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Reactions and structural investigation of chlorpromazine radical cation

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Abstract

Experimental and theoretical studies have been carried out to understand pro-oxidant behaviour of chlorpromazine radical cation ($CPZ^{\cdot+}$). Pulse radiolysis studies have shown that $CPZ^{\cdot+}$ oxidizes physiological antioxidants (uric acid and bilirubin), and biomolecules like, tyrosine and proteins (bovine serum albumin and casein), thereby acting as a pro-oxidant. Ab-initio quantum chemical calculations suggest structural and electronic changes on oxidation of CPZ. The calculations with Hartree–Fock and density functional methods show that ring nitrogen atom is the site of electron removal from CPZ and sulfur atom is the site of maximum spin in $CPZ^{\cdot+}$. The calculations also suggest that oxidation of CPZ leads to increase in planarity of the tricyclic ring as well as tilting of alkyl side chain towards chlorine containing ring. The structural changes on oxidation of CPZ and spin delocalization in $CPZ^{\cdot+}$ fairly explain the pro-oxidant activity of CPZ.

Keywords: Chlorpromazine radical cation; Pro-oxidant; Pulse radiolysis; Ab-initio calculations

1. Introduction

Phenothiazine derivatives are widely used as tranquilizers, psychotropic drugs and sensitizers for utilization of solar energy [1,2]. Phenothiazines (PTZ) are also known to photoionize in aqueous solution by UV-light in photovoltaic action to produce stable radical cation ($PTZ^{\cdot+}$) [1–3]. The widespread use of PTZs has resulted in a large number of studies on their chemical properties and reactivity [2,4–6]. Among phenothiazines, chlorpromazine (CPZ) is a representative one and is also used as a drug in psychopharmacology (Scheme 1). The nature of substituents on the tricyclic ring at 2-position and alkyl side chain on nitrogen atom (N_{13}) of PTZ drugs have been shown to result in variations in their color, solubility, psychotropic activity, reduction potential of their radical cation [7] and radical stability [8–13]. Free radical cations derived from phenothiazine derivatives are relatively stable and are also suggested as important intermediate in their biochemical activity, metabolism and side effects [4,14–17].

The tricyclic ring structure of CPZ is hydrophobic making it soluble in the bulk hydrocarbon phase of membrane bilayer systems, while the hydrophilic tertiary propylamine tail region is soluble in the polar headgroup region of membrane bilayers. Moreover, the propylamine chain of the CPZ is cationic ($pK_a = 9.3$) at physiological pH resulting in electrostatic binding with the anionic phospholipids of the cell membrane. Therefore, in addition to blockage of specific cellular receptors, the diverse effects of CPZ may also be attributable to the amphipathic nature of the drug. Any structural change of CPZ can affect its binding with membrane bilayer resulting in different physicochemical behaviour [3,4].

The $PTZs^{\cdot+}$ produced in aqueous as well as non-aqueous systems can be characterized by their absorption maximum at ~520 nm [18–20]. In aqueous solution, reaction of CPZ with hydroxyl radical has been reported to produce absorption bands at 270, ~310, 440, 530 nm and also a broad absorption in 700–900 nm region [18–20]. Recently,

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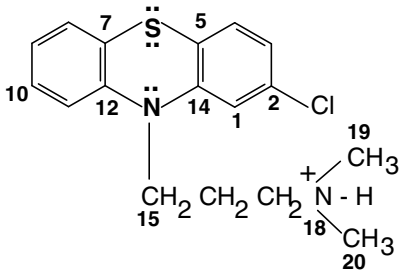

Scheme 1. Chlorpromazine (CPZ).

phenothiazine derivatives have also been reported to have anti-infective and anticancer activities suggesting a pro-oxidant behaviour [21,22]. The reduction potential value of $CPZ^{\cdot+}$/CPZ pair (860 ± 10 mV at pH 5–7 [23]) also suggests its pro-oxidant behaviour. This has prompted us to study the reactions of $CPZ^{\cdot+}$ with biomolecules to understand its pro-oxidant behaviour. In this context, theoretical calculations have also been performed to understand the charge and spin distribution on CPZ and $CPZ^{\cdot+}$, respectively, and structural changes on oxidation of CPZ. Structural and electronic changes on oxidation of CPZ have been studied because structure and binding of the PTZ drugs are known to play an important role in their pharmacological action [3,4].

