The Aurachins, Naturally Occurring Inhibitors of Photosynthetic Electron Flow through Photosystem II and the Cytochrome b_6/f-Complex

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Photosystem II, Cytochrome b_6/f-Complex, Cytochrome b/c_1-Complex, Quinoline Antibiotics, *Stigmatella aurantiaca*

A third group of antibiotics, the aurachins, have been isolated from the myxobacterium *Stigmatella aurantiaca*. The aurachins chemically are quinolones, and four of them (aurachins A–D) have been tested for their inhibitory activity in photosystem II and cytochrome b/c-complexes. Aurachin C is the best inhibitor in photosystem II (pI_{50}-value 7.2); its biochemical behaviour being the same like that of other photosystem II herbicides. Both aurachins C and D are also excellent inhibitors in the cytochrome b_6/f-complex (pI_{50}-values of 7.00 and 7.49, respectively). In its mechanism of action, aurachin C resembles antimycin, whereas aurachin D is different from either antimycin or myxothiazol.

Introduction

The myxobacterium *Stigmatella aurantiaca* produces two structurally unrelated antibiotics, a mixture of myxalamids [1, 2] and stigmatellin [3, 4] (for structural formulas, see Fig. 1). Stigmatellin has been recognized as an inhibitor of the electron flow in the respiratory chain of bovine heart submitochondrial particles at the site of the cytochrome b/c_1-complex [5]. There it blocks the ubiquinol oxidation at the Q_o-center by binding to the heme b_{566} domain of the cytochrome b as well as to the Rieske iron-sulfur protein [6]. In addition, stigmatellin inhibits photosynthetic electron flow through photosystem II [7] and, simultaneously, through the cytochrome b_6/f-complex [7], at a site similar to that in the cytochrome b/c_1-segment [8].

More recently, a third group of biologically active compounds, the so-called aurachins (for structures, see Fig. 1) have been isolated from the biomass of *Stigmatella aurantiaca* [9]. Chemically, the aurachins are quinoline derivatives and show close relationship to the well known mitochondrial electron transport inhibitor 2-heptyl-4-hydroxyquinoline *N*-Oxide (HQNO; Fig. 1) [10]. Indeed, the aurachins were shown to inhibit NADH oxidation in submitochondrial particles from beef heart [9, 11].

As we wish to report here, aurachins A–D are also inhibitors of photosynthetic electron transport through photosystem II and the cytochrome b_6/f-complex, though to a different extent. In photosystem II, aurachin C was found to be the most potent inhibitor. The mode of action of the aurachins in photosystem II exhibits close resemblance to the diuron type herbicides. In the isolated cytochrome b_6/f-complex aurachins C and D were the most active ones; the latter one even exceeds the inhibitory activity of stigmatellin, which so far has been recognized as the most potent inhibitor of the Q_z-site in the cytochrome b_6/f-complex [7].

Abbreviations: Chl, chlorophyll; DCIP, dichlorophenolindophenol; DNP-INT, 2-Iodo-2′,4,4′-trinitro-3-methyl-6-isopropyldiphenyl-ether; DQH_2, duroquinol; MV, methylviologen.

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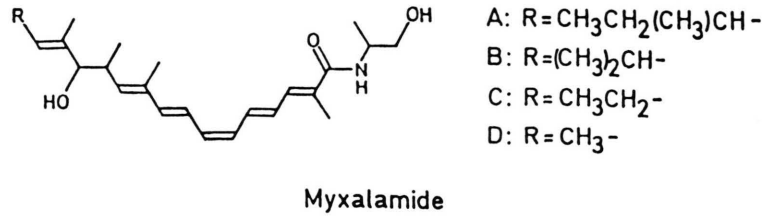
Materials and Methods

Chemicals

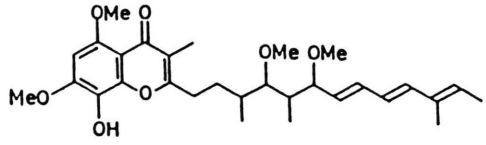
The isolation and purification of aurachins A–D has been described in [9]. The synthesis of the aurachin analogues from Table II will be published elsewhere.

