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Whole Organism ^{31}P Nuclear Magnetic Resonance Spectroscopy: A Potential Application in Aquatic Toxicity Evaluations

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Experiments were undertaken to determine if ^{31}P nuclear magnetic resonance spectroscopy (NMR) could be used to obtain well-defined phosphorus spectra from Chironomus tentans larvae and Daphnia magna. Because phosphorus plays such a basic role in bioenergetics and ATP has been shown to be a sensitive indicator of certain types of toxicants (BREZONIK & PATTERSON 1972; BREZONIK et al. 1975; HOLM-HANSEN 1970), the ability to monitor mobile phosphorus may prove useful in evaluating the effects of toxicants on aquatic organisms.

The major advantage of NMR is that it is non-specific, permitting measurement of all mobile phosphorus metabolites in living organisms. Because NMR is non-invasive investigators will have the opportunity to observe changes in mobile phosphorus compounds through time, on the same organisms, under a variety of environmental conditions. At this time the major disadvantage of NMR, from the standpoint of routine application, appears to be capital and operational costs. In addition, commercially available NMR instruments are limited to 20mm diameter maximum tube size. This limitation determines the size of organisms which can be monitored. It is our intent to investigate NMR as a tool to aid in understanding the relationship between toxicants and aquatic organism bioenergetics.

METHODS

Organisms from laboratory cultures of both species were restricted to the bottom 44mm of a 20mm OD NMR tube using a modified vortex plug with a nylon mesh screen attached to the bottom. A 3mm OD glass tube extending the length of the NMR tube, passing through the center of the vortex plug and screen was used to circulate aerated water (~14 mL/min) into the sample. A variable speed peristaltic pump connected to the 3mm OD tube and a second 3mm OD glass tube positioned near the top of the NMR tube permitted recirculation of ~300 mL of water containing 10% D_2O for locking purposes. All chemical shifts are referenced to an external 85% phosphoric acid sample. Approximately 225 chironomid larvae and several hundred D. magna were used to obtain the spectra reported. We have not at this time determined the minimum biomass necessary to obtain well-defined spectra within reasonable time frames. All spectra reported were recorded without spinning the NMR tube.

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The 60.74 MHz ^{31}P NMR spectra of live aquatic organisms C. tentans larvae and D. magna, are shown in Figure 1.

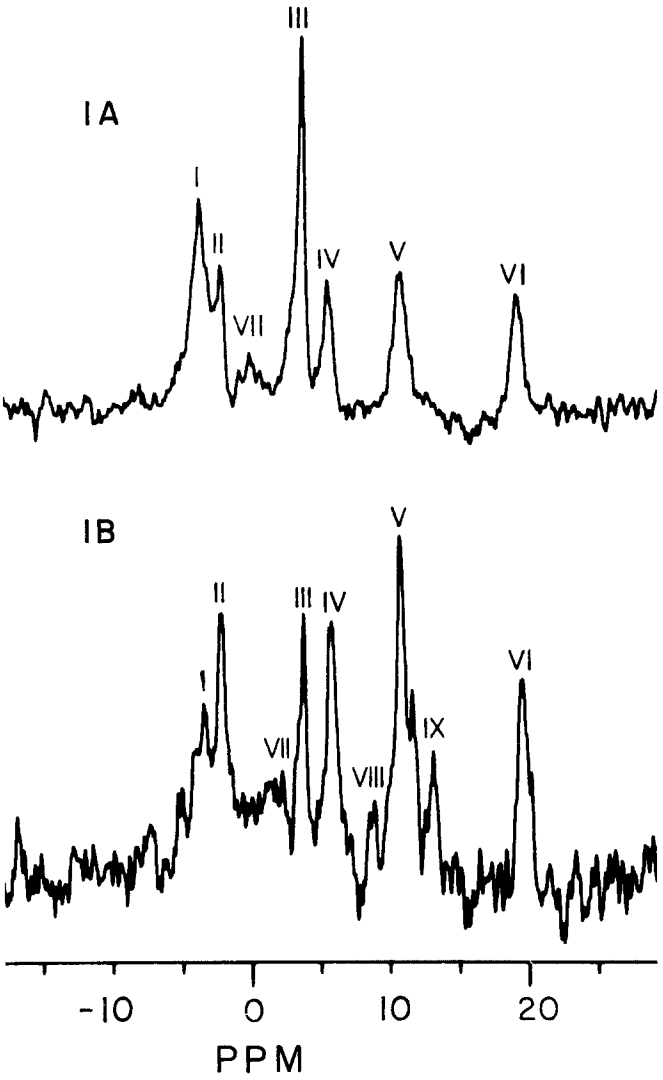

Figure 1. Spectrum 1-A of Chironomus tentans larvae; Spectrum 1-B of Daphnia magna larvae.

Both spectra 1-A and 1-B were recorded under conditions of broad band proton decoupling using a 45 degree pulse and 100 msec delay between pulses.

