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Stigmatellin. A dual type inhibitor of photosynthetic electron transport

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Stigmatellin, an antibiotic produced by the myxobacterium *Stigmatella aurantiaca*, is a powerful inhibitor of photosynthetic electron transport. In the photosynthetic electron-transport chain, it has two different inhibition sites. One is located at the reducing side of Photosystem II (I_{50} value, 52.5 nM) and identical to the inhibition site of inhibitors of the 3-(3',4'-dichlorophenyl)-1,1-dimethylurea type. The second one is located at the cytochrome b_6/f-complex (I_{50} value, 59.0 nM) and corresponds to the inhibition site of 2.5-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone. Stigmatellin is the most potent inhibitor of spinach cytochrome b_6/f complex known so far.

Stigmatellin was recently isolated from cultures of the myxobacterium *Stigmatella aurantiaca* [1,2]. It was shown to inhibit electron flow in the respiratory chain of bovine heart submitochondrial particles at the site of the cytochrome b/c_1-segment [3]. Here we wish to report that stigmatellin is also a powerful inhibitor of photosynthetic electron transport. Stigmatellin inhibits photosynthetic electron transport at two different sites. One is located at the reducing side of Photosystem II, the other one at the cytochrome b_6/f complex. At the latter site, stigmatellin is the most potent inhibitor known so far.

Abbreviations: azido-atrazine, 2-azido-4-(ethylamino)-6-(isopropylamino)-*s*-triazine; Chl, chlorophyll; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone; DCIP, 2,6-dichlorophenolindophenol; DCMU, 3-(3',4'-dichlorophenyl)-1,1-dimethylurea, DNP-INT, 2-iodo-2',4,4'-trinitro-3-methyl-6-isopropyl-diphenylether, Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mes, 2-(*N*-morpholino)ethanesulfonic acid; metribuzin, 4-amino-6-*t*-butyl-3-(methylthio)-1,2,4-triazin-5-(4H)one; UHDBT, 5-*n*-undecyl-6-hydroxy-4,7-dioxobenzothiazole

Chloroplasts from spinach were prepared according to Ref. 4 and spinach cytochrome b_6/f complex according to Ref. 5.

Photosynthetic DCIP-reduction was followed spectrophotometrically at 600 nm in a Zeiss PMQII spectrophotometer, equipped for cross-illumination with actinic light (0.02 $W \cdot cm^{-2}$) and light-dependent methyl viologen catalyzed oxygen consumption in a Clark type oxygen electrode. Electron-transport activity of isolated cytochrome b_6/f complex was measured as plastocyanin catalyzed cytochrome *c* reduction. 2.5-di-*tert*-butyl-1,4-benzohydroquinone [6] served as the donor. The reduction was followed in an Aminco DW-2 at 550 versus 540 nm.

Binding of [^{14}C]metribuzin (specific activity 25.7 mCi/mmol) in the absence and presence of stigmatellin was performed according to method B in Ref. 7.

The inhibition by stigmatellin of photosynthetic DCIP-reduction with water as the electron donor is shown in Fig. 1. 50% inhibition is achieved at a concentration of stigmatellin of 230 nM, which

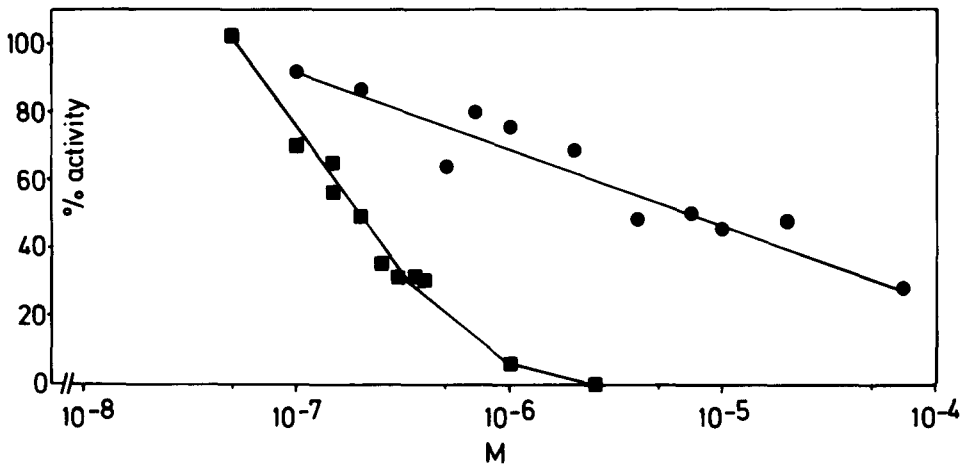

Fig. 1. Inhibition of photosynthetic electron transport in the systems H_2O > DCIP (■- - - -■) and duroquinol > methyl viologen (●- - - -●) by stigmatellin. In the DCIP assay, the reaction mixture contained in a volume of 2 ml 50 mM Hepes (pH 7.0), 5 mM $MgCl_2$, 30 μM DCIP, 10 μg gramicidine, and chloroplasts corresponding to 9 μg Chl (control rate, 117 μmol DCIP reduced per mg Chl/h). In the methyl viologen assay the reaction mixture contained in a volume of 2 ml 33 mM Hepes (pH 7.0), 3.3 mM $MgCl_2$, 0.1 mM methyl viologen, 0.15 mM NaN_3, 4 mM duroquinol, 10 μM DCMU, 10 μg gramicidine, and chloroplasts corresponding to 70 μg Chl (control rate, 370 1 nmol O_2 consumed per mg chl/h)