Biochemical methods

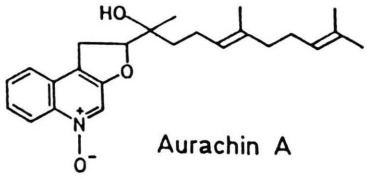
Thylakoids from spinach were prepared according to ref. [12]. Photosynthetic activity in the sys-



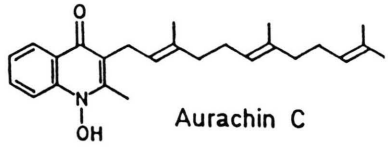
Myxalamide



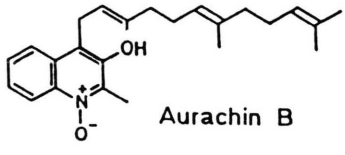
Stigmatellin



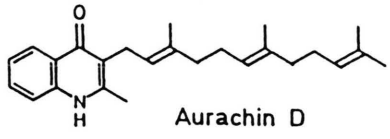
Aurachin A



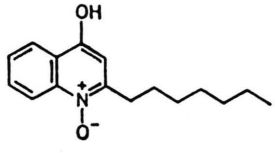
Aurachin C



Aurachin B



Aurachin D



HQNO

Fig. 1. Structural formulas of myxalamides, stigmatellin, aurachins A–D, and HQNO.

tems H_2O > DCIP and DQH_2 > MV was assayed as in [7]. Displacement experiments with [^{14}C]metribuzin (spec. act. 25.7 mCi/mmol; a generous gift by Bayer AG, Pflanzenschutzzentrum Monheim, F.R.G.) and [^{14}C]ioxynil (spec. act. 12.2 mCi/mmol; a generous gift by May & Baker Ltd., Ongar, England) were performed according to a protocol by Tischer and Strotmann [13].

The cytochrome b_6/f-complex from spinach was prepared according to Hurt and Hauska [14] with

some slight modifications. The ammonium sulfate precipitations were performed from 10–35%, 35–45%, and 45–60%. Octyl glucoside was used instead of Triton X-100. Electron transport in the cytochrome b_6/f-complex was measured in a dual wavelength spectrophotometer (Sigma ZWSII, Biochem, Munich, F.R.G.) at 550 *versus* 540 nm with cytochrome *c* (from horse heart) as the terminal electron acceptor. The reaction medium contained 30 mM MES, pH 6.5, 0.4 μM plastocyanin, 10 μM cytochrome *c*, and 50 μM 2,5-di-*tert*. butylbenzohydrochinon as the electron donor. The reaction was started by addition of 20 nM cytochrome b_6/f-complex.

Cytochrome b/c_1-complex from *Rhodospirillum rubrum* was purified according to Ljungdahl *et al.* [15] with slight modifications. Activity was measured as described in Berry and Trumpower [16] using ubiquinol-2 as substrate. Steady state measurements were carried out with the dual wavelength spectrophotometer at 562 *versus* 577 nm. The reaction mixture contained 50 mM MES, pH 6.5, 1 mM $MgSO_4$, 0.1 mg/ml dodecyl-β-D-maltosid and cytochrome b/c_1-complex at a concentration of 3.5 μM cytochrome *b*. The complex was preoxidized by addition of 2 μM ferricyanide. Additions were made to a stirred cuvette during the experiment. Inhibitors were added in 5 μM, ubiquinol-2 in 125 μM, and ferricyanide in 20 μM concentrations. Inhibitors were added in methanolic or ethanolic solution, but the maximum amount of alcohol never exceeded 1%.

Results

The inhibitory activities of the aurachins A–D are summarized in Table I. As judged from the pI_{50}-values in the system $H_2O > DCIP$, which measures only photosystem II inhibition (because of the presence of DNP–INT, an inhibitor of electron transport through the cytochrome b_6/f-complex [17]), aurachins A, B and D are only weak inhibitors. Aurachin C exhibits a pI_{50}-value of 7.20, which is in the same order of magnitude compared to other powerful photosystem II herbicides.

