In the Name of God

Razi University
Faculty of Science
Department of Biology

M.Sc.Thesis

Title of the Thesis

Study of DNA interaction with 2-Tert-Butylhyroquinone and 3-Butylated Hydroxyanisol food additives

Supervisor :

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By:

Jafar Ezzati Nazhad Dolatabadi

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Abstract

The interaction of native calf thymus DNA with butylated hydroxyanisole (BHA) and tertiary butyl hydroquinone (TBHQ) in 10 mM Tris HCl aqueous solutions at neutral pH=7.4, has been investigated by spectrophotometric, circular dichroism (CD), spectrofluorometric, viscosimetric and voltammetric techniques. It is found that both BHA and TBHQ molecules could intercalate between base pairs of DNA as are evidenced by: hyperchromism in UV absorption band of DNA, sharp increase in specific viscosity of DNA, induced CD spectral changes, decrease in the fluorescence of BHA and TBHQ solutions in the presence of increasing amounts of DNA, and decrease in the both the anodic and cathodic CV peak current heights of the BHA and TBHQ and positive shift in the CV peak potentials of TBHQ and BHA. All the results suggest that the BHA and TBHQ interact with calf thymus DNA by intercalative mode of binding.

Key Words: CT-DNA; BHA; TBHQ; Intercalation;

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CHAPTER ONE

INTRODUCTION

1. Introduction

1.1 General [1-2]

Food antioxidants in the broadest sense are all of the substances that have some effects on preventing or retarding oxidative deterioration in foods. They can be classified into a number of groups. Primary antioxidants terminate free radical chains and function as electron donors. They include the phenolic antioxidants, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ), alkylgalates, usually propylgallate (PG) (Fig 1.1), and natural and synthetic tocopherols and tocotrienols. Oxygen scavengers can remove oxygen in a closed system. The most widely used compounds are vitamin C and related substances, ascorbyl palmitate, and erythorbic acid (the D-isomer of ascorbic acid). Chelating agents or sequestrants remove metallic ions, especially copper and iron, that are powerful prooxidants. Citric acid is widely used for this purpose. Amino acids and ethylene diamine tetraacetic acid (EDTA) are other examples of chelating agents. Enzymic antioxidants can remove dissolved or head space oxygen, such as glucose oxidase. Superoxide dismutase can be used to remove highly oxidative compounds from food systems [1].

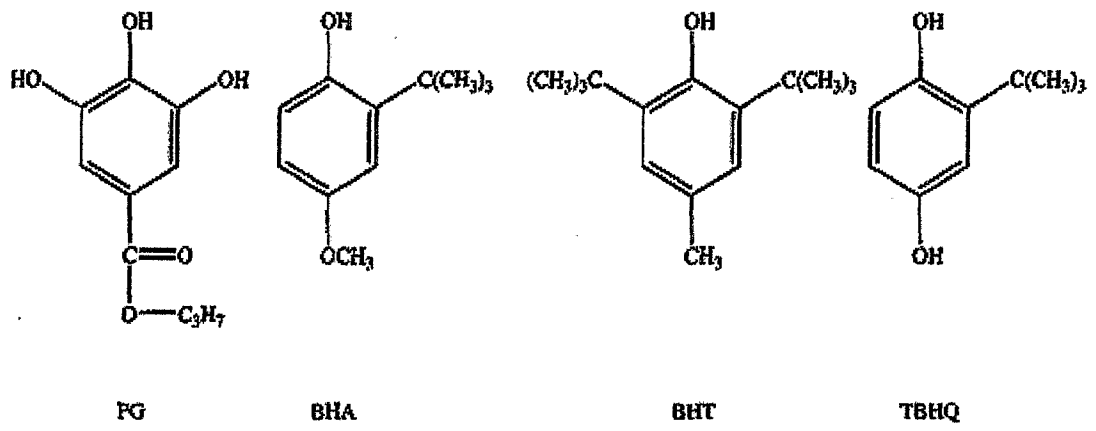

Fig 1.1 Structure of Propyl Gallate (PG), Butylated Hydroxyanisole (BHA), Butylated Hydroxy Toluene (BHT), and Tert-Butyl Hydroquinone (TBHQ)

Some antioxidants used to prevent the chemical break down of food have also been shown to be involved in the prevention of human illnesses. Antioxidants are important substances because they are used to protect oils and fats against lipid peroxidation or oxidative rancidity. Foods rich in lipids and polyunsaturated fatty acids are extremely sensitive to oxidation, which results in changes of color, odor, taste, and nutritional value [2].

