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DNA Cleavage Protection Ability of Ortho and Para Methyl Derivatives of N-1-Naphthylbenzohydroxamic Acid

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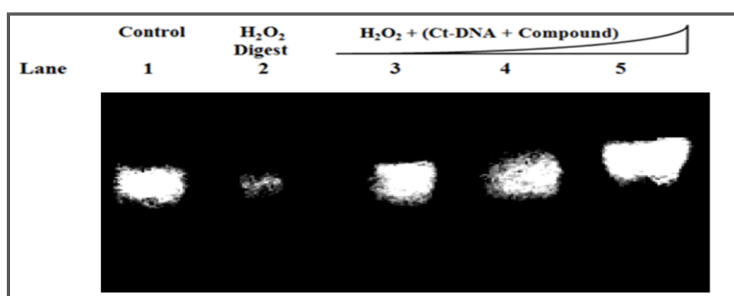
ABSTRACT

The antioxidant behaviour of ortho and para methyl derivatives of N-1-naphthylbenzohydroxamic acid has been studied through DNA cleavage protection ability. Gel electrophoresis technique was used.0.8% agarose gel was used for loading the samples along with loading buffer. Electrophoresis was performed using TAE buffer and ethidium bromide dye was used for visualizing the DNA samples through the UV transilluminator. The results obtained showed that both the derivatives were capable of inhibiting hydrogen peroxide mediated DNA damage. This can be attributed to the hydrogen bond donor nature of hydroxamic acids.

Graphical Abstract



Highlights

- DNA cleavage protection ability of ortho and para methyl derivatives of N-1-naphthyl benzohydroxamic acid has been studied.
- Gel electrophoresis technique was used.
- Both the derivatives were capable of inhibiting hydrogen peroxide mediated DNA damage.
- This can be attributed to the hydrogen bond donor nature of hydroxamic acids.

Keywords: DNA cleavage protection, Free radical scavenging activity, N-1-naphthyl-o-benzohydroxamic acid.

INTRODUCTION

Free radicals and reactive oxygen species capable of damaging nucleic acids, proteins and lipids are constantly being created in biological systems [1]. This leads to occurrence of degenerative diseases [2, 3]. Compounds capable of scavenging these free radicals such as peroxide or hydroxyl radicals are called antioxidants [4-7]. The antioxidant strength of a compound depend primarily on the ease of releasing its H-atom to neutralize the radical [8, 9].Since, N-arylhydroxamic acids possess hydrogen bond donor ability [10], they also show anti-cancer/anti-tumor behaviour [11]. The parent compound of the N-arylhydroxamic acids series, phenylbenzohydroxamic acid (PBHA) has been reported to possess antioxidant property along with other hydroxamic acids [12-15]. Therefore, DNA cleavage protection ability of ortho and para methyl derivatives of N-1-naphthylbenzohydroxamic acid has been studied.

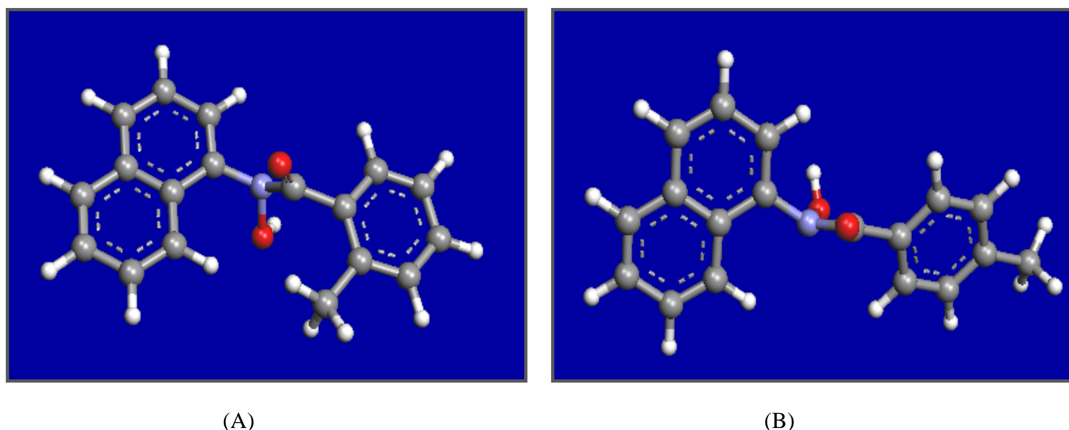
Electrophoresis is extensively used in molecular biology and medicine field for separation and purification of biological macromolecules such as proteins, nucleic acids [16]. This technique is applied for size estimation of DNA molecules after restriction enzyme digestion, analysis of polymerase chain reaction products, genetic finger printing and separation of restricted genomic DNA before Southern transfer, or separation of RNA before Northern transfer. It can be used in combination with other methods depending on the nature and type of analysis.

