Ferredoxin-mediated reduction of 2-nitrothiophene inhibits photosynthesis: Mechanism and herbicidal potential

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Running head: Ferredoxin-mediated reduction of 2-nitrothiophene inhibits photosynthesis

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Abstract
Searching for compounds that inhibit growth of photosynthetic organisms highlighted a prominent effect at micromolar concentrations of the nitroheteroaromatic thioether, 2-nitrothiophene, applied in the light. Since similar effects were reminiscent to those obtained also by radicals produced under excessive illumination or by herbicides, and in light of its redox potential, we suspected that 2-nitrothiophene was reduced by ferredoxin, a major reducing compound in the light. In silico examination using docking and tunneling computing algorithms of the putative interaction between 2-nitrothiophene and cyanobacterial ferredoxin has suggested a site of interaction enabling robust electron transfer from the iron sulfur cluster of ferredoxin to the nitro group of 2-nitrothiophene. ESR and oximetry analyses of cyanobacterial cells (Anabaena PCC7120) treated with 50 μM 2-nitrothiophene under illumination revealed accumulation of oxygen radicals and peroxides. GCMS analysis of 2-nitrothiophene treated-cells identified cytotoxic nitroso and non-toxic amino derivatives. These products of the degradation pathway of 2-nitrothiophene, which initializes with a single electron transfer that forms a short-live anion radical, are then decomposed to nitrate and thiophene, and may be further reduced to a nitroso hydroxyl amine and amino derivatives. This mechanism of toxicity is similar to that of nitroimidazoles (e.g. ornidazole and metronidazole) reduced by ferredoxin in anaerobic bacteria and protozoa, but differs from that of ornidazole in planta.
Introduction

Chemical herbicides (weed killers) are effective, cheap and widely used in agriculture and landscape management for weed control [1, 2]. They disrupt plant growth by mimicking natural plant hormones or by inhibiting key processes in plant life like seed germination or essential metabolic pathways, such as photosynthesis or lipid and amino acid biosynthesis [3-5]. Still, herbicides do not completely obliterate weeds, and their continual use imposes selection pressures that often lead to resistance buildup [2, 4, 6]. In addition, the massive use of hardly degradable herbicides contaminates the environment with accumulation of toxic compounds in soil and water that endanger humans, livestock, and the entire biota [2, 6]. These problems raise a need for novel, safer herbicides with different modes of action, whose alternative use would lift the pressure and omit resistance buildup among weeds [6].

The photosynthetic apparatus is a major target for herbicides that either inhibit its biogenesis or its activity, resulting in plants starvation or stimulation and production of detrimental secondary cytotoxic compounds (e.g. radicals). For example, inhibitors of pigment biosynthesis (e.g. norflurazon) and electron transfer blockers (e.g. diuron and atrazine), or artificial electron acceptors (e.g. paraquat) that drain electrons from photosystem I and ferredoxin [3, 7-9].

Ferredoxins are small soluble iron-sulfur proteins (~100 amino acid residues) that serve as electron carriers of redox reactions in a wide range of organisms. They are classified by the nature of their iron-sulfur clusters and by sequence similarity [10]. In the plant-type ferredoxin, the iron sulfur-cluster is organized as an Fe_2-S_2 tetrahedron coordinated by both inorganic sulfur atoms and sulfurs of 4 conserved cysteine residues [11]. The ferredoxin species involved in photosynthesis resides within the thylakoids in the chloroplast or at their cytoplasmic side in cyanobacteria, and is a key component of the photosynthetic electron transport chain. It accepts electrons from photosystem I and mediates them via its redox center to diverse reactions in the chloroplast stroma of plants or in the cyanobacterial cytosol [11]. Ferredoxin reduces NADP^+ thus forming NADPH molecules required for carbon fixation, and it also supplies electrons for nitrogen and sulfur assimilation and for chlorophyll, fatty acids and hormones biosynthesis [10]. Ferredoxin is a main donor of electrons to the regulatory redox protein thioredoxin [12], and also mediates electrons to O_2 (Mehler reaction) and to some of the cyclic electron transport pathways [13-15]. In algae and cyanobacteria ferredoxin also contributes electrons to hydrogenase leading
to hydrogen production [16-18]. Its key role in the photosynthetic electron transfer chain denotes ferredoxin a target in attempts to arrest plant growth by disruption of their photosynthetic capacity. Still, the only commercial herbicides nowadays that interact with ferredoxin are members of the bipyridinium family, like diquat, paraquat and related compounds [3].

