Journal of Molecular Structure 1033 (2013) 171–175

Contents lists available at SciVerse ScienceDirect

Journal of Molecular Structure

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

The pH behavior of a 2-aminoethyl dihydrogen phosphate zwitterion studied with NMR-titrations

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HIGHLIGHTS

- 2-Aminoethyl dihydrogen phosphate ($AEPH_2$) exists as zwitterion in water solutions.
- $AEPH_2$ has the zwitterionic form in the pH range of between 1 and 5.
- Phosphate group of $AEPH_2$ deprotonates at pH 1.0 and 5.9.
- Amino group of $AEPH_2$ deprotonates at pH 11.0.
- $AEPH_2$ exists in the zwitterionic structure in solid state.

ARTICLE INFO

Article history:
Received 11 May 2012
Received in revised form 20 August 2012
Accepted 20 August 2012
Available online 29 August 2012

Keywords:
Organic phosphate
Amino
Zwitterion
pH dependent
NMR-titration
X-ray diffraction

ABSTRACT

In this study a bifunctional 2-aminoethyl dihydrogen phosphate ($AEPH_2$) was ^1H and ^{31}P NMR characterized in a pH range of 1–12 in order to determine the zwitterion properties in different pH regions in H_2O and D_2O solutions. NMR was also used to determine the pH range where $AEPH_2$ exists as a zwitterion. The phosphate group has two deprotonation points, around pH 1 and 6, while the amino group deprotonates at pH 11. The zwitterion form of $AEPH_2$ ($NH_3^+—CH_2—CH_2—OPO_3H^-$) exists as the main ion between pH 1 and 6 in water solutions and also in the solid state.

1. Introduction

Most molecules have specific signals in the NMR spectrum, depending on the chemical structure. However, the chemical shift of the signal is affected by ionic concentration and the pH of the solution they are measured in. Changes in chemical shift are usually induced by changes in the electronic structure of the molecule or interaction with a solvent e.g. accepting an electron or proton. These interactions are usually dependent upon the solution pH and only happen in a specific and narrow pH range. By monitoring certain signals in NMR as a function of a solution pH it is possible to pinpoint solvent interactions to a specific pH area, but also to a specific functional group on a multifunctional compound.

Some multifunctional molecules have functional groups that can either accept or donate protons, but there are also amphoteric groups that can do both. When a molecule has functional groups that are capable of donating and accepting protons there is a chance for the formation of a zwitterion. 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]. One example of such a molecule is the common amino acid, glycine ($NH_2—CH_2—COOH$), which exists as zwitterions in a solid state, water solution [2], and gas phase. [3] Amino acids have both a proton donating carboxylic acid group (—COOH) and an electron accepting primary amino group ($—NH_2$). In neutral aqueous solutions, the amino acid forms a carboxylic acid anion ($—COO^-$) and an ammonium cation ($—NH_3^+$), effectively producing zwitterions, which are stabilized by the water molecules [4].

The formation of zwitterions via proton transfer can happen in many different ways: through intramolecular proton transfer, proton donation/acceptance via a protic solvent [5] and intermolecular proton transfer between molecules of a dimeric complex [6]. With NMR titration, it is possible to track the pH range of actual zwitterions and to determine the ionic forms in other pH

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http://dx.doi.org/10.1016/j.molstruc.2012.08.033

regions. Because protonation and proton transfer play an important role in biological processes between amino acids and proteins, the use of proton NMR for the determination of a sample pH has been studied extensively in biological systems. In biological samples, the pH is usually tracked with amino acids or simple proteins, e.g. glycine [2,4], histidine [7], and carnosine [8], which have pH-dependent reactions and are commonly found in tissue samples.

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 [9]. Aminophosphate zwitterions are known to exist in a solid state [10], the phosphate group is known to interact with free cationic NH_3^+ groups, [11] and the protons of the phosphate group dissociate easily in water solutions [12], so it is possible for such zwitterions to exist in water solutions.

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 [13], $AEPH_2$ is a bifunctional short-chained organic molecule that has both a phosphate group and an amino group. The phosphate group is, in general, useful for binding with various metal oxides like TiO_2 [14–16] and iron oxides [17,18] and the amino group is useful for bonding with several metal ions [19], complexes [20], organometallic compounds [21], and biomolecules via hydrogen bonds [22]. The short alkyl chain length between the phosphate and the amino group promotes the solubility in protic solvents [23]. $AEPH_2$ has also been successfully bonded to an anatase form of TiO_2 powder in two different pHs [16]. A study of the zwitterionic nature of $AEPH_2$ can offer valuable information about its bonding behavior in very acidic and very basic environments. $AEPH_2$ could also be used for pH measurements in biological systems, as it has been found in brain samples as a metabolic side-product [24,25]. There might be a small difference between NMR results achieved in biological samples and in a pure solvent used in this study due to the various solute–solute interactions present in a typical metabolite or sucrose solution [26].

