INHIBITION OF CHLOROPLAST ADENOSINE TRIPHOSPHATASE ACTIVITY BY ADENOSINE TRIPHOSPHATASE INHIBITOR FROM BEEF HEART MITOCHONDRIA

Hiroshi Hasebe, Shojiro Yamazaki, Aurea M. Almazan, Yutaka Tamaura, and Yuji Inada

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

Received June 22,1977

The Ca^{2+}-dependent ATPase activity of chloroplast coupling factor 1(CF_1), which is activated by heat or by digestion with trypsin, is non-competitively inhibited by an ATPase inhibitor(F_1-inhibitor) obtained from beef heart mitochondria. This inhibition is retained even after digestion of F_1-inhibitor with trypsin. The Mg^{2+}-dependent ATPase activity of CF_1 is also inhibited by F_1-inhibitor. The difference in inhibitory action of chloroplast ATPase inhibitor(CF_1-inhibitor) and of F_1-inhibitor on heat- and trypsin-activated CF_1 is discussed in relation to the subunit structure of the CF_1 molecule.

ATPase inhibitors were isolated from mitochondria(1), chloroplasts(2), membranes of Escherichia coli(3) and myofibrils(4). The effects of endogenous ATPase inhibitors on the activity of various ATPases are being studied extensively to clarify the regulation mechanism of energy transformation. These inhibitors have similar physico-chemical properties such as molecular weight, isoelectric point and amino acid composition and similar physiological properties in terms of heat-stability, trypsin-sensitivity and urea-insensitivity(1-4).

This paper constitutes the sixth in a series of articles dealing with the action of ATPase inhibitors on ATPase obtained from different sources with a system of heterogeneous combination of them. In the previous reports(5-9), it was demonstrated that troponin component TN-I, which is an inhibitor of actomyosin ATPase, strongly inhibits the activities of ATPases from mitochondria and from chloroplasts by binding to the ATPase molecule. Mitochondrial

ISSN 0006-291X

ATPase inhibitor(F_1-inhibitor) also inhibits actomyosin ATPase activity and its inhibitory action is a similar to that of Component TN-I.

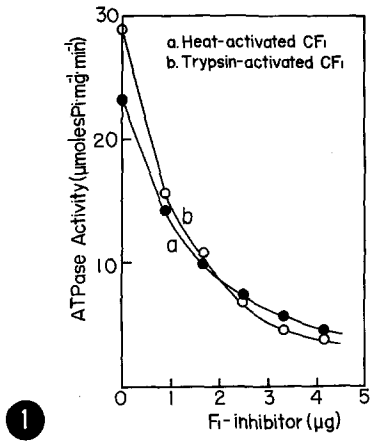
The experiments described below deal with the inhibition of the ATPase activity of chloroplast coupling factor 1(CF_1) by F_1-inhibitor obtained from mitochondria in beef heart.

MATERIALS AND METHODS

Mitochondrial ATPase inhibitor(F_1-inhibitor) was isolated from beef heart mitochondria by the method of Horstman and Racker(10). Chloroplast ATPase inhibitor(CF_1-inhibitor) was prepared using the procedure of Nelson et al.(2). Chloroplast coupling factor 1(CF_1), obtained by the method of Lien and Racker (11), was activated by heat at 60°C for 4 min or by digestion with trypsin at 30°C for 6 min. The heat- and trypsin-activated CF_1 had an ATPase activity of 20-30 μmoles Pi/mg of protein/min in the presence of Ca^{2+}(10 mM). To test the inhibitory effect of digested F_1-inhibitor on ATPase activity, F_1-inhibitor (13.2 μg) was digested with trypsin(50 μg) at 37°C for 10 min. Digestion was stopped by adding 100 μg of trypsin inhibitor(Miles Lab. Ltd.). Ca^{2+}-dependent ATPase activity was determined by adding activated CF_1(1.25 μg) to 0.5 ml of 40 mM Tricine-NaOH buffer(pH 8.0) containing 8 mM ATP, 10 mM $CaCl_2$ and F_1-inhibitor(0-16.5 μg), and by incubating for 10 min at 37°C. Reaction was stopped by adding 2.0 ml of ice-cold 3% trichloroacetic acid. The amount of inorganic phosphate liberated from ATP was determined by the Martin-Doty method(12). Assay of the Mg^{2+}-dependent ATPase activity of activated CF_1 was performed by following the same procedure except that 3.13 μg activated CF_1, 2 mM $MgCl_2$ and 60 mM sodium maleate were used in the system. Mitochondrial ATPase activity was measured as described previously(5). Protein concentration was estimated by the method of Lowry et al.(13), using bovine serum albumin as the protein standard.

