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Reactivity of 12-tungstophosphoric acid and its inhibitor potency toward Na^+/K^+-ATPase: A combined ^{31}P NMR study, *ab initio* calculations and crystallographic analysis

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ABSTRACT

Influence of 12-tungstophosphoric acid (WPA) on conversion of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) in the presence of Na^+/K^+-ATPase was monitored by ^{31}P NMR spectroscopy. It was shown that WPA exhibits inhibitory effect on Na^+/K^+-ATPase activity. In order to study WPA reactivity and intermolecular interactions between WPA oxygen atoms and different proton donor types (D = O, N, C), we have considered data for WPA based compounds from the Cambridge Structural Database (CSD), the Crystallographic Open Database (COD) and the Inorganic Crystal Structure Database (ICSD). Binding properties of Keggin's anion in biological systems are illustrated using Protein Data Bank (PDB). This work constitutes the first determination of theoretical Bader charges on polyoxotungstate compound *via* the Atom In Molecule theory. An analysis of electrostatic potential maps at the molecular surface and charge of WPA, resulting from DFT calculations, suggests that the preferred protonation site corresponds to WPA bridging oxygen. These results enlightened WPA chemical reactivity and its potential biological applications such as the inhibition of the ATPase activity.

1. Introduction

Polyoxometalates (POMs) are a class of metal-oxygen cluster compounds with unique structural and physicochemical properties [1]. They have attracted great attention for many years due to their strong acidity and redox properties, which have led to their broad applications in industry as homogeneous and heterogeneous catalysts [2–5]. POMs are generally nontoxic to normal cells and exhibit *in vitro*, and *in vivo* biological activities [1,6–10]. Such activities include highly selective inhibition of enzyme functions, *in vitro* and *in vivo* antitumoral [11–15], antiviral [16] and antiretroviral activities [17] against human immunodeficiency virus (HIV) infections.

Biomedical importance of POMs, as confirmed by numerous studies is mostly based on their interactions with proteins [10,14,18–21]. These studies illustrate the importance of noncovalent interactions between POM and peptides. Negative charge of POMs is thought to be one of the key features for their interactions with proteins, as POMs are binding primarily to the positively charged protein region [22]. It is consistent with the experimental results obtained by Zhang et al. indicating that the binding of POM to proteins may cause the protein molecule to undergo an unfolding process [23].

Among the POMs, 12-tungstophosphoric acid ($H_3PW_{12}O_{40}$ abbreviated WPA) occupies a special position. It is recognized as a promising catalyst in biomass fuel production [24–25]. The anion has also various biological properties [26–33], including inhibition of ATPase. Primary structure of WPA is the Keggin unit, which is composed of a central PO_4^{3-} tetrahedron surrounded by 12 WO_6 octahedra as a polyanion $(PW_{12}O_{40})^{3-}$ with three negative charges that are neutralized by three protons. Fig. 1 represents the anion structure. According to a representation of oxygen octahedron (Fig. 1b), 4 oxygen types can be defined; 4 central oxygen atoms (Oa), 12 oxygen atoms that bridge two tungsten atoms sharing a central oxygen atom (edge-sharing Oc), 12 oxygen atoms that bridge tungsten atoms not sharing a central oxygen atom (Ob) and 12 terminal oxygen atoms (Od) bound to a single

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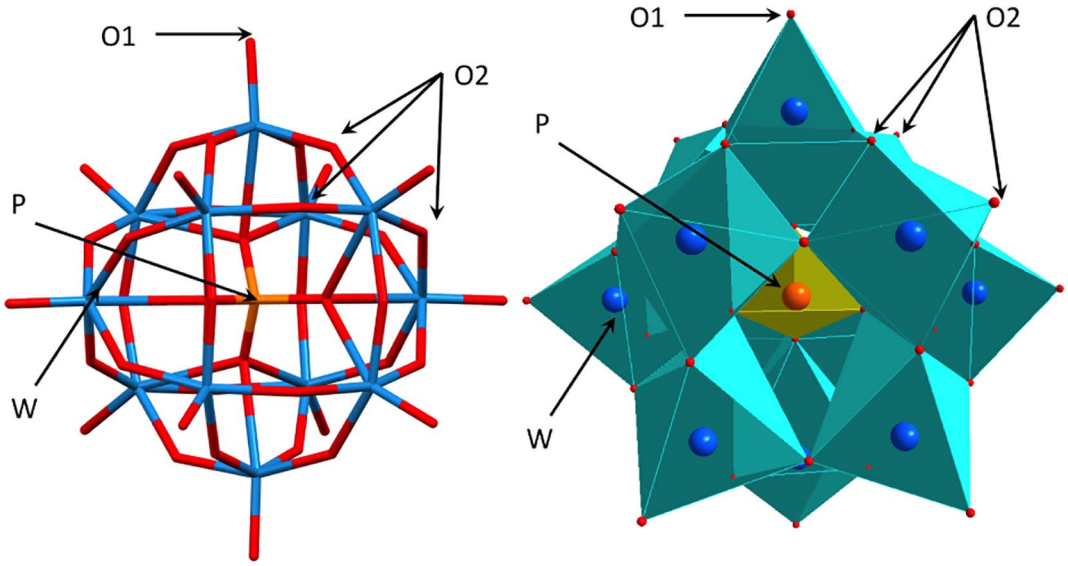