In a search for compounds that upon reduction by ferredoxin would produce toxic derivatives in planta we have previously examined ornidazole, a member of the 5-nitroimidazole drug family used to treat humans and animals infected by protozoa (e.g., *Trichomonas vaginalis*) and anaerobic bacteria [19]. Since becoming active requires preactivation of ornidazole by reduction of the nitro group, we hypothesized that reduction by ferredoxin in the light might produce toxic derivatives that inhibit photosynthesis and kill plants, in a similar mechanism claimed for metronidazole [20-21]. Unexpectedly, rather than attract electrons from ferredoxin and their transfer to molecular oxygen to form devastating radicals and peroxides, ornidazole hardly affected the electron transfer from ferredoxin, but instead abolished photosynthesis by inhibition of two Calvin cycle enzymes, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and triose-phosphate isomerase (TPI) [22]. The different mode of action of nitroimidazoles in planta and in anaerobic bacteria and protozoa implied that they interacted differently with various ferredoxins [22]. This implication is strongly supported by the findings of Vidakovic et al [23], who have shown that the rate of nitroimidazole reduction by cyanobacterial ferredoxin is two orders of magnitude lower than that obtained by the ferredoxin from the protozoan *Trichomonas vaginalis*. Structural modeling of such interactions revealed differences in the association sites and the putative electron transfer pathways from the iron sulfur cluster of ferredoxin to nitroimidazole [22]. Based on these findings we searched for other nitroheteroaromatic molecules that would be efficient electron acceptors from the plant-type ferredoxin. This search raised 2-nitrothiophene as a potential electron mediator from ferredoxin to O₂. Under illumination, 2-nitrothiophene stimulated in cyanobacteria radical and peroxide production, and inhibited photosynthesis and growth. Analysis of the reduction derivatives of 2-nitrothiophene uncovered the mechanism of its activation and degradation.
Methods

Growth and photosynthetic measurements: Cells of *Anabaena* PCC7120 were grown in BG11 medium at 30°C under 10 to 20 μmol photons/m²/sec, as was previously described [24]. The aquatic plant *Lemna minor* was collected from a pond in the Botanical Garden of Tel Aviv University. Oxygen exchange rate in cyanobacteria was determined in the growth media using a Clark-type O₂ electrode (Rank Brothers, UK) as was previously described [22, 24].

Simulation of 2-nitrothiophene interaction with ferredoxin: Putative docking of 2-nitrothiophene and ornidazole at ferredoxin from the cyanobacterium *Synechocystis* PCC6803 was performed using the molecular docking algorithm PatchDock (http://bioinfo3d.cs.tau.ac.il/PatchDock) and the 3D structures of ferredoxin (PDB code 1OFQ), ornidazole and 2-nitrothiophene from the ZINC database (http://www.zinc.dockink.org). The pathways with highest electron transfer rates from the Fe-S cluster of ferredoxin to 2-nitrothiophene were selected by quantum mechanics calculations of the coupling factors and distances of donor-acceptor pairs using the tunneling pathway model of electronic coupling in proteins [25] and ‘The Pathways’ software [26]. All models were drawn using the Chimera software (http://www.cgl.ucsf.edu/chimera/).

Extraction of 2-nitrothiophene and its degradation products: Treated cells of *Anabaena* were disrupted with hot ethanol (60°C), and the extract was centrifuged at 15,000g for 5 min. 2-Nitrothiophen and its putative degradation derivatives were determined in the supernatant as delineated below.

GC-MS Analyses: One μl aliquots of cell extract were injected to Agilent 6890/5977A GC-MS system and chromatographed on Agilent 30 m × 0.25 mm i.d. HP-5MS UI column (5% Phenyl/Methylpolysiloxane, 0.25 μm film thickness) using Helium (99.999%) as a gas carrier. The injector temperature was 250°C and the initial oven temperature was 50°C, held 2 min prior to increase to 300°C at a rate of 20°C/min. MS was performed in the EI positive ion mode. MS data were collected in full-scan mode (m/z 45500) and analyzed with Agilent Chemstation software.
**ESR measurements:** Spectra were recorded on a Bruker ELEXSYS 500 X-band spectrometer equipped with a Bruker ER4102ST resonator in a Wilmad flat cell for aqueous solutions (WG-808-Q) at room temperature. Experimental conditions were, 1024 points, with microwave power of 20 mW, 0.1 mT modulation amplitude and 100 kHz modulation frequency. Sweep range was 10 mT. Accumulation of oxygen radicals was estimated in the presence of the membrane permeable stable spin probe TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl) [27, 28].

