Reaction of substituted pyrimidines with photochemically generated \( t\)-BuO\(^\bullet\) radicals

L Charitha and M Adinarayana*
Department of Chemistry, Osmania University, Hyderabad 500 007, India

Received 7 March 2005; revised 2 September 2005

Humans are exposed to various organic peroxides through chemical, pharmaceutical and cosmetic products. On photolysis, these peroxides produce alkoxyl radicals and hydroxyl radicals. The reaction of \( ^*\)OH radicals with DNA and its constituents have been extensively studied, but very little is known about the reactions of alkoxyl radicals with DNA and its constituents. In view of this, the oxidation of pyrimidine bases viz., thymine, uracil, cytosine, 5-bromouracil, 6-methyluracil and 1, 3-dimethyluracil by \( t\)-BuO\(^\bullet\) radicals in aqueous solution at pH 7.5 has been carried out. The reaction between pyrimidine and \( t\)-BuO\(^\bullet\) is followed by measuring the absorbance of pyrimidine at the respective \( \lambda_{\text{max}} \). The rates of oxidation of pyrimidines are calculated from the plot of absorbance vs time. The rates of oxidation of pyrimidines have been found to increase with increase in [\( t\)-BuOOH], [pyrimidine] and light intensity. The quantum yields are calculated from the initial rates of oxidation of pyrimidine and the measured light intensity at 254 nm the wavelength at which \( t\)-BuOOH is activated to give radicals. The quantum yields are found to depend on [pyrimidine] as well as on [\( t\)-BuOOH] while they are independent of light intensity. The product analysis was carried out on HPLC with UV-visible detector. The corresponding 5,6-dihydroxypyrimidine and isobarbituric acid have been identified by comparing the retention times of the authentic samples.

On the basis of experimental results and product analysis, it is suggested that \( t\)-BuOOH on photolysis gives \( t\)-BuO\(^\bullet\) radical, which initiates the reaction by adding to C (5) or C (6) position of pyrimidine base, leading to the formation of pyrimidine base radical via hydrolysis. The pyrimidine radical further reacts with \( t\)-BuO\(^\bullet\) radical to give the final product. This study predicts the probable transient pyrimidine radicals.

**Keywords:** tert-butyl hydroperoxide, \( t\)-BuO\(^\bullet\) radical, pyrimidine bases, oxidation of pyrimidines by \( t\)-BuO\(^\bullet\)

**IPC Code:** C07D 239/00, C07H 1906

Metabolic degradation of endogenous and exogenous peroxides is thought to play a role in the etiology of several diseases including cancer\(^1\)-\(^4\). The one-electron reduction of peroxide gives alkoxyl radicals, which is significant in the presence of high concentrations of the hydroperoxide. Relatively little is known about the biological effects of these radicals and the key cellular targets for these species. It is not known whether strand breakage is the major form of DNA damage or attack at the base moiety is also important\(^5\)-\(^6\).

Previous studies on the reactivity of tert-butoxy radicals (\( t\)-BuO\(^\bullet\)) suggest that this species might be expected to attack both the sugar and the base moiety of DNA\(^7\). In order to understand the mechanism of oxidation of pyrimidine, we have carried out the kinetics of reaction of \( t\)-BuO\(^\bullet\) radicals with substituted pyrimidines viz., uracil, cytosine, thymine, 5-bromouracil, 6-methyluracil and 1,3-dimethyluracil.

In the present work, \( t\)-BuO\(^\bullet\) radicals have been generated by steady state photolysis of tert-butyl hydroperoxide (\( t\)-BuOOH) in the presence of \( t\)-butanol (\( t\)-BuOH) to scavenge \( ^*\)OH radicals in aqueous solution\(^8\).

**Materials and Methods**

Pyrimidine bases viz., uracil, cytosine, thymine, 5-bromouracil (5-BrU), 6-methyluracil (6-MU) and 1,3-dimethyluracil (1,3-DMU) were purchased from Sigma Chemical Co., St. Louis, USA. All solutions were prepared afresh using double distilled water. tert-Butyl hydroperoxide (\( t\)-BuOOH) was from Merck-Schuchardt, Germany. No contamination of other peroxides was found in the assay of the sample. \( t\)-BuOOH was estimated by iodometric method\(^9\).

The photochemical reactor used was of Srinivasan Griffin Rayonet type, which contained four 18 W medium pressure mercury lamps arranged in a circular way. The quartz cuvette containing the sample was placed in the middle of the well of the reactor using an external support and the cuvette was exposed to light from both sides of the transparent...
surfaces and the irradiations were uniform throughout the bulk of the solution. This was confirmed by the reproducibility of results in duplicate runs. The irradiations were interrupted at regular intervals of time and the absorbance was noted. The light intensity corresponding to the irradiating wavelength (254 nm) was measured using peroxysulphate chemical actinometry. On photolysis, t-BuOOH was activated to generate 'OH and t-BuO• radicals by homolytic cleavage of O-O bond. The 'OH radicals produced were completely scavenged using 1.0 M concentration of t-BuOH, which was calculated from competition kinetics. The 'OH radicals are more deleterious over t-BuO• radicals to the integrity of pyrimidines, if they are not scavenged by t-BuOH. In a typical reaction, the aqueous reaction mixture containing desired concentration of pyrimidine, t-BuOH and t-BuOOH was taken in a 1 cm path length quartz cuvette, suitable for both irradiations and absorbance measurements. The absorbance measurements were made at the \( \lambda_{\text{max}} \) of pyrimidine on a Chemito 2100 UV-visible spectrophotometer. The initial rate of oxidation of pyrimidine was calculated from absorbance-time data using microcal origin computer program on a personal computer. The rates were found to be within ± 5%.

