INHIBITION OF CHLOROPLAST ADENOSINE TRIPHOSPHATASE ACTIVITY BY BASIC PROTEINS AND PEPTIDES

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1. Introduction

Physicochemical and functional properties of ATPases and their inhibitors from different sources such as chloroplasts [1], mitochondria [2], membranes of *Escherichia coli* [3] and myofibrils [4] have been extensively studied to clarify the mechanism of energy transducing systems. In our laboratory, we demonstrated that troponin component TN-I, which is an inhibitor of actomyosin ATPase, strongly inhibits the mitochondrial and chloroplast ATPase activities [5–7]. Mitochondrial ATPase inhibitor (F_1-inhibitor) also inhibited the chloroplast and actomyosin ATPase activities [8,9] as well as the mitochondrial ATPase activity. These results showed the similarity of the action of ATPase inhibitors on ATPases obtained from different sources with a system of heterogeneous combination.

The actomyosin ATPase activity was reported [10] to be inhibited by basic proteins such as salmine, lysozyme and cytochrome *c*. It is demonstrated here that the chloroplast ATPase activity is inhibited by basic proteins and peptides and that the inhibitory action is due to amino groups in the molecule of inhibitor proteins.

2. Materials and methods

Histone(lysine-rich subgroup f_1) from calf thymus, putrescine, spermidine, spermine and gramicidin S were purchased from Sigma Chemical Co. Crystalline lysozyme from hen egg-white, protamine from salmon sperm and bacitracin were obtained from Seikagaku Kogyo Co., Tokyo Kasei Co. and Ono Pharmaceutical Co., respectively. Poly(L-lysine) and poly(L-glutamic acid) were purchased from the Institute of Protein Research, Osaka University, and fluorescamine, 4-phenyl-spiro[furan-2(3H)-1′-phthalan]-3,3′-dione, was obtained from Roche Inc. The other reagents were of analytical grades.

One of troponin component, TN-I, was isolated from rabbit skeletal muscle by the method in [11]. Actomyosin was prepared by mixing myosin and actin in 4:1 weight ratio, which were obtained in a pure state from rabbit skeletal muscle [8]. Chloroplast coupling factor 1 (CF_1) was prepared as in [12] and was activated by heat at 60°C for 4 min. The heat-activated CF_1 had an ATPase activity of 25–35 μmol P_i/mg protein/min in the presence of Ca^{2+}.

The Ca^{2+}-dependent chloroplast ATPase activity was determined as follows: 20 μl heat-activated CF_1 (2 μg) solution was added to 0.5 ml 40 mM Tricine–NaOH buffer (pH 8.0) containing 8 mM ATP and 10 mM $CaCl_2$ in the presence and absence of basic proteins or peptides. The mixture was incubated for 10 min at 37°C and the reaction was stopped by the addition of cold 3% trichloroacetic acid. The actomyosin ATPase activity was determined as in [8]. The amount of inorganic phosphate liberated from ATP was determined as in [13]. Modifications of amino groups in troponin component TN-I and gramicidin S were carried out with fluorescamine [14] and succinic anhydride [15], respectively. Succinylated gramicidin S, in which the total two amino groups in the molecule were modified, was used in the present experiment. Degree of modification of amino groups in troponin component TN-I

with fluorescamine was determined fluorometrically as in [14]. Protein concentration was estimated by the Lowry method [16], using bovine serum albumin as a protein standard.

3. Results and discussion

Figure 1 represents the inhibitory action of histone, protamine, poly(L-lysine), lysozyme and poly(L-glutamic acid) on the chloroplast ATPase activity. In the case of histone (curve A) and protamine (curve B), the ATPase activity of CF_1 was markedly decreased with increasing concentration of each protein and was almost completely lost at 10 μg protein concentration. Inhibitory action of poly(L-lysine) curve C, or of lysozyme, curve D, was not so strong, and the ATPase activity was reduced to 45% by poly(L-lysine) and to 74% by lysozyme, at 50 μg protein concentration. Poly(L-glutamic acid) did not inhibit at all, curve E.