1.2 BHA [3-4]

Butylated hydroxyanisole (BHA) (Fig 1.2) is very commonly used antioxidants in the food industry. BHA is generally regarded as safe (GRAS) substances, limited by a total antioxidant content of not more than 0.02% of the oil or fat content of the food. It is also found as additives in dry cereals, shortenings, potato products, dessert mixes, and in beverages and desserts made from dry mixes [3].

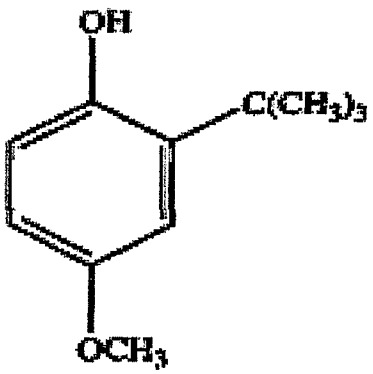

Fig 1.2 Structure of Butylated Hydroxyanisole (BHA).

The major food groups contributing to dietary intake of BHA are cakes, cookies and pies, other fine bakery ware and emulsified sauces with the maximum permitted level of 400 mg/kg being allowed in dietary supplements and chewing gum. The ADI for BHA is 0.5 mg/kg body weight/day.

BHA that is GRAS by the US Food and Drug Administration (FDA). By 1987, after BHA was shown to be a rodent carcinogen, its use had declined six-fold; this was due to voluntary replacement with other antioxidants, and to the fact that the use of animal fats and oils, in which BHA is primarily used as an antioxidant, has consistently declined in the USA. The mechanistic and carcinogenicity results on BHA indicate that malignant tumors were induced only at a dose above the maximum tolerable dose (MTD) at which cell division is increased in the forestomach, which is the only site of tumorigenesis; the

proliferation is only at high doses, and is dependent on continuous dosing until late in the experiment. Humans do not have a forestomach[2].

A review article on BHA described the metabolism and the mechanism of toxicity of BHA, which is considered to become carcinogenic after metabolizing to more reactive compounds. Tert-Butylhydroquinone (TBHQ) is a major metabolite in vivo in dogs, rats and man and oxidative demethylation of BHA to TBHQ was detected in vitro with rat liver microsomes [4].

1.3 TBHQ [5-6]

The compound tert-butyl hydroquinine (TBHQ) (Fig 1.3) is used for its effectiveness in increasing oxidative stability of polyunsaturated oils and fats. It also provides carry-through protection for fried foods. Antioxidants are frequently used in combination or together with synergists [1].

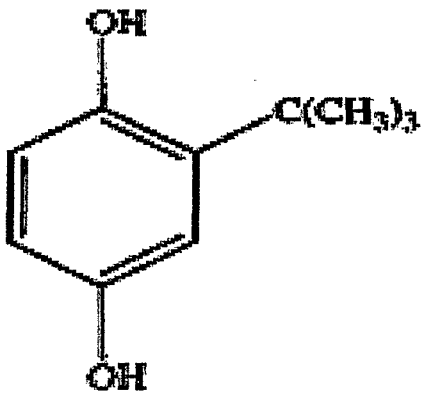

Fig 1.3 Structure of Tert-Butyl Hydroquinone (TBHQ).

TBHQ is a highly effective preservative for unsaturated vegetable oils, many edible animal fats and meat products. It does not cause discoloration even in the presence of iron, and does not change flavor or odor of the material it is added to. Metabolically, TBHQ is formed from 3-tert-butyl-4-hydroxyanisole (BHA), another widely used food additive, by O-demethylation. And it is further oxidized to 2-tert-butyl-1, 4-benzoquinone (TBQ). TBHQ administered to rats was excreted mostly in urine and feces. Although TBHQ was not considered to be carcinogenic in rats or mice (IPCS, 1998), a high dose (400 mmol/kg body weight, i.v.) of glutathione conjugates of TBHQ, a metabolite of the urinary tract, were found to be toxic to kidney and bladder. TBHQ at the dose of 2 mg/kg to mice

