INHIBITION OF MUSCLE ACTOMYOSIN ATPase ACTIVITY BY MITOCHONDRIAL ATPase INHIBITOR

Shojiro Yamazaki, Hiroshi Hasebe, Haruhiko Takisawa, Yutaka Tamaura and Yuji Inada

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

Received March 1,1977

SUMMARY

The mitochondrial ATPase inhibitor(F_1-inhibitor) from beef heart mitochondria strongly inhibits the muscle actomyosin ATPase activity. This inhibitory action by F_1-inhibitor is not affected by the addition of tropomyosin or troponin component TN-C. These results obtained by F_1-inhibitor are discussed in relation to the inhibitory action of one of troponin components, TN-I, on the actomyosin ATPase activity.

During the course of investigations of the energy transducing processes of oxidative phosphorylation, it was found that the activity of chloroplast ATPase(CF_1) or of mitochondrial ATPase (F_1) is non-competitively inhibited by one of the troponin components, TN-I(1, 2, 3), which inhibits the ATPase activity of actomyosin in the presence of tropomyosin. It was also found that once inhibited activities of mitochondrial and chloroplast ATPases were restored by the addition of another troponin component, TN-C(4), which plays an important role in regulation of the energy transducing system.

Pullman and Monroy(5) isolated an inhibitor(F_1-inhibitor) with the molecular weight of 11,000(ref. 6) of mitochondrial ATPase from beef heart mitochondria. A similar inhibitor was obtained from spinach chloroplast by Nelson et al.(7) and its inhibitor(CF_1-inhibitor) was one of the CF_1 subunits, ε, with

ISSN 0006-291X

solution saturated with diethyl ether (5). This communication describes another inhibitor, high concentration of guanidine hydrochloride (Guan-HCl), and circular dichroism (CD) of the 410-nm intermediate.

MATERIALS AND METHODS

Cells and purple membranes were obtained as described (6). The solution of purple membranes in Guan-HCl was prepared by adding solid Guan-HCl to the concentrated suspension of purple membranes in a graduated glass tube. After buffer and KCl solution were added, the volume was adjusted by water. The resulting solution was sonicated for 10 sec to homogenize the fragments of purple membranes. This solution was stable for months at 4° or at room temperature.

Absorption spectra were recorded with a double-beam spectrophotometer (Shimazu UV-200) with scanning speed 200 nm per min from 700 nm to 300 nm. Decay of the 410-nm intermediate in the dark was measured with a single-beam spectrophotometer attached with an actinic light source, 650 W quartz halogen lamp filtered through a 10 cm water layer and a cut-off filter (Toshiba,VO-55). The illumination (5 sec) was started and terminated with a shutter (COPAL,No.0). The changes of transmittance at 410 nm after closing the shutter were memorized by a transient recorder (Datalab,Model DL 905) and recorded on a chart recorder.

CD spectra were measured in a Jasco J-20 recording spectrometer with a quartz cuvette placed in a low temperature cell holder. The sample was cooled slowly under illumination by dry ice-aceton (-86°) and then measured in the dark. The absorption maximum around 560 nm had completely disappeared throughout measurement and the 410-nm intermediate did not transform back to the purple complex for overnight in the dark at this temperature. The sample for the purple complex was cooled without illumination and the other conditions were the same as those of the illuminated one.

RESULTS AND DISCUSSION

Bacteriorhodopsin was found to be capable of photochemical reaction in 8 M Guan-HCl solution. However, we noticed that purple membrane solution containing 8 M Guan-HCl changed the color from purple to yellow under illumination by actinic light. When the sample was returned to the dark, purple color reappeared spontaneously. Fig.1 shows a time series of the regeneration of the purple complex in the dark. Upon turning

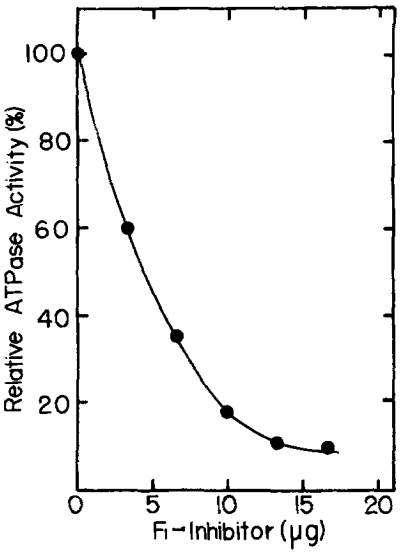

Fig. 1. Inhibition of the actomyosin ATPase activity by F_1-inhibitor. The reaction mixture (1.0 ml) contains 25 mM Tris-HCl(pH 7.8), 25 mM KCl, 5 mM $MgCl_2$, 4 mM ATP and 0.076 mg actomyosin with various amounts of F_1-inhibitor. The absolute value of the ATPase activity, 0.37 μmoles Pi/mg/min, was taken as 100%.