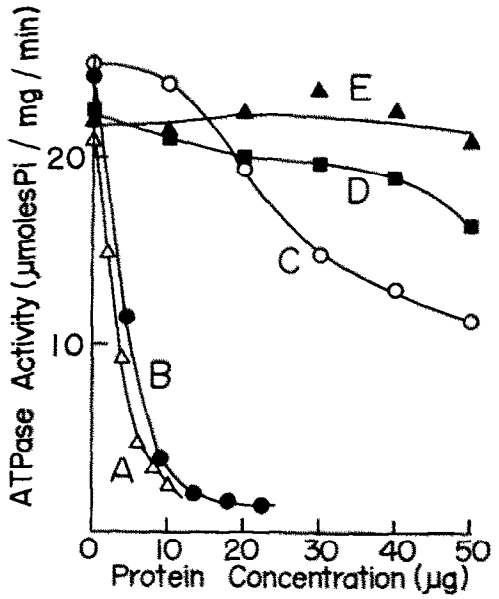

Fig.1. Inhibition of the chloroplast ATPase activity by proteins and polypeptides. Curves A, B, C, D and E: histone, protamine, poly(L-lysine), lysozyme and poly(L-glutamic acid), respectively. The reaction mixture (0.57 ml) contains: 8 mM ATP, 10 mM $CaCl_2$, 40 mM Tricine–NaOH (pH 8.0) and 2 μg heat-activated CF_1 with various amounts of each protein and peptide. The reaction mixture was incubated at 37°C for 10 min.

In the next series of experiments, the effect of amino acids and polyamines on the inhibition of the chloroplast ATPase activity was determined (table 1). The degree of inhibition of various amino acids and polyamines at ~20 mM is shown by the upper column of the table. Spermine was the most effective in inhibiting the ATPase activity, compared with ethylenediamine, diethylenetriamine, triethylenetetramine, putrescine and spermidine. The inhibitory action of polyamines was markedly enhanced by increasing the number of amino groups in the molecule. No inhibition of the ATPase activity was observed for amino acids, and poly(L-glutamic acid). The lower column in table 1 shows the amount (μg) of various inhibitors such as poly(L-lysine), gramicidin S, protamine, histone, CF_1-inhibitor [1], F_1-inhibitor [9] and troponin component TN-I [7], in which 50% inhibition of the enzyme activity takes place using 1 μg chloroplast ATPase. Inhibitory ability of basic proteins such as protamine (2.0 μg) and histone (1.9 μg) on the chloroplast ATPase activity was only 2 or 3 times smaller than that obtained for the homologous inhibitor, ε subunit of CF_1 [1].

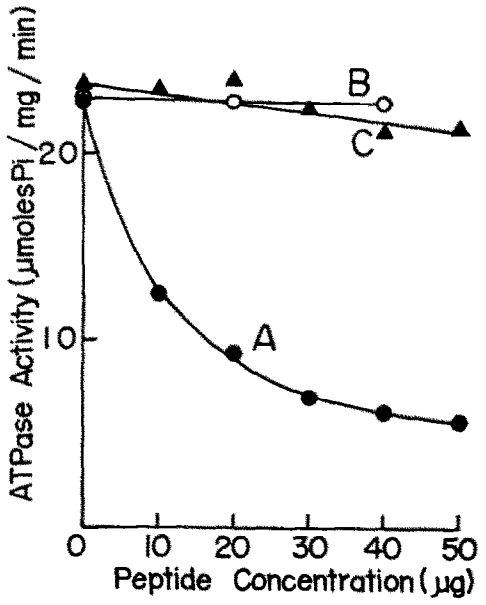

Fig.2. Inhibition of the chloroplast ATPase activity by bacitracin and gramicidin S. Curve A, gramicidin S. Curve B, gramicidin S modified with succinic anhydride, in which 2 amino groups in the molecule were succinylated. Curve C, bacitracin. Experimental conditions as in fig.1.

Table 1
Inhibitory action of various amino acids, amines, peptides and proteins on the chloroplast ATPase activity

Amino acids, amines, peptides and proteins		mM	ATPase activity (μmol P_i/mg/min)	Inhibition (%)
None			31.6	0
Glutamic acid	$HOOC(CH_2)_3CH(NH_2)COOH$	20	32.0	0
Ornithine	$H_2N(CH_2)_3CH(NH_2)COOH$	20	32.5	0
Lysine	$H_2N(CH_2)_4CH(NH_2)COOH$	20	31.9	0
Arginine	$HN{=}CNH(NH_2)(CH_2)_3CH(NH_2)COOH$	20	31.3	0
Ethylenediamine	$H_2N(CH_2)_2NH_2$	21	26.2	17
Diethylenetriamine	$H_2N(CH_2)_2NH(CH_2)_2NH_2$	21	23.1	27
Triethylenetetramine	$H_2N(CH_2)_2NH(CH_2)_2NH(CH_2)_2NH_2$	21	18.3	42
Putrescine	$H_2N(CH_2)_4NH_2$	21	16.7	47
Spermidine	$H_2N(CH_2)_3NH(CH_2)_4NH_2$	21	13.5	57
Spermine	$H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$	21	11.7	63
Poly(L-glutamic acid)		50 μg		0
Bacitracin		50 μg		10
Lysozyme		50 μg		26

