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SYNERGISTIC ACTIVATION OF AN Mg-SPECIFIC ATPase ACTIVITY IN CHLOROPLAST COUPLING FACTOR BY OCTYLGLUCOSIDE AND TENTOXIN

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(1) Octylglucoside stimulates an Mg^{2+}-specific ATPase activity with CF_1 preparations from different higher plants and the alga *Chlamydomonas reinhardii*. (2) Tentoxin at high concentrations (10^{-4}–10^{-3} M) in the presence of octylglucoside further stimulates the Mg^{2+}-ATPase activity of CF_1 from tentoxin-sensitive species and inhibits the activity of CF_1 from tentoxin-resistant species. The extent of tentoxin stimulation and inhibition varies among species. A maximal stimulation of over 2-fold was obtained with spinach CF_1 and a maximal inhibition of 50% was obtained with *C. reinhardii* CF_1. In *Nicotiana* spp., tentoxin had only a marginal effect on the Mg^{2+}-ATPase activity induced by octylglucoside.

Introduction

The interactions of tentoxin with chloroplast coupling factor 1 (CF_1) can be characterized by two different effects: At low concentrations (10^{-9}–10^{-6} M) tentoxin acts as a species-specific energy-transfer inhibitor of photophosphorylation; it blocks the synthesis as well as hydrolysis of ATP by both membrane-bound and purified CF_1 [1,2]. Conversely, high concentrations of tentoxin (10^{-4}–10^{-3} M) stimulate both a Ca^{2+}- and an Mg^{2+}-ATPase activity of CF_1 isolated from tentoxin-sensitive plants [3–5].

We have recently reported that the neutral detergent, octylglucoside (1-*O*-(*n*-octyl)-β-D-glucopyranoside), at concentrations above the critical micellar concentration activates an Mg^{2+}-specific ATPase in solubilized lettuce CF_1 [6]. The striking advantage of the octylglucoside-activation procedure over other activation procedures [7–10] is that the catalytical properties of the octylglucoside-activated enzyme appear to be similar to light-activated, membrane-bound CF_1 with respect to the divalent metal ion specificity [11].

In this paper, we demonstrate that tentoxin and octylglucoside synergistically activate an Mg^{2+}-specific ATPase activity in CF_1 from some tentoxin-sensitive species and that tentoxin inhibits octylglucoside-stimulated Mg^{2+}-ATPase activity in CF_1 from tentoxin-resistant species. These results imply that octylglucoside and tentoxin activate ATP hydrolysis by chloroplast CF_1 via different mechanisms.

Materials and Methods

CF_1 was isolated from spinach leaves by the EDTA extraction procedure [12], from petunia, radish and *Nicotiana* species by the chloroform extraction procedure [13], and from *Chlamydomonas reinhardii* according to the method of Selman-Reimer et al. [14].

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Mg^{2+}-ATPase activity was measured by the release of ^{32}P from [γ-^{32}P]ATP [15]: The reaction was carried out at 37°C for 5 min in a medium containing 30 mM Na-Tricine (pH 8.0), 4 mM ATP, 1 mM $MgCl_2$, 40 mM octylglucoside and 4 μg/ml CF_1 unless otherwise indicated. Trypsin-activated Ca^{2+}-ATPase activity was similarly measured in the presence of 30 mM Na-Tricine, 4 mM [γ-^{32}P]ATP, 8 mM $CaCl_2$ and 25 μg/ml chymotrypsin-free trypsin [9]. Tentoxin was purified from culture filtrates of *Alternaria alternata* [16]. Enzymes and chemicals were obtained from Sigma Chemical Co.

Results

Octylglucoside at concentrations above the critical micellar concentration (10–15 mM [6]) markedly stimulated an Mg^{2+}-specific ATPase activity in CF_1 preparations purified from spinach and from *C. reinhardii* (Fig. 1). The data also demonstrate that 300 μM tentoxin, when added to octylglucoside, caused a further 2-fold stimulation of ATP hydrolysis by spinach CF_1 (a tentoxin-sensitive species) but inhibited the activity of *C. reinhardii* CF_1 (a tentoxin-resistant species) by approx. 50%.

The stimulation of octylglucoside-induced Mg^{2+}-specific ATPase activity of spinach CF_1 by tentoxin required high concentrations (10^{-4}–10^{-3} M) of this cyclic tetrapeptide, whereas tentoxin in the micromolar concentration range slightly inhibited the activity (Fig. 2). A similar dual effect of tentoxin on the trypsin-activated Ca^{2+}-ATPase activity was reported before [3,4]. The comparison demonstrates that even though the extent of the inhibition and stimulation of the two activities by tentoxin are quite different, the apparent affinities for tentoxin in both the inhibition site and the stimulation site are very similar for both the octylglucoside-induced Mg^{2+}-ATPase and trypsin-activated Ca^{2+}-ATPase. Therefore, they probably involve the same tentoxin-binding sites as will be discussed below.

