Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 173 (2017) 727–732

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Monitoring wheat mitochondrial compositional and respiratory changes using Fourier transform mid-infrared spectroscopy in response to agrochemical treatments

Matthew Pedersen [a], Casey Wegner [b], Piyaporn Phansak [c], Gautam Sarath [d], Roch Gaussoin [a], Vicki Schlegel [e,*]

[a] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE, USA
[b] Zomedic Pharmacceuticals, 3928 Varsity Drive, Ann Arbor, MI 48103, USA
[c] Division of Biology, Faculty of Science, Nakhon Phanom University, Muang, Nakhon Phanom 48000, Thailand
[d] Grain, Forage and Bioenergy Research Unit, USDA-ARS, 251 Filley Hall, East Campus, UNL, Lincoln, NE 68583-0937, USA
[e] Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE, USA

ARTICLE INFO

Article history:
Received 24 May 2016
Received in revised form 25 September 2016
Accepted 16 October 2016
Available online 18 October 2016

Keywords:
Fourier transform mid infrared
Mitochondria respiration
Fungicides
High-throughput

ABSTRACT

Fungicides and plant growth regulators can impact plant growth outside of their effects on fungal pathogens. Although many of these chemicals are inhibitors of mitochondrial oxygen uptake, information remains limited as to whether they are able to modify other mitochondrial constituents. Fourier transform mid-infrared spectroscopy (FT-mIR) offers a high sample throughput method to comparatively and qualitatively evaluate the effects of exogenously added compounds on mitochondrial components. Therefore the objective of this study was to determine the ability of FT-mIR to detect effects mitochondrial fractions isolated from wheat (*Triticum aestivum* L.) seedlings in response to several agrochemical treatments, with an emphasis on fungicides. The accessed need was to develop FT-mIR analytical and statistical routines as an effective approach to differentiate spectra obtained from chemically-treated or untreated mitochondria. An NADH-dependent oxygen uptake approach was initially used as a comparative method to determine whether the fungicides (azoxystrobin, boscalid, cyazofamid, fluazinam, isopyrazam, and pyraclostrobin) and the plant growth regulator, (trinexapac-ethyl) reduced respiration inhibition on isolated mitochondria. Pyraclostrobin was the most effective inhibitor, whereas amisulbrom did not impact oxygen uptake. However, hierarchical clustering of FT-mIR spectra of isolated mitochondria treated with these different compounds separated into clades consistent with each of their expected mode of action. Analysis of the FT-mIR amide protein region indicated that amisulbrom and pyraclostrobin interacted with the isolated wheat mitochondria. Both chemicals were statistically different from the control signifying that respiration was indeed influenced by these treatments. Moreover, the entire FT-mIR region showed differences in various biological bands thereby providing additional information on mitochondria responses to agrochemicals, if so warranted.

Published by Elsevier B.V.

1. Introduction

Agrochemicals, such as fungicides, often have secondary activities in plants outside of their targeted pathogen or primary mode of action. Many fungicides are inhibitors of the highly conserved mitochondria electron transport proteins [1]. Strobilurins, a relatively new class of fungicide, which can target mitochondrial proteins, have been reported to positively impact cereal grain yields [2]. However, the secondary effects of many agrochemicals on plant growth remains limited. Therefore, the objective of this study was to develop a FT-mIR method capable of obtaining biochemical information from chemically treated or untreated mitochondria as a means to classify agrochemicals based on their mode of cellular respiration inhibition.

Mid-Infrared (mIR) spectroscopy is considered a metabolic fingerprinting method that is able to simultaneously monitor carbohydrates, lipids, fatty acids, DNA, RNA, proteins, phosphate containing molecules and polysaccharides of intact cells, cellular organelles and other biological samples [3–14]. For a molecule to absorb IR light of a specific frequency (wavenumber 4000–200 cm^{-1}), it must undergo a net change in dipole moment resulting in bond stretching, rocking and/or bending [15]. Molecules can then be identified based on their mIR fingerprint produced by these vibrations, which typically results in multiple and convoluted, bands representing one molecule.

