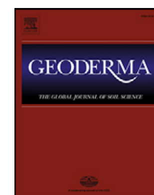
Geoderma 257–258 (2015) 102–114

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma



Improved peak identification in ^{31}P-NMR spectra of environmental samples with a standardized method and peak library

Barbara J. Cade-Menun

Agriculture and Agri-Food Canada, Semiarid Prairie Agricultural Research Centre, Box 1030, Gate 4, Airport Road, Swift Current, SK S9H 3X2, Canada

A R T I C L E I N F O

Article history:
Received 30 June 2014
Received in revised form 24 September 2014
Accepted 23 December 2014
Available online 8 January 2015

Keywords:
P-NMR
Chemical shifts
Peak identification
Standardization

A B S T R A C T

The technique of ^{31}P-NMR spectroscopy has done more to advance the knowledge of organic P forms in environmental samples than any other method. Early ^{31}P-NMR work limited identification to peaks that were clearly separated, such as orthophosphate and pyrophosphate, grouping the remaining peaks into broad categories such as orthophosphate monoesters and orthophosphate diesters. Advances in ^{31}P-NMR methodology for environmental samples now produce clearer spectra, providing the potential to identify more peaks. However, there is at present no standard method for peak identification, and no library of chemical shifts of P forms analyzed under standardized, easily replicated conditions. Various research groups have conducted spiking experiments and have developed their own peak libraries. However, because the chemical shifts of P forms are affected by sample conditions such as pH and salt concentration, it can be difficult to use the work of one lab group to identify P forms in samples analyzed by another lab group under different conditions. For this paper, more than 50 P compounds were analyzed under standardized conditions that can easily be repeated by other research groups. These compounds include phosphonates, orthophosphate monoesters, orthophosphate diesters, polyphosphates, pyrophosphate, and a compound with an N–P bond. The chemical shifts of P forms analyzed for this P compound library were compared to those identified elsewhere, if available. In addition, recommendations are given for standardized spiking experiments to improve peak identification.

Crown Copyright © 2014 Published by Elsevier B.V. All rights reserved.

1. Introduction

The technique of ^{31}P-NMR spectroscopy has done more to advance the knowledge of phosphorus (P) cycling, particularly of organic P forms, in environmental samples than any other method. Since its first use on soil samples (Newman and Tate, 1980), advances have been made to refine the technique, including optimizing extraction procedures and experimental parameters (e.g., Cade-Menun and Liu, 2014). One result of these refinements is spectra with increased signal-to-noise and reduced line-broadening; in these spectra, peaks are sharper and overlapping is minimized, providing the potential to identify more peaks, especially in the orthophosphate monoester region. However, there is at present no standard method for peak identification, and no standardized library of chemical shifts of P forms.

In the first published study using ^{31}P-NMR to characterize P forms in soil extracts, Newman and Tate (1980) used a combination of techniques to identify P compounds in their spectra of soil samples prepared by extracting soils with 0.5 M aqueous NaOH and then concentrating the extracts to 5–7 mL by rotary evaporation. Orthophosphate, pyrophosphate, and polyphosphate were identified in ^{31}P-NMR spectra by spiking experiments, whereby spectra were obtained after $NaH_2PO_4 \cdot 2H_2O$, $Na_4P_2O_7 \cdot 10H_2O$ and $(KPO_3)_n$ were added directly to previously analyzed samples, and then the spectra with and without added compounds were compared to look for changes to peaks. Newman and Tate (1980) also prepared model compounds dissolved in 1 M aqueous NaOH plus D_2O, which were analyzed separately from soil samples. These model compounds were used to identify peaks for *myo*-inositol hexaphosphate (*myo*-IHP, phytate), choline P, and 2-aminotheylphosphonate. Finally, Newman and Tate (1980) identified other peaks as alkyl phosphonic esters, phospholipids, and DNA based on the literature (Glonek et al., 1970; Henderson et al., 1974; Hanlon et al., 1976). Subsequent studies used these peak assignments and built on them, with Tate and Newman (1982) adding glucose 1-phosphate and ethanolamine phosphate, and Condron et al. (1990) adding teichoic acid, all based on spectra of model compounds analyzed in 1 M aqueous NaOH by R.H. Newman, as for Newman and Tate (1980). Spiking experiments were not conducted to confirm these peak identifications in spectra of soil extracts.