**Peroxide measurements:** Determination of hydrogen peroxide liberated into the medium upon 2-nitrothiophen reduction was performed by addition of 0.1 mg/ml catalase (decomposes hydrogen peroxide to water and molecular oxygen) to the medium and monitoring the oxygen level with an oxygen electrode.

**Results**

**Computational search for suitable nitroheteroaromatic molecules**

To identify a compound that is efficiently reduced by plant-type ferredoxin, we analyzed nitroheteroaromatic molecules that show similarity to ornidazole [22], yet with slightly lower redox potential (e.g., nitro derivatives of thiophene, pyrrole and furan). The analysis was primarily based on structural modeling of the putative interaction with ferredoxin using the Docking algorithm that employs the 3-dimensional structure of the molecules involved. Based on the 3-dimensional coordinates of the complexes using the Pathways software, which calculates the coupling factors of electron tunneling\(^1\) and distances of the donor-acceptor pairs [25, 26], the putative electron transfer pathways from the iron sulfur cluster of ferredoxin to the nitro group of nitroheteroaromatic molecules were obtained. By this approach, we found that 2-nitrothiophene, a nitroheteroaromatic compound composed of an intramolecular thioether-containing 5-atom ring (thiophene) and a nitro group at position 2 of the ring, interacts with the cyanobacterial (plant-type) ferredoxin at a site that partially overlaps that of ornidazole (Fig. 1A). However, while two putative pathways with low rate electron transfer, were suggested for ornidazole, (either from the S1 atom of Fe-S via Ser39, Tyr38 and Pro37, or from Fe2 via Cys48 and Thr47;

\(^1\) Coupling is a quantum mechanics parameter reflecting the probability of electron transfer to a higher energetic state (tunneling). The electron transfer rate is proportional to the square of the coupling of the tunneling [29].
(Fig. 1B; ref. 22), a single high rate electron transfer pathway to the nitro group of 2-nitrothiophene only via Fe2, Cys48 and Thr47 was uncovered (Fig. 1B). This rate is comparable to that predicted for ornidazole reduction by trypanosomal ferredoxin (Fig. 1C).

**Product analysis following 2-nitrothiophene reduction**

Reduction of 2-nitrothiophene by ferredoxin may produce the anion radical, nitroso, hydroxyl-amine and amine derivatives depending on the redox potential and pH (Fig. 2; ref. 30). In addition to these derivatives, the nitrothiophene anion radical may be decomposed into thiophene and nitrite radicals (Fig. 2), or stimulate oxygen radical production in a single electron transfer step [31]. The oxygen radical can be converted to hydrogen peroxide by the enzyme superoxide dismutase (Fig. 2). To discern among these predictions, we examined the putative products of 2-nitrothiophene reduction in an extract of illuminated *Anabaena* culture using gas chromatography mass spectrometry (GCMS). As shown in Fig. 3, 2-nitrothiophene, its nitroso and amino derivatives, and thiophene were identified by their mass, whereas two putative intermediates in the reduction pathway of 2-nitrothiophene, the short-live anion radical and hydroxylamine derivatives (Fig. 2), could not be detected. To validate that the radical production was stimulated by 2-nitrothiophene reduction, the ESR spectra of illuminated or darkened *Anabaena* cells were determined in the presence or absence of 2-nitrothiophene, and the stable spin probe TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl) was used to detect oxygen radicals [28]. Whereas in the absence of nitrothiophene, the typical triplet spectrum of nitro anion radical [32] was not detected either in dark- or light-grown cells, a time-dependent increase in the amplitude of this signal was observed in cells held under illumination in the presence of 50 µM 2-nitrothiophene and 1 mM TEMPOL (Fig. 4).

To identify oxygen radicals formed under illumination in the presence of 2-nitrothiophene, a monitoring system was established taking in consideration the fact that *Anabaena* cells lack catalase [33]. Instead, they normally decompose H2O2 by peroxiredoxin [34], which is reduced by thioredoxin. However, in the presence of 2-nitrothiophene that drains electrons from ferredoxin, thioredoxin is not reduced, and thus peroxiredoxin remains oxidized. Peroxiredoxin is also sensitive to H2O2 stimulated by 2-nitrothiophene and so all together this enzyme is most likely inactive. Consequently, the oxygen radicals are converted to hydrogen peroxide by
superoxide dismutase and the non-charged hydrogen peroxide diffuses outward of the cells, and so is amenable to catalase added to the cell suspension.