RESULTS AND DISCUSSION

The inhibitory effects of mitochondrial ATPase inhibitor(F_1-inhibitor) on



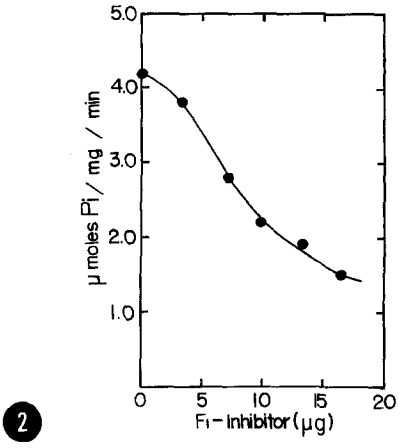


Fig. 1. Inhibition of the Ca^{2+}-dependent ATPase activities of the heat- and trypsin-activated CF_1 by F_1-inhibitor. The reaction system is as follows: 0.5 ml of sample solution containing 8 mM ATP, 40 mM Tricine-NaOH (pH 8.0), 10 mM $CaCl_2$ and activated CF_1 in the presence of various amounts of F_1-inhibitor, at 37°C. Curves a and b; heat-activated CF_1 (1.25 μg) and trypsin-activated CF_1 (1.20 μg), respectively.

Fig. 2. Inhibition of the Mg^{2+}-dependent ATPase activity of the heat-activated CF_1 by F_1-inhibitor. The reaction system is as follows: 0.5 ml of sample solution containing 8 mM ATP, 2 mM $MgCl_2$, 60 mM sodium maleate, 40 mM Tricine-NaOH (pH 8.0) and 3.13 μg heat-activated CF_1 with various amounts of F_1-inhibitor.

the Ca^{2+}- and the Mg^{2+}-dependent ATPase activities of heat-activated CF_1 are shown in Figs. 1 and 2. Ca^{2+}-ATPase activity(curve a in Fig. 1) decreases with increasing amount of F_1-inhibitor and is almost completely inhibited by 4 μg F_1-inhibitor. Fifty percent inhibition of Ca^{2+}-ATPase activity takes place in the presence of 1.5 μg F_1-inhibitor which corresponds to 100 pmoles/μg of CF_1. This value is 2 times greater than 50 pmoles/μg of CF_1 for the inhibition by CF_1-inhibitor reported by Nelson et al.(2). The Mg^{2+}-ATPase activity(Fig. 2) also decreases with increasing amount of the inhibitor and 50% inhibition occurs at a concentration of 10 μg of F_1-inhibitor(300 pmoles/μg of CF_1).

The next series of experiments determines whether F_1-inhibitor digested with trypsin is still capable of inhibiting the activity of ATPases from

Table I. Effect of F_1-inhibitor digested with trypsin on the ATPase activities of mitochondria and chloroplasts. Incubation system for inhibition of chloroplast ATPase is 0.5 ml of sample solution containing 8 mM ATP, 40 mM Tricine-NaOH(pH 8.0), 10 mM $CaCl_2$ and 1.25 μg heat-activated CF_1 in the absence and in the presence of F_1-inhibitor(2.65 μg) or digested F_1-inhibitor (2.65 μg).

