J. Chem. Thermodynamics 97 (2016) 290–296

Contents lists available at ScienceDirect

J. Chem. Thermodynamics

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

Thermodynamic study of the interaction between calcium and zoledronic acid by calorimetry

Mohamed A. Mostefa Side Larbi [a,b,c,*], Christophe Sauzet [a,*], Philippe Piccerelle [a,b], Pierre Cau [c,d], Nicolas Levy [c,e], Philippe Gallice [b,f], David Berge-Lefranc [b,f,*]

[a] Laboratoire de Pharmacie Galénique Industrielle et Cosmétologie, Faculté de Pharmacie, 27 Boulevard Jean Moulin, 13385 Marseille Cedex 5, France
[b] IMBE, UMR CNRS 7263, IRD 237, Aix-Marseille Université, Marseille, France
[c] INSERM, UMR S 910, Génétique Médicale et Génomique Fonctionnelle, Faculté de Médecine, 27 Bd Jean Moulin, 13385 Marseille Cedex 5, France
[d] AP-HM, Service de Biologie Cellulaire, Hôpital La Timone, 264 Rue Saint Pierre, 13385 Marseille Cedex 5, France
[e] AP-HM, Département de Génétique Médicale, Hôpital d'enfants Timone, 264 Rue Saint Pierre, 13385 Marseille Cedex 5, France
[f] Unité Chimie Physique & Prévention des Risques et Nuisances Technologiques Biogénotoxicologie – Santé Humaine et Environnement, Faculté de Pharmacie Aix Marseille Université, 27 Boulevard Jean Moulin, 13385 Marseille Cedex 5, France

ARTICLE INFO

Article history:
Received 16 June 2015
Received in revised form 29 January 2016
Accepted 4 February 2016
Available online 10 February 2016

Keywords:
Bisphosphonate
Zoledronic acid
Zolendronate
Calcium
Interactions
ITC study
Thermodynamic

ABSTRACT

Bisphosphonates (BPs) are widely used to treat calcium disorders because of their structural and functional similarities with the organic pyrophosphates present in plasma and urine. BPs are well known for their strong interactions with calcium, and they have been shown to bind to hydroxyapatite or bone; however, no model exists for studying in greater detail how BPs and particularly amino-bisphosphonates (N-BPs) such as zolendronate (Zol) bind to free calcium. The aim of this work was to determine the effect of pH on Ca^{2+}/Zol complex formation using isothermal titration calorimetry (ITC) because these effects might have important implications for the future development of a solid dosage form. In this study, using a predictive model, we can observe, the existence of three Ca^{2+}/Zol complexes. Knowledge of the binding constant for each complex is helpful for predicting the predominance of the different species at different Ca^{2+}/Zol ratios. Binding is due to ionic interaction between Ca^{2+} and the negative charges formed by dissociated Zol as a function of the *pKa*. Ca^{2+} fixation induces a strong rearrangement of the surrounding water molecules and causes proton release or uptake. The pH-dependent affinity of calcium for each site based on the model used in this work is proposed in detail, which might facilitate the development of new bisphosphonates and enable further elucidation of their mode of action.

1. Introduction

Bisphosphonates (BPs) are used to treat calcium disorders due to their structural and functional similarities with the organic pyrophosphates present in plasma and urine, which have been shown to be the major inhibitor of calcium phosphate. Pyrophosphate has been shown to prevent the in vitro formation of calcium phosphate crystals and their dissolution. In vivo, pyrophosphate plays a physiological role as an inhibitor of biomineralization [1].

Although the rapid hydrolysis of pyrophosphate considerably limits its application in the medical field, researchers have developed pyrophosphate structural analogs that exhibit physicochemical activities similar to those of pyrophosphate. The structural analogs are resistant to enzymatic hydrolysis and are therefore not metabolized.

Abbreviations: BPs, bisphosphonates; non N-BPs, non amino-bisphosphonates; Ca^{2+}, calcium; GLY, glycine–NaOH; HEPES, 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid; ITC, isothermal titration calorimetry; PIPES, piperazine-*N*-*N*′-bis(2-ethanesulfonic acid; $\Delta G°$, standard Gibbs energy change; $\Delta H°$, standard enthalpy change; $\Delta S°$, standard entropy change; TRIS, 2-amino-2-hydroxymethyl-propane-1,3-diol; Zol, zoledronic acid.

