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Electron paramagnetic resonance study of the radiation damage in phosphoryethanolamine single crystal

Betul Caliskan [a, *], Ali Cengiz Caliskan [b]

[a] Department of Physics, Faculty of Arts and Science, Pamukkale University, 20070, Kinikli, Denizli, Turkey
[b] Department of Chemistry, Faculty of Science, Gazi University, 06500, Ankara, Turkey

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ABSTRACT

Gamma irradiated phosphoryethanolamine ($C_2H_8NO_4P$) single crystals were investigated at 140 K using Electron Paramagnetic Resonance (EPR) technique. Phosphoryethanolamine single crystals have been irradiated with ^{60}Co-γ rays, at room temperature. The electron paramagnetic resonance (EPR) spectra of gamma irradiated phosphoryethanolamine single crystals have been studied for different orientations of crystals in a magnetic field. The spectra have been found to be temperature independent. The structure of the radical produced by γ-irradiation of a single crystal of phosphoryethanolamine was discussed. The investigation of EPR spectra of gamma-irradiated single crystals of phosphoryethanolamine showed the presence of two radicals. One of the radicals is the $H_2\dot{P}O_4$ neutral radical formed by the breakdown of the O(7)–C(8) bond. The other is the $HPO_4^{\cdot-}$ anion radical formed by cleavage of the O(7)–C(8) bond and the attachment of an electron to O(2) by breaking H(1) bound to O(2). The principal values of the hyperfine coupling tensor of the unpaired electron and the principal values of the g-tensor and direction cosines of the radiation damage centers have been determined. The results were found to be in good agreement with the existing literature.

1. Introduction

A zwitterion is a molecule that has both positively and negatively charged functional groups, but maintains an overall neutral charge. Zwitterionic structures exist extensively in biological systems such as amino acids and peptides [1]. Zwitterions containing amino and phosphate groups are much less studied, even though the interaction between the zwitterionic form of amino acids and phosphate groups of the DNA strand has been found [2]. Phosphoryethanolamine (2-Aminoethyl dihydrogen phosphate ($AEPH_2$)) has managed to remain a fairly unknown compound despite its potential as a surface modification agent. Made by esterification of 2-aminoethanol and orthophosphoric acid [3], phosphoryethanolamine is a bifunctional short-chained organic molecule that has both a phosphate group and an amino group. The short alkyl chain length between the phosphate and the amino group promotes the solubility in protic solvents [4]. A study of the zwitterionic nature of phosphoryethanolamine can offer valuable information about its bonding behavior in very acidic and very basic environments. Phosphoryethanolamine could also be used for pH measurements in biological systems, as it has been found in brain samples as a metabolic side-product [5,6].

The form of phosphoryethanolamine in aqueous solution was studied by means of NMR titration. A potentiometric titration study done earlier [7] on phosphoryethanolamine shows two deprotonation steps, but the compound has three labile protons in the pH range of 1–12.

The first deprotonation point can be found around pH 1, where the phosphate group $-OPO_3H_2$ releases a proton and becomes an ionic $-OPO_3H^-$ group (phosphoryethanolamine $pK_{a1} = 1.0$). The ^{31}P NMR chemical shift of the phosphate group as a function of pH shows a small equivalent point between pH 1 and pH 2. The amino group is in a $-NH_3^+$ state at pH 1, making the molecule a zwitterion [8]. The second deprotonation point is found at pH 5.9 where the ionic phosphate group $-OPO_3H^-$ releases its last proton and becomes a $-OPO_3^{2-}$ group (phosphoryethanolamine $pK_{a2} = 5.9$) while the amino group remains as $-NH_3^+$. A very large change in the chemical shift of phosphorus can be seen at pH 5.9, which denotes the second deprotonation of the phosphate group and an equivalent point between $-OPO_3H^-$ and $-OPO_3^{2-}$ forms. Such a large increase in the ^{31}P chemical shift can be explained by the increased

* Corresponding author.
E-mail addresses: bcaliskan@pau.edu.tr, bcaliska@gmail.com (B. Caliskan).

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polarization of the $P-O$ bonds [9] that derives from the increased electronic density of the oxygen atoms after the deprotonation. Due to an increased electron density surrounding the phosphorus atom, the ^{31}P resonance shifts downfield. Owing to the overall negative charge, the molecule can no longer be considered a zwitterion at this point. Because of the zwitterionic nature the phosphoryethanolamine is a both weak acid and a conjugate base, simultaneously able to donate and accept protons in multiple functional groups, this can affect results of potentiometric titration where concentrations are calculated using the consumed titrant base. In NMR titration the net effect of simultaneous proton donation/acceptance does not interfere with the observation of equivalent points because they are determined from the changes in a single functional group [8].

