HEAT FRAGMENTATION PATTERN OF DNA TREATED WITH TWO DIFFERENT FUROCOUMARINS ISOLATED FROM *ANETHUM GRAVEOLENS* LEAVES

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Abstract - Prolonged local consumption of Anethum graveolens leaves, as an antihyperlipimic and antihypercholesterolemic agent, has been associated in some patients with skin disorders such as darkening and pigmentation mainly in the exposed areas such as face and hands. Anethum graveolens belongs to the umbelliferae family which is believed to be rich in photoactive furocoumarins. An oraganic extract of the powdered leaves was analysed for the photosensitive compounds. At least six different photosensitive components were detected in the TLC chromatogram of the extract. Two of the most photosensitive components were purified. Under UV irradiation, significant intercalations of the purified compounds into two different purified plasmid DNAs were obesrved. In addition, photoreacted DNA samples were easily fragmented by heat treatment in comparison to control samples not treated with the furocoumarins.

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INTRODUCTION

The ancient Turks, Hindus, and Egyptians have used the extract of some plants for the treatment of leukodenna (skin depigmentation, vitiligo) (1). In 1940's, it was established that the photosensitizing and pigment stimulating agents were a group of compounds known as linear furocoumarins or psoralens (2) which are three- ringed heterocyclic compounds. The details of the biochemical basis for light-induced psoralens reactions with skin is reviewed by Scott and co-workers (3). In today's medicine a variety of skin disorders such as psoriasis, mycosis fungoides, polymorphous light eruption and vitiligo, are treated by topical or oral administration of furocoumarins followed by irradiation of the patients with far UV light, UVA (320 - 400nm) (4-7). It is reported that some of these diseases are characterized by hyperproliferative conditions, e.g. psoriasis, while others like vitiligo, are manifested by skin depigmentation (4).

It is believed that furocoumarins intercalate into DNA and induce intrastrand cross- links (8,9). In addition, oxidative damage in cell membranes as well as DNA /protein cross- links have also been reported (10,11). Despite numerous and considerable efforts the mechanism of the lethal action of photoexcited furocoumarins is still not yet fully understood. However, it is suggested that furocoumarins first intercalate into DNA helix noncovalently, and then, with the absorption of UV light, they photoreact with pyrimidine bases, mainly thymine residues, to form pyrimidine- furocoumarine monoadducts.

The study reported here was conducted to show the effects of two of the isolated photosensitive furocoumarins from A. graveolens leaves on two different plasmid DNAs and to evaluate the final effect on heat denaturation of the treated DNA samples.

MATERIALS AND METHODS

Plant material

A. graveolens was purchased from the farm lands around Tehran. The plant material was dried at room temperature away from direct light. The dried leaves were pulverized and stored in a closed container at 4°C pending further investigation.

Chemicals

All chemicals used were of the best analytical grade available and purchased from Aldrich (UK) or Merck (Germany) and were used without further purification. Double distilled water was used throughout this investigation.

Extraction and isolation

Powdered leaves were extracted three times with petroleum ether followed by three times of extraction with chloroform. The accumulated extract was concentrated under reduced pressure. The residue was

subjected to coulum chromatography (silica gel, diethyl ether with gradual increase of $CHCl_3$ from 10% to 60%). Four different fractions were collected which upon further purification steps resulted in the isolation of five different photoactive furocoumarins I to V. The interaction of two of these components, III and IV, with two different plasmid DNA is reported here.

Photoactivation

To 5 μl aliquots of the plasmid DNA (50 μg/ml) in Tris buffer :10 mMTris, 1mM EDTA, pH 7.4, different volumes (0, 10, 20, and 30 μl) of each of the photosensitive components (III and IV) were added. The furocoumarins have been dissolved in 96% ethanol solution (10^{-3} μg/ml) and the plasmid DNA RY8 (22) was prepared as described previosely (12). A purified sample of plasmid $p^{bluskrip}$ was also obtained from the National Research Center for Genetic Engineering and Biotechnology of Iran. The DNA/Drug mixtures were kept at 8°C overnight and then irradiated for two hours at room temperature. Irradiation was achieved in a UV light box (Ultrviolet products Inc. Chromato-Vue Model CC- 20) at 365nm. The light intensity at the sample surface was not determined. Each irradiated sample was equally divided into two eppendorf tubes. The even- numbered tubes were then heated in a boiling water bath for 5 minutes then cooled immediately by inserting in dry ice bath. The odd- and even-numbered DNA samples were then subjected to agarose gel electrophoresis (1% agarose in buffer containing 50 mM Tris, 50 mM boric acid and 1 mM EDTA) and the electrophoresis was run at 50 volt in a dark room. The agarose gels were stained with ethidium bromide and photographed under long wavelength UV light.