induced a positive SCE frequency. TBHQ was weakly genotoxic in hepatocye-mediated assay with V79 Chinese hamster lung cells and TBQ was cytotoxic but not genotoxic to V79 cells. Based on the results of alkaline elution assay, TBHQ administered by the oral route did not cause DNA damage in the forestomach epithelium of male F344 rats, but TBQ at low dose induced DNA damage, and it was suggested that TBQ and its thiol conjugates are active metabolites that cause DNA damage. The concentration of TBHQ required to cause DNA damage was much higher than that of TBQ. tert-Butylsemiquinone anion radical is formed from TBHQ and from TBQ in rat liver microsomes and the semiquinone dependent superoxide formation may contribute to the toxic actions. Okubo et al demonstrated that TBHQ caused DNA cleavage in vitro and the formation of 8-hydroxydeoxyguanosine in calf thymus DNA due to the generation of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide and hydroxyl radicals [5]. It is also used industrially as a stabilizer to inhibit autopolymerization of organic peroxidase. In high doses, it has some negative health effects on lab animals, such as precursors to stomach tumors and damage to DNA [5].

TBHQ has a clastogenic effect on chromosomes in mice, and exhibits genotoxicity and cytotoxicity. Toxicological data were summarized by van Esch in order to evaluate TBHQ as a food additive and to calculate the safety margin. He reported that negative mutagenicity was observed in the Ames test without S9 and that 20-month feeding of rats with 0.5% TBHQ had no adverse effect. However, F344 rats fed with 1% TBHQ in the diet showed a low level of cell proliferation in the basal cell layer of the forestomach epithelium. Metabolic studies showed that BHA is absorbed from the gastrointestinal tract and excreted almost totally as 4-O-sulfate or 4-0-glucuronide conjugate forms and little unconjugated TBHQ was found in the bile in rats. 2,5-Di-tert-butylhydroquinone (DTBHQ) may be derived from 2,5-di-tert-butylhydroanisole (DTBHA), which is one of the contaminants of commercial BHA. DTBHQ, as well as BHA, caused the development of forestomach papilloma in Syrian golden hamsters in 24 weeks when added to the diet at the 1% level [6].

1.4 DNA Intercalators [7-16]

Intercalation as a mode of DNA binding was first proposed by Lehman in 1961[7]. It is defined as a non-covalent association in which a planar, heteroaromatic molecule slides between the base pairs of DNA [8, 9]. The physical effects of this interaction on the DNA helix are profound. The DNA base pairs and helical backbone become unwound, resulting

in helical unwinding and an increase in helix length and rigidity. Overlap between DNA helix and intercalator exists at the unwinding site, rigidly holding the intercalator and orienting it perpendicular to the helical axis. By virtue of this tight "sandwiching" between the DNA bases, the intercalator is electronically stabilized by π - π stacking and dipole-dipole interations [10, 11].

DNA binding by intercalation has been demonstrated for a wide variety of drugs, carcinogens and dyes [11-15]. Intercalating drugs can cause mutations in DNA, and several of these compounds such as (doxorubicin, daunomycin, adriamycin) are used as drugs in the clinic [16]. Due to their potent activity, the development of DNA-intercalating drugs continues to be at the forefront of medicine. Examples of some common intercalating drugs and dyes are shown in Fig 1.4.