Maillard reactions, which occur between an amino group and a reducing sugar, are the most important among the chemical and oxidative reactions occurring in foods and biological samples; they contribute to food deterioration and the pathophysiology of human diseases. Although protein glycation has been investigated thoroughly, little attention has been paid to lipid glycation [10]. Nonenzymatic glycation of biomolecules by reducing sugars and other carbonyl compounds causes irreversible changes in their properties. Glycation occurs naturally in the human body during aging and is the main culprit of some hyperglycemia related diseases [11,12]. Whereas the nonenzymatic glycation of proteins has been the subject of much study [11,13,14], that of aminophospholipids has received considerably less attention. An amino group of phosphoryethanolamine (PE) is considered as a target for nonenzymatic glycation, and the potential involvement of lipid glycation in the pathogenesis of diabetic complications has generated interest [15]. In 1993, Bucala et al. [16] showed the aminophospholipid phosphoryethanolamine (PE), which is present in mammal cell membranes, to react with glucose and initiate its glycation. Subsequent in vitro studies showed the reactions of PE and phosphatidylserine (PS) with glucose to develop via the general glycation mechanism previously proposed for proteins [17,18]. The first step in the process is the reversible formation of a Schiff base that subsequently rearranges to form more stable compound: a ketoamine known as an “Amadori compound”. Amadori compounds can give various reactions leading to the formation of a heterogeneous body of compounds known as “advanced glycation products” (AGEs). Oxidation of Schiff bases and/or Amadori compounds produce free radicals capable of peroxidizing lipids and giving advanced lipoxidation end-products (ALEs) [16,17,19,20]. Ravandi et al. [17,21] and Pamplona et al. [22] provided experimental evidence of aminophospholipid glycation in vivo. Subsequently, Fountain et al. [23] quantified glucose-linked PE and PS in samples from diabetic patients. In 2001, Breitling-Utzmann et al. [24] identified and quantified Schiff–PE and Amadori–PE adducts in erythrocytes from healthy and diabetic individuals by liquid chromatography–electrospray mass spectrometry. In recent years, Miyazawa and co-workers [10,15,19] have improved the analysis of Amadori-glycated phosphoryethanolamine in human erythrocytes and blood plasma by mass spectrometry. Because of the high biological importance of vitamin B6 to inhibit biomolecular glycation, the effect of aminophospholipids was investigated using O-phosphoryethanolamine and O-phospho-D,L-serine [25,26]. Some studies demonstrated that Amadori-PE is capable of generating reactive oxygen species, and thereby triggers lipid peroxidation in the presence of ferrous ions [20]; moreover, it is an important compound that promotes vascular disease as a result of its angiogenic activity on endothelial cells [27].

In a model study on γ-irradiated α-glycerolphosphate, the PO_4^{2-} radical has been assigned, which results from $C-O$ bond rupture with, $a_{P-iso} = 30$ G, indicating an oxygen-centered radical [28]. In a single crystal EPR study of X-irradiated *o*-phosphorylethanolamine, phosphonyl *P* radicals have been detected which are obviously formed by $P-O$ bond cleavage [29]. It is not clear whether this process is induced by electron capture at the ester bond resulting in dissociation, or that *H* radicals are involved [30].

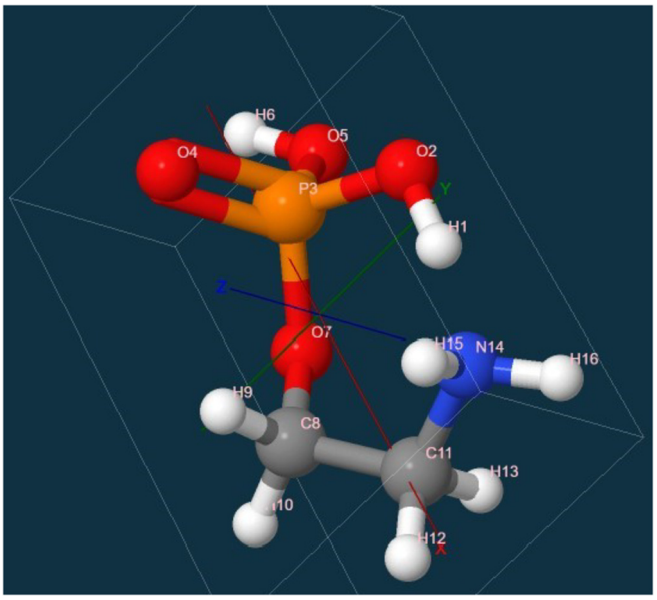

Fig. 1. Molecular structure of phosphoryethanolamine.

In the present study, the radiation damage center in gamma-irradiated phosphoryethanolamine has been characterized by Electron Paramagnetic Resonance method at 140 K. There is no report in literature on the EPR studies of gamma-irradiated phosphoryethanolamine single crystals. Since we performed both experimental and simulation study of phosphoryethanolamine single crystal by EPR spectroscopy method.

2. Experimental design

The single crystals of phosphoryethanolamine were grown in the laboratory by slow evaporation of concentrated pure water solution. A slow evaporation technique was used to amplify the crystals. The solution was prepared by adding pure water and compound into a beaker of appropriate size. The solution was clarified by heating. The solution was filtered through a filter paper with slow flow rate. The beaker was covered with aluminum foil and holes were drilled on several places. Thus, slow evaporation is achieved. The solution was placed wherever possible to avoid any vibrations and crystals were obtained in this calm environment.

X-ray diffraction result of phosphoryethanolamine agrees well with the crystallographic data previously measured with X-ray photographic method [31] and neutron diffraction [32]. Phosphoryethanolamine has a monoclinic $P2_1/c$ space group: a = 9.0065(2) Å, b = 7.7389(2) Å, c = 8.7756(2) Å, b = 102.4840(10) Å, Z = 4 [8]. Phosphorus-oxygen bond lengths are 1.56 Å for a single $P-O$ bond and 1.45 Å for $P=O$ double bond [33].

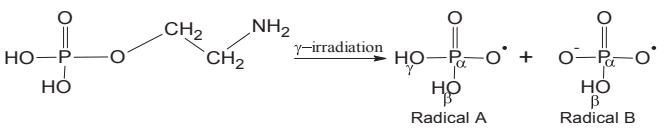

Fig. 2. Structure of two radicals observed in phosphoryethanolamine single crystal.