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Review

Use of NMR techniques for toxic organophosphorus compound profiling☆

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ABSTRACT

This review presents with selected examples the versatility of nuclear magnetic resonance (NMR) spectroscopy in the analysis of toxic organophosphorus (OP) compounds, i.e. OP pesticides and chemical warfare agents (CWAs). Several NMR applications of biological importance, like studies on inhibition mechanism, metabolism, and exposure determination, are presented. The review also concerns with the environmental analysis of OP compounds by NMR spectroscopy. Residue analysis of environment and food samples as well as characterization of degradation in environment is discussed. Some of the NMR studies that have been done to support the Chemical Weapons Convention, i.e. the development of suitable CWA detoxification means and the method development of verification analysis for CWAs and their degradation products, are outlined.

Contents

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1. Introduction

Organophosphorus (OP) compounds are derivatives of phosphorus that have at least one organic (alkyl or aryl) group attached to the phosphorus atom either directly or indirectly by means of another element (e.g. oxygen, sulfur or nitrogen) [1]. OP compounds are in many cases highly toxic, and some of these toxic OP compounds have importance as pesticides. Pesticide is a broad term, covering a range of products that are used to control pests: insect killers (insecticides), mould and fungi killers (fungicides), weedkillers (herbicides), slug pellets (molluscicides), plant growth regulators, bird and animal repellents, and rat and mouse killers (rodenticides) [2]. National regulations control the availability of pesticides on market, and define the acceptable upper limits of the amounts of pesticide residues in food products and animal feed. A part of the toxic OP compounds has gained notorious reputation due to their potential as chemical warfare agents (CWAs) [3,4]. The CWAs have usually been discovered in connection with the development of pesticides. The G-series nerve agents, tabun (ethyl dimethylphosphoramidocyanidate, GA), sarin (isopropyl methylphosphonofluoridate, GB), soman (pinacolyl methylphosphonofluoridate, GD), and cyclosoman (cyclohexyl methylphosphonofluoridate, GF), were discovered during the 1930s and 1940s, and the V-series nerve agents like VX (*O*-ethyl *S*-(2-diisopropylaminoethyl) methylphosphonothiolate) and its isomer Russian-VX (*O*-isobutyl *S*-(2-diethylaminoethyl) methylphosphonothiolate) later during the 1950s [3,4]. The international concern over the threat of CWA culminated in 1993 to an agreement, the Chemical Weapons Convention (CWC) [5], which prohibits the development, production, acquisition, stockpiling, retention, transfer and use of chemical weapons. The Technical Secretariat of the Organisation for the Prohibition of Chemical Weapons (OPCW) [6] is the governing body that implements the CWC internationally. While general pesticides are not included as Scheduled Chemicals in the CWC [5], they can be as harmful as CWA to humans, and occasionally they have been weaponized [7,8]. However, according to Article VI of the CWC [5], States Parties must adopt measures to ensure that any toxic chemicals and their precursors are only used for purposes not prohibited by the CWC. The States Parties which have chemical plants or other facilities producing certain amount of toxic OP compounds must declare their production to the OPCW. The State Party must grant to the OPCW inspectors access to facilities as required in the Verification Annex of the CWC [5].

The introduction of pesticide residues in the environment through agricultural processes is a major public concern [9]. Abandoned CWA munitions can also pollute the environment [10]. This can result in that humans, domestic animals, as well as wildlife can be exposed to harmful doses of OP compounds. Valid analytical techniques are needed to monitor that the level of toxic OP compounds in the environment follows the regulations, and to determine the cause of poisoning when a harmful level of exposure has occurred. Separation techniques hyphenated to detectors with a high sensitivity, like gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (LC–MS), have usually been the methods of choice [11]. Nuclear magnetic resonance (NMR) spectroscopy as one of the most important structural elucidation techniques has also been employed in the OP compound analyses. The strength of NMR spectroscopy has been in characterization of the chemical structures [12], and by that giving information about the OP compound degradation processes in the environment as well as the OP compound metabolism in organisms. Because NMR is also a quantitative technique [13], it has been applied in quality control of the OP pesticides and other agrochemical products [14]. Finally, NMR is nondestructive, meaning that the sample can be analyzed without consuming it during the process like with GC–MS or LC–MS techniques, and the sample can be stored after the analysis for later studies.

The NMR spectroscopy of phosphorus-containing chemicals can be considered to begin from the discovery of the nuclear resonance of phosphorus [15]. First notion about the characteristic J_{PF} coupling of phosphorus–fluoride compounds was then reported by Gutowsky and McCall [16]. Muller et al. [17] reported ^{31}P shifts of 63 different OP compounds, and the relation between the chemical shift and the structure was discussed on theoretical basis. Since then, ^{31}P NMR spectroscopy [18,19] has established its usefulness in the analysis of OP compounds.

One of the reasons for the popularity of ^{31}P NMR spectroscopy is the relatively good sensitivity of phosphorus. Phosphorus-31, a half-spin nucleus, exists on 100% natural abundance. Its receptivity is roughly 400 times higher compared to carbon-13 on 1.1% abundance. Furthermore, the chemical shift of phosphorus is very sensitive to its chemical environment, and offers a reliable way to identify the OP compounds even in complex mixtures. The chemical shift range of phosphorus is quite broad (ca. 2000 ppm) [20], and background signals do not usually obscure the relevant OP compound peaks like in the ^{1}H NMR analyses. The ^{31}P detection can be used also with solid samples using magic angle spinning (MAS) techniques [21], thus offering a way for direct analysis of soil samples.

On some occasions the amount of OP compound can be scarce, and ^{1}H NMR has been found useful due to its higher sensitivity compared to ^{31}P detection, although the background signals can hamper compound identification. There are also demonstrations how ^{1}H-^{31}P correlation spectroscopy can be used for both sensitive and selective screening of the OP compounds. Two-dimensional (2D) NMR [22], when used with a mixture, can act as a "separation" technique to distinguish different components as well as isomers in the mixture. There are also some recent examples how an established separation technique, liquid chromatography [23], can be hyphenated to NMR in OP compound-related analyses.

This review will not go into the details of the NMR experiments used, as there are many good text books about the NMR theory and techniques (e.g. [24,25]). The main focus of this review is to highlight with selected examples how NMR has been applied in the analysis of toxic OP compounds, e.g. pesticides and CWAs (Table 1). The topic has been divided on the basis of the applications. First the text will focus on the biological aspects of OP compounds, e.g. the enzyme inhibition mechanism, metabolites, biomonitoring, and antidotes, and will outline some of the NMR studies on the topics. The environmental fate of the OP compounds has been of concern for some time due to raising environmental awareness. While the application of NMR spectroscopy in environmental chemistry is well described elsewhere [26], some of the applications in detection of the OP compounds in environmental and food samples, as well as characterization of OP compound degradation in environment will be presented. The last sections are more concerned with CWAs, and will outline some of the NMR investigations that have been conducted during the development of suitable CWA detoxification means, as well as in the verification of CWAs and their degradation products in environmental samples and urban matrices.

2. Applications

2.1. Enzyme inhibition mechanism

The high toxicity of OP compounds is due to a cascade of reactions that begins with inhibition of acetylcholinesterase (AChE), a serine hydrolase responsible for processing the neurotransmitter acetylcholine. The inhibition is caused by formation of a stoichiometric (1:1) covalent conjugate with the active site serine. This