Fig. 1. Chemical structure and atom labeling of WPA a) wireframe bond style b) oxygen octahedral representation.

tungsten atom. However, a precise analysis of the WPA geometry shows that there is no significant difference (as deduced from classical X-ray diffraction) between Ob and Oc oxygen atoms in terms of bond lengths and bond angles. It remains than in solution the accessibility of the Ob and Oc oxygen atoms could be distinguished as demonstrated by Lopez et al. *via* molecular dynamic simulations [34]. Therefore, two types of "external" oxygen atoms, those which are accessible to a noncovalent interaction, will be used, according to their tungsten coordination: O1x (bonded to only one tungsten atom) and O2x (shared between two tungsten atoms). They will be referred to as O1 and O2 in this paper (Fig. 1a).Oxygen atoms of a WPA anion are attractive sites, and can therefore form intermolecular hydrogen bonds. These interactions bear a particular importance because they are responsible for their biological actions toward viral enzymes or viral cell envelopes.

Precise proton positions and acid strength of tungstophosphoric acid, which are essential for understanding of the nature of the catalytic sites and reactivity, are still under active debate in the community. Kozhevnikov et al. [35] concluded from ^{17}O NMR spectroscopy that the protons are located on the terminal oxygen atoms (O1). Ganapathy et al. [36] reached a similar conclusion based on the results from $^{1}H/^{31}P$ and $^{31}P/^{1}H$ Rotational Echo Double Resonance (REDOR) NMR experiments and density functional theory (DFT) based quantum chemical calculations of the proton affinity for a single proton. ^{31}P NMR analysis and DFT calculations indicated that the trimethylphosphine oxide complexes are associated with protons located at three different terminal oxygen (O1) sites of the WPA polyanion [37]. Solid-state NMR technique [38] was used to argue that the anhydrous protons reside on the bridging oxygen atoms. The most energetically favorable site for the acidic proton in anhydrous heteropolyacids was determined by quantum chemical calculations to be a bridging oxygen atom [39]. DFT calculations, which considered the position of all three protons, indicated that the energy preference for either oxygen type is small and that protons are likely to be distributed among all oxygen types [40]. REDOR NMR experiments combined with quantum chemical DFT calculations demonstrated that acidic protons in anhydrous WPA are localized on both bridging (O2) and terminal (O1) atoms of the Keggin unit [41].

Recently, we found that WPA exhibits potential bioactivities, especially the inhibition of Na^+/K^+-ATPase [31]. In a number of enzyme reactions involving phosphates, the most direct method for monitoring interaction between WPA and enzyme and identification of generated chemical species is ^{31}P NMR spectroscopy [42–44]. The possibility of readily distinguishing and assigning each phosphorus atom of adenosine triphosphate (ATP) and adenosine diphosphate (ADP) by ^{31}P NMR was early recognized and demonstrated in 1960 [45]. Therefore we applied ^{31}P NMR to follow ATPase activity in the presence of WPA.

The understanding of the interaction, between WPA and enzyme (ATPase), at a molecular level is essential for the interpretation and the development of potent compounds with selective enzymatic affinity. In order to understand the reactivity of WPA, the OH···O, NH···O and CH···O interactions between different organic parts and WPA were analyzed using the Cambridge Structural Data Base (CSD) [46], Crystallographic Open Database (COD) [47] as well as the Inorganic Crystal Structure Database (ICSD) [48] or the Protein Data Bank (PDB) [49]. This analysis was performed according to the oxygen atom type (O1 or O2). Consequently, it explains the interaction of organic molecules with Keggin anion through hydrogen bonding. Hydrogen bonds, weak interactions and their environment have a well–documented geometry in the crystalline state. CSD provides a large amount of experimental data that allows a better description of hydrogen bonds geometry [50–53]. This method has already been applied to different POMs compounds such as decavanadate [54] and functionalized hexavanadate [55].

The aim of this paper is a contribution to a better understanding of the WPA anion reactivity and its inhibitor potency toward Na^+/K^+-ATPase. These interactions have been studied through a combined approach using both theoretical and experimental tools. We have investigated the action of the ATPase inhibitor WPA on the breakdown of extracellular ATP. The influence of WPA binding to Na^+/K^+-ATPase activity in conversion of ATP to ADP is supported by an analysis of ^{31}P NMR spectroscopy data. To achieve this goal, a theoretical analysis of various interactions of WPA, has been provided, with an emphasis on the changes of bonding nature depending on the oxygen position in the molecules (O1 or O2). Binding properties of Keggin anion in a biological system are illustrated using the PDB. A more efficient and elegant method to predict ligand binding sites involves the use of DFT calculations and the subsequent determination of the electrostatic potential (EP) values at the molecular surface of WPA.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical grade. Na^+/K^+-ATPase from porcine cerebral cortex and ATP were purchased from Sigma Chemicals Co. (Germany), as well as some chemicals for assay medium (magnesium chloride and Tris–HCl). WPA was prepared according to the literature