Abstract

The interactions of native calf thymus DNA with Ethylenediaminetetraaceticacid (EDTA) in Tris-HCl buffer at pH 7.8 (at this pH, EDTA altered to its disodium salt) and sesamol in Tris-HCl buffer at pH 7.4 have been investigated. EDTA and sesamol are widely used in food technology and chemical industry.

The DNA binding mode of EDTA was monitored by absorption spectrophotometry, circular dichrosim (CD), viscometry and gel electrophoresis. UV spectra of DNA showed small hyperchromicity with the increase in EDTA concentration. The CD signals at 245 and 275 nm indicated structural changes in DNA structure and shifting the B-DNA to a more A-like DNA, and no significant effect on DNA viscosity was observed in the presence of increasing amounts of EDTA. Results were indicative of an outside, non-intercalative binding mode of EDTA to DNA. Moreover gel electrophoresis studies showed considerable oxidative cleavage effect of EDTA on plasmid DNA. It should be noted that, in the presence of selenium, DNA cleavage effect of EDTA was inhibited.

The interaction of native calf thymus DNA (CT-DNA) with sesamol was monitored by absorption spectrophotometry, viscometry, spectrofluorometriy and circular dichrosim (CD). It was found that sesamol molecules could interact with DNA via outside and/or groove binding modes, as were evidenced by: hyperchromism in UV absorption band, very slow decrease in specific viscosity of DNA, and small increase in the fluorescence of methylene blue (MB)-DNA solutions in the presence of increasing amounts of sesamol, which indicates that it is able to partially release the bound MB. CD changes, thermodynamic data and its DNA binding constant are other evidences to support non-intercalative mode of binding of sesamol to DNA.

Study of DNA interaction with Ethylenediamine tetraaceticacid (EDTA) and Sesamol food additives

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Study Proposes

Investigation of EDTA/DNA and sesamol/DNA interactions by a variety of physicochemical techniques including spectrophotometry, spectrofluorimetery, viscometry, CD Spectropolarimeter and gel electrophoresis.

CHAPTER ONE

INTRODUCTION

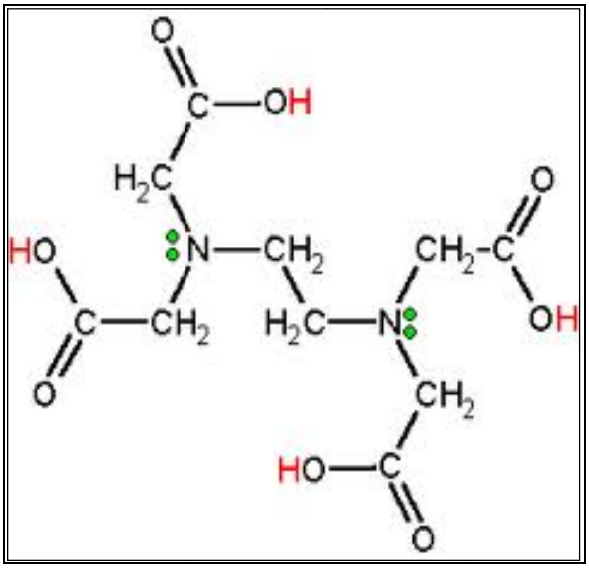
1. Introduction

1.1 General

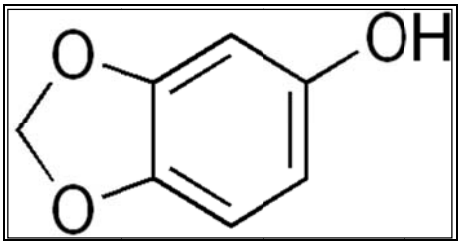
Food additives such as antioxidants, colors, chelating agents and sequesterants have been extensively applied in recent decades in food industry throughout the world. Food antioxidants such as Ethylenediaminetetraaceticacid (EDTA) and 3,4-methylenedioxyphenol (sesamol) (Fig 1.1) which have some effects on preventing or retarding oxidative deterioration in foods. The most widely used compounds are vitamin C and related substances, ascorbyl palmitate, and erythorbic acid (the D-isomer of ascorbic acid).

The susceptibility of lipid to oxidation is one of the major causes of quality deterioration in many types of natural and processed foods. Lipid oxidation leads to changes in the quality of food, such as taste, texture, appearance, and nutritional value. Effects of lipid oxidation are also major causes of many pathological effects, such as cardiovascular disease, cancer, and brain dysfunction as well as the aging process. Since lipid oxidation is a chain process, there are two mechanistically distinct classes of antioxidants which can be used to retard lipid oxidation, one group controls the radical chain-breaking mechanism and the other group involves prevention of the introduction of chain-initiating radicals, includes butylated ascorbic acid and EDTA (Jittrepotch *et al*., 2006). Meat colour changes from the acceptable cherry red to unattractive brown color as a result of oxymyoglobin oxidation and the formation of metmyoglobin (Ismail *et al*., 2008). Interest exists in the manufacture of functional meat products whereby synthetic antioxidants are replaced with naturally-sourced compounds such as sesamol may aid

in reducing the risk of various human chronic diseases and, hence, may represent plausible functional ingredients in meat products (Daly *et al*., 2010).