Throughout the late 1980s and 1990s, researchers used a variety of extractants and methods for ^{31}P-NMR of soils and environmental

Abbreviations: 2-AEP, 2-aminoethylphosphonic acid; ADP, adenosine diphosphate; ATP, adenosine triphosphate; CWAs, chemical weapons agents; DNA, deoxyribonucleic acid; EDTA, ethylenediaminetetra-acetic acid; GTP, guanosine triphosphate; IHP, inositol hexakisphosphate; LB, line-broadening; MDP, methylene diphosphonic acid; MP, monophosphate; NAD, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate hydrate; NaOH, sodium hydroxide; NMR, nuclear magnetic resonance spectroscopy; RNA, ribonucleic acid; SW, spectral window or sweep width.

E-mail address: Barbara.Cade-Menun@agr.gc.ca.

http://dx.doi.org/10.1016/j.geoderma.2014.12.016
0016-7061/Crown Copyright © 2014 Published by Elsevier B.V. All rights reserved.

samples [see Cade-Menun (2005) for more details]. In the majority of publications, peaks were grouped into broad categories such as orthophosphate monoesters and diesters (e.g., Hawkes et al., 1984; Forster and Zech, 1993; Makarov et al., 1995), or were specifically identified based on previous publications such as Newman and Tate (1980). The exceptions were Adams and Byrne (1989), who conducted spiking experiments to assign peaks in concentrated water plus Chelex extracts of soils to orthophosphate, glycerophosphate, inositol phosphate, glucose 6-phosphate, ribose 6-phosphate, choline phosphate, and pyrophosphate, and Bedrock et al. (1994), who spiked concentrated 0.5 M NaOH extracts with "known compounds" of aromatic phosphate diesters and orthophosphate monoesters, but do not specify which forms were used.

In 1996, Cade-Menun and Preston (1996) introduced the use of a 1:1 mix of 0.5 M NaOH + 0.1 M Na_2EDTA as a single-step extractant for ^{31}P-NMR, based on the Bowman and Moir (1993) extractant for soil organic P. This has subsequently become the standard extraction method for ^{31}P-NMR work with environmental samples (Cade-Menun, 2005; Cade-Menun and Liu, 2014). Early users of this procedure relied on the literature for peak identifications, and peak identifications were for the most part limited to broad categories or the chemical shifts reported in the literature for other extractants (Dai et al., 1996; Cade-Menun et al., 2000a, 2000b, 2002; Turner et al., 2003a). In 2003, Turner et al. (2003b) recognized that relying on the literature for peak identifications was unsatisfactory, because chemical shift is strongly influenced by sample pH, ionic strength and paramagnetic ion concentration. They determined the chemical shifts of 34 model compounds analyzed in a standardized soil extract. This consisted of soil extracted in NaOH–EDTA as per Cade-Menun and Preston (1996), freeze-dried, and redissolved in NaOH plus D_2O for NMR analysis. The analyzed model compounds covered a range of P compound classes (inorganic phosphates, phosphonates, organic polyphosphates, and orthophosphate monoesters and diesters), and included most of the model compounds previously used by other research groups. Each model compound was analyzed in a separate aliquot of the redissolved soil extract. More recently, Turner and Richardson (2004) identified the peak for scyllo-inositol hexaphosphate (*scyllo*-IHP) by spiking purified material into a soil extract, and by brominating NaOH–EDTA extracts to remove all but the inositol phosphates before reanalysis with ^{31}P-NMR. Their results suggested that peaks previously identified as choline phosphate were likely *scyllo*-IHP. Turner et al. (2012) identified *chiro*- and *neo*-inositol hexaphosphates (*chiro*-and *neo*-IHP) in a similar fashion, and suggested that peaks with chemical shifts in this region had been misidentified previously as aromatic phosphate diesters (Bedrock et al., 1994; Turner et al., 2003b).