	ATPase Activity	
	Mitochondria** (μmoles Pi/10 min)	Chloroplast* (μmoles Pi/mg/min)
Complete system	0.40(100%)	15.6(100%)
+ F_1-inhibitor	0.05(12%)	4.2(27%)
+ Digested F_1-inhibitor	0.53(134%)	3.9(25%)

* Ca^{2+}-dependent ATPase activity.
** Data taken from previous report(9).

different sources or not(Table I). Mitochondrial ATPase activity is inhibited by F_1-inhibitor but not by digested F_1-inhibitor. Inhibition of the chloroplast ATPase activity by F_1-inhibitor is, however, retained even after digestion of the inhibitor. This suggests the presence of a site with a specific sequence in the F_1-inhibitor which interacts with CF_1. A similar phenomenon was observed for the inhibition of actomyosin ATPase by digested troponin component TN-I(14) and F_1-inhibitor(9). Syska et al.(14) showed that a peptide containing basic amino acid residues in troponin component TN-I interacts with actomyosin ATPase. It was suggested(9) that a site with basic amino acid residues in F_1-inhibitor may be responsible for its inhibitory action on actomyosin ATPase activity also.

The activation mechanism of the CF_1 molecule by heat or by trypsin digestion had been demonstrated by Nelson et al.(15). CF_1 molecule has five subunits (α, β, γ, δ and ε). Heating dissociates ε subunit, which is also called CF_1-inhibitor, whereas trypsin digestion releases γ, δ and ε subunits from the rest of the molecule to generate the ATPase activity.

Table II. Inhibitory action of CF_1- and F_1-inhibitors, on the Ca^{2+}-dependent ATPase activities of CF_1 activated by heat and by trypsin digestion. Incubation system is the same as that described in Table I with the exception of CF_1-inhibitor(1.4 μg) and F_1-inhibitor(2.5 μg).

	Ca^{2+}-ATPase Activities(μmoles Pi/mg/min)	
	Heat-activated CF_1 ($\alpha_2\beta_2\gamma\delta$)	Trypsin-activated CF_1 ($\alpha_2\beta_2$)
Complete system	29.0(100%)	29.3(100%)
+CF_1-inhibitor	8.7(30%)	29.7(101%)
+ F_1-inhibitor	9.2(32%)	6.9(24%)

Table II shows the inhibitory action of CF_1- and of F_1-inhibitor on the Ca^{2+}-dependent ATPase activities of chloroplast ATPase activated by heat and by digestion with trypsin. CF_1-inhibitor inhibits the activity of heat-activated CF_1 but not that of trypsin-activated CF_1, as reported by Deters et al.(16). On the other hand, F_1-inhibitor inhibits the ATPase activities of both the heat- and trypsin-activated CF_1. The degree of inhibition increases with increasing concentration of F_1-inhibitor (Fig. 1) in both cases. Fifty percent inhibition takes place at 1.5 μg and 1.4 μg F_1-inhibitor concentration for the heat-activated and trypsin-activated CF_1, respectively. Presumably, the F_1-inhibitor has an affinity for the α and/or β subunit(s) in the chloroplast ATPase molecule activated by trypsin ($\alpha_2\beta_2$) resulting to the inhibition of its ATPase activity. In the case of the heat-activated CF_1, a similar mechanism for inhibition probably occurs. Nelson (15) demonstrated that the interaction between CF_1-inhibitor and γ subunit in the heat-activated CF_1 ($\alpha_2\beta_2\gamma\delta$) inhibits ATPase activity. Quercetin (3,3',4',5,7-pentahydroxyflavone) (16) and troponin component TN-I(7) also inhibit both activities of CF_1 activated by heat and by digestion with trypsin.