* Corresponding authors at: Faculté de Pharmacie Aix Marseille Université, Laboratoire de Pharmacie Galénique Industrielle et Cosmétologie, Faculté de Pharmacie, 27 Boulevard Jean Moulin, 13385 Marseille Cedex 5, France. Tel.: +33 4 91 83 55 48; fax: +33 4 91 78 75 75 (M.A. Mostefa Side Larbi). Tel.: +33 4 91 83 56 69; fax: +33 4 91 78 75 75 (C. Sauzet). Faculté de Pharmacie Aix Marseille Université, Unité Chimie Physique & Prévention des Risques et Nuisances Technologiques Biogénotoxicologie – Santé Humaine et Environnement, 27 Boulevard Jean Moulin, 13385 Marseille Cedex 5, France. Tel./fax: +33 4 91 83 56 70 (D.B. Lefranc).

E-mail addresses: mostefamohamed@ymail.com (M.A. Mostefa Side Larbi), christophe.sauzet@univ-amu.fr (C. Sauzet), david.berge-lefranc@univ-amu.fr (D. Berge-Lefranc).

http://dx.doi.org/10.1016/j.jct.2016.02.005
0021-9614/

The replacement of an oxygen atom in the molecular structure of pyrophosphate (P–O–P) with a carbon atom (P–C–P) generates a group of molecules resistant to pyrophosphatase hydrolysis while maintaining the physicochemical properties of pyrophosphate (figure 1). The P–C–P structure allows a large number of possible variations by changing the two side chains (R1, R2) on the carbon atom. Thus, two major categories of bisphosphonates exist (figure 1): non-amino bisphosphonates (non-N-BPs), also called first-generation compounds, and amino-bisphosphonates (N-BPs). One N-BP side chain bears an amine function as an aminoalkyl group (second-generation derivatives) or as a heterocycle with one or more nitrogen atoms (third-generation derivatives).

The early development of chemistry in this area was largely empirical and based on modifying R2 groups in a variety of ways. Apart from the general ability of BPs to chelate calcium ions (Ca^{2+}) and thereby target the calcium phosphate mineral component of bone, attempts to refine clear structure–activity relationships have led to ambiguous or seemingly contradictory results.

The first artificial non-N-BP, etidronate, was used to treat myositis ossificans [2]. Several BPs have been subsequently developed during the four last decades for the treatment of various diseases related to bone, teeth and calcium metabolism.

As pyrophosphates, BPs inhibit in vitro formation and aggregation of calcium phosphate crystals and decelerate their dissolution [3]. All these effects are related to the affinity of BPs toward the calcium phosphate solid phase (hydroxyapatite) on whose surface they strongly adsorb [4,5]. This property is the basis for using BPs in medicine because of their selective accumulation in bone.

In addition to their stabilizing role in bone hydroxyapatite mineral crystals, BPs also elicit cellular effects. In the acidic environment of the bone resorption lacuna, which is generated by the secretion of lysosomal contents by osteoclasts [6], BP are released from bone hydroxyapatite, then endocytosed and transferred from endosomes into the osteoclast cytosol, as demonstrated in studies using radiolabelled or fluorescent BP derivatives [7–10].

Within the cell cytosol, non-N-BPs are catabolized by aminoacyl-tRNA synthetases [11] into non-hydrolyzable derivatives of adenosine triphosphate (ATP) that can block mitochondrial ATP/AdenosineDiPhosphate translocase and trigger cell apoptosis, at least in vitro [12].

The observation that pamidronate, a BP with R1 = OH and R2 = $CH_2CH_2NH_2$, exhibits a higher potency than previously known BP drugs represented the first step toward the later recognition of the critical importance of having nitrogen in the R2 side chain (figure 1). A large number of nitrogen-containing BPs were synthesized and biologically evaluated in the 1980s, in particular, although these studies were still conducted with an incomplete understanding of their structure–activity relationships.

A major advance was the discovery that the anti-resorptive effects of N-BPs (including alendronate, risedronate, ibandronate, and zoledronate) on osteoclasts appear to be caused by their potency as inhibitors of the enzyme farnesyl pyrophosphate synthase (FPPS), which is a key branch-point enzyme in the mevalonate pathway resulting in the biosynthesis of isoprenoid groups and cholesterol [13,14].