RESULTS AND DISCUSSION

The mitochondrial ATPase inhibitor(F_1-inhibitor) strongly inhibits the actomyosin ATPase activity as shown in Fig. 1. The ATPase activity decreases with increasing F_1-inhibitor concentration and remains at a constant level of about 10% of the original activity at more than 13.2 μg F_1-inhibitor. Fifty percent inhibition of the ATPase activity by F_1-inhibitor takes place at a concentration of 400 pmoles F_1-inhibitor per 76 μg actomyosin, which is four times higher concentration than 100 pmoles Component TN-I per 76 μg actomyosin in the presence of tropomyosin(14).

Table I shows the effect of F_1-inhibitor on the activity of myosin ATPase or of actomyosin ATPase. The activity of actomyosin ATPase is inhibited by F_1-inhibitor, while that of

Table I. Effect of F_1-inhibitor on the activities of myosin ATPase and actomyosin ATPase.

	ATPase Activities(μmoles Pi/mg/min)	
	Myosin ATPase	Actomyosin ATPase
None	0.012 (100%)	0.31 (100%)
+F_1-inhibitor	0.011 (91%)	0.03 (10%)

F_1-inhibitor(16.5 μg) was added to the reaction mixture(1.0 ml) containing 2.0 mg myosin or 0.076 mg actomyosin.

Table II. Effect of Component TN-C or tropomyosin on the inhibition of the actomyosin ATPase activity by F_1-inhibitor.

Additions	ATPase Activity(μmoles Pi/mg/min)
Actomyosin	0.31 (100%)
+Component TN-C	0.30 (99%)
+F_1-inhibitor	0.14 (44%)
+F_1-inhibitor+Component TN-C	0.12 (38%)
Actomyosin	0.37 (100%)
+Tropomyosin	0.39 (105%)
+F_1-inhibitor	0.23 (62%)
+F_1-inhibitor+Tropomyosin	0.24 (64%)

17.6 μg Component TN-C was added to the reaction mixture(1.0 ml) containing 0.076 mg actomyosin and 6.6 μg F_1-inhibitor.
20.2 μg tropomyosin was added to the reaction mixture(1.0 ml) containing 0.076 mg actomyosin and 3.3 μg F_1-inhibitor.

myosin ATPase is not, as seen from Table I. This experimental results are identical with the result obtained for the effect of troponin component TN-I on the activity of myosin ATPase or actomyosin ATPase, which was carried out by Schraub and Perry(15). From these facts, it may be concluded that F_1-inhibitor inhibits the interaction between myosin and actin.

Table III. Effect of F_1-inhibitor digested with trypsin on the activities of actomyosin ATPase and mitochondrial ATPase. F_1-inhibitor(0.26 mg/ml) in a medium containing 10 mM Tris-HCl (pH 7.8) was digested with trypsin(1.0 mg/ml) at 37°C for 30 min, and the digestion was stopped by adding trypsin inhibitor(2.0 mg/ml).

	ATPase Activities	
	Actomyosin ATPase (μmoles Pi/mg/ml)	Mitochondrial ATPase (μmoles Pi/10 min)
None	0.31 (100%)	0.40 (100%)
+F_1-inhibitor	0.05 (17%)	0.05 (12%)
+Digested F_1-inhibitor	0.06 (20%)	0.53 (134%)

Actomyosin ATPase; 6.6 μg F_1-inhibitor was added.
Mitochondrial ATPase; 1.32 μg F_1-inhibitor was added.

It has been well-known that tropomyosin enhances the inhibition of the actomyosin ATPase activity by troponin component TN-I(16), and that troponin component TN-C reverses the inhibitory action of Component TN-I on the actomyosin ATPase activity(17). In the present experiment, the effect of tropomyosin or Component TN-C on the inhibition of the actomyosin ATPase activity by F_1-inhibitor was examined and the results are shown in Table II. No significant difference in the ATPase activity of actomyosin was observed between the inhibitory action by F_1-inhibitor alone and by F_1-inhibitor together with tropomyosin or with Component TN-C. This result may indicate that tropomyosin or Component TN-C does not affect the inhibition of the actomyosin ATPase activity by F_1-inhibitor, which is entirely different from that by Component TN-I(16, 17).

