POSSIBLE ROLE OF PHOSPHODIESTERS IN COLD TOLERANCE

Marina Marjanovic*, Barbara A. Lawrence*, Nicholas C. Wright*, Jon P. Costanzo[#] and Richard E. Lee, Jr.[#]
***Eastern Illinois University, Charleston, IL and [#] Miami University, Oxford, OH**

Introduction

Ectothermic animals living in the northern regions are well adapted to survive exposure to extreme cold. Several species of insects, amphibians and reptiles are freeze-tolerant. During the freezing process in these animals, ice crystals are forming only in the extracellular environment, while intracellular water remains in the liquid state. Although several mechanisms contributing to this phenomenon have been described, physiological basis for natural freeze tolerance is not completely understood.

This study involved representatives of each of the major taxa for which freeze-tolerance has been described: wood frog (*R. sylvatica*), tree frog (*P. crucifer*), painted turtle (*C. picta*), and goldenrod gall fly (*E. solidignis*). Leopard frog (*R. pipiens*) was used as a control, freeze-intolerant species.

Previous studies showed that some of these species have significant concentrations of cytoplasmic phosphodiesters (PDEs), that are not component of the cellular membranes. Using non-invasive 31P-Nuclear Magnetic Resonance (NMR), we investigated phosphorus metabolites in both isolated tissues and whole bodies of these animals. The goal was to establish biochemical differences that could further explain the cellular mechanisms of freeze-tolerance.

^{31}P-NMR spectroscopy

- Samples were placed in NMR tubes and spectra were obtained with the 300 MHz G.E. QE300 FT-NMR spectrometer.
- The chemical shift and relative areas under the peaks in the spectra were used to identify and determine relative concentration of the metabolites.
- The identity of the compounds was confirmed by "spiking" the extracts of the tissues with a suspected compound and comparing its chemical shift with the chemical shift of peaks in the spectrum.

		total PDEs (% total P)	
Leopard frog		1.6	(3)
Wood frog (warm acclimated)			
	muscle	9.7	(3)
	muscle after *in vitro* freezing	16.1	(2)
Wood frog (cold acclimated)			
	muscle	19.1	(6)
	muscle after *in vitro* freezing	26.6	(3)
	muscle after *in vivo* freezing	36.9	(4)
	whole body	17.8	(2)
	whole body after cooling	19.7	(2)
Tree frog (warm acclimated)			
	whole body	16.4	(2)
Tree frog (cold acclimated)			
	whole body	27.7	(1)
Painted turtle			
	whole body	48.5	(1)
Gall fly larvae (warm acclimated)			
	whole body	0.0	(6)
Gall fly larvae (cold acclimated)			
	whole body	12.4	(6)

Table 1. Levels of total PDEs expressed as percent of the total phosphorus signal. PDE values are averages of number of samples given in parenthesis.

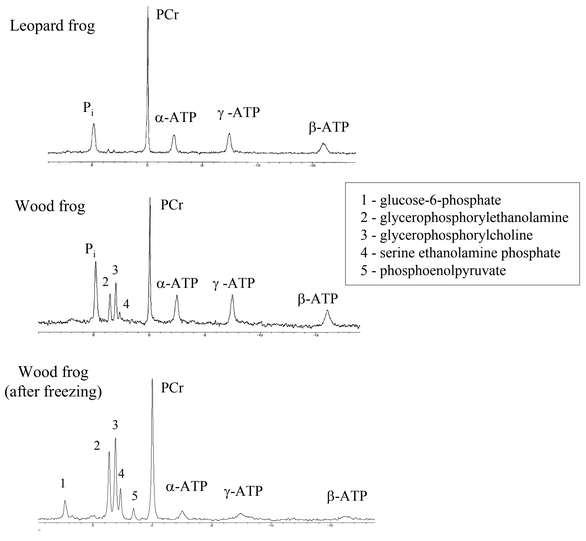

Figure 1. Phosphorus spectra of isolated skeletal muscles (*m. gastocnemii*) from leopard frog and cold-acclimated wood frog at 21ºC. Lower spectrum: wood frogs were frozen for 36-42 h at –3ºC and muscles were dissected without thawing. Numbers indicate chemical shifts of compounds given in the legend.

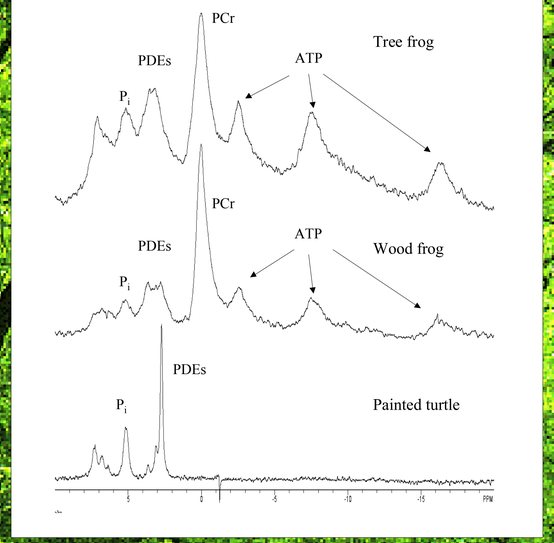

Figure 3. Whole body spectra of cold-acclimated wood frog, tree frog and hatchlings of painted turtle show the presence of cytoplasmic PDEs.

