Rational design of one-phase brain tissue extracts for highly reproducible ^{31}P MRS of phospholipids

N. W. Lutz[1], and P. J. Cozzone[1]

[1]CRMBM UMR CNRS 6612, Faculté de Médecine de la Timone, Université de la Méditerranée, Marseille, France

Introduction

Lipid extracts of brain and other body tissues are being employed to analyze phospholipid (PL) composition by in vitro ^{31}P MRS. Several PL solvents have been proposed to enable identification and quantitation of a large number of PL classes and subclasses by ^{31}P MRS. For two-phase solvent systems, the effects of varying water and methanol content on chemical shift and linewidth (LW) of PL signals have been previously determined [1]. However, little attention has been paid to the influence that other extract components may exert on signal separation. We studied both signal overlap and LW as a function of (i) sample concentration (mg tissue per ml solvent), and (ii) the concentration of chelating agent in the aqueous component of a one-phase solvent system. Measurements were carried out at two different temperatures. The joint effects of these three variables on ^{31}P MRS chemical shift and LW have not been investigated previously in tissue PL extracts, to the best of our knowledge. This study provides essential data for the generation of well-defined one-phase extracts for highly reproducible and well-resolved ^{31}P MR spectra of brain PL, and may serve to guide rational and efficient optimization of PL spectra from other body tissues, cultured cells [2] and PL-containing biofluids.

Method

Brains of Lewis rats were extracted with methanol/chloroform/water (1:1:1). Solvents were removed from the separated lower phase under a N_2 stream. The residue was redissolved in a ternary mixture of deuterochloroform, methanol, and an aqueous CDTA (*trans*-1,2-cyclohexyldiaminetetraacetic acid) solution (5:4:1). The pH of the CDTA solutions was adjusted to 7.1 ± 0.1 (means ± s.d.). CDTA concentrations in the aqueous solvent component varied between 50 and 1000 mM, and sample concentrations varied between 118 and 944 mg/ml (brain wet weight per final solvent volume). 1-D ^{31}P MR spectra were acquired for five hours at 277 or 297 K on an AVANCE 400 spectrometer (Bruker, Wissembourg), using TR=15 s, a 90° flip angle, proton decoupling, and 16K data points. Spectra were referenced to an aqueous 20 mM methylenediphosphonate (MDP) solution as a secondary external standard at 19.39 ppm.

Results

Figures 1 and 2 show ^{31}P MR spectra of brain PL extracts (236 mg/ml, 297 K) with 200 and 1000 mM CDTA, respectively. The spectrum at 1000 mM CDTA shows reduced LW (1.5 Hz for PtdC) compared to that at 200 mM CDTA (2.1 Hz). However, sphingomyelin, SM, and phosphatidylethanolamine plasmalogen, $PtdE_{plasm}$, signals are completely superimposed at 1000 mM CDTA owing to practically identical chemical shifts, while all major signals of this spectral region are reasonably well separated at 200 mM CDTA.

PtdC LW increased with extract concentration, as shown in Figs. 3 and 4 for three different CDTA concentrations and two sample temperatures (correlation coefficients, r, ranged between 0.98 and 1.00). At 297 K (Fig. 4), LW increased to a greater extent and in a less linear fashion than at 277 K (Fig. 3), and were more affected by CDTA concentrations. Smallest LW were achieved for combinations of low sample temperature and high CDTA concentrations, although a CDTA increase from 200 to 1000 mM did not result in a marked change in LW at 277 K. In contrast, one-sided pairwise t tests confirmed that LW was significantly decreased for 200 vs. 50 mM CDTA, at both temperatures ($p < 0.0001$). Similar LW trends were observed for numerous less abundant PL classes and subclasses.

Fig. 1

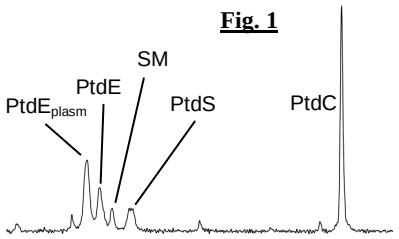
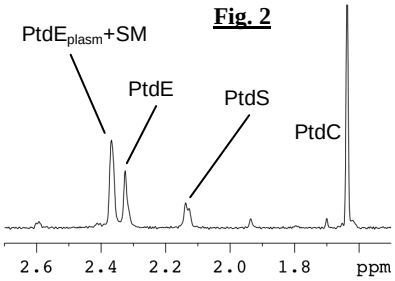


Fig. 2



Discussion

We demonstrated that ^{31}P MRS chemical shifts and relative peak positions of PL vary as a function of extract and CDTA concentration, and that these variations depend on the sample temperature. Therefore, chemical shift can be manipulated to maximize signal separation for reliable quantitation of individual PL groups and subgroups.

LW measurements in brain lipid extracts suggested that minimal ^{31}P MRS LW can be achieved when high CDTA concentrations and low extract concentrations are used for PL analysis, preferably at low sample temperatures. The increase in PL LW with rising extract concentration may be explained by growing concentration of paramagnetic ions at constant CDTA concentration. In addition, interactions of the PL head group with polar solvent molecules and diamagnetic metal ions such as Ca^{2+} influence LW through exchange processes that are both concentration and temperature-dependent. Formation of micelles/vesicles and aggregation of PL with other neutral lipids may also play a role [3], as may head group interactions with other soluble cell compounds, e.g. residual proteins.

It is obvious from our results that the quality of PL analysis by ^{31}P MRS of chloroform/methanol/water extracts is strongly influenced by the concentrations of the extract itself and of the chelating agent, due to *predictable* LW and chemical-shift effects. Judicious adjustment of these experimental parameters can be employed as an optimization strategy for extracts of brain tissue and other tissue types.

Fig. 3

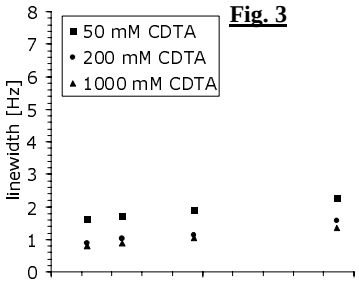
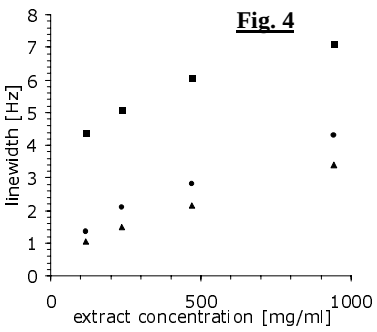


Fig. 4



References

1. Edzes HT et al. (1992) *Magn Reson Med Rev* 16: 307-315. 2. Lutz NW et al. (2002) *NMR Biomed* 15: 356-366. 3. Gorenstein DG, ed. (1984) Phosphorus-31 NMR, Academic Press, Orlando.