UNCOUPLING OF PHOTOPHOSPHORYLATION IN SPINACH CHLOROPLASTS BY THE IONOPHOROUS ANTIBIOTIC A23187

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1. Introduction

Ionophorous antibiotics have been intensely used in the study of energy transfer and ion transport processes.

Recently, the action of a new ionophorous antibiotic, A23187, on mitochondrial functions [1, 2] and on ion transport across other biological membranes [3, 4] has been reported.

A23187 is a carboxylic antibiotic that binds divalent cations [1] and uncouples oxidative phosphorylation in rat liver mitochondria [1, 2]. Ca^{2+} but not Mg^{2+} enhances the uncoupling effect [1, 2]. Reed and Lardy [1] proposed that a cyclic, energy-dissipating flux of mitochondrial calcium accounts for uncoupling by A23187.

The present report deals with the action of A23187 on photophosphorylation, photosynthetic electron transport, proton uptake and Ca^{2+} transport in spinach chloroplasts. The data show that A23187 is an uncoupler of photophosphorylation and suggest that the antibiotic increases the H^+ permeability of the thylakoid membrane. Ca^{2+} enhanced the uncoupling effect of A23187 and was transported into the chloroplasts by the antibiotic.

2. Experimental

Chloroplasts were isolated from market spinach leaves (*Spinacea olearacea L*) as previously described [5].

Photophosphorylation, electron transport from water to ferricyanide and the pH changes of chloroplasts suspensions were determined as described [6].

Total chlorophyll was determined as described [7].

A23187 was the generous gift of Dr Donald R. Brannon, The Lilly Research Laboratories, Indianapolis.

3. Results and discussion

Fig. 1A shows the titration of the inhibition of cyclic and non-cyclic photophosphorylation in spinach chloroplasts by the antibiotic A23187. The phosphorylation associated with ferricyanide reduction was more sensitive to the ionophore than the phenazine methosulphate-mediated phosphorylation. The basal electron transport rate from water to ferricyanide was stimulated by the ionophore to the level of the electron transport rate observed in the presence of ADP and Pi (fig. 1B). Neither the latter nor the uncoupled electron transport was affected by A23187 (fig. 1B). Therefore, inhibition of photophosphorylation by the antibiotic was due to uncoupling as was that of oxidative phosphorylation [1, 2].

Table 1 shows that A23187 inhibited the light-dependent proton uptake of unbuffered suspensions of chloroplasts at concentrations similar to those that uncoupled cyclic photophosphorylation.

10 μM EGTA or EDTA affected neither the light-dependent proton uptake nor the inhibition induced by 5 μM A23187.

The inhibition of the light-dependent pH rise by low concentrations of A23187 was enhanced by the presence of Ca^{2+} in the reaction medium as shown in fig. 2A. $CaCl_2$ had no effect on the proton uptake by itself but strongly increased the inhibition induced by a low concentration of the antibiotic (fig. 2B).

