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Modulation of Mutagenicity of Various Mutagens by Shrimp Flesh and Skin Extracts in Salmonella Test

[1]S. Mehrabian and [2]E. Shirkhodaei
[1]Depertment of Biology, Teacher Training University of Tehran, Iran
[2]Department of Microbiology, Azad Islamic University, Branch North of Tehran, Iran

Abstract: Many mutagens and carcinogens may act through the generation of Reactive Oxygen Species (ROS) in cells that maybe related to cancer. The carotenoid astaxanthin–the red pigment for the attractive coloration in the skin and flesh of shrimp-has attracted considerable interest in recent years because of its superior antioxidative activity to most of the hydrophobic antioxidants. According to the important role of carotenoids in human health, the main carotenoid pigment in flesh and by-products of the different shrimp species from penaeidae family-Astaxanthin-was extracted and separated by thin layer chromatography using silica gel, subsequently. The identifications were confirmed by fourier transform infrared (ET-IR) spectroscopy. The effect of these extracts on mutagenicity and carcinogenicity induced by Sodium Azide and potassium permanganate was investigated in bacterial assay system, i.e., the Ames test with *salmonella typhimurium* TA100, TA104. These strains have a certain mutation in their histidin operon, which are related to the external histidin source. Such a mutation can be reversed through contact with a diagnostic mutagen, but in shrimp extracts prevented from effect of this mutagen in which, after repeated test series under standardized condition, the anticarcinogenic effect of flesh and skin of shrimp was proven and the shrimp skin showed stronger anticarcinogenic effect in comparison with shrimp flesh. Different shrimp species from penaeidae family indicated similar antricariongenic effects.

Key words: Astaxanthin, Ames test, antimutagenic effect

INTRODUCTION

Chemical carcinogens are of major importance in the induction of human cancer and are therefore significant for public health. One of the most obvious for cancer to be followed by mutation is that most of carcinogens are also mutagens. Ames test is a short-term assay to evaluate mutagenicity of chemical and employed for primary screening of suspicious carcinogens. Dietary habits are important for human health. Many mutagens and carcinogens may act through the generation of Reactive Oxygen Species (ROS). ROS may play a major role as endogenous initiators of degenerative processes, such as DNA damage and mutation (and promotion), that maybe related to cancer, heart disease and aging (Ames, 1983). Besides the endogenous defenses, the consumption of dietary antioxidants, play a vital role in protecting against ROS. Also many antioxidants are being identified as anticarcinogens Gerber *et al*. (2002). The carotenoid astaxanthin (3, 3', dihydroxy-β, β-carotene-4, 4'-dione) is a red pigment responsible for the attractive coloration in many fish, crustaceans and birds Hernadz *et al*. (2002). It has attracted considerable interest in recent years because of its superior antioxidative activity to most of the hydrophobic antioxidants (Mercke *et al*., 2003). The skin and the flesh of shrimp contain substantial amount of astaxanthin (Vitaglion and Fogliano, 2004).

Today, however, there are more than one hundred different testing methods for collecting evidence of carcinogenic and mutagenic activity. Many of them are based on this principle that genotoxicity or mutagenicity serves as an indicator for the carcinogenic potential of the substance. Ames plate incorporation test has been recommended by various workers as a valid indicator of mutagenicity/genotoxicity of various substances present in the environment (Rosen Kraz, 2003).

The objective of the present study was to determine antimutagenic and anticarcinogenic effect of the flesh and by-products of shrimp.

MATERIALS AND METHODS

Materials: Tow species of Penaeidae family caught along the Persian Gulf in Ahwaz city: 1-*P.semisulcatus*, 2-*P. merguiensis* were transferred to the laboratory on winter 2004. The flesh and skin were grounded in a meat grinder and frozen at -10°C until utilization. Bacterial

Corresponding Author: S. Mehrabian, No. 6.1, Toopchi St., Sohrevardi Ave., Post Code 15588, Tehran, Iran
Tel: 0098-21-88766690 Fax: 0098-21-88825580

strains-*Salmonella typhimurium* strains TA100 and TA104, which were received directly from Professor Ames (University of Berkeley, CA). The tester strains genotype should be confirmed, so fresh overnight Nutrient broth cultures were used for this purpose. Strains of *Salmonella typhimurium* has defense in dark repair of mutations (UVRB) and unable to synthesize a protein of the cell wall (rfa). The strains were tested for the presence of the Ampicillin resistance factor; it is a convenient marker that makes it possible to test for the presence of the R-factor plasmid.

Preparation of shrimp skin and flesh extracts: Ten gram portion of test samples separately were placed into glass flasks. Petroleum ether: acetone: water was added at a ratio of 1.5:7.5:1 to the samples and left under cover for two hours. Then the solvents were evaporated in rotary evaporator until sample dryness. The resulting pigments were re-dissolved in an adequate volume of diethyl ether. These solutions were analyzed by TLC analyses and FT-IR spectroscopy. In TLC, mixture 25% acetone in n-hexane was used as developers (Kobayashi and Yoshiktani, 2001). In additional, the values of Astaxanthin in those extracts were assayed. To quality analyze and separate Astaxanthin, we used TLC chromatography and column chromatography, respectively. Then we proved this test IR in these extracts.

