PROBING MITOCHONDRIAL DISORDERS BY NMR SPECTROSCOPY

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Introduction

The traditional approach to diagnosing inborn errors of metabolism has relied on: 1) clinical suspicion of a disease phenotype, 2) quantitation of readily measurable, but relatively nonspecific, surrogate disease markers such as the blood lactate concentration, and 3) enzymological and/or molecular genetic techniques to identify the primary defect. However, very little is known of the quantitative or qualitative consequences of such defects on vital processes, such as the flux through the tricarboxylic acid cycle or the overall intracellular energy charge. Our long-term objective is to investigate the biochemical consequences of primary genetic defects of mitochondrial function. Experiments performed in this study are designed to establish the utility of alginate encapsulation to maintain mitochondrial defective human fibroblasts viable for prolonged periods of time.

Experimental

Human fibroblasts obtained from skin biopsy samples of patients with mitochondrial defects were cultured and examined by ^{13}C and ^{31}P NMR spectroscopy following exposure to uniformly labeled ^{13}C-glucose for 20 hours prior to a dual phase extraction. Cells were examined in the presence and absence of dichloroacetate (DCA) treatment. All NMR spectra were acquired at 11.75 T vertical bore Bruker Avance-500 spectrometer located at the AMRIS facility of UF. Isotopomer analysis of the glutamate resonances was used to assess relative fluxes through the TCA cycle.

Results and Discussion

To date we have examined 5 patients with an E1α defect and one patient with an E1β defect. In addition toi these patient samples we have also examined fibroblasts obtained from 5 healthy volunteers. ^{31}P NMR spectra acquired from the aqueous phase of the extract show that the ATP/ADP ratio was statistically reduced in the E1α patient population. Analysis of the ^{1}H decoupled ^{13}C NMR spectra (Figure 1) with a Wilcoxon signed rant test it was also determined that the labeled pyruvate pool increased while the flux through pyruvate carboxylase compared to pyruvate dehydrogenase decreased with DCA treatment.

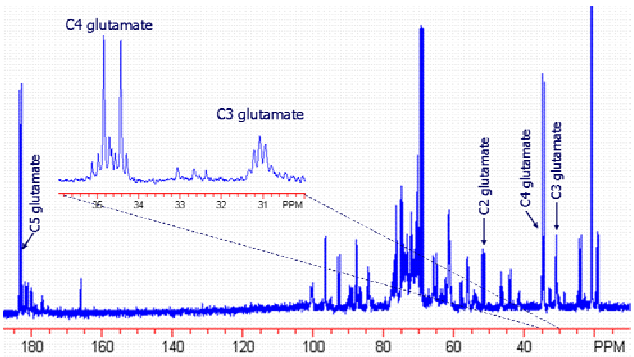

Figure 1: ^{1}H decoupled ^{13}C NMR spectrum of human fibroblasts obtained from a patients with an E1a defective pyruvate dehydrogenase complex.

Conclusions

Our data show that treatment with DCA increased the energy charge of the E1α defective cells and altered TCA cycle flux. Specifically, ^{13}C NMR spectra showed that the activity of pyruvate carboxylase was markedly reduced and that the cells favored the pyruvate dehydrogenase pathway. Thus, NMR spectroscopic techniques applied to cellular extracts of fibroblast cultures is a useful technique to study the biochemical consequences of inborn errors of metabolism.

Acknowledgements

This work was supported by NIH grants DK556890 and DK47858. This financial support is greatly appreciated.