Ca^{2+} potentiated also the inhibition of photophos-

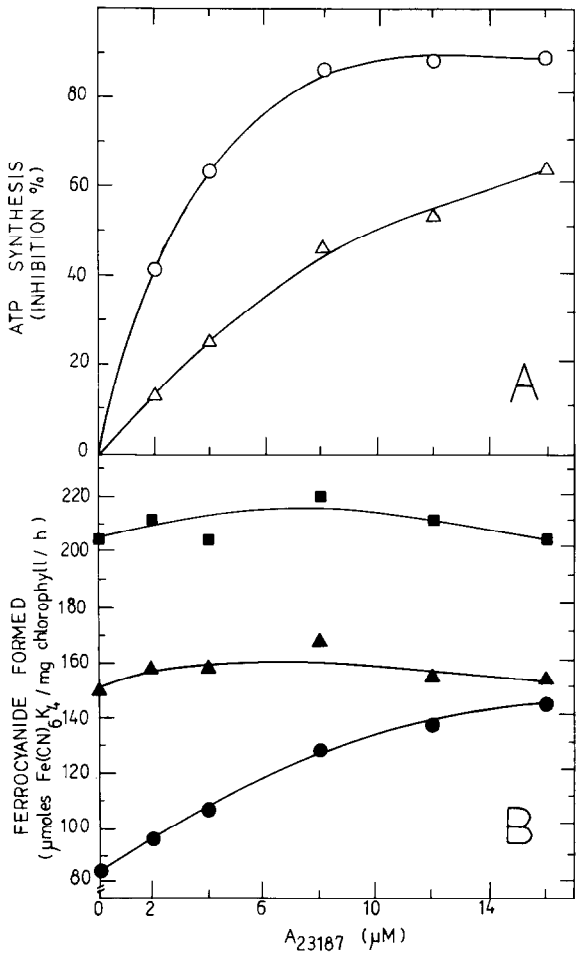

Fig. 1. Effects of A23187 on photophosphorylation and electron transport in spinach chloroplasts. The reaction medium (1 ml) was 250 mM sucrose, 20 mM N-Tris(hydroxymethyl)-methyl-2-aminoethanesulphonic acid-NaOH buffer (pH: 7.8), 3 mM $MgCl_2$ and either 1.2 mM ferricyanide or 33 μM phenazine methosulphate. ADP and potassium phosphate were 2 mM when added. An amount of chloroplasts equivalent to 10 μg of chlorophyll was used per test tube. Measurements were as described in the text. A) cyclic (△---△) and non cyclic (○---○) ferricyanide associated photophosphorylation. Control values were 391 and 83 μmoles ATP/mg chlorophyll/hr respectively. B) electron flow from water to ferricyanide was determined in basal conditions (●---●), in the presence of ADP and P_i (▲---▲) and in the presence of 10 mM methylamine (■---■).

Table 1
Effect of A23187 on the light-dependent proton uptake by spinach chloroplasts

Additions (μM)		Proton uptake (μmoles H^+/mg chlorophyll)
None		0.767
A23187	(1.6)	0.674
A23187	(3.3)	0.555
A23187	(5.0)	0.495
A23187	(10.0)	0.307
A23187	(15.0)	0.153

The reaction medium (3 ml) was 50 mM KCl, 20 μM pyocyanine and chloroplasts (40 μg of chlorophyll/ml) prepared as usual but suspended in unbuffered 50 mM KCl. Other experimental conditions were as described [6].

phorylation by A23187. Table 2 shows that 1 μM A23187 inhibited by 28% the synthesis of ATP associated with reduction of ferricyanide and by 54% in the presence of 3 mM $CaCl_2$ which, by itself, had no action. The uncoupling effect of the ionophore was not prevented by 100 μM EGTA (table 2).

Several ionophorous antibiotics have been described as uncouplers of photophosphorylation [8–14]. However, their mechanisms of uncoupling are not identical.

Nigericin and other related carboxylic ionophores are potent uncouplers of photophosphorylation in chloroplasts [8–11]. They require the presence of a suitable alkali metal cation. Uncoupling was proposed to occur by dissipation of the proton gradient through a cation/H^+ exchange induce by antibiotics.

Low concentrations of valinomycin, the first ionophore described that uncouples oxidative phosphorylation by increasing the mitochondrial membrane permeability to K^+ [15], has little effect on photophosphorylation but together with low concentrations of nigericin, 2–4 dinitrophenol or FCCP uncouples synergically [12,13]. According to Karlish et al. [12] this synergistic uncoupling is of the same type as that induced by nigericin, i.e., both of them were proposed to increase the membrane permeability to both cations and protons. However, the action of valinomycin in chloroplasts seems rather complex since it has been described as an energy transfer inhibitor [16] and more recently [14] it was shown that concentrations of valinomycin higher than 10^{-6} M inhi-

