurred; the reason for this is not clear.

Component TN-I was digested with trypsin and the inhibitory effect of the digested component TN-I on the enzymic activity of Mg^{2+}-stimulated ATPase was tested. As seen in figure 2, after a 2-min-digestion of component TN-I with trypsin, the inhibitory action was not observed and the ATPase activity was completely restored. However, the actomyosin ATPase activity was inhibited by the digested component TN-I. This is the same result as that reported by Shigekawa and Tonomura[16]. The disc-electrophoretic pattern of the digested component TN-I had no band corresponding to that of native component TN-I and had bands with smaller molecular weights than that of component TN-I. This indicates the significance of the conformation of the component TN-I molecule for its inhibitory action on partially purified *E. coli* ATPase. It is well known that one of the troponin components, TN-C, which has binding-ability for component TN-I, reverses the inhibitory action of the component TN-I on the enzymic activity of actomyosin ATPase. When component TN-C (34.8 µg) was added to the reaction mixture containing the partially purified *E. coli* ATPase (2.3 µg) and component TN-I (31.7 µg), no inhibition of the ATPase activity was observed. This shows that binding of component TN-C to component TN-I causes the loss of the inhibitory ability of component TN-I on the partially purified *E. coli* ATPase.

We have reported so far the inhibitory action caused by the interaction between ATPase and their inhibitors from different sources[8-11]. Component TN-I inhibits mitochondrial, chloroplast and bacterial ATPases, and F_1-inhibitor inhibits muscle, chloroplast and bacterial ATPases. For the muscle, mitochondrial and chloroplast ATPases, the ATPase activities were inhibited by component TN-I and also restored by component TN-C as reported previously[17,18]. These ATPases, including the bacterial ATPase, seem to be inhibited in a similar interaction between the ATPase and component TN-I.

1 M.E. Pullman, H.S. Penefsky, A. Datta and E. Racker, J. biol. Chem. *235*, 3322 (1960).
2 L.L. Horstman and E. Racker, J. biol. Chem. *245*, 1336 (1970).
3 V.K. Vambutas and E. Racker, J. biol. Chem. *240*, 2660 (1965).
4 N. Nelson, H. Nelson and E. Racker, J. biol. Chem. *247*, 7657 (1972).
5 K.M. Nauss, S. Kitagawa and J. Gergely, J. biol. Chem. *244*, 755 (1969).
6 J.A. Spudich and S. Watt, J. biol. Chem. *246*, 4866 (1971).
7 M.L. Greaser and J. Gergely, J. biol. Chem. *246*, 4226 (1971).
8 Y. Tamaura, S. Yamazaki, S. Hirose and Y. Inada, Biochem. biophys. Res. Commun. *53*, 673 (1973).
9 S. Yamazaki, H. Takizawa, Y. Tamaura, S. Hirose and Y. Inada, FEBS Lett. *56*, 248 (1975).
10 S. Yamazaki, H. Hasebe, H. Takizawa, Y. Tamaura and Y. Inada, Biochem. biophys. Res. Commun. *75*, 1104 (1977).
11 H. Hasebe, S. Yamazaki, A.M. Almazan, Y. Tamaura and Y. Inada, Biochem. biophys. Res. Commun. *77*, 932 (1977).
12 M. Futai, P.C. Sternweis and L.A. Heppel, Proc. nat. Acad. Sci. USA *71*, 2725 (1974).
13 F.J.R.M. Nieuwenhuis and A.R.J. Bakkenist, Biochem. biophys. Acta *459*, 596 (1977).
14 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
15 J.B. Martin and D.M. Doty, Analyt. Chem. *21*, 965 (1949).
16 M. Shigekawa, Y. Tonomura, J. Biochem. *72*, 957 (1972).
17 E. Eisenberg and W.W. Kielly, J. biol. Chem. *249*, 4742 (1974).
18 S. Yamazaki, H. Takizawa, Y. Tamaura, S. Hirose and Y. Inada, FEBS Lett. *66*, 23 (1976).

Effect of the ionic strength on the kinetic properties of the mitochondrial L-malate dehydrogenase

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Summary. Increase of the ionic strength inhibits the catalytic activity of the mitochondrial MDH, reduces substrate inhibition and decreases the affinity of substrates for the enzyme.

The catalytic activity of the mitochondrial L-malate dehydrogenase (MDH) is strongly affected by changes in the ionic environment, while the cytoplasmic MDH is insensible to such changes[3]. It was shown[3] that nature and extent of the ionic strength effect on the mitochondrial MDH depend on the oxaloacetate concentration. The mitochondrial MDH shows substrate inhibition[4] at high oxaloacetate (or NADH) concentrations, and one would expect that this concentration dependence of the ionic strength action reflects mainly a kinetical effect. It is the purpose of this study to investigate this kinetical effect and to examine the ionic strength action on the kinetical parameters of the enzyme.

Materials and methods. The mitochondrial L-malate dehydrogenase (EC 1.1.1.37) from beef-heart was purified according to the procedure described by Englard and Siegel[5]. Mitochondria were prepared according to the methodology of Crane et al.[6]. The purified enzyme was stored at low temperature and appropriately diluted with tris-acetate buffer (pH 8.0 and 0.05 M in acetate) at the moment of use. The experimental data were obtained by monitoring the NADH (reduced nicotinamide adenine dinucleotide) oxidation at 340 nm during the first min of reaction. The minimal ionic strength was 0.05 M and can be attributed to the components of the buffer (tris-acetate, 0.05 M in acetate). Further increments in the ionic strength were obtained by the addition of several strong electrolytes, but all data presented in this paper were obtained in the presence of KCl. The experimental data presented in this paper are the average values of 3 independent estimates.