This inhibition prevents the prenylation of several proteins, including small regulatory G proteins (GTPases Ras, Rab, Rho, Rac, etc.) involved in several intracellular signaling processes, and inhibits their function. FPPS inhibition and the resultant protein prenylation blockade lead to osteoclast cytoskeletal disorganization, disappearance of the ruffled brush border, and inactivation, followed by apoptosis [15]. FPPS inhibition also results in the production of another toxic ATP derivative [16].

Zoledronic acid (Zol), or zolendronate, is one of the most powerful BPs [17–19]. Its main structure has a P–C–P core with a hydroxyl group attached at the R1 position [20]. A heterocyclic imidazole group is attached at the R2 position and differentiates the chemical structure of Zol from those of other BPs [21] (figure 2).

Zol is available for therapy in parenteral dosage forms because the oral form exhibits low bioavailability. This low bioavailability is certainly due to the BP/Ca^{2+} interaction; it is also likely due to in vivo formation of insoluble BP/Ca^{2+} complexes [22]. The improvements in oral bioavailability have yielded a good understanding of the interactions between Zol and Ca^{2+} as a function of the different physiological pH and calcemia. We studied this phenomenon using isothermal titration calorimetry (ITC) and measured the binding affinities of Zol with Ca^{2+} at different pH levels. The pH range used was chosen in order to reach the different dissociation states of phosphonates groups. The knowledge of the affinity evolution with pH will be useful in order to understand the behavior of Zol–Ca^{2+} complexes under in vivo conditions (pH around 2 to 4 and 7 to 8 for gastric and intestine conditions respectively).

2. Experimental section

2.1. Reagents

Calcium chloride, $CaCl_2$, $2H_2O$ was purchased from Prolabo (purity > 98%). Zoledronic acid, Zol, H_2O was purchased from Sigma-Aldrich (purity ⩾ 98%). The number of coordinated water molecules was determined using thermogravimetric analysis (monohydrate drug). All of the buffers —piperazine-*N*-*N*′-bis(2-ethanesulfonic acid) (PIPES), 4-(2-hydroxyethyl)-1-piperazineetha nesulfonic acid) (HEPES), glycine–NaOH (GLY) and 2-amino-2-hy droxymethyl-propane-1,3-diol (TRIS)— were obtained from Sigma-Aldrich (P1851-25G, H4034-25G, 410225-50G and T5030-50TAB, respectively). Buffers were chosen for their range of ionization enthalpy. The sodium chloride was also purchased from Sigma-Aldrich (S7653-250G). Water was purified using a Millipore Simplicity® UV filtration system (resistivity = 18.2 MΩ·cm).

$CaCl_2$ and Zol powders were dissolved in solutions containing 50 mM buffer and 150 mM sodium chloride. Solution pH was adjusted from 7 to 9.5 when necessary by adding concentrated microvolumes of NaOH or HCl.

2.2. Isothermal titration calorimetry

Isothermal titration calorimetry (ITC) was performed on an ITC200 instrument (Microcal, Northampton, United States) at 25 °C in PIPES, HEPES, GLY and TRIS buffers. Forty microliters of Ca^{2+} solutions ranging in concentration from 19 to 22 mM were added by syringe to the sample cell containing Zol solutions with concentrations ranging from (0.9 to 1.1) mM. The Ca^{2+} solution was added sequentially after an initial injection of 0.5 μL (not used in data fitting [23]) by adding 39 1-μL injections at 90 s intervals. The reference power varied from (13 to 33) μW, and the feedback gain was modified according to the thermal power of the peaks. Each experiment was repeated three times. We corrected the signal by subtracting blank titrations to remove dilution and mixing enthalpies. The data were analyzed using a "successive binding sites model". The binding constants and enthalpy variations of binding were estimated by fitting experimental values with the "successive binding sites model". Fitting was performed using the Levenberg–Marquardt non-linear curve-fitting algorithm in Microsoft Excel®.

Proton-exchange processes were evidenced by performing ITC experiments in different buffers possessing different ionization enthalpies. The enthalpy variation observed ($\Delta H°_{app}$) is the sum