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Primary antioxidant and metal-binding effects of tiopronin: A theoretical investigation of its action mechanism

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ABSTRACT

In this work, we have carried out a quantum chemistry and computational kinetics study on the reactivity of tiopronin, a synthetic compound used mainly to combat cystinuria. We show that, as proposed earlier by several authors (Date et al., 2002; Horwitz et al., 1994) tiopronin is also an excellent antioxidant. We have found that it is an exceptionally good scavenger of free radicals, with rate constants ranging from 2.87×10^7 for reaction with the OOH radical, to 3.57×10^8 for reaction with OCH_3. It is also a good chelating agent that is capable of forming complexes with copper, thus reducing the formation of hydroxyl radicals through the Fenton reaction. The amide moiety in the vicinity of the SH group plays a crucial role in this behavior by reducing the pK_a and therefore facilitating the formation of the tiopronin S^- anion, which is an excellent electron donor. On the other hand, the carboxylate moiety forms a strong hydrogen bond with an OOH radical, thus helping H atom abstraction from a neutral SH group.

1. Introduction

Reactive Oxygen Species (ROS) can be formed as sub-products of cellular respiration or by exposure to external factors such as solar radiation or pollutants. ROS can cause the oxidation of DNA, proteins and lipids, and they appear to play an important role in the pathogenesis of many neurodegenerative processes such as Alzheimer's and Parkinson's diseases [3,4], as well as other important diseases like cancer and atherosclerosis. Cells have sophisticated regulatory antioxidant systems that balance the formation and destruction of free radicals. However, when this equilibrium is broken, oxidative stress and injuries to tissues may occur [5,6].

Several studies have shown that metals like iron, chromium, copper, lead, mercury, nickel and vanadium generate ROS [7]. The metal ions contribution to ROS generation is commonly thought to occur by Fenton-type reactions, where endogenous metals such as Fe^{2+} or Cu^+ react with hydrogen peroxide to generate the hydroxyl radical (·OH) [8].

There is a wide variety of non-enzymatic antioxidant compounds as polyphenols, flavonoids, and molecules containing sulfur and selenium. The relationship between structure and antioxidant activity has been shown to be very important in the search for new potent antioxidants [9,10]. Several studies have focused on the use of sulfur compounds in the treatment and prevention of diseases, and they have established their antioxidant effect. Besides ROS scavenging, sulfur compounds tend to bind with metals, thus providing additional protection against cellular oxidative damage [11–16].

Tiopronin (Tpn) or N-(2-mercaptopropionyl)-glycine, Fig. 1, is a low molecular-weight synthetic analogue of glutathione, which is an important endogenous antioxidant. Tpn is used to treat cystinuria [17,18], and rheumatoid arthritis [19]. It has been demonstrated that the level of oxidative stress increases in an *in vivo* murine model of pressure overload hypertrophy, and that therapy with Tpn inhibits cardiac hypertrophy [1]. In a study in cultured cardiac myocytes, Tpn was found to be more effective than dimethylthiourea in preventing cytotoxicity caused by hydrogen peroxide, while being non-toxic even at high concentrations [20]. Horwitz et al. [2] have shown that Tpn is an excellent hydrogen peroxide scavenger, which contributes to the protection of myocytes during myocardial ischemia and reperfusion. Tiopronin, is reported to be able to suppress lipid peroxidation, thus attenuating tissue damage [21]. Tpn may find use as a nephroprotective agent to reduce the toxicity associated with cisplatin treatment [22].

Tpn is also used in mercury and copper poisoning [23,24]. The inhibition of peptidylglycine α-amidating, monooxygenase (PAM) by homocysteine-extended peptides [25] and by captopril ((2S)-1-(3-mercapto-2-methylpropionyl)-L-proline) [26] has been attributed to the interaction of a sulfur atom with an enzyme-bound copper. Similarly, inhibition of dopamine β-hydroxylase (DbM) by captopril, cysteine, and glutathione has been ascribed to in situ

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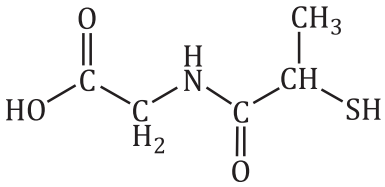

Fig. 1. Tiopronin.

chelation of the enzyme-bound coppers [27,28]. McIntyre and coworkers [29] have demonstrated that two sulfur-containing drugs, tiopronin and thiorphan, inhibit PAM.

In this paper, the antioxidant activity of tiopronin has been studied. Since the primary activity of an antioxidant is free radical scavenging, we tested its reactivity against five different free radicals through two reaction mechanisms: electron transfer and hydrogen transfer. In addition, Tpn's secondary antioxidant activity was evaluated by studying the formation of a tiopronin–copper complex as well as the ability of these complexes to reduce the formation of hydroxyl radicals through Fenton-type reactions.