The HPLC system used for analysis of products was Shimadzu LC-10AT equipment with a dual piston-pump system, a solvent programmer and a Reodhyne injector model 7725 fitted with 20 μl loop. A prepacked octadeclsilil gel ODS hypersil column 25 cm × 0.46 cm, mean particle size 5 μm was used. The column effluents were monitored at 220 nm, using variable wavelength SPD-10A UV-visible detector equipped with an 8 μl flow cell and attached to a computer through communications bus module (CBM) 101. The 20 μl of reaction mixture or authentic samples were injected into the 20 μl loop. Samples were eluted with aqueous solutions containing 5% (v/v) methanol and buffered with 10 mM KH₂PO₄ solution adjusted to pH 7.0. Before use, the phosphate buffer was filtered through a millipore type HA 0.45 μm membrane filter. All mobile phases were degassed using a vacuum pump. The solvent flow rate was kept constant at 1.0 ml/min and all the HPLC runs were carried out at ambient temperature.

Results and Discussion

Oxidation of pyrimidines has been carried out, by irradiating the aqueous solution containing t-BuOOH, substrate and t-butanol (t-BuOH), and the rates of oxidation have been measured under different experimental conditions. The rates of oxidation of pyrimidines by t-BuO• are found to increase with increase in [pyrimidine] and also [t-BuOOH] at constant intensity. The irradiated aqueous reaction mixture has been analyzed on HPLC using UV-visible detector. In the oxidation of thymine by t-BuO•, three peaks were observed. The peaks with retention times 10.3, 3.2 and 7.2 min correspond to thymine and 5, 6-dihydroxythymine and substituted isobarbituric acid, respectively, which were further confirmed by authentic samples (Fig. 1A). Similarly, in the oxidation of uracil by t-BuO•, three peaks were observed. The peaks with retention times of 4.9, 4.1 and 3.2 min correspond to uracil, isobarbituric acid and 5,6-dihydroxyuracil, respectively, which were further confirmed by authentic samples (Fig. 1B). Also, in the oxidation of cytosine by t-BuO•, three peaks were observed. The peaks with retention times 4.1, 3.8 and 3.2 min correspond to isobarbituric acid, cytosine, and 5,6-dihydroxyuracil, respectively (Fig. 1C). The HPLC profile of the various standards is given in the Fig. 1D. Earlier study reported that t-BuO• radical attacks C5-C6 double bond of pyrimidine moiety of nucleic acid.

t-BuOOH is activated to radical reaction by the absorption of light at 254 nm. In the present work, it is proposed that t-BuO•, produced on photolysis of t-BuOOH in the initiation step, reacts with pyrimidine bases at C5/C6 double bond. The influence of light intensity on quantum yields of oxidation of pyrimidines (in the oxidation of thymine by t-BuO• when the intensity is changed from 3.510 × 10⁻³ to 3.384 × 10⁻³) suggests that the role of light is mainly restricted to the generation of t-BuO• from t-BuOOH in the initiation step. The oxidation of pyrimidines is not observed in the absence of t-BuOOH on shining the light. The increase in quantum yield (Table 1) with increase in [pyrimidine] suggests that excited pyrimidine might be acting as a sensitizer to transfer its energy to t-BuOOH. Such sensitizing effect in similar systems was proposed earlier. As the reaction rates are dependent on [pyrimidine] and [t-BuOOH], t-BuO• radical might react with pyrimidine in a slow step yielding either reducing (C5) or oxidizing (C6) radicals, which in turn react with t-BuO• radical to give final products.
The order of reactivity of pyrimidine bases by $t$-BuO• is found to be: uracil (U) >1,3-dimethyluracil (1,3-DMU) >6-methyluracil (6MU) > thymine (5-methyluracil) >5-bromouracil (5BrU). Substitution at C5 or C6 decreases the probability of $t$-BuO• attack at the site of substitution and favours addition at the adjacent carbon. The above proposal receives support, as the rates of oxidation of uracil and 1,3-DMU are more than 6MU, thymine and 5BrU. The higher rate of oxidation of 6MU by $t$-BuO•, compared to thymine
(5-methyluracil) indicates that \( t\)-BuO\(^{•} \) might be preferentially attacking at C5 position of pyrimidine leading to C6-yl radical. In the case of thymine, attack at both ends of C5-C6 double bond appears to occur due to steric hindrance of C5 methyl group. The lower rate for 5-BrU, compared to thymine (5-methyluracil) could be due to the fact that bromine is bulkier over methyl group. The higher rate of oxidation of uracil and substituted uracils, compared to cytosine could be due to \( \alpha,\beta \)-unsaturated carbonyl group, which increases the electron density at C5 position. The electron rich C5 position in uracils reacts faster with \( t\)-BuO\(^{•} \) radicals than cytosine.

In view of experimental results and the above discussion, the scheme of oxidation, taking thymine as an example may be written as above.

**Conclusion**

The study of rates of oxidation of substituted pyrimidines by \( t\)-BuO\(^{•} \) radical suggests preferential attack of \( t\)-BuO\(^{•} \) radical at C5 position. Product analysis shows the formation of corresponding 5,6-dihydroxypyrimidines and isobarbituric acid.

**Acknowledgement**

One of us (M A) is thankful to UGC, New Delhi, for awarding a Major Research project. The authors thank Prof. P Jayaprakash Rao, Department of Chemistry, Osmania University for helpful discussions.

**References**

3 Marnett L J (1987) *Carcinogenesis* 8, 1365-1373
4 Chance B, Sies H & Boveris A (1979) *Physiol Rev* 59, 527-605
8 Asmus K D, Mockel H & Henglein A (1973) *J Phys Chem* 77, 1218-1221