(a)



(b)

Fig1.1. structure of Ethylenediaminetetraaceticacid (a) and sesamol (b).

1. 2 EDTA

EDTA (Fig 1.2) is widely used in medicine, chemical industry, food technology, agriculture and pharmaceutical technology. In foods, vegetables, canned mushrooms, mayonnaise and salad dressings EDTA is added to prevent deteriorative changes, and to preserve color, odor and flavor (Krokidis *et al*., 2005).

The omega-3 fatty acids found in fish oil have been found to be clinically beneficial to health, although they are extremely sensitive to lipid oxidation, EDTA has been shown to dramatically retard lipid oxidation in salmon oil-in-water emulsions by removing iron from the droplet surface, high concentrations of EDTA in relation to iron will inhibit lipid oxidation by surrounding the metal and preventing interaction with peroxides (Alamad *et al*., 2006). The effects of EDTA on postharvest pathogens of apple and peach, and on improving the efficacy of the biocontrol product Aspire were evaluated (Droby *et al*., 2003). EDTA is a safe, economical metal chelator which sequesters divalent cations (Ca^{2+} and Mg^{2+}) which contribute to the stability of the outer membrane of Gram-negative bacteria by providing electrostatic interactions with proteins and lipo-polysaccharides (Sivarooban *et al*., 2008).

EDTA affects the inhibition of DNA synthesis in primary cultures of mammalian cells, this may be due to impairment of enzymes involved in DNA replication, some early studies have shown that EDTA leads to morphological changes of choromatin and chromosome structure in plant and animal cells, this alteration consist of dispersion or swelling of chromosomes or a loss of interphase chromatin structure,

for several test systems, a low chromosome-breaking activity of EDTA has been reported, a weak activity in the induction of gene mutations has also been observed, (Heindorff *et al*., 1983).

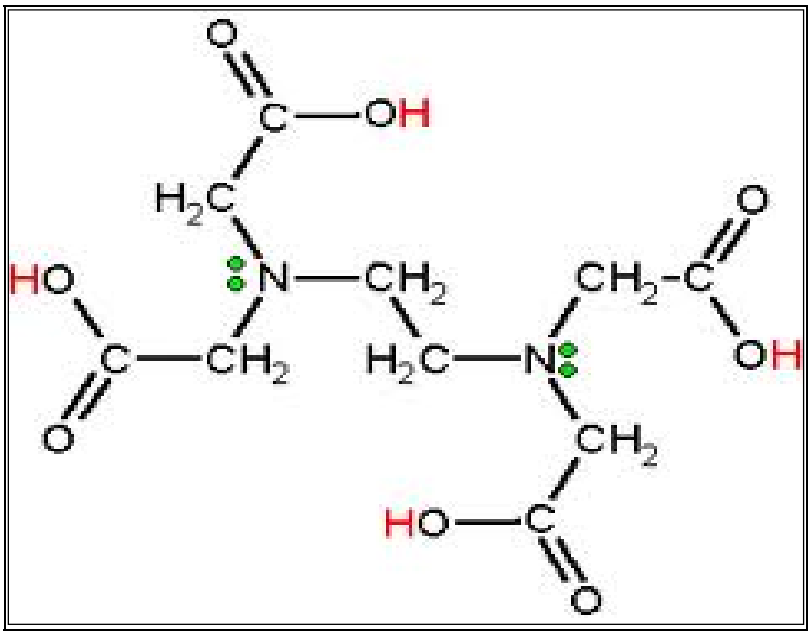


Fig.1.2. structure of Ethylenediaminetetraaceticacid(EDTA)

1. 3 sesamol

Sesame (*Sesamum indicum L*.) is cultivated in several countries such as India, Sudan, China and Burma which are considered as the major producers (Elleuch *et al*., 2007). Sesamol (Fig 1. 3) is a dietary phytochemical (Kanimozhi *et al*., 2009), and the major constituent of sesame seed oil; it is more resistant to oxidative deterioration than other vegetable oils. Sesamol scavenges hydroxyl and lipid peroxyl radicals and reduces radiation-induced deoxyribose degradation. It is a powerful antioxidant and has been

shown to possess neuroprotective, hepatoprotective, anti-inflammatory, chemo-preventive and anti-aging properties (Chopra *et al*., 2010; Chang *et al*., 2010). However, recently two cytotoxic products were isolated, trimer and tetramer, from oxidation of sesamol which showed cytotoxic effect of these products in rat thymocytes and human leukemia K562 cells (Fujimoto *et al*., 2010). Sesamol was associated with squamous cell carcinoma in male and female mice, naturally occurring antioxidants sesamol and caffeic acid have also been shown to induce hyperplasias in rat and hamster forestomach epithelium in short-term experient, as well as papillomas in this organ of rats in a 60-week experiment (Hiros *et al*., 1990).