Since catalase decomposes peroxide to H_2O and O_2, the oxygen concentration can be measured with an oxygen electrode. We have previously shown in this way that ornidazole hardly stimulated radical production in Anabaena [22]. Here however, incubation of the cells in the presence of 100 μM 2-nitrothiophene led to massive production of radicals and peroxides (Fig. 5). Intriguingly, addition of 1 mM ornidazole, which by itself was a poor electron acceptor from cyanobacterial ferredoxin [22, 23], to nitrothiophene-treated cells, inhibited the nitrothiophene-stimulated radical production (Fig. 5). This competitive inhibition of 2-nitrothiophene reduction indicates that ornidazole and 2-nitrothiophene share, at least in part, a common interaction site at the surface of ferredoxin, as was predicted by the structural model (Fig. 1).

Based on these findings we examined the effect of 2-nitrothiophene on photosynthesis and growth of Anabaena. Strikingly, 50 μM 2-nitrothiophene in the cell medium reduced instantaneously the rate of photosynthesis. The effect of the inhibitor on photosynthesis increased along with elevated irradiance (Fig. 6). Similarly, 50 μM 2-nitrothiophene abolished the growth of Anabaena cells, whereas 5 μM decreased the growth rate by 40% (Fig. 7). These results raised the question of whether 2-nitrothiophene would have similar effects on a higher plant, where ferredoxin involved in photosynthesis is very similar to that in cyanobacteria. Indeed, application of 0.5-1 mM 2-nitrothiophene to a culture of the aquatic plant Lemna minor, used here as a convenient higher plant representative, resulted in degradation of the photosynthetic machinery (Fig. 7) and bleaching of the photosynthetic pigments, indicating photo-oxidative damages that eventually caused plant death.

Discussion
Devastating radicals, formed by electron excitation or transfer that create atoms or molecules with unpaired electron in the valence shell [35], are naturally used by many organisms to eradicate invading pathogens as well as in a variety of developmental processes, e.g. during apoptosis [36]; in the immune system [37]; in plant responses to pathogens [38]; and in some medical procedures such as radiotherapy [31]. Inspired by these natural mechanisms we searched
for novel herbicides capable of producing free radicals acting in a similar way to that of 2-nitroimidazoles, such as ornidazole and metronidazole in anaerobic bacteria and protozoa, where they are being reduced by the bacterial-type ferredoxin forming toxic derivatives and radicals that kill the pathogens [19]. Our approach was to identify nitroimidazole-like molecules, which upon reduction in the light by the plant-type ferredoxin (the multi-potent electron carrier in the photosynthetic electron transfer chain of plants, algae and cyanobacteria; refs. 10, 11), would stimulate the formation of cytotoxic radicals. To serve as an herbicide, such a molecule should be permeable, be reduced specifically by the electron carrier, and degrade quickly to decrease toxicity and environmental contamination. The plant-type ferredoxin differs from the bacterial one in sequence (only 21% amino acid residues in *Synechocystis* PCC6803 and *Trichomonas* ferredoxins are identical; ref. 22) and the predicted interaction site of ornidazole at the surface of the bacterial ferredoxin is missing in the photosynthetic ferredoxin [22]. Moreover, the *in vitro* rate 2-nitroimidazoles reduction by the plant-type ferredoxin is extremely low [23]. For these reasons, our first step in identifying efficacious nitroheteroaromatic compounds that interact with plant-type ferredoxin was by computer-based simulations of their putative interactions. Assuming that electron transfer from the iron sulfur cluster of ferredoxin to the nitro group of the nitroheteroaromatic molecule occurs by tunneling, according to the Marcus and Sutin theory [29], the electron transfer rate can be assessed and the site allowing the highest reduction rate may be considered the interaction site. Examination of the putative interaction of ornidazole and 2-nitrothiophene with ferredoxin (Fig. 1; ref. 22) predicted some overlap of the interaction sites, yet ornidazole appeared to be a poor electron acceptor in comparison to 2-nitrothiophen.

Although metronidazole (closely related to ornidazole) was reported as an electron acceptor from plant-type ferredoxin, our simulations as well as the study of Vidakovic *et al* [23] have suggested that 2-nitroimidazoles are actually poor oxidants of the plant-type ferredoxin, and their inhibitory effect *in planta* could result from inhibition of Calvin cycle enzymes [22].