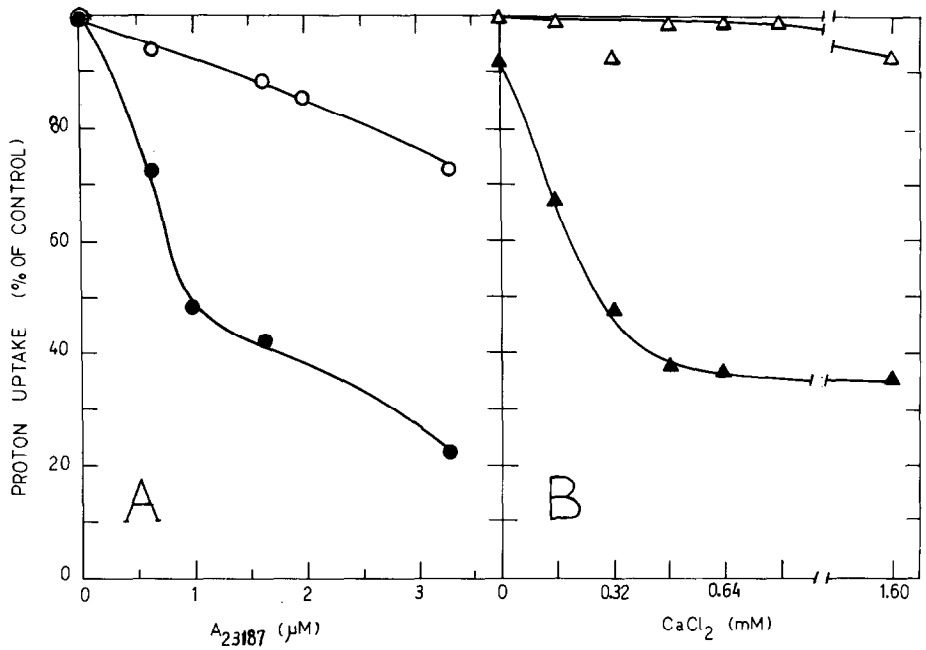

Fig. 2. Effect of A23187 and $CaCl_2$ on the light-dependent proton uptake by chloroplasts. Experimental conditions were as described in the legend to table 1. A) Effect of A23187 in the absence (o---o) or in the presence (•---•) of 0.32 mM $CaCl_2$. B) Effect of $CaCl_2$ in the absence (△---△) or in the presence (▲---▲) of 1 μM A23187.

Table 2
Effects of $CaCl_2$ and EGTA on the inhibition of noncyclic photophosphorylation by A23187

Additions	Non cyclic photophosphorylation (μmoles ATP/mg chlorophyll/hr)
None	70.64
A23187 (1 μM)	50.86
$CaCl_2$ (3 mM)	71.35
A23187 (1 μM); $CaCl_2$ (3 mM)	32.49
A23187 (5 μM)	17.66
EGTA (100 μ M)	72.05
A23187 (5 μ M); EGTA (100 μM)	16.25

Experimental conditions as described in the legend to fig. 1.

bited electron flow in chloroplasts uncoupled by nigericin or NH_4Cl but they stimulated basal electron transport. Both effects were observed in the absence of added K^+ although the presence of K^+ facilitated the actions of valinomycin.

As described in this paper, A23187 inhibited ATP synthesis and proton uptake by chloroplasts and stimulated basal electron transport without affecting active or uncoupled electron flow. This uncoupling behavior does not seem related to the antibiotic ability to transport divalent cations [1–4] since, for instance, the inhibition of proton uptake occurred in the absence of any added divalent cation.

The possibility that the uncoupling may be mediated by a cyclic transport to endogenous Ca^{2+} or Mg^{2+} as described for mitochondria [1], seems unlikely because of the lack of effect of chelating agents. Therefore, the present results suggest that A23187 uncouples chloroplasts by increasing the permeability of the thylakoid membrane to protons and thus collapsing the proton gradient even in the absence of any divalent cation. However, addition of Ca^{2+} to the reaction medium potentiates the uncoupling effect of the antibiotic (fig. 2 and table 2). This enhancement may be explained by an exchange of protons for calcium across the thylakoid membrane catalyzed by A23187 as has been shown in other membranes [3].

Table 3
Effect of A23187 on Ca^{2+} uptake by spinach chloroplasts

Additions (μM)	Uptake of $^{45}Ca^{2+}$ (nmoles ^{45}Ca/mg chlorophyll)	
	Light	Dark
None	6.8	7.2
A23187 (3)	9.4	8.3
A23187 (6)	11.5	6.3
A23187 (12)	11.4	7.3

Reaction medium (1 ml) was that described in the legend to table 1 with the addition of 0.32 mM $CaCl_2$ and 1 μC of $^{45}CaCl_2$. After 1 min of incubation at 25°C in the dark or in saturating light an aliquot of 0.2 ml was filtered through a Sartorius membrane filter (pore size 0.8 μ). The filter was washed with 50 mM KCl and the radioactivity counted in a Beckman LS-233 liquid scintillation counter.

Actually, as shown in table 3, A23187 catalyzed a light-dependent Ca^{2+} uptake by chloroplasts in experimental conditions similar to those of proton uptake. Under these experimental conditions there is not a light-dependent Ca^{2+} uptake by chloroplasts (see ref. 17). The lack of effect in the dark suggests that a light-dependent proton gradient is necessary for the induction of an electroneutral exchange of calcium for protons by A23187.

In conclusion, it has been shown that the antibiotic A23187 uncouples chloroplasts and it is proposed that in the absence of added Ca^{2+} A23187 uncouples by increasing the proton efflux from the chloroplasts. In the presence of Ca^{2+}, lower concentrations of the antibiotic are needed for uncoupling because it catalyzes a light-dependent Ca^{2+} uptake that probably occurs through a calcium/proton exchange.

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