* Corresponding author at: 1909 N. 21 St., Food Innovation Center, Department of Food Science and Technology, University of Nebraska, Lincoln, USA.
E-mail addresses: pedersenm@huskers.unl.edu (M. Pedersen), cjwegner@umich.edu (C. Wegner), pphansak@npu.ac.th (P. Phansak), Gautam.Sarath@ars.usda.gov (G. Sarath), rgaussoin1@unl.edu (R. Gaussoin), vschlegel3@unl.edu (V. Schlegel).

http://dx.doi.org/10.1016/j.saa.2016.10.025
1386-1425/Published by Elsevier B.V.

Fourier transform mid infrared spectroscopy (FT-mIR) has been used to monitor plant physiological processes [16,17]. One such study showed that this technique was able to detect changes in the redox properties involved in oxidative phosphorylation [18]. Here, the effectiveness of FT-mIR spectroscopy for detecting and classifying respiration inhibitor induced mitochondrial changes was evaluated by performing side-by-side studies with the NADH-dependent oxygen uptake assay. Eight chemicals were selected with a diverse range of target sites (Table 1). These chemicals were selected as they are commonly used as agricultural fungicides with the exception of trinexapac-ethyl, which is a plant growth regulator [1,19]. Based on the results, the FT-mIR approach was not only able to classify changes in mitochondrial function, but also was able to discriminate between agrochemical-treated and untreated samples while providing additional spectral information of other potential biochemical changes.

2. Materials and Methods

2.1. Mitochondria Isolation

Mitochondria were isolated from shoots of 3-day-old etiolated 'Overland' wheat seedlings using a modified Hanson [20] procedure. Grinding media consisted of 350 mM mannitol, 30 mM 3-[*N*-morpholino]propane sulfonic acid (MOPS) and 1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.6. Following pH adjustment, 1.5 g of polyvinylpolypyrrolidone and 0.32 g L-cysteine were added to the grinding media. Centrifuge speeds were 5110*g* for the debris cleaning phase and 19,150*g* for mitochondria collection. Higher centrifugation speeds were used to more efficiently clean and collect mitochondria. The pellet was washed and resuspended with media containing 300 mM mannitol, 20 mM MOPS and 1 mM EDTA, pH 7.2. For the final cleaning phase, the suspension was centrifuged at 5110*g* for 2 min and the pellet was discarded. The final suspension media consisted of 250 mM sucrose and 30 mM MOPS, pH 7.2. Isolated mitochondria intended for the NADH-dependent oxygen uptake assay were immediately stored at −80 °C until analysis. Isolated mitochondria to be used for FT-mIR analysis were diluted using the suspension media to an optical density of ~1.5 absorbance units (AU) at 600 nm to ensure high and consistent signal to noise ratios, and then immediately stored at −80 °C until analysis.

2.2. NADH-dependent Oxygen Uptake

Isolated mitochondria were sonicated to disrupt membranes. Oxygen consumption was measured polarographically by using a Rank Brothers O electrode (Rank Brothers, Ltd., Cambridge, UK) at 25 °C in 1 mL of reaction medium containing 30 mM MOPS adjusted to pH 7.2. The oxygen content of air-saturated water was estimated according to Estabrook [21]. The reaction was initiated with 10 μL of 250 mM NADH. Mitochondria were treated with 1, 2.5, and 5 mM chemical treatments (Table 1) dissolved in solvent (DMSO, DCM or ethanol). Solvent controls were assessed to ensure all inhibition effects were specifically attributable to chemical treatments.

2.3. FT-mIR Spectroscopy (Sample Preparation and Data Collection)

Isolated mitochondria were briefly thawed and divided into 100 μL aliquots. One microliter of 100, 250 or 500 mM inhibitor in solvent (DMSO, DCM, or ethanol) was dissolved in dimethyl sulfoxide (DMSO) and added to the mitochondrial solution to produce final treatment concentrations of 1, 2.5 and 5 mM. Treated mitochondria were lightly vortexed before plating. For FT-mIR analysis, 30 μL aliquots of treated and untreated mitochondrial preparations were pipetted onto a zinc selenide (ZnSe) plate and dried under vacuum (28 Hg) for 45 min at 35 °C. Chemical controls (treatments without mitochondria in MOPS) were also prepared and analyzed for use in subtraction procedures.