RESULTS AND DISCUSSION

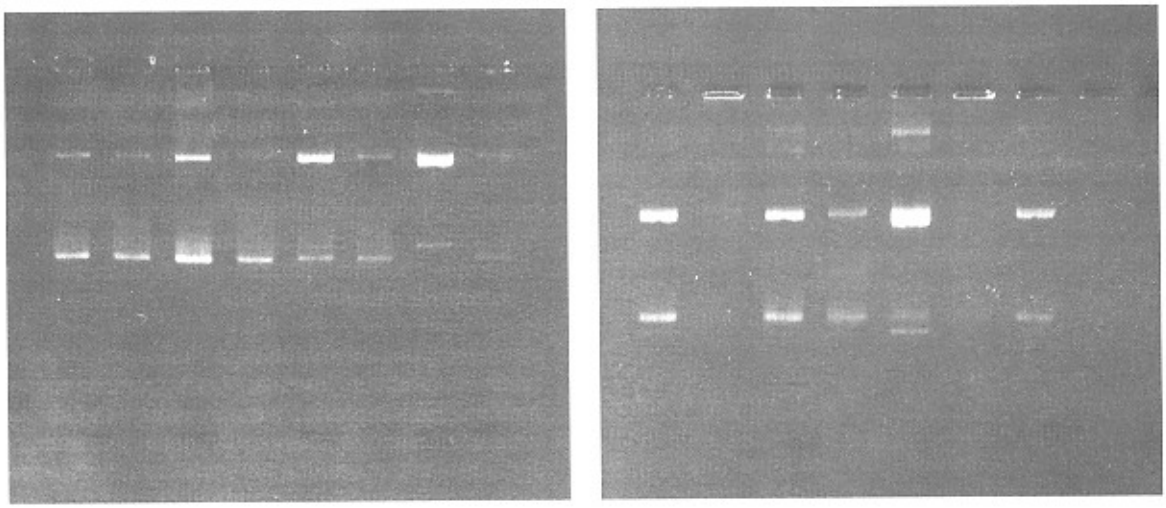
Five different photosensitive components have been purified from the non-aqueous extract of A. graveolens leaves which were proved to be furocoumarins (13). In this investigation, the effects of compound III and IV, two of these components, on two different plasmid DNAs were studied in the presence of UV irradiation.

Figure 1 indicates the simultaneous effects of compound III and IV light on plasmid DNA $p^{bluskrip}$, a, and the plasmid DNA RY8(22), b. As it is evident from lanes 1, 3, 5, and 7 of Fig. 1a, DNA interacts with compound III in a dose dependant manner: The intensity of the DNA bands in lanes 1, 3, 5, and 7 increases as the amount of compound III varies from zero to 10^{-3} μg per test. Plasmid DNA RY8(22) reacts similarly up to 10^{-4} μg of compound III per test but at 10^{-3} μg, the intensity of the interaction declines. This may explain a lower interaction rate between plasmid DNA RY8(22) and compound III in comparison to the same reaction between plasmid $p^{bluskrip}$ and compound III. Under these circumstances and the presence of UV light, compound III photoreacts with other furocoumarins (14).

Comparison of the intensity of band in lane 2 with intensity of the corresponding bands in lanes 6 and 8 (Fig. 1b) clearly indicates that the effected DNA molecules, with compound III, fragment more upon heat treatments.