In order to decide whether the aurachins inhibit at a site common to other photosystem II inhibitors, displacement experiments have been performed. Fig. 2 shows a displacement experiment, where thylakoids are incubated with 1 μM [^{14}C]metribuzin and then increasing concentrations of the aurachins A–D are added. Aurachin C competes efficiently with metribuzin for the binding site; at 10 μM concentration almost no residual metribuzin is detected within the membrane. Contrary, to achieve the same effect for aurachins A, B and D concentrations are required which are several orders of magnitude higher as

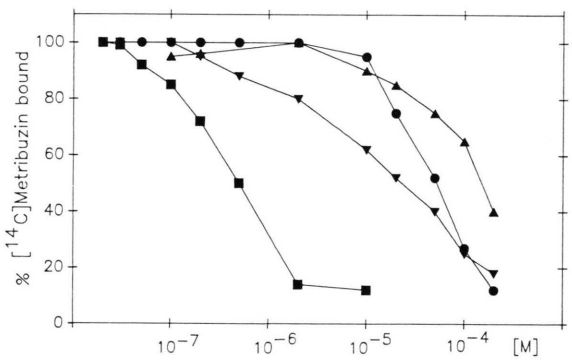


Fig. 2. Displacement of 10^{-6} M [^{14}C]metribuzin by (●——●) aurachin A, (▲——▲) aurachin B, (■——■) aurachin C, and (▼——▼) aurachin D in isolated spinach thylakoids.

Table I. pD_{50}-values for displacement of [^{14}C]metribuzin in thylakoids and pI_{50}-values for inhibition of photosynthetic electron transport through photosystem II and the cytochrome b_6/f-complex.

Aurachin	pD_{50}-value metribuzin	pI_{50}-value $H_2O > DCIP$	pI_{50}-value $DQH_2 > MV$	pI_{50}-value isolated cytochrome b_6/f-complex
A	4.38	4.38	4.10	4.40
B	3.87	3.00	3.42	4.74
C	6.41	7.20	5.52	7.00
D	4.65	4.28	6.12	7.49

compared to aurachin C. This reflects a close relationship between displacement activity and pI_{50}-value. A pD_{50}-value can be defined, which is the −log of the concentration necessary, to displace 50% of the originally bound [^{14}C]metribuzin from the thylakoid membrane. The pD_{50}-values for the four different aurachins are also listed in Table I. It is obvious that there exits a close numerical relationship between pI_{50}- and pD_{50}-value.

The competition behaviour of aurachin C, the most potent inhibitor of the aurachin series, was analyzed in more detail. The Eadie-Scatchard plot of the binding of [^{14}C]metribuzin at various aurachin C concentrations exhibits a common abscissa intercept of the regression lines (Fig. 3). This indicates an identical number of binding sites at different aurachin C concentrations which is considered as a competitive displacement mechanism. The same response is observed in the displacement of the phenolic herbicide [^{14}C]ioxynil by aurachin C (Fig. 4). In this respect, aurachin C behaves like all other photosystem II herbicides of either the diuron or phenol type.

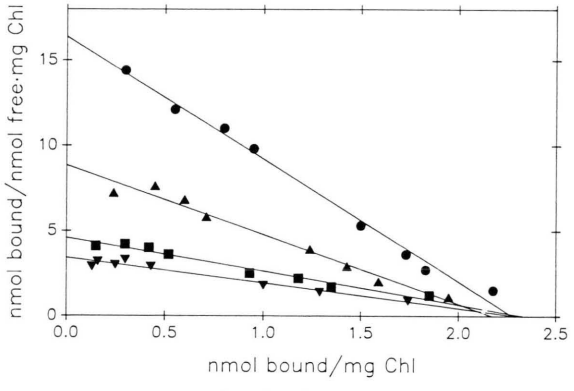


Fig. 3. Eadie-Scatchard plot for binding data of [^{14}C]metribuzin in the presence of aurachin C. (●——●) control, (▲——▲) +0.25 nmol, (■——■) +0.5 nmol, and (▼——▼) +0.75 nmol aurachin C.

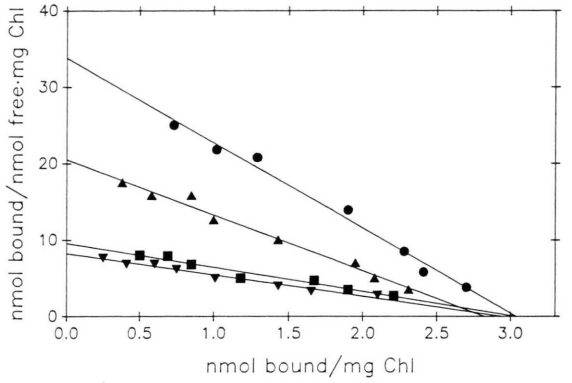


Fig. 4. Eadie-Scatchard plot for binding data of [^{14}C]ioxynil in the presence of aurachin C. (●——●) control, (▲——▲) +0.25 nmol, (■——■) +0.75 nmol, (▼——▼) +1 nmol aurachin C.