Proteins (mol. wt)	Amount of each inhibitor (50% inhibition/1 μg CF_1)
Poly(L-lysine)	18
Gramicidin S(1142)	6
Protamine(from salmon sperm)	2.0
Histone(lysine-rich)	1.9
CF_1-inhibitor(13 000)	0.73[a]
F_1-inhibitor(10 500)	0.96[b]
Troponin component TN-I(23 000)	3.9[c]

[a,b,c] [1], [9] and [7], respectively

Figure 2 shows the inhibition of the chloroplast ATPase activity by gramicidin S (curve A), a cyclic decapeptide antibiotic, and bacitracin (curve C), a cyclic dodecapeptide antibiotic. The chloroplast ATPase activity was remarkably inhibited by higher levels of gramicidin S and ~76% inhibition took place at 50 μg gramicidin S concentration. On the other hand, bacitracin did not inhibit the chloroplast ATPase activity. Effectiveness of inhibition by gramicidin S may be due to its basic property since it has 2 amino groups and no carboxyl group. On the other hand, bacitracin has 2 amino groups and 2 carboxyl groups. No inhibition of the chloroplast ATPase activity by bacitracin may be, therefore, due to the non-basic property of the molecule. In order to confirm the importance of amino groups in gramicidin S on the inhibition of the ATPase activity, the 2 amino groups in the gramicidin S molecule were modified with succinic anhydride. No inhibition of the ATPase activity by the succinylated gramicidin S was observed (fig.2, curve B).

Troponin component TN-I inhibits the activity of chloroplast ATPase [7] as well as that of actomyosin ATPase. Modification of amino groups in the troponin component TN-I with fluorescamine led to a complete loss of the ability to inhibit the chloroplast ATPase activity. The result is shown in fig.3. Curve A represents the degree of inhibition of the chloroplast ATPase activity by troponin component TN-I modified with fluorescamine. By increasing the degree of modification of amino groups in troponin component TN-I, the inhibitory action was sharply reduced. The modification of ~3 out of the total 29 amino groups in the troponin component TN-I molecule caused the com-

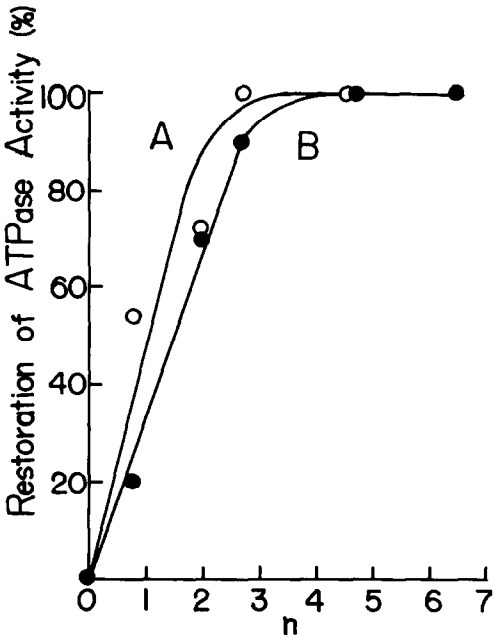

Fig.3. Effect of NH_2-modified troponin component TN-I on the enzymic activity of chloroplast ATPase (CF_1) or actomyosin ATPase. Curve A, heat-activated CF_1 (2.2 μg). Curve B, actomyosin (67 μg). Experimental conditions as detailed in section 2. *n* stands for the number of amino groups in the troponin component TN-I molecule modified with fluorescamine.

plete loss of the inhibitory action on the ATPase activity. The same experiment as above was carried out for actomyosin ATPase with the modified troponin component TN-I. As is seen by curve B in fig.3, the restoration of the ATPase activity was also observed when 3 amino groups were modified with the reagent. From these results, it may be concluded that amino groups in the protein inhibitor molecule play an important role in the inhibition of ATPase activity.

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