Nitrothiophenes were rarely used in biological systems. They were tested for their anti trypanozomal, bacterial and fungicidal capabilities and as radiosensitisers of cells in hypoxic regions of solid tumors in experimental animals [31]. Following these experiments, the bactericidal and fungicidal activities of nitrothiophenes were attributed to nucleophilic attacks (e.g., by thiols) at the aromatic ring [39]. Among various nitrothiophenes tested, the bactericidal
and fungicidal activities of 2-nitrothiophene were negligible [39], and there is no report on its herbicidal potency. Here we show, however, a substantial inhibitory effect of 2-nitrothiophene on cyanobacterial photosynthesis and growth resulting presumably from radical formation in the light (Figs. 4-7) consequent to its reduction by ferredoxin (Fig. 1). This conclusion is further supported by the competitive inhibition of 2-nitrothiophene action by ornidazole (Fig. 5), as was predicted by the simulation model (Fig. 1).

Because 2-nitrothiophene has not been commonly used as a cytotoxic drug, its mode of action and degradation pathway were not studied. Yet, based on the consensual mechanism of nitroaromatics reduction [19], we postulated that 2-nitrothiophene is reduced upon a single electron transfer, producing a short-live anion radical that might be further reduced to its nitroso, hydroxylamine and amino derivatives depending on the redox conditions in the cell. The 2-nitrothiophene anion radical may in addition be decomposed to thiophene and nitrate or stimulate production of oxygen radicals, while being reoxidized in a futile cycle (Fig. 2). Evidently, not all these derivatives were found in this study (Figs. 3, 4), but thiophene and its nitroso and amino derivatives were detected using GCMS (Fig. 3), and evidence for the production of oxygen radicals was obtained by using ESR (Fig. 4) and monitoring the derived peroxides employing catalase and an oxygen electrode (Fig. 5). Further indirect evidence for oxidative damage induced by 2-nitrothiophene was obtained by the bleaching of the water plant, *Lemna minor*, incubated in its presence (Fig. 7b). A possible reason for the lack of detection of the ESR signal of 2-nitrothiophene anion radical is its short lifetime and quenching by the oxygen produced at the photosynthetic apparatus resulting in very low concentrations. We assume that the other undetectable intermediates probably also existed in very low concentrations. Notably, detection of the ESR signal of the anion radical derivative of nitroimidazoles required a dense anaerobic culture of *Tritrichomonas foetus* in the presence of high drug concentration (8-12 mM) [32].

Overall, the instantaneous inhibition of photosynthesis by 2-nitrothiophene (Fig. 5) results presumably from electrons abstraction from ferredoxin and their transfer to oxygen, thus interfering in the natural electron allocation in the photosynthetic apparatus. The oxidative stress most likely accompanies this arrest and occurs consequent to the radical and peroxide accumulation. This mode of action engaged in 2-nitrothiophene degradation, which prevents environmental pollution, might be advantageous in weed control strategies. Of note is the fact
that despite genotoxicity generally attributed to nitroaromatics [40], Ames tests of nitrothiophenes revealed lack or low mutagenicity [31], and therefore reduced environmental and health risks in their use are anticipated.

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Figure legends

Fig. 1: Simulation of the putative interactions of 2-nitrothiophene or ornidazole with the cyanobacterial ferredoxin. (A) Docking sites of 2-nitrothiophene and ornidazole (in sticks; CPK or grey color, respectively) at ferredoxin (green). Ferredoxin residues involved in electron transfer are in sticks and CPK colors. (B) The most probable electron-transfer pathways from the Fe-S cluster of ferredoxin to the nitro group of the two nitroheteroaromatic compounds. (C) Calculated coupling of the electron transfer pathways from the Fe-S cluster of ferredoxin to ornidazole (pattern) or 2-nitrothiophene (black) and from the trypanozomal ferredoxin to ornidazole (white) (Fig. 1B). Simulations were performed with the molecular docking algorithm PatchDock (http://bioinfo3d.cs.tau.ac.il/PatchDock) using the 3D structures of ferredoxin from the cyanobacterium Synechocystis PCC6803 (PDB code 1OFF) and Trichomonas vaginalis (PDB code 1L5P), and those of 2-nitrothiophene and ornidazole using the ZINC database (http://www.zink.docking.org). Putative electron transfer pathways were identified using 'The Pathways' software to calculate the coupling of residue pairs based on the three-dimensional structure of ferredoxin-nitroheteroaromatic complex [26]. Ferredoxin-nitroimidazole complexes were drawn using the Chimera software (http://www.cgl.ucsf.edu/chimera).