In addition to their inhibition site at the acceptor side of photosystem II the aurachins exhibit a second inhibition site at the cytochrome b_6/*f*-complex. This is judged from the inhibition of duroquinol mediated methylviologen reduction (Table I). In this system, duroquinol feeds electrons directly into the cytochrome b_6/*f*-complex and photosystem II is inhibited by diuron [18]. This time, aurachin D is more active than aurachin C. With isolated cytochrome b_6/*f*-complex the activity of the aurachins gets even better (Table I). For aurachin D a pI_{50}-value of 7.49 is found which exceeds the pI_{50}-value of the so far best inhibitor stigmatellin (pI_{50}-value of 7.23 [7]).

In addition to the naturally occurring aurachins A–D, some synthetic analogues have been tested (Table II). They proved to be only weak photosystem II inhibitors, but were considerably more active in the cytochrome b_6/*f*-complex.

Table II. pI_{50}-values of some synthetic aurachin D analogues for electron transport through photosystem II and the cytochrome b_6/*f*-complex.

R^1	R^2	$H_2O > DCIP$	$DQH_2 > MV$
CH_3	*n*-$C_{12}H_{25}$	3.81	5.70
n-$C_{12}H_{25}$	CH_3	3.98	5.60
CH_3	$CH_2CH{=}C(CH_3)_2$	4.53	5.01

R^1 is in the 2-position and R^2 in the 3-position of the quinolone-(4).

In order to decide which quinone binding site is inhibited by the aurachins, *i.e.* the Q_c- or Q_z-site (or Q_i- and Q_o-site, respectively), we have investigated the influence of aurachins C and D on the "oxidant-induced reduction" of cytochrome *b* in the cytochrome b/c_1-complex from the photosynthetic bacterium *Rhodospirillum rubrum*. When in the complex cytochrome c_1 and the iron-sulfur protein are reduced and the Q_i-site is inhibited by antimycin, reduction of cytochrome *b* by ubiquinol is not possible. When cytochrome c_1 and the iron-sulfur protein are oxidized by ferricyanide, cytochrome *b* will be rapidly reduced. This "oxidant-induced reduction" of cytochrome *b* will be inhibited by inhibitors of the Q_o-site, like myxothiazol (for a detailed description of the method and the inhibitors mentioned, see [19]). The influence of aurachins C and D and in combination with antimycin and myxothiazol on oxidant-induced reduction of cytochrome *b* is demonstrated in Fig. 5. Trace a serves as a control where after addition of ubiquinol and the oxidant ferricyanide no extra reduction of cytochrome *b* is observed. Addition of antimycin before ferricyanide induces extra cytochrome *b* reduction (trace b). An identical behaviour is observed for aurachin C (trace c) but not for aurachin D (trace d). In this respect aurachin D behaves like myxothiazol (trace e). Addition of myxothiazol before antimycin abolishes the extra reduction of the cytochrome *b* (trace f). However, the combination of aurachins C and D leads to extra reduction of cytochrome *b* (traces g and h). This result is not fully understood yet. Clearly, aurachin C shows identical properties like antimycin and can be classified as a Group III inhibitor according to [19], blocking ubiquinone reduction at the Q_i-site. Aurachin D has some similarities to myxothiazol, but because of the experimental results as observed in traces g and h, Fig. 5, cannot be classified definitely.

Discussion

The native resident within the Q_B binding niche of the D1/D2 photosystem II reaction center core complex of photosystem II is plastoquinone, a 1,4-