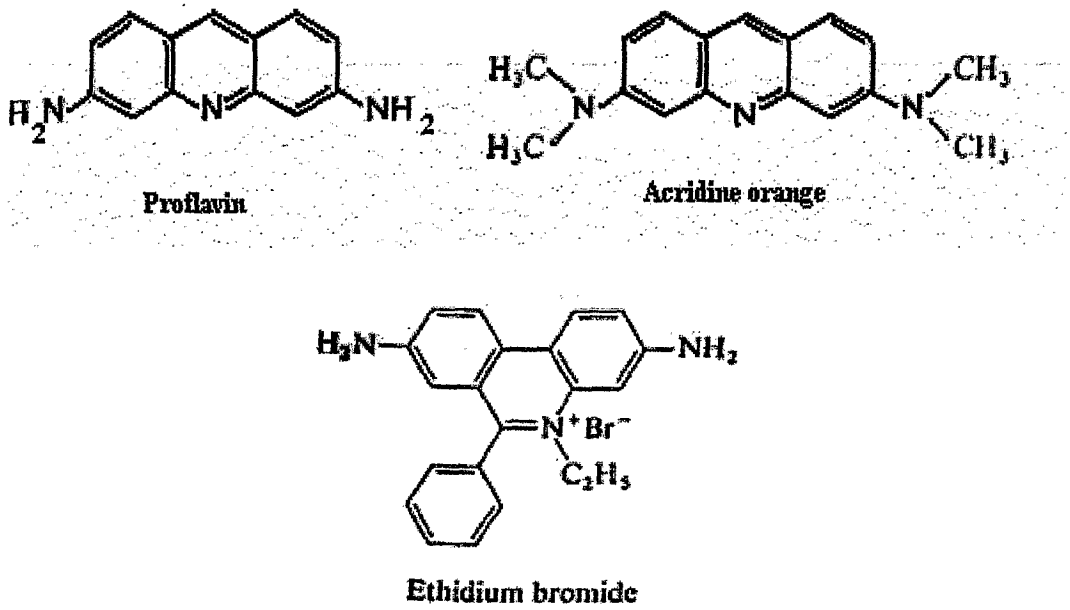

Fig 1.4 Structures of DNA intercalating dyes.

1.5 Characteristics of DNA

The phosphorus- and nitrogen-containing materials that came to be known as nucleic acids were first isolated from cells around 1870 by Friedrich Miescher but were long regarded as something of a curiosity.

Nevertheless, the structures of the monomer units, the nucleotides, were established by 1909 and the correct polynucleotide structure of the chains of DNA and RNA was proposed by Levene and Tipson in 1935.

1. One of the pyrimidine or purine "bases": uracil, cytosine, adenine, or guanine (Fig.1.3).

All four of these bases are present in RNA, while DNA contains thymine instead of uracil. Atoms in the bases are numbered 1–6 or 1–9.

2. A sugar, either D-ribose or D-2-deoxyribose. Carbon atoms in sugars are numbered 1′–5′.

3. Phosphoric acid

Although the biological synthesis is indirect, we can imagine that nucleotides are formed from these parts by elimination of two molecules of water. In nucleic acids the nucleotides are combined through phosphodiester linkages between the 5'-hydroxyl of the sugar in one nucleotide and the 3'-hydroxyl of another. Again, we can imagine that these linkages were formed by the elimination of water.

The structures of a pair of short polynucleotide strands in DNA are shown in Fig. 1.6. That of a segment of double-helical DNA is shown in Fig. 1.7.

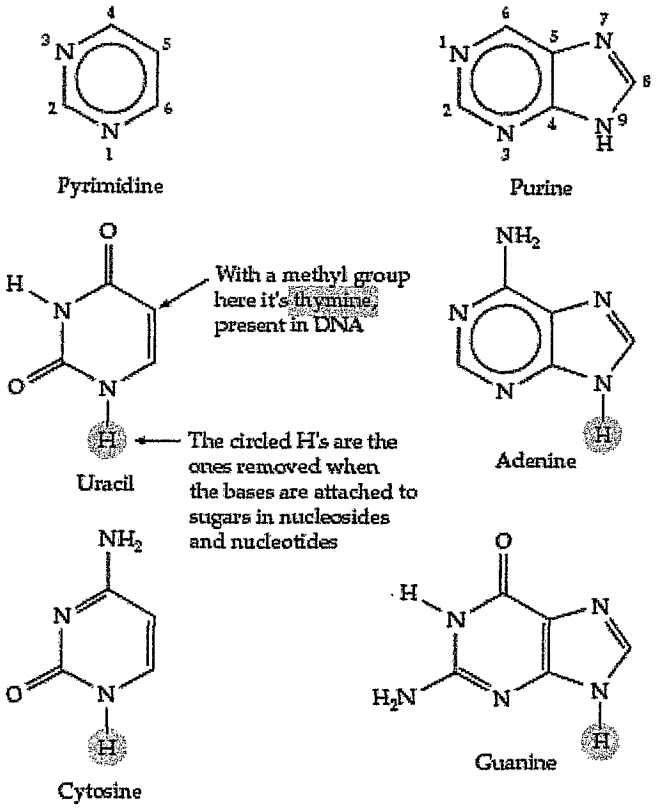

Fig 1.5 Structures of the major pyrimidine and purine bases of DNA and RNA