Fig. 2: Putative pathway of 2-nitrothiophene reduction and degradation. Abbreviations: R, thiophene; SOD, superoxide dismutase.

Fig. 3: Identification of 2-nitrothiophene degradation products in Anabaena cells. Filaments of Anabaena were incubated in the presence of 50 μM 2-nitrothiophene under 100 μmol photons x m⁻² x sec⁻¹ for 60 min. 2-Nitrothiophene and its derivatives were extracted using hot ethanol (60°C). The extracts were centrifuged and 1 ml aliquots of the supernatant were analyzed using GCMS.

Fig. 4: ESR spectra of Anabaena cells (500 μg chlorophyll) following incubation in the light or dark in the presence or absence of 0.1 mM 2-nitrothiophene and 1 mM TEMPOL under illumination (250 μmol photons x m⁻² x sec⁻¹) or in the dark. Inset: The time-dependent
amplitude of the ESR signal under illumination defined as the difference in the ESR signal at 3330.4G and 3310.3G. G designates Gauss.

**Fig. 5:** Stimulation of peroxide production by 2-nitrothiophene and ornidazole. The O$_2$ exchange rate in *Anabaena* cells was determined as described in Fig. 4. Onset of illumination and points of the addition of 50 μM 2-nitrothiophene or 1 mM ornidazole and 0.1 mg/ml catalase are designated.

**Fig. 6:** The effect of 2-nitrothiophene on net photosynthesis of *Anabaena* PCC7120. O$_2$ exchange rates of *Anabaena* cells incubated in the presence or the absence of 50 μM 2-nitrothiophene under various irradiance conditions were determined in BG11 growth medium at 30°C using a Clark O$_2$ electrode.

**Fig. 7:** The effect of 2-nitrothiophene on growth of *Anabaena* (A), and on *Lemma minor* (B). *Anabaena* cells were grown in the presence of the indicated concentrations of 2-nitrothiophene in BG11 medium under 100 μmol photons x m$^{-2}$ x sec$^{-1}$. The aquatic plant *Lemma minor* was incubated in the presence of the indicated concentrations of 2-nitrothiophene for 24 hrs.
Fig. 1
Fig. 2

$\text{SOD} \quad \text{O}_2^- \quad \text{H}^+ \quad \frac{1}{2} \text{H}_2\text{O}_2 + \frac{1}{2}\text{O}_2$

$\text{R-NO}_2 \quad \text{e}^- \quad \text{e}^- \quad \text{e}^- \quad \text{R-NHOH} \quad \text{R-NH}_2$

$\text{R-NO}_2^- \quad \text{R-NO} \quad 2\text{R}^- + \text{NO}_2^-$

$\text{2-nitrothiophene}$
| Retention time (min) | Molecular mass (m/e) | Molecule                  |
|---------------------|----------------------|---------------------------|
| 4.4                 | 113                  | 2-nitrosothiophene        |
| 5.89                | 84                   | thiophene                 |
| 5.89                | 99                   | 2-aminothiophene          |
| 5.89                | 129                  | 2-nitrothiophene          |

**Retention time (min)**

- Cells + 2-nitrothiophene: 5.89 min
- Cells: 4.4 min
Fig. 4

First derivative of ESR signal

Symbol 2-NT Illumination Atmosphere TEMPOL

- Light Air +
- Dark Air +
- Light Air +
- Light N₂ -

Magnetic field, G

Δ(3330.4-3310.3 G)

Cells, TEMPOL, 2-NT
Cells, 2-NT
Cells
Fig. 5

- Light
- 0.1 mM 2-nitrothiophene
- 10 µg/ml catalase
- 1 mM ornidazole

µmol O₂ / mg chl
500 sec
Fig. 6

Net O₂ exchange, mmol O₂ x mg⁻¹ chl x hr⁻¹

Irradiance, µmol photons x m⁻² x sec⁻¹

2-Nitrothiphene

Control
Fig. 7

A

2-nitrothiophene, μM

0 0.5 1 5 25 50 100 5000 1000

0 3 6 9 12 15 18

µg chl/ml

Time, hrs

B

0 0.5 mM 1.0 mM

0