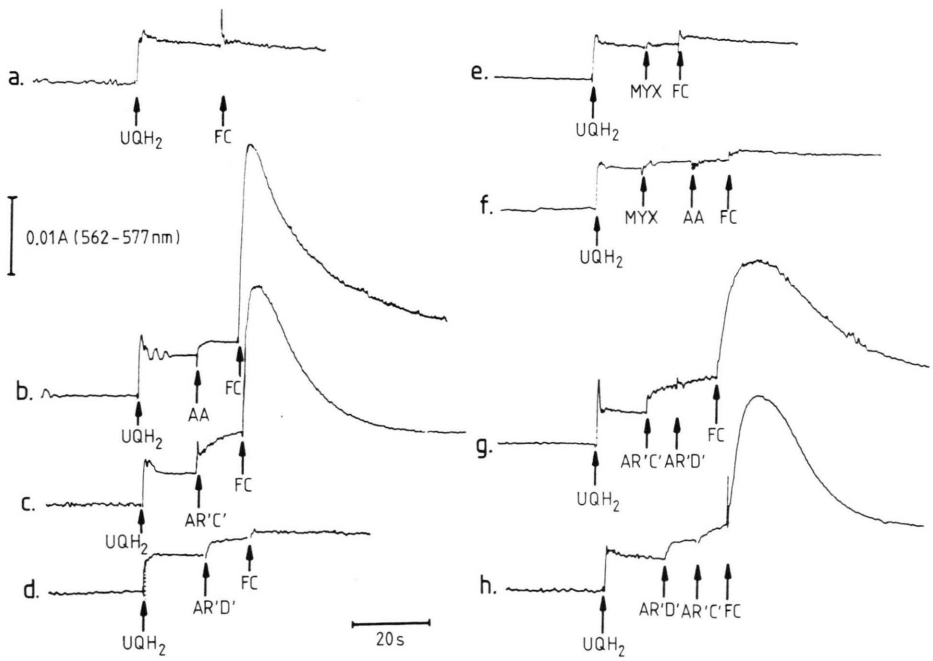


Fig. 5. Oxidant-induced reduction of cytochrome *b* in isolated cytochrome b/c_1-complex from *Rhodospirillum rubrum*. a) Control (UQH_2, ubiquinol; FC, ferricyanide); b) +antimycin (AA); c) +aurachin C (AR'C'); d) +aurachin D (AR'D'); e) +myxothiazol (MYX); f) +myxothiazol, then +antimycin; g) +aurachin C, then +aurachin D; h) +aurachin D, then +aurachin C.

benzoquinone. It is not surprising, therefore, that other 1,4-benzoquinones, especially halogen-substituted ones, can efficiently compete with plastoquinone for the binding site and thus interrupt the photosynthetic electron transport chain [20–22]. Competition is not restricted to 1,4-benzoquinones, 1,4-naphthoquinones [23, 24], and 9,10-anthraquinones [25] are equally effective. Furthermore, one carbonyl group in the 1,4-benzoquinone moiety may be replaced by either an oxygen or a nitrogen bridge, and the resulting pyrones and pyridones also inhibit electron transport through photosystem II [26, 27]. Addition of an aromatic ring to the pyrones and pyridones to give chromones and chinolones, respectively, also leads to active photosystem II inhibitors (stigmatellin [7], and aurachins, this paper). The inhibitory properties in photosystem II of quinolones, containing a trifluoromethyl group, has been reported [28]. The biological activity of compounds of the xanthon and acridon series, were a second aromatic moiety is attached, remains to be established.

The reason for the low activity of aurachins A and B is probably the lack of the carbonyl group. It is more difficult to rationalize the difference between aurachin C and D either in photosystem II and in their different mechanism of inhibition in the cytochrome b_6/f- or the cytochrome b/c_1-complex. Principally, aurachins C and D can exist in two forms, either the quinolone-4 or the tautomeric 4-hydroxyquinoline (this tautomeric form is shown for HQNO in Fig. 1). Though ^{13}C NMR has established the quinolone species to be predominant in organic solvents (G. Höfle, unpublished), it cannot be decided in which form the aurachin is bound within the receptor site. The binding enthalpy for the binding of diuron has been determined to be −50 kJ/mol [29]. A similar value has to be expected for aurachins C and D. This enthalpy will be sufficient to allow for the tautomerization to the hydroxy form within the binding site if suitable hydrogen bridges are formed. This would indicate that the aurachins belong to the phenol type or histidine (215) family [30]. Aurachin C is the *N*-oxide of aurachin D and, consequently, exhibits two negative charges at the oxygens in the 1- and 4-positions. Therefore, for aurachin C two possible orientations within the binding site seem feasible. If aurachin C orients itself within the receptor site in a way that bounding occurs to the oxygen in position 1, the isoprenoidal side chain is in the *meta* position to this oxygen, a situation which is identical for plastoquinone. Contrary, if aurachin D is bound with the oxygen in position 4, the isoprenoidal side chain would be in the *ortho* position, which might lead to steric hindrance. This might account for the differences in biological activities of aurachins C and D in photosystem II. We have no satisfactory explanation yet for the different mechanism of action of aurachins C and D in the cytochrome b/c-complexes.

Acknowledgement

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