The goal of our study was to examine whether the $AEPH_2$ molecule exists in a zwitterionic form in water solutions and in a solid state. ^1H and ^{31}P NMR titration were carried-out in order to characterize the acid base reactions of this multifunctional aminophosphate, and X-ray diffraction measurement was used to determine the solid state structure. Special emphasis was placed on the pH range of the three depronation steps of $AEPH_2$, namely the two deprotonations of phosphate, and the deprotonation of the NH_3^+ group.

2. Experimental

2.1. Materials

Ethanol 99.5% (Altia Etax Aa), D_2O 99.9% (Aldrich), DCl 99% (Aldrich), NaOD 99.5% (Aldrich), and $CDCl_3$ 99.8% (Euriso-Top) were used as received from the supplier. $AEPH_2$ powder 98% (Aldrich) was recrystallized and filtered from a 1:3 water/ethanol mixture and then crystallized from a supersaturated water solution. NaOH and H_2SO_4 solutions were made by dilution from 1.00 M to 5.00 M storage solutions, respectively. All non-deuterated water used in the research was 18 MΩ cm^{-1} Milli-Q water.

2.2. Instruments and methods

The pH values were measured with an electronic Consort P901 pH-meter using a combination electrode, temperature correction, and a 3-point calibration in buffer solutions at pH 4.005, pH 7.000, and pH 10.012.

All NMR measurements were made with a Bruker Avance 400 NMR spectrometer with 64 scans per sample. Delays between pulses were 2 s for ^1H samples and 10 s for ^{31}P samples. The solvent was D_2O for the titration samples and $CDCl_3$ for the aminoethanol verification. An airtight capillary tube with 0.03% of TMS dissolved in $CDCl_3$ was the external standard for the ^1H measurements (0.00 ppm) and 85% orthophosphoric acid was the external standard for ^{31}P measurements (0.00 ppm).

Single crystal X-ray diffraction was conducted with a Bruker Smart ApexII diffractometer. A single crystal of $AEPH_2$ was immersed in cryo-oil, mounted in a nylon loop and measured at 100 K using an X-ray beam wavelength of 0.71073 Å.

2.3. Titration of NMR samples

First, a 10 ml batch of 0.1 M $AEPH_2$ solution was prepared in D_2O, which was then divided into two separate solutions with an initial pH of 3.5. The first D_2O solution of $AEPH_2$ was titrated using 5 M, 1 M and 0.1 M H_2SO_4 solutions in order to make a solution pH 1 and a new NMR sample was taken every 0.5 pH mark in the pH range of 1–3.5. The second D_2O solution was titrated using 1 M and 0.1 M NaOH solutions, in order to make a solution pH 12 and a new NMR sample was taken every 0.5 pH mark between the pH range of 3.5 and 12.

Second, another 10 ml batch of titrations was done with fully deuterated chemicals in a 0.1 M D_2O solution of $AEPH_2$. The solution was the divided into two separate solutions with an initial pH of 3.5. This time the titrations were conducted using a 35 wt% DCl in D_2O for a pH range of 1–3.5 and a 40 wt% NaOD in D_2O for a pH range of 3.5–12 and a new NMR sample was taken every 1.0 pH mark.

2.4. Extraction of aminoethanol

A sample of the $AEPH_2$ solution was extracted for 2-Aminoethanol in order to see if it de-esterificates at a low pH. At pH 1 the 0.1 M $AEPH_2$ solution was extracted with three times 50 ml of diethylether. Diethylether was removed by evaporation at room temperature and the presence of 2-aminoethanol was evaluated with ^1H NMR measurements in a $CDCl_3$ medium. The expected signals of 2-aminoethanol (triplet δ 3.59 ppm and triplet δ 2.90 ppm) were not observed in the extracted sample.

3. Results and discussion

The $AEPH_2$ was expected to be in zwitterionic form in a solid state, based on the high melting point (246.6 °C) and its good solubility in water [16]. A single crystal X-ray diffraction analysis confirmed this hypothesis. X-ray diffraction result of $AEPH_2$ agrees well with the crystallographic data previously measured with X-ray photographic method [27] and neutron diffraction [28]. $AEPH_2$ has a monoclinic $P2_1/c$ space group: $a = 9.0065(2)$ Å, $b = 7.7389(2)$ Å, $c = 8.7756(2)$ Å, $\beta = 102.4840(10)°$, $Z = 4$, and a zwitterionic $NH_3^+—CH_2—CH_2—OPO_3H^-$ configuration (Fig. 1), resulting in a three dimensional hydrogen bonded network of cross-linked sheets, similar to α-aminopropanephosphonic acid [29]. Phosphorus–oxygen bond lengths are 1.56 Å for a single P—O bond and 1.45 Å for P═O double bond [30]. The measured P—O bond distances of the phosphate group (P1—O3 and P1—O4) are very similar (1.493 Å and 1.508 Å, respectively) and they are between the reference bond lengths of single and double bonds, so they both have a bond order of 1.5 with a delocalized negative charge capable