Detection of Pentose Phosphate Pathway Flux Using Hyperpolarized [1-^{13}C]Gluconolactone in Mouse Livers

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Introduction

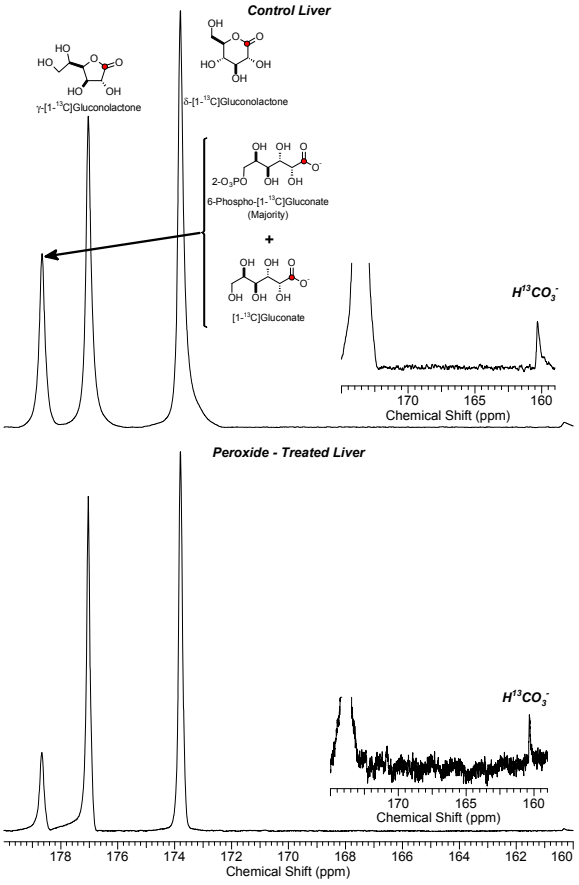
The pentose phosphate pathway (PPP) produces ribose-5-phosphate that may be used for the synthesis of nucleotides. This pathway also generates NADPH as needed for regeneration of reduced glutathione during oxidative stress, and to support fatty acid synthesis. Thus, the PPP has high activity in liver, mammary and adrenal glands, and adipose tissues (1). The PPP is also stimulated in brain trauma (2,3) and it may be increased in cancerous tissues (4). The conversion of glucose-6-phosphate to 6-phosphogluconolactone, the first characteristic intermediate of the PPP, by glucose-6-phosphate dehydrogenase reduces $NADP^+$ to NADPH. This reaction is followed by a second reduction and release of carbon 1 of the lactone as CO_2. Because of the biological and potential clinical importance of measuring flux in the PPP, tracer methods have been developed. Net flux through the PPP is typically assessed by administration of [1,2-^{13}C]glucose and detection of singly-labeled lactate indicating flux through a decarboxylation pathway. The appearance of hyperpolarized $^{13}CO_2$ and $H^{13}CO_3^-$ has been used to detect decarboxylation of hyperpolarized [1-^{13}C]pyruvate. In these studies we examined the use of δ-[1-^{13}C]gluconolactone to detect flux through a single reaction in the PPP by observation of hyperpolarized $H^{13}CO_3^-$. Hydrogen peroxide was used in our studies as an oxidative stress in perfused livers and CCl_4 as a model of hepatic injury *in vivo*.

Methods

D-δ-[1-^{13}C]Gluconolactone was synthesized from the oxidation of D-[1-^{13}C]glucose. Livers were cannulated through the portal vein and excised from anesthetized mice (20 – 25 g) and perfused using a Krebs-Henseleit (KH) medium containing 0.4 mM octanoate only. All mice were fasted overnight. Three groups were examined for PPP flux: 1) Control livers; 2) Acute oxidative injury through hydrogen peroxide perfusion; 3) Sub-acute oxidative injury through CCl_4 adminstration (3 mL/kg) 24 hrs to the NMR study. All livers were perfused by a constant flow (8 mL/min) and received an 8 mL injection of 4 mM HP [1-^{13}C]gluconolactone. The H_2O_2–treated livers were exposed with 10 mM peroxide in KH medium containing 0.4 mM octanoate for 5 min and switched to KHs with octanoate only a few minutes prior to injection of HP [1-^{13}C]gluconolactone.

Results

Four resonances were identified with most experiments: 6-phospho-[1-^{13}C] gluconate and [1-^{13}C]gluconate, γ-[1-^{13}C]gluconolactone, δ-[1-^{13}C]gluconolactone, and $H^{13}CO_3^-$ found at 178.6, 177.0, 173.8 and 160.3 ppm, respectively. γ-[1-^{13}C] Gluconolactone is the result of spontaneous interconversion in the medium with δ-[1-^{13}C]gluconolactone. A control liver produced a significant amount of $H^{13}CO_3^-$ in relation to the peroxide-treated livers. The ratio of gluconate to bicarbonate, taken from peak areas of the respective spectral summations (15 scans), is ~ 29 : 1 for the control liver and ~ 59 : 1 for peroxide-treated livers. 6-Phospho-[1-^{13}C]gluconate or [1-^{13}C]gluconate was observed in CCl_4-treated livers but bicarbonate was not observed even though liver enzymes were dramatically elevated: AST ~ 1800 U/L; ALT ~ 2700 U/L, as expected for CCl_4 injury.



^{13}C NMR Spectra of Control (upper panel) and Peroxide-treated liver (lower panel). Each spectrum is the sum of 15 scans following an injection of hyperpolarized [1-^{13}C]gluconolactone.

Conclusions

Detecting flux through the PPP via δ-[1-^{13}C]gluconolactone is important in understanding oxidative stress in the liver. These studies show that the lactone is incorporated within the hepatocyte, phosphorylated and metabolized through the PPP. Although the amount of $H^{13}CO_3^-$ observed in a peroxide damaged liver was less than that observed in controls, it has been shown that peroxide perfusion inhibits glucose uptake by ~ 50 % due to cell membrane oxidation (5). The lactone is metabolized to 6-Phospho-[1-^{13}C]gluconate or [1-^{13}C]gluconate even in severe acute liver injury due to CCl_4.

References

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