In the absence of octylglucoside, high concentrations of tentoxin also significantly stimulated an Mg^{2+}-ATPase activity of purified spinach CF_1. However, the maximal rate of ATP hydrolysis obtained in the absence of octylglucoside was

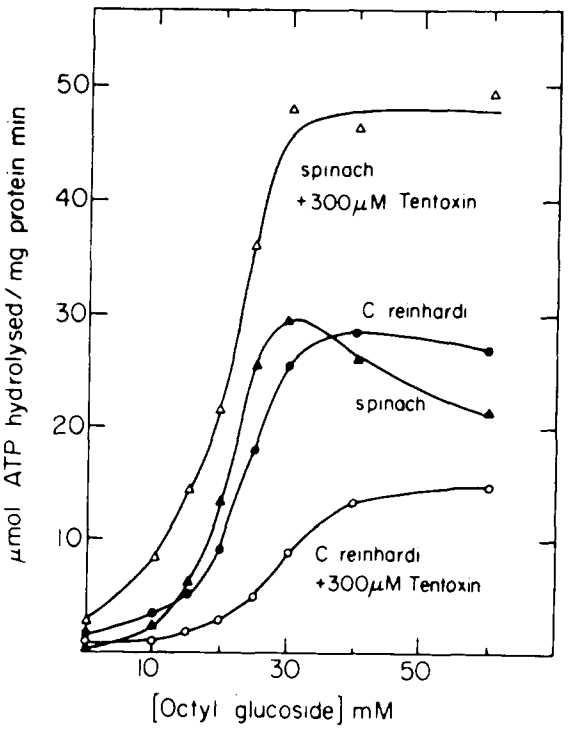

Fig. 1. Octylglucoside activation, in the presence and absence of tentoxin, of Mg^{2+}-specific ATPase with CF_1 from spinach and *C. reinhardii*.. ATPase activity of spinach (▲, △) and of *C. reinhardii* (●, ○) CF_1 preparations was measured in the absence (▲, ●) or presence (△, ○) of 300 μM tentoxin and different octylglucoside concentrations.

only about 10% of the rate in the presence of both tentoxin and octylglucoside. Very low rates of ATP hydrolysis were obtained when Mg^{2+} was replaced by 8 mM Ca^{2+}, indicating that the octylglucoside + tentoxin activation is Mg^{2+} specific (data not shown).

The interactions of tentoxin and octylglucoside with CF_1 preparations from different sources were investigated and are summarized in Table I. The stimulation of Mg^{2+}-ATPase activity by octylglucoside seems to be a general phenomenon observed in all the species tested. The rates of ATP hydrolysis obtained by this activation procedure were at least 2-fold higher than trypsin-activated Ca^{2+}-ATPase activities catalysed by the same CF_1 preparations. Tentoxin (300 μM) in the absence of octylglucoside activated an Mg^{2+}-ATPase activity only in the tentoxin-sensitive species (spinach, petunia, *Nicotiana glutinosa*) but had no significant effect on tentoxin-resistant species (*Nicotiana*

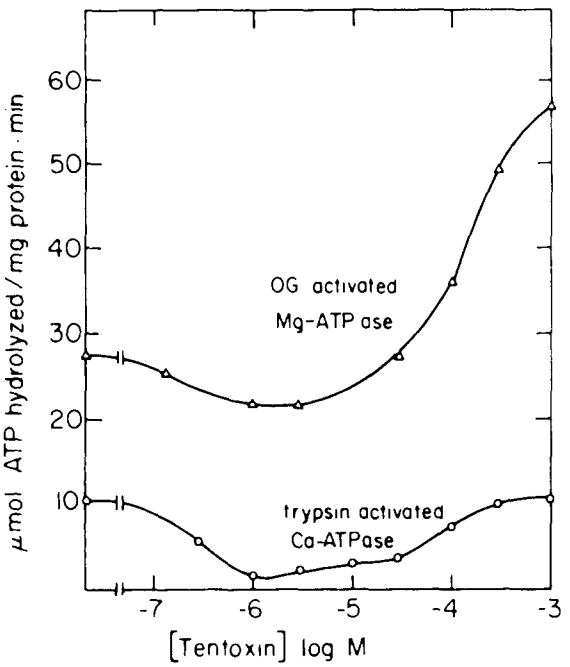

Fig. 2. The dual effect of tentoxin on CF_1-ATPase activity. The rate of ATP hydrolysis by spinach CF_1 was measured after trypsin activation in the presence of tentoxin and 8 mM Ca^{2+} (○) or 40 mM octylglucoside (OG) and 1 mM Mg^{2+} (△) and with the indicated tentoxin concentrations.

tabacum, radish, *C. reinhardii*) in agreement with Conrad et al. [5].