corresponds to a pI_{50} value of 6.64. Extrapolation of I_{50} and pI_{50} values to zero Chl concentration [8], yields values of 52.5 nM and 7.27, respectively. If, in addition to stigmatellin, 1 μM DNP-INT [9] is present in the medium (same conditions as in Fig. 1), the pI_{50} value slightly drops from 6.64 to 6.36 (data not shown). This indicates that under these conditions almost exclusively Photosystem-II-dependent DCIP-reduction is inhibited.

This notion is further corroborated by a displacement experiment with a 'DCMU-type' Photosystem II inhibitor. As Fig. 2B shows, the radioactively labeled triazinone herbicide [^{14}C]-metribuzin can be displaced by stigmatellin from its binding site; about 50% displacement can be achieved at 0.5 μM stigmatellin. Furthermore, binding of [^{14}C]metribuzin has been determined in the absence and in the presence of various concentrations of stigmatellin. An Eadie-Scatchard plot of the binding data (Fig. 2A) indicates that the binding affinity of metribuzin (as expressed by the binding constant) decreases with increasing stigmatellin concentrations (control: 46.5 nM; 0.25 μM stigmatellin/130 nM; 1 μM/424 nM; 2.5 μM/1.18 μM). Simultaneously, the number of binding sites shows a small increase from 1.93 nmol/mg Chl (control) to 2.92 nmol/mg Chl (25 μM stigmatellin). This represents a typical example of a competitive interaction between both compounds; i.e., metribuzin and stigmatellin compete for an identical binding site in the thylakoid, as do other 'DCMU-type' inhibitors [8]. This binding site has been identified as the 34 kDa herbicide binding protein by the photoaffinity labeling technique [10,11]. In a photoaffinity labeling experiment with [^{14}C]azido-triazinone [12] (2 nmol/mg Chl) a total amount of 292 cpm radioactivity is found in the 34 kDa herbicide binding protein. If 20 nmol stigmatellin are added prior to illumination, this radioactivity is decreased to 145 cpm. This indicates that stigmatellin like other 'DCMU-type' inhibitors binds to the 34 kDa herbicide binding protein.

For 'DCMU-type' inhibitors a structural element $-N-C=X$, where $X=O$ or N, has been recognized to be essential for inhibitory activity [13]. However, this structural element is missing in the benzopyranone structure of stigmatellin [1,2]. In this respect stigmatellin resembles the cyanoacrylate inhibitors [14,15], where the structural element is expanded by a vinyl group.

In addition to its inhibition site at the acceptor side of Photosystem II, stigmatellin has an additional inhibition site in the photosynthetic elec-

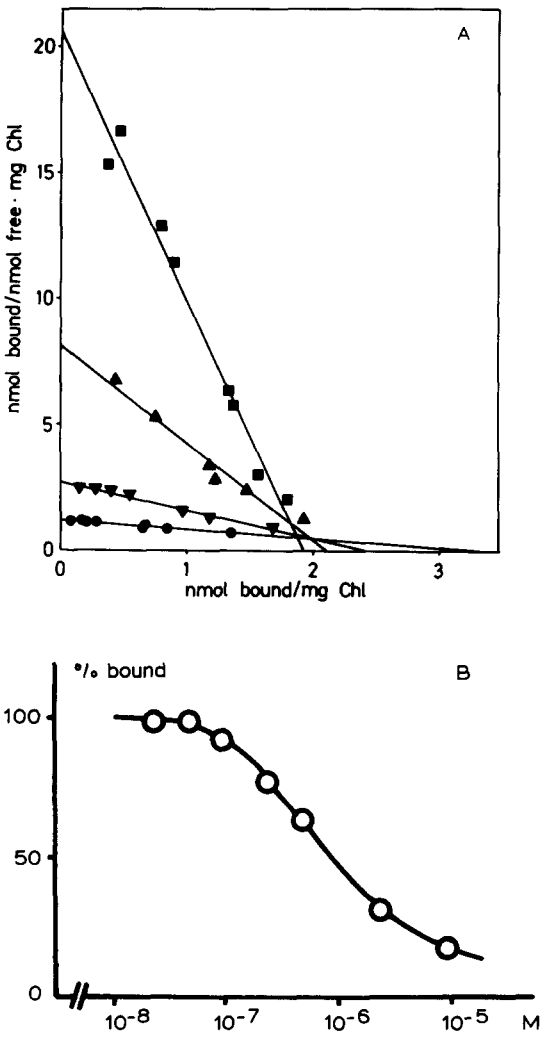

Fig. 2. (A) Eadie-Scatchard plot for displacement of [^{14}C]metribuzin from the thylakoid membrane by stigmatellin ■- - - -■, control; ▲- - - -▲, +0.25 μM; ▼- - - -▼, +0.5 μM; ●- - - -●, +1 μM stigmatellin (B) displacement of [^{14}C]metribuzin (0.1 μM) by stigmatellin The concentration of chloroplasts in all experiments corresponded to 100 μg Chl.

tron-transport chain. This is evident from methyl viologen catalyzed oxygen consumption using duroquinol as the donor system. Duroquinol feeds electrons into the cytochrome b_6/f complex and bypasses the Photosystem II inhibition site [16,17]. This system is inhibited at 3.6 μM stigmatellin (Fig. 1; note the different Chl concentrations for the DCIP and methyl viologen assay).

