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Inhibition of photophosphorylation by tentoxin, a cyclic tetrapeptide

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SUMMARY

Tentoxin, a fungal toxin which is known to cause seedling chlorosis, was found to inhibit cyclic photophosphorylation but not reversible proton accumulation by isolated chloroplasts. The toxin, at low concentrations, also inhibited coupled electron flow (in the presence of ADP and phosphate) but did not effect basal electron flow (in the absence of ADP and phosphate) or uncoupled electron transport. It is suggested that tentoxin is an energy transfer inhibitor which acts at the terminal steps of ATP synthesis.

A large number of fungal phytopathogens are known to secrete toxic compounds which affect growth of the host plant[1–5]. Some of these compounds are termed pathotoxins since they can induce all of the characteristic symptoms of disease in the absence of the living parasite[5]. Several species of the fungus genus *Alternaria* produce plant toxins when grown in pure culture[6]. One of these fungi is *Alternaria tenuis* Auct. which causes a seedling disease of several plant species which is characterized by the appearance of an irreversible variegated chlorosis. The chlorotic areas which appear are sharply demarcated from green regions of the seedling[6,7]. Culture filtrates of *A. tenuis* grown in artificial media were found to contain a phytotoxic compound which has been identified as a cyclic tetrapeptide: cycloleucyl-*N*-methylalanylglycyl-*N*-methyldehydrophenylalanyl (trivial name, tentoxin)[6,8]. Seedling chlorosis was observed if seeds were soaked or germinated in tentoxin[6]. It has been suggested that tentoxin plays a role in the infection process during plant parasitism by *A. tenuis*.

The mode of action of tentoxin in disrupting cellular metabolism is incompletely understood. Halloin *et al.*[9] have reported that tentoxin does not inhibit protochlorophyll to chlorophyll photoconversion in dark-grown cucumber seedlings although there is a reduction in the rate of chlorophyll synthesis. Since cellular biosynthetic metabolism

involved in chloroplast development must certainly require an energy supply, and since normal grana formation in chloroplasts has been suggested to have a high energy requirement[10], we have begun an investigation of the direct effects of tentoxin on cellular energy coupling reactions.

A. tenuis was grown in still culture as described by Saad *et al.*[11]. Tentoxin was purified from culture filtrates as previously reported by Arntzen *et al.*[12] for *Helminthosporium maydis* pathotoxin. Tentoxin concentration was determined using a molar extinction coefficient of 12 241 at 285 nm[6]. Experimental procedures for assays and for isolation of chloroplasts were as previously described[13].

Preliminary experiments with tentoxin revealed that it caused nearly total inhibition of cyclic photophosphorylation (catalyzed by phenazine methosulfate) at concentrations as low as $4 \cdot 10^{-7}$ M. In contrast, light-induced proton uptake (measured in the presence of ADP and sodium arsenate) was stimulated by the addition of the same concentrations of tentoxin. These results suggested that tentoxin was acting as an energy transfer inhibitor much like Dio-9 or phlorizin[14]. These inhibitory agents are thought to interfere with the terminal steps of ATP synthesis but do not directly regulate electron transport or energy-linked processes such as ion (H^+) transport.

Direct evidence suggesting that tentoxin acts as an energy transfer inhibitor in chloroplasts come from studies of its effect on electron transport. West and Wiskich[15] and McCarty *et al.*[16] have previously demonstrated that chloroplast electron transport is stimulated in the presence of ADP and phosphate (shown in Trace a of Fig. 1). Under these conditions, the energy transfer inhibitor Dio-9 had no effect on the basal rate of electron transport (in the absence of a complete ATP generating system) but did inhibit coupled electron transport (in the presence of ADP and phosphate)[15,16]. The same pattern of inhibition was seen for tentoxin. Addition of tentoxin (final concn of $4 \cdot 10^{-7}$ M) to a

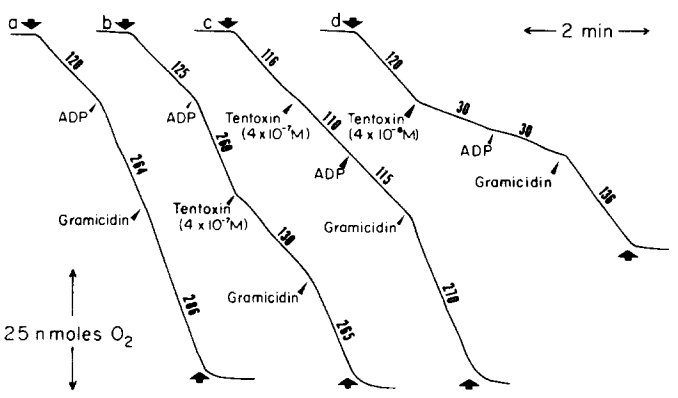

Fig. 1. Light-induced oxygen uptake by lettuce chloroplasts. Reaction mixtures contained the following in μmoles per total volume of 1.5 ml: sodium tricine (pH 8.0), 25; KCl, 25; $MgCl_2$, 5; methyl viologen, 1.25; NaN_3, 1.25; $NaPO_4$, 5; and 31 μg chlorophyll per assay. ADP (4.5 μmoles/assay) was added where indicated. Gramicidin was added to give a final concentration of $1 \cdot 10^{-6}$ M. Large arrows indicate the time the light was turned on (downward arrow) or off (upward arrow). Numbers next to each trace give the rate of methyl viologen reduction in μmoles methyl viologen reduced per mg chlorophyll per h.

phosphorylating reaction mix inhibited electron flow back to the basal level (Trace b, Fig. 1). If tentoxin was added to the basal reaction mixture prior to the addition of ADP, no stimulatory effect of ADP on the rate of electron transport was detected (Trace c, Fig. 1). Addition of the uncoupling agent gramicidin to the reaction mixture either in the presence or absence of $4 \cdot 10^{-7}$ M tentoxin resulted in a large stimulation of the rate of electron flow (Fig. 1). These data indicate that low levels of tentoxin very effectively inhibit the phosphorylation coupling mechanism but have little or no direct influence on electron transport. Higher concentrations of tentoxin (Trace d, Fig. 1) were found to have an inhibitory effect on both basal and coupled electron flow and on uncoupled electron transport. This suggests that at higher concentrations tentoxin has a secondary effect which results in direct inhibition of electron flow.

Purified tentoxin preparations at concentrations as high as $1 \cdot 10^{-5}$ M have been found to have no effect on electron transport or phosphorylation reactions with mitochondria isolated from corn shoots (Koeppe, D., personal communication). Chloroplasts isolated from corn leaves are affected in the same way and over the same concentration range as are the lettuce chloroplasts described above (unpublished observations). Tentoxin may therefore provide a valuable tool for the study of phosphorylating systems in green cells since it may allow for the selective inhibition of light-dependent, but not oxidative phosphorylation.

Earlier reports have demonstrated that tentoxin solutions inhibit chlorophyll accumulation and normal plastid membrane formation in germinating seedlings[6,9]. Since tentoxin was shown above to selectively inhibit the terminal steps in photophosphorylation it can be suggested that ATP synthesis in the plastid is required for normal chloroplast development.

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