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Fatty Acid Oxidation In Isolated Chloroplasts From The Tropical Marine Chlorophyte, *Anadyomene stellata*

by

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ABSTRACT

Fatty Acid Oxidation in Isolated Chloroplasts from the Tropical Marine Chlorophyte, *Anadyomene stellata*

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The marine Chlorophyte, *Anadyomene stellata*, was utilized as a model to study fatty acid oxidation and resultant oxylipin production. Two oxidation reactions were observed. Initially, we identified the presence of five conjugated tetraene containing fatty acids by UV spectrophotometry and GC/MS suggesting fatty acid oxidase activity. Structural analyses of two novel compounds, determined by NMR experiments, led to identification of bosseopentaenoic acid (20:5) and stellaheptaenoic acid (22:7), a previously undescribed 22 carbon fatty acid. Further work pointed to the chloroplast as the subcellular organelle with high enzymatic activity largely responsible for the formation of these unique metabolites. Biosynthesis studies with six fatty acid substrates (palmitoleic, 6,9,12,15-octadecatetraenoic, arachidonic, eicosapentaenoic, 7,10,13,16-docosatetraenoic, and 4,7,10,13,16,19-docosahexaenoic acids) were carried out with the isolated chloroplast preparation, and revealed the capability of all substrates to support conjugated tetraene synthesis. Interestingly, the 22 carbon

substrates were the only substrates able to support the biosynthesis of all five conjugated tetraenes identified. Kinetic analyses with all substrates revealed the enzyme preparation had the highest affinity towards arachidonic acid (20:4), but the greatest V_{max} was observed with 4,7,10,13,16,19-docosahexaenoic acid (22:6).

A semi-purified enzyme preparation was obtained following anion exchange chromatograph and gel filtration that showed high lipoxygenase activity, as measured by an increase in absorbance at 234 nm following the addition of linoleic acid. TLC analyses of the reaction products suggested the presence of 9-HODE based on comparison of R_f values obtained from standards. Furthermore, products from chloroplasts incubated with arachidonic acid were derivatized with 9-anthryldiazomethane (ADAM). These derivatives were analyzed by LC/MS ($APcI^-$), and resulted in the identification of a compound with a molecular weight consistent with that of a dihydroperoxy eicosatrienoic acid. The presence of these oxygenated metabolites are indicative of lipoxygenase catalysis of the substrate.

Endogenous fatty acid concentrations in the chloroplasts were examined by GC/MS to determine the endogenous PUFAs present as potential substrates to these oxidative pathways. Chloroplasts isolated from algae collected in both the Florida Keys and the Mediterranean were analyzed and their resultant fatty acid profiles were compared. Interestingly, all samples contained high levels of 20

carbon PUFAs, comprising approximately 18 – 20% of total fatty acids. This is a unique finding for a Chlorophyte which typically have levels closer to 5% of total fatty acids. Significant differences were noted between algae collected at different sites, which may result from environmental factors.

To examine the relationship between these fatty acid oxidative pathways and their physiological role, arachidonic acid was added to the sea water in a controlled environment while oxygen flux from the thalli were measured. In both light and dark conditions, oxygen flux was significantly reduced suggesting that this fatty acid was able to enter the algal cells and elicit an effect. The order of magnitude of this effect was consistent with the theoretical net uptake of oxygen required for the various fatty acid oxidation reactions described.

This work has addressed a few key factors involved in the elucidation of fatty acid metabolic pathways in the chloroplasts of *A. stellata* resulting in unique oxylipins with apparent physiological significance.

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