In vivo IR mapping of micro-algal cells using synchrotron sources

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Depletion of major nutrients such as phosphorus commonly occurs in natural aquatic ecosystems. [1,2]. Microalgae typically respond to these nutrient perturbations by massive reallocation of carbon fixed by photosynthesis away from protein into molecular pools such as carbohydrates and lipids [3]. This can adversely impact on higher trophic levels in the ecosystems because of changes in the food quality of the producers induced by the nutrient fluctuations [1,2]. Hitherto, these responses have been detected in single algal cells by changes in bands in the IR spectrum, however these techniques have been too crude to ascertain changes in the sub-cellular distribution of molecules [3]. This study explores the possibility of detecting these processes within living algal cells utilising the high brilliance of IR radiation provided by synchrotron sources.

The spatial resolution of a IR microscope coupled to a synchrotron source is diffraction limited to about 5-10 micron and is dependent on the wavelength of the IR photons. Hence, large cells are required to have a good chance of resolving finer differences in spatial distribution of macromolecules within single cells. We have been using cells from the algal family *Desmidae* that are often very large (up to 300 μm in diameter), but thin, making them excellent subjects for intracellular studies employing IR synchrotron microspectroscopy. Cells from a strain of *Micrasterias hardyi* were grown for 3-5 days on either replete nutrient medium or the same medium without phosphorus. The cells were placed between two IR transparent windows that were drilled to allow the flow of nutient medium across the enclosed algal cell. Cells were then continuously supplied with replete nutrient medium for a number of hours. This experiment was repeated using the IR beamlines on synchrotrons in the UK (SRS, Daresbury) and Germany (ANKA, Karlsrhue). Spectra were acquired at 10 μm spacing across cells using a range of aperture sizes on the IR microscopes down to 20 μm. Spectra were recorded every 30 min at a 8 cm^{-1} resolution with 64 or 128 scans co-added. The aims of the experiment were two-fold: to determine whether macromolecular composition varies spatially across the cells and whether this distribution changes temporally after P is re-supplied to the deficient cells.

Preliminary results indicate that in vivo synchrotron IR microspectroscopy is able to detect changes in macromolecular composition in cells that were deprived of P compared with cells grown on replete medium, with a large build up in lipid relative to protein that occurs when cells deprived of P divert carbon to storage products. Second, there were spatial differences detected in the distribution of different molecular classes across the cells. We believe these differences reflect changes in the distribution of cellular components such as cell walls, chloroplasts and nucleus. Third, it appears possible that temporal changes in molecular distribution can be detected following re-supply of P to P-starved *Micrasterias* cells.

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