For the most part, subsequent ^{31}P-NMR studies have either continued to group P forms into broad compound classes, or have identified peaks, particularly *myo*-IHP and *scyllo*-IHP, by assigning peaks with similar chemical shifts to those compounds identified by Turner et al. (2003b) and Turner and Richardson (2004) (e.g., Turner and Newman, 2005; Leytem et al., 2006; Smith et al., 2006; Hill and Cade-Menun, 2009). However, Smernik and Dougherty (2007) noted that relying on the literature alone to identify peaks can be misleading, particularly for samples prepared in different solvents for ^{31}P-NMR than were used for the original reference, such as freeze-dried extracts dissolved in water rather than NaOH [see Cade-Menun and Liu (2014) for more details on this]. Spiking was subsequently recommended as the only way to specifically identify P forms (McDowell et al., 2007; Smernik and Dougherty, 2007; Doolette et al., 2009), and was subsequently used in studies of soil (Cade-Menun et al., 2010; Vincent et al., 2012), animal manure (He et al., 2009), humic acids (He et al., 2011) and lake sediments (Jørgensen et al., 2011; Shinohara et al., 2012). However, it is important to note that spiking with one compound does not confirm the identification of other P species in ^{31}P-NMR spectra: Cade-Menun et al. (2010) identified myo-IHP by spiking, but misidentified β-glycerophosphate by relying on the literature (as demonstrated with spiking experiments in He et al., 2011).

In 2012, Vestergren et al. (2012) used two-dimensional (2D) ^{1}H–^{31}P NMR to identify 17 P species in NaOH–EDTA extracts of soils, after sulfide precipitation of the extracts to remove paramagnetic ions. Peak identifications were based on similarly prepared and analyzed reference compounds. The ^{31}P chemical shifts were consistent with those previously identified by others with 1D ^{31}P-NMR experiments, although they were slightly offset due to differences in solution chemistry. The major advancement from these 2D experiments was the ability to distinguish P species with overlapping ^{31}P chemical shifts but different ^{1}H chemical shifts, such as the mononucleotides uridine 3′ monophosphate (MP) and guanosine 3′ MP.

Identifying as many P species as possible in ^{31}P-NMR spectra is essential for advancing our knowledge of P cycling in the environment. It is well-established that P forms within the broad categories of orthophosphate monoesters and diesters differ in their bioavailability and reactivity in the environment (Condron et al., 2005). As such, lumping P forms together into these broad categories provides little if any useful information about their cycling, especially compared to the knowledge that can be gained from identifying the specific P forms under different environmental conditions. It is also well-established that some orthophosphate diesters (e.g., RNA, phospholipids) are degraded to orthophosphate monoesters during ^{31}P-NMR analysis (e.g., Turner et al., 2003b; Doolette et al., 2009), with the degree of this degradation varying with NMR experimental length and conditions (Cade-Menun, 2011; Cade-Menun and Liu, 2014). Correcting for these degradation products not only provides for better calculations of the total concentrations of orthophosphate monoesters and diesters in the original soil samples, it can also improve statistical analysis of P NMR forms with other soil parameters (Liu et al., 2013; Young et al., 2013). However, correcting for degradation peaks requires the proper identification of these peaks in spectra.

As previously demonstrated, relying on published chemical shifts can introduce errors if there are any differences among NMR experiments, particularly in sample preparation. Spiking experiments are essential to confirm peak identifications. However, the cost of spiking all known P compounds into all NMR spectra would be prohibitively expensive with respect to purchasing the compounds and analyzing samples spiked with these compounds by ^{31}P-NMR, and not all identified P forms in environmental samples are commercially available for spiking (e.g., *scyllo*-IHP). A more cost-effective method would be to combine spiking, using a selection of compounds, with a reference library of other P compounds that were prepared and analyzed in a standardized fashion, with each research group following a standardized spiking and identification protocol for consistency among research groups. This includes analyzing reference P compounds in a matrix that mimics freeze-dried and dissolved soil extracts with respect to pH and salt content, and including one P compound with a fixed chemical shift with each analyzed P compound.

Thus, the objective of this research was to develop a library of more than 50 P compounds, analyzed by ^{31}P-NMR spectroscopy under standard conditions that could be easily replicated by any research group for peak identification or to add new compounds to this library. Recommendations are given to use this library effectively, and to spike samples effectively for peak identification.

2. Materials and methods

2.1. Standardized blanks

Blank sodium hydroxide–disodium ethylenediaminetetra-acetic acid (NaOH–EDTA) plus orthophosphate samples (hereafter referred to as "blanks") were prepared by adding a 1-mL aliquot of 1000 ppm P (certified standard, SPEX CertPrep) to 25 mL of 0.5 M NaOH plus 0.1 M Na_2EDTA in a 50-mL disposable centrifuge tube. Samples were then frozen on a slant to maximize surface area. Frozen samples were lyophilized by replacing the caps of each tube with parafilm (poking a

Download English Version:

https://daneshyari.com/en/article/4573133

Download Persian Version:

https://daneshyari.com/article/4573133

Daneshyari.com