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Protonation–deprotonation and structural dynamics of antidiabetic drug metformin

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ABSTRACT

Since the late 1950s, metformin is the worldwide first-line pharmacologic treatment for type 2 diabetes. Beyond the fact that the mode of action of this drug has always been very difficult to elucidate, little is known about its physicochemical properties in aqueous solution. Herein, we focus on the protonation–deprotonation features of metformin by using jointly Raman scattering and theoretical calculations. Vibrational markers evidence the fact that within a wide pH interval extended at either side of the physiological one, i.e. $\sim 7 \pm 4$, metformin is mainly monoprotonated. Although the biprotonated form appears as major population at very low pH values (<1.5), Raman markers of neutral species do not dominate even at very high pH values (>13), presumably because of the extreme basicity of metformin as described by recent NMR measurements. Density functional theory calculations using both explicit and implicit hydration models, have led to presume a possible coexistence of two possible monoprotonated forms in aqueous environment. In conclusion, the biophysical features of this molecule and the amount used in clinical practice might certainly explain the pleiotropic actions toward several targets where metformin could be a permanent cationic partner, a proton donor/acceptor, as well as a good candidate for stabilizing the so-called $\pi \rightarrow \pi$ interactions.

1. Introduction

Metformin (*N*,*N*-dimethylbiguanide), hereafter referred to as *Metf*, is one of the structurally simplest biguanide compounds (Scheme 1). Metf has become since the late 1950s a worldwide first-line pharmacologic treatment for type 2 diabetes [1]. It has also pleiotropic actions as anticancer agent [2]. Nevertheless, its mode of action has always been very difficult to elucidate. Recently, the inhibition of the mitochondrial isoform of glycerophosphate dehydrogenase, transferring a pair of electrons to the electron transport chain restraining the use of glycerol and lactate, as gluconeogenic precursors and decreasing the level of blood glucose, was demonstrated [3]. In contrast to many other drugs such as statin, an antihypertension prescribed at a dosage of <50 mg a day, Metf is used at a daily dose as high as 2000 mg in millions of people, with a consequent environmental impact on water [4].

Despite the increasing biological and therapeutic importance of Metf, there is a manifest lack of information on the biophysical properties of this drug in aqueous media, where it is supposed to interact with its targets. More specifically and in relation with this report, the major part of the existing literature data on the structural and vibrational features of Metf correspond to solid phase, as obtained by X-ray diffraction [5–7], UV absorption [7,8], FT-IR [8–11] and Raman [8,11] spectra. Nevertheless, a few number of recent investigations were devoted to the aqueous solution physicochemical properties of Metf by analyzing (i) its adsorption on silver nanoparticles as followed by surface-enhanced Raman spectroscopy [12], (ii) its oxidative reactions as a function of pH by gamma irradiation and HPLC/mass spectrometry [13], and (iii) its pH-dependent chemical shifts by ^{1}H NMR measurements [14]. During the recent years, ground-state quantum mechanical calculations were carried out in order to probe (i) the structural and vibrational features of Metf in an isolated (in vacuum) neutral form (Scheme 1A) by means of Hartree–Fock (HF) and density functional theory (DFT) approaches [11] and (ii) the conformational properties of the implicitly hydrated species by DFT method [13]. Particularly,

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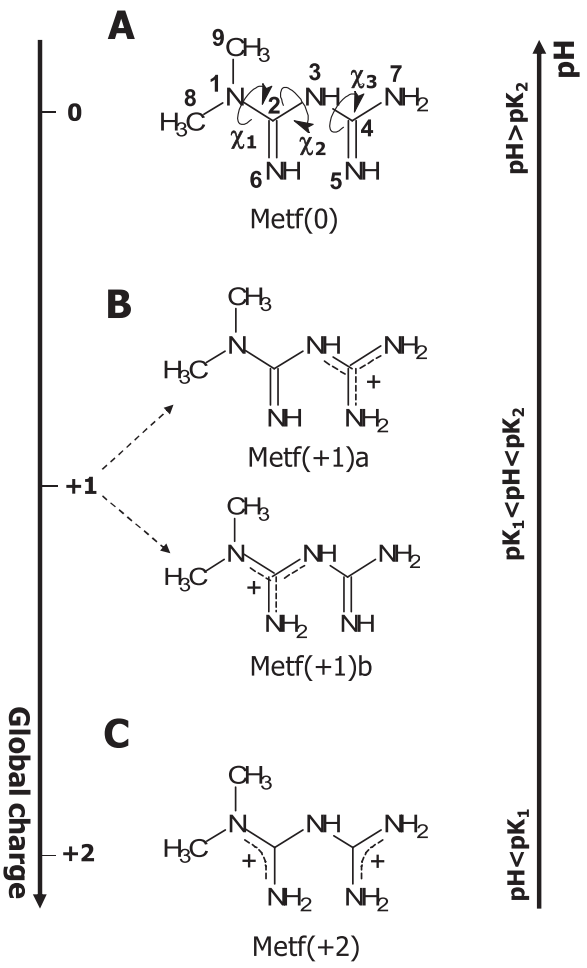

