INHIBITION OF SPINACH CHLOROPLASTS PHOTOPHOSPHORYLATION BY THE ANTIBIOTICS LEUCINOSTATIN AND EFRAPEPTIN

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1. Introduction

The ATPases that catalyze the synthesis of ATP in oxidative and photosynthetic phosphorylation are rather similar with respect to molecular weight, subunit distribution, amino acid composition and coupling activity (for a review see [1]). However, they are immunologically different and do not replace each other as coupling factors. They also differ in their sensitivity towards some inhibitors. For instance, Lardy and coworkers have introduced several antibiotics as specific inhibitors of oxidative phosphorylation [2]. The best known and more widely used are oligomycin and aurovertin [3,4]. Although both antibiotics inhibit oxidative phosphorylation their binding sites are different and only aurovertin inhibits the ATPase activity of soluble F_1 [4]. On the other hand, they do not affect photophosphorylation except for a weak uncoupling by high concentrations of oligomycin [5].

Recently, Lardy et al. [2] have postulated four mitochondrial binding sites for antibiotics that inhibit phosphoryl transfer. Two of them are in F_1 and correspond to aurovertin and to efrapeptin. The other two are located on the membrane component of the ATPase complex; one of them is the oligomycin site and the fourth corresponds to leucinostatin.

Since photophosphorylation is not affected by oligomycin and aurovertin, we thought it would be interesting to test the effects of leucinostatin and efrapeptin on spinach chloroplasts.This paper shows that both antibiotics inhibited photophosphorylation although by different mechanisms: leucinostatin is an uncoupler of photophosphorylation while efrapeptin inhibited phosphoryl transfer probably by acting on the chloroplast coupling factor 1 (CF_1).

2. Experimental

Chloroplasts were isolated from spinach leaves (*Spinacea oleracea* L) as described [6] and suspended in 250 mM sucrose, 20 mM Tris-HCl (pH 7.8), and 5 mM $MgCl_2$.

Total chlorophyll [7] photophosphorylation [6] electron transport from water to methylviologen [8] and proton uptake [9] were determined as described. CF_1 was purified from spinach chloroplasts and heat-activated as described [10]. Ca-ATPase was measured at 37°C in 1 ml, containing 40 mM Tris-HCl (pH 8), 5 mM ATP and 5 mM $CaCl_2$. The reaction was started by addition of 3 μg of heat activated CF_1. After 10 min the reaction was stopped by 0.1 ml trichloroacetic acid 50% (P/V) and the P_i liberated was determined in the supernatants according to Sumner [11].

Leucinostatin (A20668) and efrapeptin (A23871) were generous gifts from Dr Robert L. Hamill, Lilly Research Laboratories, Indianapolis.

All other chemicals were of analytical degree.

3. Results and discussion

Figure 1 shows the inhibitory effects of the antibiotics leucinostatin and efrapeptin on photophosphorylation in spinach chloroplasts. The former

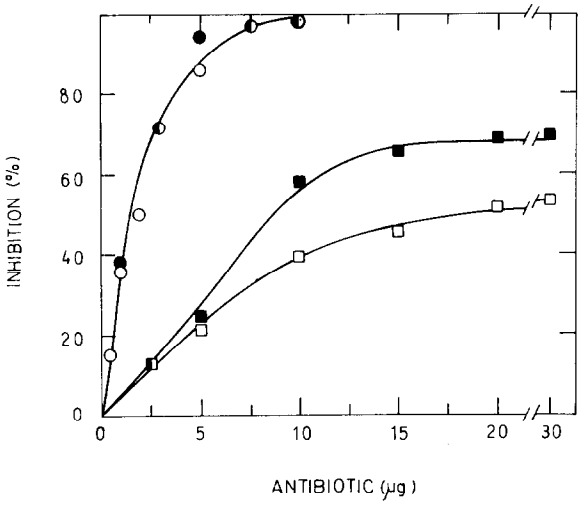

Fig.1. Inhibition of photophosphorylation by leucinostatin and efrapeptin. Cyclic, phenazine methosulfate catalyzed, and non-cyclic, methylviologen associated, photophosphorylation in spinach chloroplasts were determined as described in the text. (□, ■), efrapeptin, (○, ●), leucinostatin. Control values were 376 and 108 $\mu mol \cdot h^{-1} \cdot mg^{-1}$ chlorophyll for cyclic (open symbols) and non-cyclic (closed symbols) photophosphorylation respectively.

is a more effective inhibitor than the latter. Both cyclic and non cyclic photophosphorylation were similarly diminished by leucinostatin. Fifty per cent inhibition was achieved with about 2 μg/ml.