Temporal changes in the FT-mIR spectra of treated isolated mitochondria were evaluated against untreated mitochondria. Specifically, a Bruker Equinox 55 FT-MIR equipped with a deuterated triglycine sulfate detector was used to acquire FT-mIR spectra. Samples were applied to a ZnSe BioWheel, which contained 15 sample windows. A spectrum was recorded directly from a dried sample biofilm using the absorbance mode. Each spectrum was recorded in the mid IR range (4000–500 cm^{-1}) at a resolution of 4 cm^{-1} with 128 interferograms co-added, averaged and apodized with the Blackman-Harris 3-Term function and then Fourier-transformed.

Recording of spectra, data storage and all other spectral manipulations were performed using the Bruker Opus 4.0 Software. The spectra were independently and consistently baseline corrected, normalized to the amide I peak and Fourier self-deconvoluted before applying other spectral manipulations. A total of three biological replications with 3 technical replications per biological replication were collected and averaged to determine treatment means. Sample plating techniques followed a randomized complete block design, which were blocked for replicates. Averaged fungicidal control spectra were subtracted from the corresponding averaged mitochondrial treatments in the amide I region to ensure spectral differences reflected treatment-induced conformational shifts. Spectral areas were monitored in the amide I region (~1710–1600 cm^{-1}) and compared to the control through hierarchal cluster analysis (Ward's algorithm) and presented in the form of dendrograms [22].

2.4. Statistical Analysis of FT-mIR Spectral Data

Further analysis of the FT-mIR spectra from amisulbrom, pyraclostrobin and the control groups was completed to determine statistically significant differences in the spectra between treatments.

Table 1
Common and IUPAC name of chemicals used in oxygen uptake experiments, fungicide resistance action used and target site for respiration.

Common name	IUPAC name	FRAC code	Target site
Amisulbrom	3-(3-Bromo-6-fluoro-2-methylindol-1-yl)sulfonyl-*N*,*N*-dimethyl-1,2,4-triazole-1-sulfonamide	C4	Complex III: cytochrome *bc*1 at quinone inside site [1]
Azoxystrobin	Methyl (*E*)-2-[2-[6-(2-cyanophenoxy)pyrimidin-4-yl]oxyphenyl]-3-methoxyprop-2-enoate	C3	Complex III: cytochrome *bc*1 at quinone outside site [1]
Boscalid	2-Chloro-*N*-[2-(4-chlorophenyl)phenyl]pyridine-3-carboxamide	C2	Complex II: succinate-dehydrogenase [1]
Cyazofamid	4-Chloro-2-cyano-*N*,*N*-dimethyl-5-(4-methylphenyl)imidazole-1-sulfonamide	C4	Complex III: cytochrome *bc*1 at quinone inside site [1]
Fluazinam	3-Chloro-*N*-[3-chloro-2,6-dinitro-4-(trifluoromethyl)phenyl]-5-(trifluoromethyl)pyridin-2-amine	C5	Uncoupler of oxidative phosphorylation [1]
Isopyrazam	3-(Difluormethyl)-1-methyl-*N*-[1,2,3,4-tetrahydro-9-(1-methylethyl)-1,4-methanonaphthalen-5-yl]-1H-pyrazol-4-carboxamid	C2	Complex II: succinate-dehydrogenase [1]
Pyraclostrobin	Methyl *N*-[2-[[1-(4-chlorophenyl)pyrazol-3-yl]oxymethyl]phenyl]-*N*-methoxycarbamate	C3	Complex III: cytochrome *bc*1 at quinone outside site [1]
Trinexapac-ethyl	Ethyl 4-[cyclopropyl(hydroxy)methylidene]-3,5-dioxocyclohexane-1-carboxylate		Complex III: cytochrome *bc*1 [19]