Scheme 1. Protonation–deprotonation of metformin. (A) Neutral form, Metf(0), with heavy atom numbering and definition of the three skeletal torsion angles χ_1(C_8-N_1-C_2-N_3), χ_2(N_1-C_2-N_3-C_4) and χ_3(C_2-N_3-C_4-N_7). (B) Monoprotonated forms, Metf(+1)a and Metf(+1)b. (C) Biprotonated form, Metf(+2).

the latter theoretical investigation revealed one of the two possible monoprotonated forms, i.e. referred to as Metf(+1)a (Scheme 1B), as the lowest energy one. This form was thus suggested as the most populated one in aqueous environment around neutral pH. However, it is a matter of fact that an implicit hydration model based on a solvent continuum is well adapted for reflecting the so-called bulk effects on a solute, i.e. those effects arising from remote water molecules. A better description of solvent effects needs the presence of explicit water molecules that are known for their particular role in decreasing the energy barriers separating different forms of a given species.

It should be stressed that Metf has two distant pK_a values, referred to here as pK_1 and pK_2, corresponding to the biprotonated/monoprotonated, and monoprotonated/neutral form conversions, respectively (Scheme 1A–C). Although the widely spread values of these parameters are known as: pK_1 = 2.8 and pK_2 = 11.6 [7,13], a recent investigation based on the chemical shift measurements as a function of pH has suggested higher values such as pK_1 = 3.1 and pK_2 = 13.8 [14]. Recently, the strength of the solution Raman scattering to follow the protonation–deprotonation in the glycine backbone [15], as well as in the cysteine side chain and backbone [16], was emphasized. In addition, pH-dependent Raman markers could be analyzed by means of a multiconformational analysis [15–18], which consists in constructing the Raman spectrum of a given molecular species by the thermal average of the calculated spectra arising from its low-energy conformers. Herein, this combined experimental/theoretical methodology has been applied to probe the protonation–deprotonation and structural dynamics of Metf.

2. Material and method

2.1. Sample preparation

Metformin.HCl powder (lot MB250305-REFP) was a courtesy from Merck (Lyon, France). It was dissolved in water taken from a Millipore filtration system. Sample concentration was maintained between 50 and 70 mM (~8–12 g/L). D_2O, 100% purity, was from Euriso-top (Saclay, France). pH was measured with an accuracy of ±0.1 just before and after each Raman experiment. Upon dissolution of Metf in H_2O, pH was ca. 7. Lower pH values (down to 1) were reached by adding drops of HCl to the samples. Conversely, higher pH values (up to 12) were reached by adding NaOH to the solution. To make sure the achievement of neutral species (Scheme 1A) in solution, Raman spectrum was measured on a sample containing Metf dissolved directly in NaOH (1 N). The pH value of the latter sample was estimated to be >13. Special care was taken to avoid contamination of NaOH containing solutions by carbonate ions. This could be checked by the absence of the intense band at ~1067 cm^{-1} (carbonate ion marker) in Raman spectra.

2.2. Raman scattering measurements

Samples containing 50 mL of Metf were placed in a suprasil quartz cell (5 mm path length) and excited by the 488 nm line of an Ar^+ laser (Spectra Physics, Santa Clara, CA, USA) with a ~200 mW power at the sample. Scattered light at right angle was analyzed on a Jobin-Yvon T64000 (HORIBA Jobin-Yvon, Lonjumeau, France) in a single spectrograph configuration with a 1200 grooves per mm holographic grating and a holographic notch filter. Stokes Raman data were collected by means of a liquid nitrogen-cooled CCD detection system (Spectrum One, Jobin-Yvon). The effective spectral slit width was set to ~5 cm^{-1}. Each recorded spectrum corresponds to a total acquisition time of 1200s. Buffer subtraction and smoothing of the observed Raman spectra could be performed using the GRAMS/AI Z.00 package (Thermo Galactic, Waltham, MA, USA). The final presentation of these spectra was performed by means of SigmaPlot package 6.10 (SPSS Inc., Chicago, IL, USA).

2.3. Theoretical framework

Energetic and geometrical data as well as harmonic wavenumbers and Raman activities of Metf conformers were estimated by DFT approach [19]. Hybrid B3LYP functional [20,21], along with polarized triple-zeta Gaussian atomic basis sets, referred to as 6-311++G(d,p), were used. The adequacy of this level of theory was previously confirmed by the calculations on various organic compounds of biological interest [15–18]. To take into account the hydration effects, two different models were considered. (i) A purely implicit approach by placing the solute in a cavity within the solvent reaction field. The used hydration model is based on the so-called integral equation formalism variant of the polarizable continuum model (IEFPCM) [22,23]. The relative permittivity of the solvent continuum was supposed to be that of water, i.e. ε_r = 78.39. (ii) A combination of implicit and explicit hydration models realized by embedding in the aforementioned continuum the solute which is surrounded by n water molecules (here n = 1 or 3) interacting with its imino or amino groups. Prior to calculations in the presence of explicit solvent, the deepest energy minima were explored in continuum through the variation of the electronic energy (E_e) versus skeletal torsion angles (χ_1, χ_2 and χ_3) defined in Scheme 1. To do this, successive rotations of a torsion angle