2. Computational methodology

All electronic calculations were performed with the Gaussian 09 package of programs [30]. Geometry optimizations and frequency calculations were carried out using the M06-2X [31] functional and the 6-31++G(d,p) basis set, using an ultrafine grid, in conjunction with the SMD continuum model [32] using water as solvent to mimic aqueous environments. The M05-2X and M06-2X functionals have been recommended and tested for kinetic calculations by their developers [31]. M05-2X has been successfully used by independent authors [33–38] and M06-2X was developed as an improvement over its predecessor M05-2X. It is also among the best performing functionals for calculating reaction energies involving free radicals [39]. SMD is considered to be a universal solvation model, due to its applicability to any charged or uncharged solute in any solvent or liquid medium for which only a few key descriptors are known [32]. Unrestricted calculations were used for open shell systems. Local minima and transition states were identified by the number of imaginary frequencies; local minima have only real frequencies, while transition states are identified by the presence of a single imaginary frequency that corresponds to the expected motion along the reaction coordinate. Relative energies were calculated with respect to the sum of the energies of the isolated reactants. Thermodynamic corrections at 298.15 K were included in the calculation of relative energies, which correspond to 1 M standard state, following the quantum mechanics-based test for the overall free radical scavenging activity (QM-ORSA) protocol [40]. This computational protocol has been validated by comparison with experimental results, and its uncertainties have been proven to be no larger than those arising from experiments [40].

In addition, solvent effects on entropy loss in the liquid phase have been taken into account according to the free volume theory [41]. The rate constants (k) were calculated using conventional transition state theory (TST) [42–44]:

$$k = \sigma\kappa\frac{k_B T}{h}e^{-(\Delta G^{\neq})/RT} \tag{1}$$

where k_B and h are respectively the Boltzmann and Planck constants; $\Delta G^{\neq}$ is the Gibbs free energy of activation; σ represents the reaction path degeneracy, which accounts for the number of equivalent reaction paths; and κ is the tunneling correction. The latter is defined as the Boltzmann average of the ratio of the quantum and the classical probabilities, and it is calculated using Eckart Barrier [45]. For single electron transfer reactions (SET), the barriers were estimated using Marcus theory [46,47], which relies on the transition state formalism. In this theory, the SET activation barrier ($\Delta G^{\neq}_{SET}$) is defined in terms of two thermodynamic parameters, the free energy of reaction (ΔG^{0}_{SET}) and the nuclear reorganization energy (λ)

$$\Delta G^{\neq}_{SET} = \frac{\lambda}{4}\left(1 + \frac{\Delta G^{0}_{SET}}{\lambda}\right)^2 \tag{2}$$

The reorganization energy (λ) is calculated as

$$\lambda = \Delta E_{SET} - \Delta G^{0}_{SET} \tag{3}$$

where ΔE_{SET} is the nonadiabatic energy difference between reactants and vertical products. This approach is similar to the one previously used by Nelsen and co-workers [48] for a large set of self-exchange reactions.

Some of the calculated rate constant (k) values are close to, or within, the diffusion-limit regime. Accordingly, the apparent rate constant (k_{app}) cannot be directly obtained from TST calculations. In the present work, the Collins–Kimball theory [49] is used for that purpose:

$$k_{app} = \frac{k_D k}{k_D + k} \tag{4}$$

where k is the thermal rate constant, obtained from TST calculations, and k_D is the steady-state Smoluchowski [50] rate constant for an irreversible bimolecular diffusion-controlled reaction:

$$k_D = 4\pi R D_{AB} N_A \tag{5}$$

where R denotes the reaction distance, N_A is the Avogadro number, and D_{AB} is the mutual diffusion coefficient of reactants A (free radical) and B (Tpn). D_{AB} has been calculated from D_A and D_B according to Ref. [51], and D_A and D_B have been estimated from the Stokes–Einstein equation [52,53]:

$$D = \frac{k_B T}{6\pi\eta a} \tag{6}$$

where k_B is the Boltzmann constant, T is the temperature, η denotes the viscosity of the solvent (in our case water, $\eta = 8.91 \times 10^{-4}$ Pa s), and a is the radius of the solute.

The formation constants of the copper complexes were calculated with the expression:

$$K = e^{-\frac{\Delta G^0}{RT}} \tag{7}$$

where ΔG^0 is the Gibbs free energy of formation.

3. Results and discussion

3.1. Acid/base equilibria

Tpn has three pK_a values. Only two of them are significant in biological media: 3.6 and 8.74, [54] which correspond to deprotonation of the carboxyl group and of the thiol group, respectively. It is important to point out that, in general, the thiol deprotonation pK_a is larger than 10. In Tpn, the electron withdrawing effect of the amide moiety lowers the corresponding pK_a.

With these pK_a values, it was found that, at physiological pH, the molar fraction of the neutral species of Tpn (H_3Tpn) is <0.0001, while for the anionic (H_2Tpn^-) and dianionic species ($HTpn^{2-}$) these values are 0.96 and 0.04, respectively (Fig. 2). Thus, in this research, we used only H_2Tpn^- and $HTpn^{2-}$ because the amount of neutral Tpn is negligible.