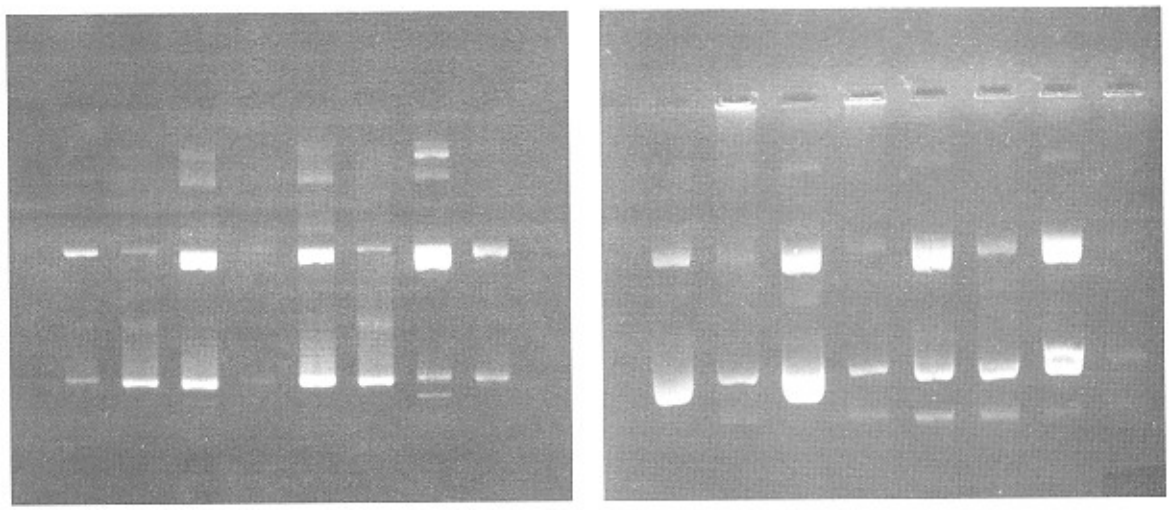
The significant structural changes in DNA molecules by furocoumarins is also observable in Figure 2a. Lane 1 indicates that the control DNA sample is mainly composed of two bands shown by arrow B and C. However, different new bands, in the area shown by arrow D, have appeared in the gel after interaction of compound IV with the DNA molecules. Induction of heat instability in the DNA molecules, after reaction with compound IV, is also shown in figure 2b (compare the intensity of bands in lanes 4, 6, and 8 with the intensity of the corresponding band in lane 2). The changes in the relative mobility of DNA molecules after treatment with compound IV under UV light, is also evident in Fig. 2b : The relative mobility of DNA molecules in position shown by arrow B in lanes 3, 5, and 7 have slightly increased compared to the control sample at the same position (lane 1, arrow B). However, the relative mobilities of DNA molecules in position shown by arrow C have decreased upon the effect of compound IV (compare lanes 1, 3, 5, and 7 at position C). Plasmid DNA $p^{bluskrip}$ reacts differently in this respect and no changes in the mobility is observable from figure 2a. However, the induced heat instability of DNA molecules by IV is clearly evident from figure 2a.

Based on these observations and regardless of the mechanism of interaction, it is clear that, some of the furocoumarins derived from Anethum graveolens, can easily effect DNA structure and interact with it under suitable conditions such as the presence of UV light. Although the consequence of these DNA effects on the total performance of the biological systems was not established in this investigation, but the destructive biological effects have been reported by others using different furocoumarins, isolated from other plants (15). Based on these global and cumulative data, it is necessary to have some concern and limitations on prescribing. A. graveolens as an antihyperlipimic and antihypercholesterolimic agent to the patients. Concerning the public health, it is necessary to produce a safer product from A. graveolens leaves or seeds, free from furocoumarins for pharmaceutical purposes. We have previosely reported that the aqueous extract of A. graveolens leaves, free from furocoumarin, has the same antihyperlipimic and antihypercholesterolimic effects as the crude extract (16).



(a) (b)

Fig. 1. The effect of compound III on plasmid DNA pBluskap a, and plasmid DNA RYS (22),b, in the presence of UV light. The DNA samples (2.5 μg per test) were incubated with different amounts of compound III overnight (lanes: μg 1,2: 0; 3,4:10^{-5} 5,6: 10^{-4} 7,8:10^{-3}). After irradiation for 2 hours at room temperature, each sample was divided equally into two aliquots. One set of aliquots was heated for 5 min in a boiling water bath, cooled immediately and then applied, along with the corresponding heat-untreated aliquots, to the agarose gel (heat treated: lanes 2, 4, 6 and 8; heat untreated : lanes 1, 3, 5 and 7). For further details see materials and method.



(a) (b)

Fig 2. The effect of compound IV on plasmid DNA pBluskap, a, and plasmid DNA RYS (22), b, in the presence of UV light. Experimental details as in figure 1.

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