Reed and Lardy [12] have reported a biphasic effect of leucinostatin in mitochondrial oxygen uptake. First, the antibiotic inhibited coupled electron transport and phosphorylation, then at higher concentration, it stimulated oxygen uptake. Table 1 shows that leucinostatin stimulated basal and coupled photosynthetic electron transport in spinach chloroplasts in the same range of concentrations it inhibited photophosphorylation while uncoupled electron transport was not affected. The data presented in table 2 show that the light-induced proton uptake by spinach chloroplasts was similarly depressed by leucinostatin. The ATPase activity of heat-activated CF_1 was not inhibit by 10 μg of leucinostatin (not shown). From these results it may concluded that, at variance with its effects in mitochondria, leucinostatin behaves only as an uncoupler in spinach chloroplasts.

Efrapeptin inhibition of photophosphorylation

Table 1
Effects of leucinostatin and efrapeptin in photosynthetic electron transport

Additions (μg)		Electron transport ($\mu Eq\ O_2 \cdot mg^{-1}\ chl \cdot h^{-1}$)		
		Basal	Coupled	Uncoupled
None		148	300	404
Leucinostatin	(1)	174	334	405
Leucinostatin	(2)	244	368	398
Leucinostatin	(4)	312	412	400
Leucinostatin	(10)	456	468	400
Efrapeptin	(5)	148	200	407
Efrapeptin	(10)	148	196	398
Efrapeptin	(30)	140	150	405

Electron transport in spinach chloroplasts from water to methylviologen was determined as described [8]. The coupled and uncoupled rates were determined in the presence of 2 mM ADP, plus 2 mM P_i and 5 mM NH_4Cl respectively.

was not complete as shown in fig.1. It reached a maximum of 60–70% with 30 μg of antibiotic per ml. Non-cyclic photophosphorylation was slightly more sensitive to efrapeptin than the cyclic one. Efrapeptin also diminished the coupled electron transport from water to methylviologen while the basal and uncoupled rates were not affected (table 1). The antibiotic did not modify the light-induced proton uptake (table 2).

It has been shown [13–15] that low concentrations of ATP (or ADP) stimulate the light-dependent proton uptake in chloroplasts while inhibiting the basal photosynthetic electron transport. These

Table 2
Effect of leucinostatin and efrapeptin in the light induced proton uptake of spinach chloroplasts

Additions (μg)		Proton Uptake ($nEq\ H^+ \cdot mg^{-1}$ chl)	
None		358	
Leucinostatin	(0.5)	285	(20)
Leucinostatin	(1)	206	(43)
Leucinostatin	(2)	114	(69)
Leucinostatin	(4)	23	(94)
Efrapeptin	(20)	344	

Experimental conditions were as described [9]. Initial pH was 6.5. Numerals in parentheses indicate inhibition per cent.

Table 3
Effect of ATP and efrapeptin on proton uptake and electron transport

Additions	Proton uptake (nEq $H^+ \cdot mg^{-1}$ chl.)	Electron transport (μEq $O_2 \cdot mg^{-1}$ chl$\cdot h^{-1}$)
None	116	112
ATP 15 μM	187	80
ATP 15 μM; Efrapeptin (30 μg)	124	112

Experimental conditions for basal electron transport (water to methylviologen) and for proton uptake (initial pH 7.8) were as described [8,9].

effects have been attributed to a conformational change of CF_1 induced by binding of ATP or ADP [14]. Table 3 shows that efrapeptin abolished both the estimulation of proton uptake and the inhibition of electron transport by ATP although it affected none of these processes in the absence of ATP (tables 1 and 3).

These results suggest that efrapeptin inhibited phosphoryl transfer by interacting with CF_1. This suggestion was confrimed by the data shown in fig.2 which represent the inhibition by efrapeptin of the Ca-ATPase of purified CF_1 activated by heat. This inhibition was non-competitive with ATP (experiment not shown) and, as happened with photophosphorylation, was not higher than 70%.

In conclusion we have shown that, like in mitochondria, both leucinostatin and efrapeptin inhibit the synthesis of ATP in chloroplasts, although the former only acts as an uncoupler

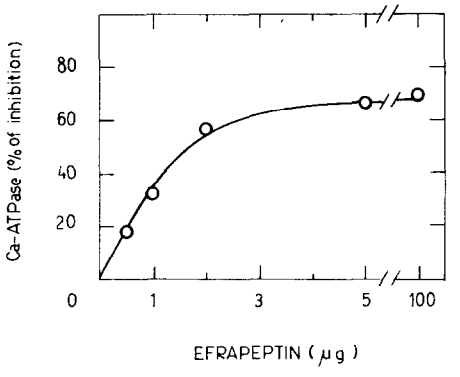

Fig.2. Effect of efrapeptin on the ATPase activity of CF_1. Heat activation of CF_1 and measurement of ATPase activity were carried out as described in the text. Control value was 17.6 μmol$\cdot$min$^{-1}\cdot$mg^{-1}.

while the latter binds to soluble CF_1 and inhibits its ATPase activity.

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