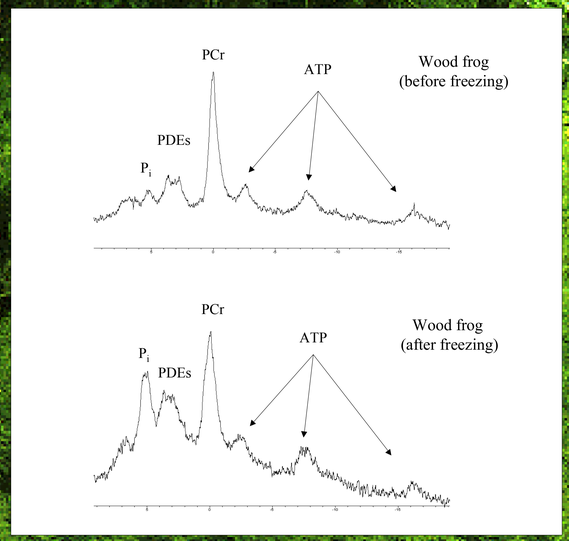

Figure 2. Phosphorus spectra of the wood frog *in vivo*. Upper spectrum: cold-acclimated wood frog. Lower spectrum: wood frog was kept frozen for 36 hours at –3ºC and then thawed at room temperature. Freezing increased the level of cytoplasmic PDEs at the whole body level.

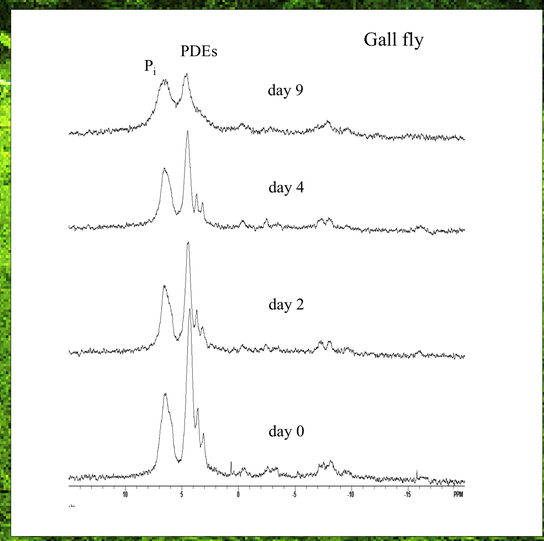

Figure 4. Phosphorus spectra of gall fly larvae after transfer from -4ºC to the environmental chamber at +20ºC for 0, 2, 4 and 9 days. PDEs show significant decrease with warm-acclimation.

Results

- Freeze-tolerant wood frog shows higher level of PDEs in the skeletal muscle compared to freeze-intolerant leopard frog (Figure 1).
- The levels of PDEs (glycerophosphorylethanolamine, glycerophosphorylcholine and serine ethanolamine phosphate) and phosphomonoesters (glucose-6-phosphate and phosphoenolpyruvate) in wood frog skeletal muscle increase with cold acclimation and with freezing (Figure 1).
- Induced freezing in cold-acclimated wood frog also increases the level of PDEs at the whole body level (Figure 2).
- All investigated freeze-tolerant species have similar PDEs (Figure 3 and 4)
- PDE levels decrease significantly in gall fly larvae with warm-acclimation within days (Figure 4).

Conclusions

Based on our findings that cytoplasmic PDEs accumulate in the cytoplasm during cold acclimation, and during freezing, they could have an important role in adaptation of organisms to low temperature.

Possible role of PDEs

- Endogenous inhibitors of lysophospholipase (by decreasing the turnover of membrane phospholipids during dormancy; Burt and Ribolow, 1984, 1994);
- Osmoregulation (non-perturbing osmolytes; Nakanishi and Burg, 1989);
- Precursors of membrane phospholipids;
- Changes in cytoplasmic PDEs may reflect alterations in membrane phospholipids during cold stress (Thebault et al., 1989);

References

- Burt C.T. and Ribolow H. Biochem. Med. 31:20, 1984
- Burt C.T. and Ribolow H. Comp. Biochem. Physiol. 108B: 11. 1994;
- Nakanishi T. and Burg M.B. Am J Physiol 257: C795, 1989
- Van den Thillart G. et al. Am J Physiol 258:R922, 1989

Supported by NSF grant (IOB 0416750)