In the presence of 40 mM octylglucoside, tentoxin generally further stimulated the rate of ATP hydrolysis by tentoxin-sensitive species and inhibited this activity in tentoxin-resistant strains. However, the extent of the tentoxin stimulations in addition to the octylglucoside-stimulated rate varied from 150% in spinach CF_1 to less than 10% in *N. glutinosa*. Similarly, the extents of inhibition in tentoxin-resistant species varied from about 10% in *N. tabacum* to about 60% in *C. reinhardii*.

Discussion

The results described in this paper indicate that the mechanism of action of octylglucoside and of high concentrations of tentoxin on CF_1 are different: (i) The activation of spinach CF_1 by octylglucoside and tentoxin is synergistic (Fig. 1, and Table I). (ii) The stimulation of ATPase by tentoxin alone or in combination with octylglucoside is species specific. In contrast, octylglucoside activation is species independent, (Fig. 1 and Table I). (iii) Finally, the observation that similar tentoxin concentrations stimulate ATP hydrolysis in latent

TABLE I

SPECIES SPECIFICITY OF CF_1 ACTIVATION BY OCTYLGLUCOSIDE AND TENTOXIN

ATP hydrolysis was measured either with no additions (second column), or in the presence of 50 μg/ml trypsin (third column), 40 mM octylglucoside (fourth column), 300 μM tentoxin (fifth column) or both octylglucoside and tentoxin (last column). Results are expressed as μmol ATP hydrolysed/mg protein per min. Numbers in brackets in the fourth and fifth columns are the -fold activation of Mg^{2+}-ATPase by octylglucoside and tentoxin, respectively. The numbers in brackets in the last column are the ratio of activity with octylglucoside + tentoxin to that with octylglucoside alone.

Source of CF_1	Control Mg^{2+}-ATPase	Trypsin-activated Ca^{2+}-ATPase	Octylglucoside-activated Mg^{2+}-ATPase	Tentoxin-activated Mg^{2+}-ATPase	Octylglucoside + tentoxin-activated Mg^{2+}-ATPase
Spinach	0.2	8.3	22.0 (110)	4.4 (22)	55.0 (2.5)
Petunia	1.4	6.7	18.2 (13)	7.9 (6)	22.2 (1.2)
N. glutinosa	0.2	–	7.6 (47)	1.8 (11)	8.0 (1.1)
N. tabacum	0.2	4.1	8.4 (42)	0 (0)	7.6 (0.9)
Radish	1.5	5.6	26.4 (18)	1.6 (1.1)	21.1 (0.8)
C. reinhardii	1.3	2.0	28.0 (21)	0.9 (0.7)	12.1 (0.4)

or in activated CF_1, irrespective of the mechanism of activation and of the divalent cation present (Fig. 2 and Refs. 3–5), suggests that octylglucoside and tentoxin probably influence different rate-limiting steps in the catalysis of ATP hydrolysis by CF_1.

In several previous papers it was demonstrated that high tentoxin concentrations which stimulate ATPase activity also modify the interaction of CF_1 with adenine nucleotides (i.e., tentoxin decreases the K_m for ATP and increases the V_{max} in trypsin-activated CF_1 [3,4] and stimulates ADP exchange at the CF_1 adenine nucleotide tight-binding sites [4,17,18]). Conversely, octylglucoside appears to reversibly modify the M^{2+}-binding properties of solubilized CF_1, converting the enzyme from a Ca^{2+}-specific to an Mg^{2+}-specific ATPase [6]. It should be noted, however, that high tentoxin concentrations seem to influence also the interactions of CF_1 with Mg^{2+}. This was indicated by the stimulation of an Mg^{2+}-ATPase activity in tentoxin-sensitive species [5].

Previous investigations have suggested a close interrelation between the low-tentoxin inhibition effect and between the high-tentoxin stimulation effect. This was indicated by the identical species specificity [3,5] and by the opposite effects of high and low tentoxin on adenine nucleotide exchange at the tight-binding sites [4,17–20].

This may suggest either binding of two forms of tentoxin to the same site or alternatively the existence of two different tentoxin-binding sites with different affinities on the enzyme.

At present, we have no direct evidence for either of these mechanisms. The observation that high tentoxin concentrations inhibit trypsin-activated Ca^{2+}-ATPase activity [4] and octylglucoside-activated Mg^{2+}-ATPase activity (Fig. 1 and Table I) in tentoxin-resistant species in the same concentration range which stimulates ATPase activity in tentoxin-sensitive strains [4] seems inconsistent with both mechanisms. Rather, it suggests the existence of a modified tentoxin-binding site in tentoxin-resistant species. The previous suggestion of Shoshan and Selman [18] that tentoxin influences two different interconversions of membrane-bound CF_1 is also in agreement with two tentoxin-binding sites on CF_1.

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