Spectrum 1-A of C. tentans larvae represents 1000 scans for a total time of 13 min, while spectrum 1-B of D. magna represents 40,000 scans for a total of 8.7 h. Peaks designated I-VI in both spectra display chemical shifts characteristic of sugar

phosphates, inorganic phosphate, phosphoarginine, and the γ, α, and β phosphorus atoms of ATP, respectively (HOULT et al. 1974; BURT et al. 1976; DWEK et al. 1977). These assignments are tentative and require further verification. The peak multiplet labeled VII in spectrum 1-A and 1-B and peaks VIII and IX in 1-B have not been assigned.

One preliminary experiment involving the exposure of C. tentans larvae to increasingly elevated concentrations of cadmium has been carried out (Figure 2). Spectrum 2-A represents a control

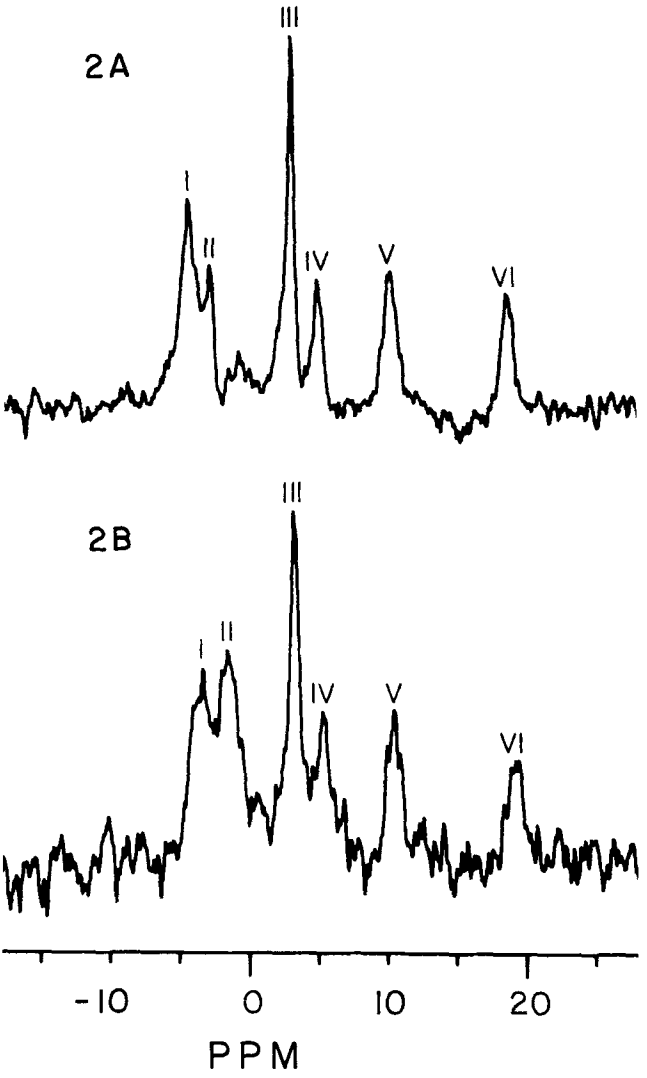

Figure 2. Spectrum 2-A of non-exposed Chironomus tentans larvae. Spectrum 2-B of the same larvae after exposure to increasingly elevated concentrations of cadmium. Peak designations same as in text.

(non-exposed) time period, while spectrum 1-B is of the same larvae after exposure to Cd (12 µg/L for 65 min; 120 µg/L for 104 min; 1.2 mg/L for 226 min; 12 mg/L for 156 min; and 120 mg/L for 117 min). This experiment was undertaken in an attempt to ascertain what types of changes in phosphorus metabolites might be expected to occur upon exposure of organisms to sublethal concentrations of Cd. The most obvious changes include an increased concentration of inorganic phosphates (peak II), upfield shifts in the sugar phosphate (peak I), inorganic phosphate (peak II) and β resonance of ATP (peak VI), and a general broadening of all the resonances with the possible exception of phosphoarginine (peak III).

Upfield shifts most probably reflect a decreased pH (BURT et al. 1976) in the exposed larvae while the line broadening reflects chemical shift nonequivalences in the sugar phosphate, inorganic phosphate, and ATP metabolites induced by the presence of Cd.

DISCUSSION

These spectra clearly demonstrate that satisfactory signal to noise ratios can be obtained using the methodology and species reported. The method permits rapid and nondestructive quantification of each of the phosphorus containing metabolites reported.

The higher signal to noise ratio obtained using the chironomid larvae when compared to D. magna was probably the result of two factors; a higher biomass of chironomids in the NMR tube, and a better distribution of larvae within the rf coil. Both factors are variables that can be manipulated.

Further studies are needed to determine how sensitive the mobile phosphorus components are to low concentrations of toxicants; within what time frame the expression of effects may be expected; and in what manner concentrations shown to cause effects in mobile phosphorus spectra relate to concentrations known to affect parameters likely to limit the success of a species within its environment. We believe the methodology used is adaptable to a wide range of aquatic organisms ranging in complexity from bacteria to fish eggs.

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