Table III represents the inhibitory effect of F_1-inhibitor digested with trypsin on the actomyosin ATPase activity and on the mitochondrial ATPase activity. The mitochondrial ATPase activity is not inhibited by F_1-inhibitor digested with trypsin,

Table IV. Inhibitory effects of mitochondrial ATPase inhibitor (F_1-inhibitor), chloroplast ATPase inhibitor(CF_1-inhibitor), and troponin component TN-I on the ATPase activities of various biological energy transducing systems.

	Component TN-I	F_1-inhibitor	CF_1-inhibitor
Molecular weight	23,000(ref.15)	11,000(ref.6)	13,000(ref.7)
Actomyosin ATPase	+ [15]	+	
Mitochondrial ATPase	+ [1]	+ [5]	- [7]
Chloroplast ATPase	+ [3]		+ [7]

+;inhibition -; non-inhibition
The numbers in parenthesis brachets are references.

which is reported by Pullman and Monroy(5). On the other hand, the actomyosin ATPase activity is inhibited by trypsin-digested F_1-inhibitor. It was reported by Shigekawa and Tonomura(18) that Component TN-I digested with trypsin inhibits the actomyosin ATPase activity and a fragment of Component TN-I has an inhibitory action on the actomyosin ATPase activity. According to Syska *et al.*(14), it is known that Component TN-I has two sites which interact with actin and Component TN-C; one site interacting with actin is a peptide from Asp to Homoserine(residues 96-117) with 9 basic amino acid residues, and the other site interacting with Component TN-C is a N-terminal peptide from AcGly to His(residues 1-47) with 11 basic amino acid residues. They also reported that basic proteins such as salmine and lysozyme inhibit the actomyosin ATPase activity. F_1-inhibitor is not so strongly basic protein(pI=7.6)(ref. 19) that a site interacting with actin may be responsible for basic amino acid residues which are located in vicinity on the primary or the tertiary structure of the F_1-inhibitor molecule.

Table IV summarized inhibitory effects of mitochondrial

ATPase inhibitor(F_1-inhibitor), chloroplast ATPase inhibitor (CF_1-inhibitor) and troponin component TN-I on the ATPase activities of various biological energy transducing systems, which were obtained in the present and the previous reports(1, 2, 3, 7, 15).

REFERENCES

1. Tamaura, Y., Yamazaki, S., Hirose, S. and Inada, Y.(1973) Biochem. Biophys. Res. Commun. 53, 673-679.
2. Takisawa, H., Yamazaki, S., Tamaura, Y., Hirose, S. and Inada, Y.(1975) Arch. Biochem. Biophys. 170, 743-744.
3. Yamazaki, S., Takisawa, H., Tamaura, Y., Hirose, S. and Inada, Y.(1975) FEBS Letters 56, 248-251.
4. Yamazaki, S., Takisawa, H., Tamaura, Y., Hirose, S. and Inada, Y.(1976) FEBS Letters 66, 23-26.
5. Pullman, M. E. and Monroy, G. C.(1963) J. Biol. Chem. 238, 3762-3769.
6. Penefsky, H. S.(1974) The Enzymes, Vol. X, pp 375-394.
7. Nelson, N., Nelson, H. and Racker, E.(1972) J. Biol. Chem. 247, 7657-7662.
8. Horstman, L. L. and Racker, E.(1970) J. Biol. Chem. 245, 1336-1344.
9. Nauss, K. M., Kitagawa, S. and Gergely, J.(1969) J. Biol. Chem. 244, 755-765.
10. Spudich, J. A. and Watt, S.(1971) J. Biol. Chem. 246, 4866-4871.
11. Greaser, M. L. and Gergely, J.(1971) J. Biol. Chem. 246, 4226-4233.
12. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J.(1951) J. Biol. Chem. 193, 265-275.
13. Martin, J. B. and Doty, D. M.(1949) Anal. Chem. 21, 965-967.
14. Syska, H., Wilkinson, J. M., Grand, R. J. A. and Perry, S. V. (1976) Biochem. J. 153, 375-387.
15. Schraub, M. C. and Perry, S. V.(1971) Biochem. J. 123, 367-377.
16. Wilkinson, J. M., Perry, S. V., Cole, H. A. and Trayer, I. P. (1972) Biochem. J. 127, 215-228.
17. Eisenberg, E. and Kielly, W. W.(1974) J. Biol. Chem. 249, 4742-4748.
18. Shigekawa, M. and Tonomura, Y.(1972) J. Biochem.(Tokyo) 72, 957-971.
19. Juntti, K., Asami, K. and Ernster, L.(1971) Abstr. 7th Meet. Fed. Eur. Biochem. Soc., Varna, No 660.