Proc. Soc. Magn. Reson. Med. **12**, 110 (1993)

Interleave $^{17}O/^{31}P$ MRS: Novel Approach for *In Vivo* Determination of Defects in Oxidative Phosphorylation (Mitochondrial Metabolism)

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Introduction

Defects in oxidative phosphorylation (OXPHOS) caused by respiration inhibitors (uncouplers) or mitochondrial DNA mutants are related to drug and degenerative processes (including late-onset diabetes, ischemic heart, Parkinson's and Alzheimer's disease, aging, etc.). So far, correlation of oxygen consumption with phosphate metabolites in the presence of OXPHOS inhibitors has been performed only ***in vitro***, on isolated mitochondria.[1-3] We report here preliminary results of the first attempt to obtain ***in vivo***, virtually simultaneous oxygen-17 and phosphorus-31 information on normal and perturbed oxidative phosphorylation.

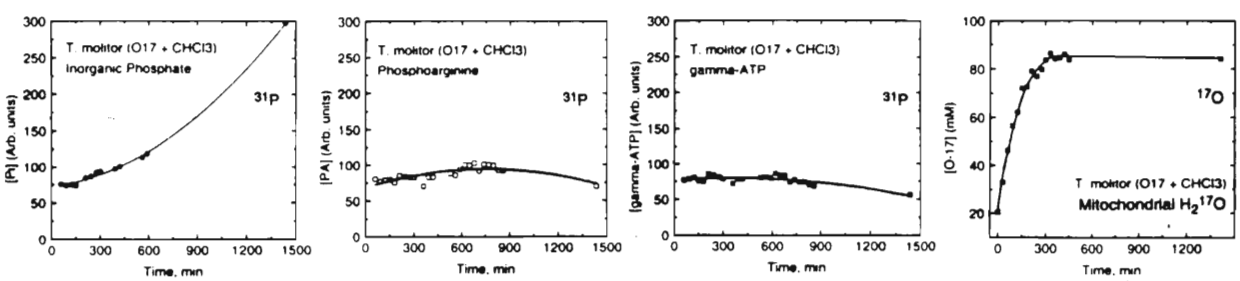
Method

Tenebrio molitor larvae (0.6-0.8 g) are placed in a specially designed dual-plunger, thin wall syringe (10 mm OD) which ensures efficient transfer and minimal losses of $^{17}O_2$ enriched air. It also allows known quantities of $CHCl_3$ to be mixed with the synthetic air. The minirespirator is immediately inserted in a 4.7 Tesla magnet. ^{17}O spectra are first taken at 27.12 MHz (6000 averages with 20 ms acquisition time, i.e., 120 s per spectrum). The probe is then tuned at 81 MHz and a ^{31}P spectrum is taken (1024 averages with 0.1 s acquisition at 0.4 s intervals, i.e., 512 s per spectrum). This sequence is repeated every 30 min.

Results and Discussion

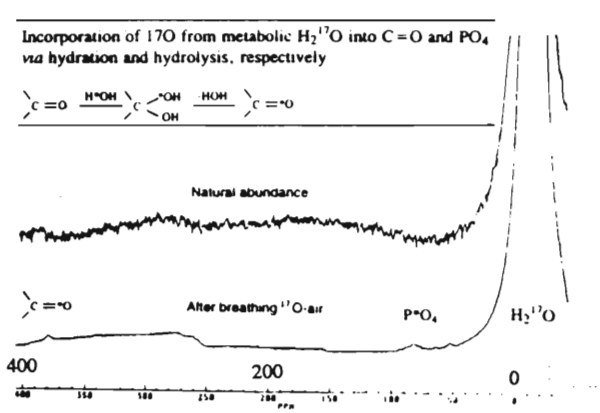
The Figures shown below show the evolution of the ^{31}P peaks of inorganic phosphate, phosphoarginine, and gamma ATP and the ^{17}O peak of the ***nascent mitochondrial water***.[4,5] It is seen that, as indicated by the ^{17}O spectra the larvae's death occurs at approximately 375 min. However, the phosphoarginine peak first shows a slight increase in concentration before following the decreasing trend of ATP. As a result of the uncouplig of the oxidative posphorylation by the added chloroform, there is a significant enhancement of oxygen consumption, as shown be the rate of formation of ^{17}O-metabolic water. In contrast to some vertebrate tissues, the ^{31}P spectra of the *T. molitor* larvae display a wider distribution of sugar phosphates. Deconvolution of the overlapping peaks is necessary in order to obtain quantitative results.

As seen in the secomd Figure, we were able to detect in the ^{17}O spectrum the incorporation of the ^{17}O label into PO_4 and C=O groups *via* hydrolysis and hydration by the metabolic water. The average metabolic rate (MR_{O2}) was 0.2 $\mu mole \cdot g^{-1} \cdot min^{-1}$. We were also able to determine the total body water of the larvae (43±5 %) by means of natural abundance ^{17}O MRS.



Conclusion

Interleave $^{17}O/^{31}P$ MRS is proposed in order to eliminate the effects of metabolism variations due to conditions such as developmental stage, temperature, pressure, humidity and diet. This makes it possible to quantitatively determine the inhibitory or uncoupling effects on OXPHOS of various agents such as anesthetics, drugs and mitochondrial DNA mutagens.[6]



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