MATERIALS AND METHODS

N-1-naphthyl-o-benzohydroxamic acid and N-1-naphthyl-p-benzohydroxamic acid (Figure 1) were prepared through standard procedure [17, 18] and their purity was confirmed by determining melting point, UV and IR spectra. Elemental analysis was performed through Vario–EL analyzer apparatus.

N-1-naphthyl-o-methylbenzohydroxamic acid (N-o-MBHA): Mol. Formula-$C_{18}H_{15}NO_2$; Mol. Weight-277.32; M.P. (°C)-153-155; Elemental Analyses-C, 77.96; H, 5.45; N, 5.05; O, 11.54; IR-(cm^{-1}): 3100 (N-OH), 1630 (C=O).

N-1-naphthyl-p-methylbenzohydroxamic acid (N-p-MBHA): Mol. Formula-$C_{18}H_{15}NO_2$; Mol. Weight-277.32; M.P. (°C)-153-154; Elemental Analyses-C, C, 77.79; H, 5.48; N, 5.16; O, 11.57; IR-(cm^{-1}): 3125 (N-OH), 1623 (C=O).



(A) (B)

Figure 1. Structure of (A) N-1-naphthyl-o-benzohydroxamic acid and (B) N-1-naphthyl-p-benzohydroxamic acid.

Stock solution of the derivatives were prepared in ethanol and stored in cool and dark place. Agarose and ethidium bromide dye were acquired from Sigma Aldrich Chem. Co., USA whereas Calf thymus DNA and hydrogen peroxide were obtained from Merck Chemical Industry, Osaka, Japan.

The chemicals used were of spectroscopic grade and doubly distilled water was used throughout for the solution preparation. Gel electrophoresis was performed through Genei submerged gel electrophoresis system. pH measurements were done using Eutech instruments Oakton digital pH meter having a combined glass-calomel electrode.

The concentration of Calf thymus DNA was determined at 260 nm through UV molar absorption coefficient ratio (A_{260}/A_{280}) which was found to be > 1.8 [19]. Calf thymus DNA was suspended in double distilled water and stored at 4°C for preparation of stock solution (1mM) and further dilutions were done with Tris-HCl buffer having 7.8 pH. Gel electrophoresis was performed with TAE buffer. Reported procedure was followed to prepare 20 X stock solution of TAE buffer. 5 μgmL^{-1} ethidium bromide was also prepared in doubly distilled water. Loading buffer containing 0.25% bromophenol + 0.25% xylene cyanol + 30% glycerol was prepared in doubly distilled water. 0.8% agarose gel was prepared in TAE Buffer [20].

The derivatives were added at different concentrations to Calf thymus DNA solution prepared in Tris-HCl buffer at pH 7.8. Hydrogen peroxide was added to the reaction mixtures followed by incubation at 37°C for 1 h. Negative and positive controls having DNA only and the solution of hydrogen peroxide treated DNA were also incubated under the same conditions. The solutions were then loaded on 0.8% agarose geltray. Lane 1 of the agarose gel tray contain control DNA, Lane 2 contains hydrogen peroxide cleaved Calf thymus DNA and Lane 3-5 contain hydrogen peroxide cleaved Calf thymus DNA in presence of 100, 200 and 300mM N-1-naphthyl-o-benzohydroxamic acid and N-1-naphthyl-p-benzohydroxamic acid. Electrophoresis was performed in a submerged gel electrophoresis system.50 mM TAE buffer was used and electrophoresis was done at 50V for about 1 h. The agarose gels were stained with 1mM ethidium bromide dye after electrophoresis and were observed through UV transilluminator and photographs were taken [20].

RESULTS AND DISCUSSION

Charged molecules migrate towards either the positive or negative pole when placed in an electric field. Agarose gel is used as a convective means. DNA bears negative charge in aqueous solution due to the phosphate group. Hence, the migration of DNA in presence of electric field is towards the anode [21]. The electrophoresis buffer provides ions to carry current. It also maintains a constant pH. Hydrogen peroxide generates hydroxyl (OH) free radical which damages the DNA. The fragments of DNA can be visualized in the agarose gel (Figure 2 and 3).