Fig. 3 shows the kinetic studies on the inhibition of the Ca^{2+}-ATPase

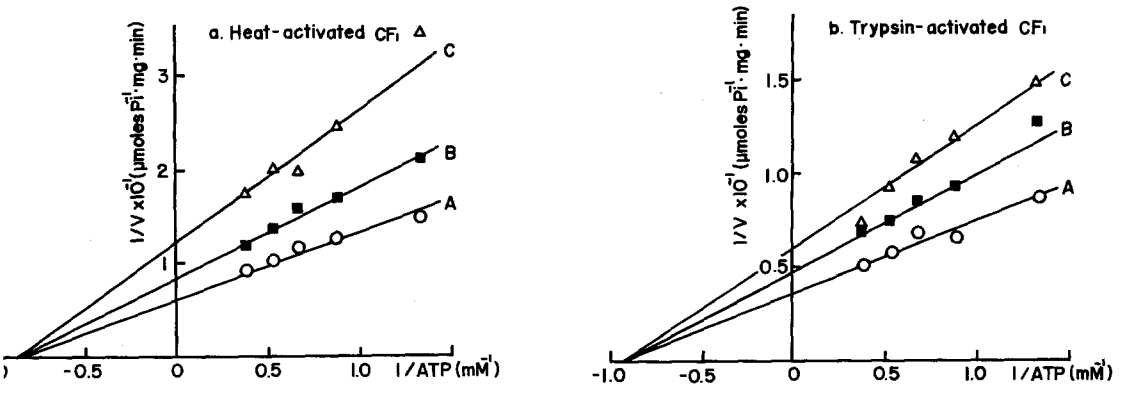


Fig. 3. Double reciprocal plots of the Ca^{2+}-dependent ATPase activities of activated CF_1 against concentration of ATP. Left panel (a): heat-activated CF_1 in the absence of F_1-inhibitor (curve A) and in the presence of 0.83 μg (curve B) and 1.66 μg F_1-inhibitor (curve C). Right panel (b): trypsin-activated CF_1 in the absence of F_1-inhibitor (curve A) and in the presence of 0.83 μg (curve B) and 1.66 μg F_1-inhibitor (curve C).

activity of heat-activated (upper panel) and trypsin-activated (lower panel) CF_1 by F_1-inhibitor. Double reciprocal plots of the ATPase activity of activated CF_1 against ATP concentration in the presence and in the absence of F_1-inhibitor give straight lines and each line intercepts at the same point on the horizontal axis. The value of Ki for the inhibition of the ATPase activity of heat- and trypsin-activated CF_1 by F_1-inhibitor is 4.3×10^{-7} M. This is smaller than the Ki value (2×10^{-6} M) for the inhibition of the ATPase activity of mitochondrial ATPase by troponin component TN-I (6). These results indicate that the ATPase activity of activated CF_1 is non-competitively inhibited by F_1-inhibitor and that F_1-inhibitor may have an affinity for a site in the CF_1 molecule which differs from the binding site of ATP.

REFERENCES

1. Pullman, M. E. and Monroy, G.C. (1963) J. Biol. Chem. 238, 3762-3769.
2. Nelson, N., Nelson, H. and Racker, E. (1972) J. Biol. Chem. 247, 7657-7662.
3. Nieuwenhuis, F. J. R. M. and Bakkenist, A. R. J. (1977) Biochim. Biophys. Acta 459, 596-604.
4. Hartshorne, D. J., Perry, S. V. and Schaub, M. C. (1967) Biochem. J. 104, 907-913.
5. Tamaura, Y., Yamazaki, S., Hirose, S., and Inada, Y. (1973) Biochem. Biophys. Res. Commun. 53, 673-679.

6. Takisawa, H., Yamazaki, S., Tamaura, Y., Hirose, S. and Inada, Y. (1975) Arch. Biochem. Biophys. 170, 743-744.
7. Yamazaki, S. Takisawa, H., Tamaura, Y., Hirose, S. and Inada, Y. (1975) FEBS Letters 56, 248-251.
8. Yamazaki, S., Takisawa, H., Tamaura, Y., Hirose, S. and Inada, Y. (1976) FEBS Letters 66, 23-26.
9. Yamazaki, S., Hasebe, H., Takisawa H., Tamaura, Y and Inada, Y. (1977) Biochem. Biophys. Res. Commun. 75, 1104-1110.
10. Horstman, L. L. and Racker, E. (1970) J. Biol. Chem. 245, 1336-1344.
11. Lien, S. and Racker, E. (1971) Methods in Enzymology Vol.XXIII, 547-555.
12. Martin, J. B. and Doty, D. M. (1949) Anal. Chem. 21. 965-967.
13. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275.
14. Syska, H., Wilkinson J. M., Grand, R. J. A. and Perry, S. V. (1976) Biochem. J. 153, 375-387.
15. Nelson, N. (1976) Biochim. Biophys. Acta 456, 314-338.
16. Deters, D. W., Racker, E., Nelson, N. and Nelson, H. (1975) J. Biol. Chem. 250, 1041-1047.