2. Materials and methods

2.1. Chemicals

CPZ (98%) was obtained from Sigma and was used as received. All other chemicals were of Analytical Reagent grade. De-ionized water (conductivity < 0.06 μS cm^{-1}) was used for preparing solutions. Fresh solutions of samples were used for the experiments. Phosphate buffer (2×10^{-3} mol dm^{-3}) has been used to prepare solutions at pH 7. High purity (>99.9%) N_2 and N_2O were used for purging solutions as per requirement.

2.2. Pulse radiolysis

The pulse radiolysis system giving pulses of 7 MeV electrons from a linear electron accelerator has been used and is described elsewhere [24]. High-energy electrons deposit energy on their passage through water/aqueous solutions to generate its reactive radicals and molecular products (Eq. (1)).

$$H_2O \rightarrow e^-_{aq}\ (0.28),\ H^{\cdot}\ (0.062),\ {}^{\cdot}OH\ (0.28),\ H_3O^+\ (0.28),\ H_2\ (0.047),\ H_2O_2\ (0.072) \quad (1)$$

The values in parentheses in Eq. (1) are radiation chemical yield in the unit of micromoles per Joule of absorbed energy. The absorbed dose was measured using an air-saturated aqueous solution containing 5×10^{-2} mol dm^{-3} KSCN ($G\varepsilon = 2.6 \times 10^{-4}$ m^2 J^{-1} at 475 nm) [25]. The kinetic spectrophotometric detection system covered the wavelength range from 250 to 800 nm. The optical path length of the cell was 1.0 cm. The width of the electron pulse was 50 or 500 ns as per requirement.

Relative absorption of radicals in the UV–vis region has been observed against time and wavelength to get kinetic and absorption characteristics, respectively. The bimolecular rate constants were calculated by plotting pseudo-first order rate of formation of the solute transient against the concerned solute concentration. The uncertainty in the measurement of wavelength, transient absorption and least square fitting to get bimolecular rate constants are ~2 nm, <10% and <5%, respectively.

2.3. Quantum chemical calculations

Unrestricted Hartree–Fock (HF) method has been used for the geometry optimization of ground state and radical cation of chlorpromazine, both in the protonated form of propylamine side chain. The ab-initio calculations have been performed using the GAMESS electronic structure program [26] with 6-31G(d,p) basis sets for all the atoms. The calculations have been performed without any symmetry restriction to get the geometry of the most stable structure of CPZ and $CPZ^{\cdot+}$. Further, the structures optimized with HF/6-31G(d,p) have been used to calculate their single point energy with DFT method using B3LYP functional and 6-31G(d,p) basis set.

3. Results and discussion

The experiments have been performed at physiological pH (~7) where the nitrogen on the propylamine side chain exists in the protonated form ($pK_a = 9.3$). $CPZ^{\cdot+}$ has been produced by the radiolysis of aqueous CPZ solution. The reactions of $CPZ^{\cdot+}$ with some biomolecules have also been studied using pulse radiolysis technique.

3.1. Reaction with oxidants and hydroxyl radical

The reaction of CPZ with oxidizing radicals (hydroxyl radical (·OH), azidyl $N_3^{\cdot}$, bromide radial anion ($Br_2^{\cdot-}$) and trichloromethyl-peroxyl ($CCl_3O_2^{\cdot}$) radicals) has been well studied and the produced transients are also assigned [11–15,18,20,23,27–31]. We have studied the reaction of CPZ with carbonate radical anion ($CO_3^{\cdot-}$) because HCO_3^-/CO_3^{2-} anion pair is known to serve as a buffer in the blood. The ·OH reacts with HCO_3^-/CO_3^{2-} anions to generate oxidizing carbonate radical anion ($CO_3^{\cdot-}$) at neutral pH (Eqs. (2)–(4)) [32].

$$HCO_3^- + {}^{\cdot}OH \rightarrow {}^-OH + HCO_3^{\cdot} \quad (2)$$

$$CO_3^{2-} + {}^{\cdot}OH \rightarrow {}^-OH + CO_3^{\cdot-} \quad (3)$$

$$HCO_3^{\cdot}\ (pK_a < 0) \leftrightarrow H^+ + CO_3^{\cdot-} \quad (4)$$

$$N_2O + e^-_{aq} \rightarrow N_2 + {}^-OH + {}^{\cdot}OH \quad (5)$$