The inhibitory effect of stigmatellin together with other inhibitors was further investigated in plastocyanin mediated cytochrome *c* reduction in isolated cytochrome b_6/f complex (Fig. 3). Of all inhibitors tested, stigmatellin exhibits the highest pI_{50} value of 7.23. We note that the pI_{50} value of stigmatellin in the isolated cytochrome b_6/f complex is more than one order of magnitude higher as compared to the thylakoid system (compare Fig. 1. with Fig. 3). We have no explanation for this different behaviour. The pI_{50} values for the other inhibitors are: DBMIB, 7.07; UHDBT, 6.46; antimycin, 4.32 (Fig. 3); DNP-INT, 6.50 (data not shown); myxothiazol, 4.30 (data not shown). It is interesting to note that UHDBT, like stigmatellin, in addition to its inhibitory potency on electron transport in the cytochrome b_6/f complex is also an efficient Photosystem II inhibitor [18].

As already mentioned, stigmatellin has been found to block electron flow in the mitochondrial cytochrome b/c_1 complex. Its inhibitory potency was identical with that of antimycin and myxothiazol [3], though their inhibition sites at the cytochrome b/c_1 complex are dissimilar. In the photosynthetic cytochrome b_6/f complex the three inhibitors are quite different: stigmatellin is an excellent, antimycin and myxothiazol are only poor inhibitors.

In photosynthetic electron transport the two inhibition sites at the acceptor side of Photosystem II and the cytochrome b_6/f complex have in common that they are presumably plastoquinone binding sites. At the acceptor side of Photosystem II plastoquinone gets reduced and is reoxidized at the cytochrome b_6/f complex. Consequently, both binding sites must be very similar in their three-dimensional structure because they accomodate an identical molecule. However, there must be also differences inasmuch as the reduction site preferentially should bind the quinone and the oxidation site the quinol.

The same is true for the inhibitors. So far, three different types can be recognized. Type I inhibitors are very efficient at the reduction site and quite unefficient at the oxidation site ('DCMU-type' inhibitors). Type II inhibitors are very efficient at the oxidation site and only poorly active at the reduction site (DBMIB, DNP-INT). Finally, type III is equally active at both sites (UHDBT, stigmatellin).

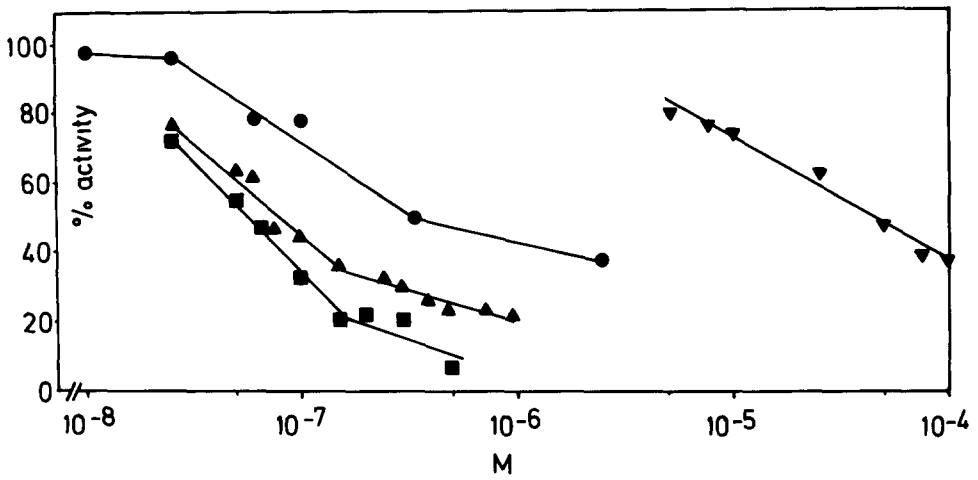

Fig. 3. Inhibition of plastocyanin-oxidoreductase activity in isolated cytochrome b_6/f-complex by stigmatellin (■- - - -■), DBMIB (▲- - - -▲), UHDBT (●- - - -●) and antimycin (▼- - - -▼) The reaction mixture contained in a volume of 1 ml 25 mM Mes (pH 6.5), 10 μM cytochrome *c*, 1 μM plastocyanin, 50 μM 2,5-di-*tert*butyl-1,4-hydroquinone and cytochrome b_6/f-complex corresponding to 50 nM cytochrome *f* The control rates were in the range of 3.4–6.0 μmol cytochrome *c* reduced per nmol cytochrome *f*/h.

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