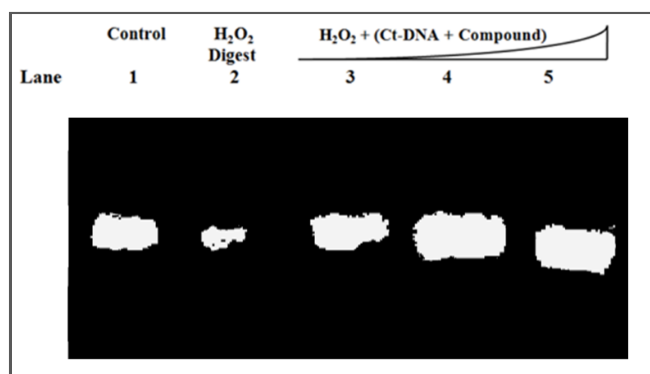


Figure 2. Hydrogen peroxide induced DNA cleavage protection activity of N-1-naphthyl-o-benzohydroxamic acid by agarose gel electrophoresis at different concentrations. Lane 1 = Control DNA, Lane 2 = hydrogen peroxide cleaved Calf thymus DNA and Lane 3-5 = Cleaved DNA protection by 100, 200 and 300 mM N-1-naphthyl-o-benzohydroxamic acid.

The band for Lane 1 containing the control DNA (without hydrogen peroxide and compounds) is the brightest. Whereas, the band of Lane 2 clearly shows the damage of Calf thymus DNA in the

presence of hydrogen peroxide. When Calf thymus DNA was treated with hydrogen peroxide for 1 hr, oxidation of DNA by hydroxyl radicals takes place. This results in uncoiling and damage of DNA leading to formation of smaller DNA fragments. The bands of Lane 3-5 in figure 2 as well as Figure 3show the protective effect of N-1-naphthyl-o-benzohydroxamic acid and N-1-naphthyl-p-benzohydroxamic acid at different concentrations. The bands show that both the compounds are capable of hydrogen peroxide mediated DNA cleavage protection effect.

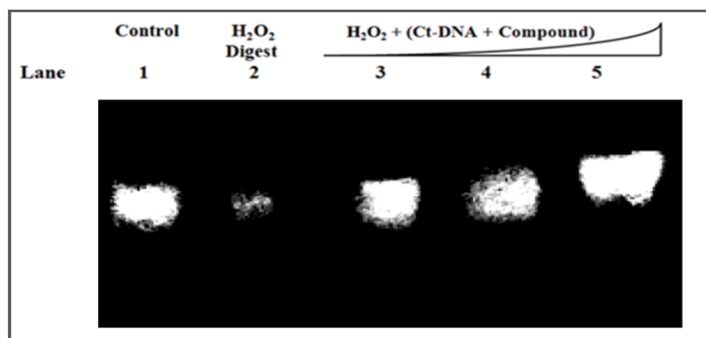


Figure 3. Hydrogen peroxide induced DNA cleavage protection activity of N-1-naphthyl-p-benzohydroxamic acid by agarose gel electrophoresis at different concentrations. Lane 1 = Control DNA, Lane 2 = hydrogen peroxide cleaved Calf thymus DNA and Lane 3-5 = Cleaved DNA protection by 100, 200 and 300 mM N-1-naphthyl-p-benzohydroxamic acid.

APPLICATION

N-arylhydroxamic acids are aromatic compounds having variety of applications in different fields. The present study is focused on the determination of H_2O_2-mediated DNA cleavage protection ability of N-1-naphthylbenzohydroxamic acid derivatives. This method provides an overview of the antioxidant potential of N-1-naphthyl-o-benzohydroxamic acid and N-1-naphthyl-p-benzohydroxamic acid.

CONCLUSION

The results reveal that both N-1-naphthyl-o-benzohydroxamic acid and N-1-naphthyl-p-benzohydroxamic acid show the inhibition of hydrogen peroxide mediated DNA damage. This can be attributed to the hydrogen bond donor nature of hydroxamic acids. Thus, it can be concluded that both N-1-naphthyl-o-benzohydroxamic acid and N-1-naphthyl-p-benzohydroxamic acid possesses antioxidant activity.

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