Magnetic Resonance Spectroscopy-based CTP:choline-phosphate Cytidylyltransferase Activity Measurement Technique

C. S. Ward[1], and S. M. Ronen[1]
[1]Radiology and Biomedical Imaging, University of California San Francisco, San Francisco, California, United States

Purpose

CTP:choline-phosphate cytidylyltransferase (CCT) is the enzyme responsible for catalyzing the addition of CTP to phosphocholine to form CDP-choline, which is generally accepted to be the rate-limiting reaction in the formation of phosphatidylcholine (1). Existing *in vitro* assays used to measure cytidylyltransferase activity employ radiolabelled ^{14}C-phosphocholine (PC) and measure product accumulation at endpoints. This study introduces a magnetic resonance spectroscopy-based means to measure the kinetics of cytidylyltransferase-catalyzed reactions and compare levels of active CCT enzyme.

Methods

This assay was based on the method described by Vance *et al.* (1981), with significant modifications. GS-2 glioma cells (~2 x 10^7) were lysed on ice in 500 µL of buffer containing 50 mM HEPES (pH 7.0), 5 mM EDTA, 5 mM EGTA, 1 µL/mL protease inhibitor cocktail (Calbiochem) and 5.5 mM sodium bisulfite. Lysate was homogenized by repeated passage through fine-tipped needle (27G 1/2) and sonication (10 x 1s @ 0°C). Cell lysate was centrifuged at 16,000 rpm for 20 min and the CCT activity of the supernatant fraction was measured immediately after addition of 100 µL reaction mixture. The reaction mixture contained 50 mM Tris-HCl (pH 8.0), 10 mM CTP, 25 mM $MgCl_2$, 5 mM DTT and 5 mM phosphocholine. NMR experiments were performed on a 600-MHz Varian spectrometer equipped with a 5-mm broadband observe probe at 35°C. ^{31}P spectra were acquired using a 30° pulse-and-acquire sequence with proton decoupling, 1 s pre-scan delay (TR = 2.6 s) and 128 transients per FID. Peak areas were determined by integration and were converted to mole values using the known initial concentration of phosphocholine and reaction volume. Values of the two CDP-choline peaks were averaged and plotted to time, after which the rate was determined by linear fit.

Results

Figure 1 shows the full spectra recorded prior to and 1.5 hr after addition of reaction mixture. Figure 2 shows ^{31}P MR spectra as a function of time illustrating the formation of CDP-choline in quantifiable amounts within 1.5 hr. The reaction kinetics were linear over the initial hour, as shown in Figure 3. The rate was determined to be 2.7 nmol/min/10^7 cells (R^2 = 0.90).

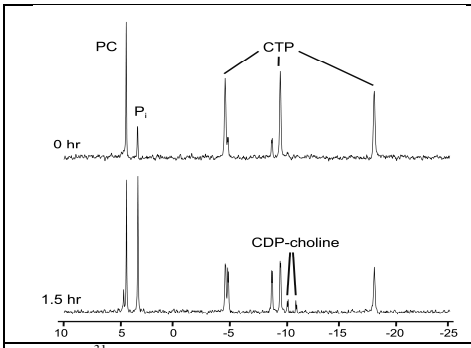

Fig. 1: ^{31}P spectra immediately after addition of reaction mixture and after 1.5 hr. Relevant metabolites are labeled.

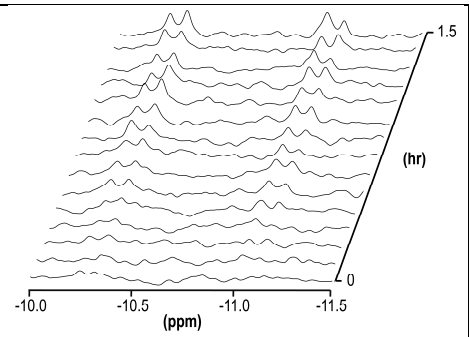

Fig. 2: ^{31}P spectral array of region of interest, showing the buildup of CDP-choline over 1.5 hr.

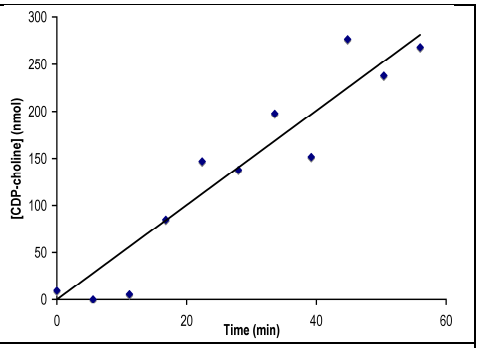

Fig. 3: Plot of normalized peak areas over time. The increase in CDP-choline is evident and a linear fit is applied to determine rate.

Discussion

We have shown that CCT enzymatic activity can be measured using dynamic ^{31}P MR. The results suggest the potential of this technique to quantitatively assess modulations in CCT activity.

References
(1) Sundler and Akesson J Biol Chem 1975, (2) Vance *et al.* Methods Enzymol 1981.

Acknowledgments
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