Futile cycling of lactate and protons through the plasma membrane of C6 glioma cells as detected by ^{2}H-^{13}C NMR

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Introduction.

Tumor cells are known to metabolize the glucose provided by the neovascularization preferentially to lactate. Intracellular lactate produced by glycolysis is then extruded to the extracellular space together with a proton using mainly the reversible monocarboxylate transporter MCT1. This process is thought to cause lactate accumulation and acidification of the extracellular space, two well known properties of the tumoral phenotype. However, because of the reversibility of MCT1 it is also possible that accumulated extracellular lactate reenters the tumor cell establishing a futile cycle of lactate and protons through the plasma membrane (Figure 1A). This report addresses futile lactate cycling and its potential consequences in the regulation of extracellular pH in cultures of C6 glioma cells.

Methods.

C6 glioma cells were grown to confluence in DMEM medium ($95\%O_2/5\%CO_2$). On the day of the experiment, DMEM was substituted by KHB (Krebs Henseleit Buffer) containing 50% (v/v) $^{2}H_2O$ and either 5 mM (3-^{13}C) lactate or a mixture of 2.5 mM (1-^{13}C) glucose and 5 mM (U-$^{13}C_3$) lactate. Aliquots of the incubation medium were taken after 0, 3, 6 and 9, 24 and 48h and were analyzed by high resolution ^{13}C NMR in a Bruker AVANCE 500WB spectrometer (125.13 MHz for ^{13}C, pH 7.2, $22^{0}C$) using ^{1}H decoupling only during the acquisition.

Results.

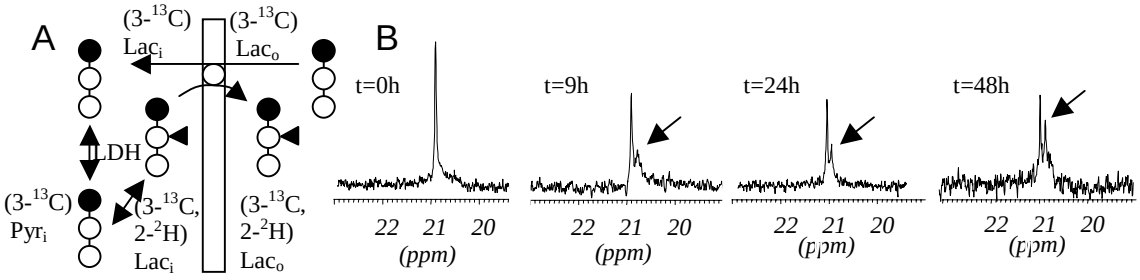

Figure 1. Demonstration of futile lactate cycling through the plasma membrane of C6 glioma cells. A: External (3-^{13}C) lactate enters the cell through MCT1 and becomes deuterated in C2 through the lactate dehydrogenase equilibrium. (3-^{13}C, 2-^{2}H)lactate abandons the cell and accumulates in the extracellular medium. B: (3-^{13}C) and (3-^{13}C, 2-^{2}H) lactate (arrow) are distinguishable by ^{13}C NMR.

Figure 1B shows representative ^{13}C NMR spectra of the lactate C3 region of the incubation medium of C6 cells containing initially (3-^{13}C) lactate in 50% $^{2}H_2O$. At time 0h, only the (3-^{13}C) lactate singlet is observed. However, as the incubation time increases (t>9h) , it is possible to distinguish easily an upfield shifted singlet (-0.1 ppm, arrows) derived from recycled (3-^{13}C,2-^{2}H) lactate molecules. Thus, lactate recycling can be demonstrated because the ^{2}H incorporation in the C2 carbon through the intracellular lactate dehydrogenase equilibrium induces a vicinal isotopic shift, only in those lactate molecules that have passed through the cytosolic space. Indeed, control experiments with 5 mM (3-^{13}C) lactate incubated in 50% $^{2}H_2O$ for the same time periods, but in the absence of cells, did not show any upfield shifted resonance, confirming the intracellular origin of the (3-^{13}C,2-^{2}H) lactate observed. In the incubations with 2.5 mM (1-^{13}C) glucose and 5 mM (U-^{13}C) lactate (not shown), it was possible to distinguish easily between the (3-^{13}C) lactate produced from (1-^{13}C) glucose (singlet at 21.0 ppm), the (U-^{13}C) lactate consumed from the incubation medium (doublet at 21.0 ppm) and the (U-^{13}C, 2-^{2}H) lactate originated from lactate recycling (shifted doublet at 20.9 ppm). Our results reveal also that the C3 resonances of undeuterated and deuterated lactates approach equilibrium with the 50% deuterated solvent, suggesting that essentially all extracellular lactate molecules recycle through the plasma membrane in the time frame of these experiments.

Discussion.

The ^{13}C-^{2}H NMR approach described herein provides a convenient method to measure lactate recycling through the plasma membrane *in vitro* and possibly *in vivo*. Our results indicate that this process occurs to a very large extent. Since lactate recycling produces and consumes the same amount of protons from the extracellular space, it is not expected to influence significantly extracellular pH. However, the present procedure may provide a useful approach to study the relative concentrations of extracellular lactate and recycled lactate *in situ*, an important aspect to understand extracellular pH homeostasis in tumors *in vivo* (García-Martín et al.Cancer Res. 2001, **61**, 6524-6531).