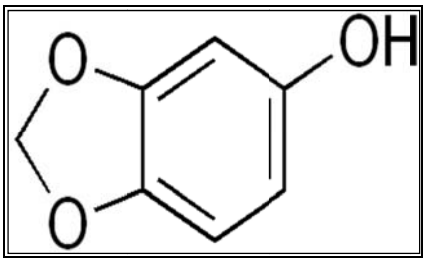


Fig.1.3. structure of Sesamol

1.4 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.1.4).

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 (Fig. 1.5). 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 (Fig 1.6).

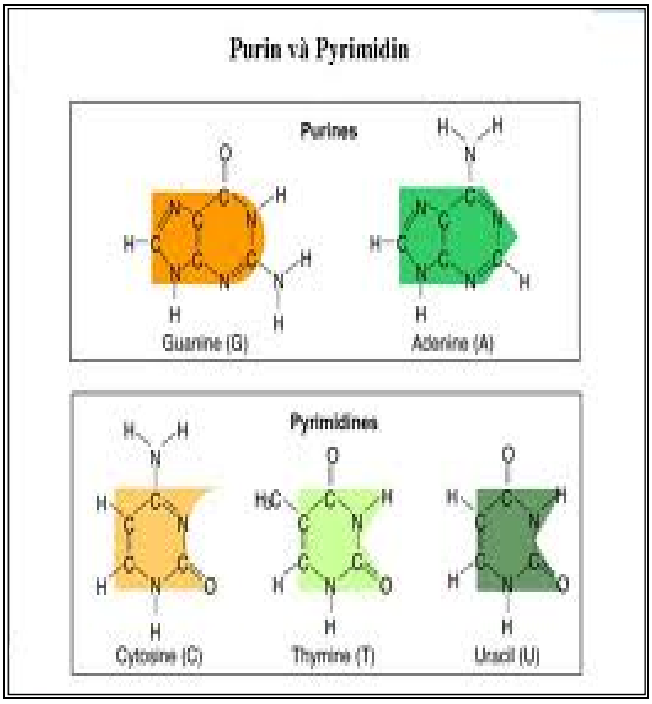


Fig 1.4. Structures of the major pyrimidine and purine bases of DNA and RNA.

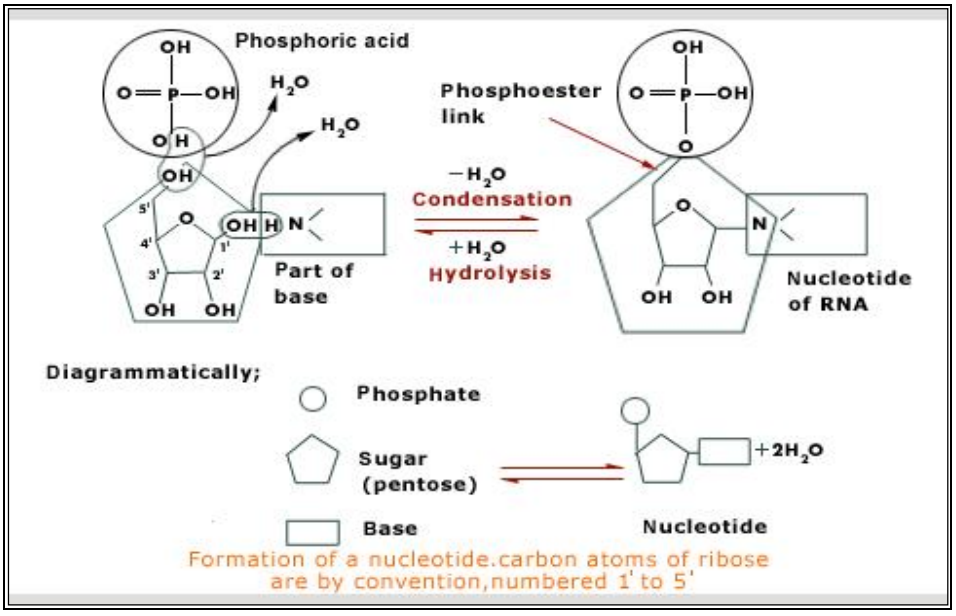


Fig 1.5. nucleotides formation by elimination of two molecules of water as indicated.