Ames test: In the antimutagenicity test, the inhibitions of mutagenic activity of Sodium Azide and potassium permanganate by the test samples were determined. One milliliter of solution of the tested compounds and 0.1 mL of an overnight bacterial culture suspension (cultivation for 16 h at 37°C, approximate cell density (2-5)×10^8 cells mL^{-1}) and 0.1 mL of solution of the positive mutagens were carefully mixed with 3 mL of melted top agar containing 50 μmol L^{-1} of histidine-biotin and pourded onto minimal glucose agar plates. Positive and negative controls were also included in each assay. Sodium Azide and potassium permanganate were used as diagnostic mutagens (0.1 mL) in the positive control and plates without mutagens and test samples were considered as negative control. His revertants were counted after incubation of the plates at 37°C for 48 h. Tester strains were checked routinely to confirm genetic features using the procedure described by Maron and Ames (1983).

RESULTS

TLC analyses: Developing of the pigment extracts from shrimp skin and flesh (Fig. 1) with TLC, it showed strong

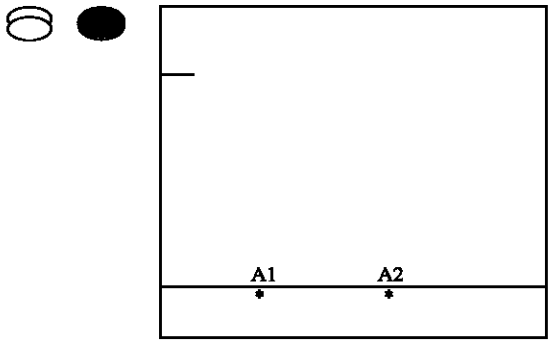

Fig. 1: Chromatography in silica gel plates of skin (A1) and flesh (A2) extracts

Table 1: Results from some Ames test experiments in presence of Sodium Azide in strains of *S. Typhimorium TA 100* and *TA104*

	No. of experiments							
	S. Typhimorium TA 100				*S. Typhimorium TA 104*			
No. of Revertants	1	2	3	4	1	2	3	4
Positive control	1680	2105	1860	1935	1450	1240	1600	2311
Negative control	478	530	398	320	502	356	555	480
Test sample (skin extract)	764	703	812	800	728	581	680	936
Test sample (flesh extract)	892	1211	1000	936	812	720	910	1233

Table 2: Results from some Ames test experiments in presence of potassium permanganate in strains of *S. Typhimorium TA 100* and *TA104*

	No. of experiments							
	S. Typhimorium TA 100				*S. Typhimorium TA 104*			
No. of Revertants	1	2	3	4	1	2	3	4
Positive control	1900	2168	1620	2020	942	1230	1490	1340
Negative control (distill water)	361	462	392	426	293	483	386	350
Test sample (skin extract)	619	830	557	630	438	720	652	513
Test sample (flesh extract)	886	1020	623	887	496	803	841	780

range band with Rf = 0.33. These main bands were identical in color and Rf value to the ones of reference astaxanthin. Two weak orange-colored bands in skin samples and one weak orange-colored band in flesh samples also appeared.

It was initially thought that those bands could have been esterified carotenoids, but this hypothesis was rejected because pigment extracts with FT-IR spectroscopy yielded identical chromatograms. In fact those bands may be artifacts due to isomerization of astaxanthin.

Antimutagenicity assay: The mutagenicity of Sodium Azide and potassium permanganate in the absence of test samples was defined as 100% or 0% inhibition. The calculation of percent inhibition was done according to

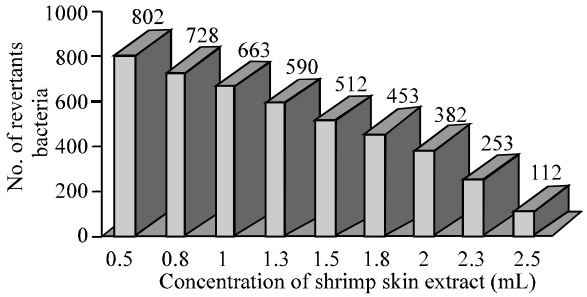

Fig. 2: The relation between numbers of revertants bacteria (*S. Typhimorium TA 100*) respect to concentration of shrimp skin extract in presence of Sodium Azide

the formula %INHIBITION = [1-T/M] × 100, where, T is the number of revertants per plate in the presence of mutagen and the test sample and M is the number of revertants per plate in the positive control. The number of spontaneous revertants was subtracted from the numerator and the denominator (Negi *et al*., 2003; Ames, 1983). The antimutagenic effect was considered moderate when the inhibitory effect was 25-40% and strong when more than 40%. Inhibitory effect of less than 25% was considered as weak and was not recognized as a positive result.

The shrimp skin and flesh extracts supplied in Ames test inhibited the mutagenicity of Sodium Azide and potassium permanganate in both strains of *Salmonella Typhimorium TA 100* and *TA 104* (Table 1 and 2). Also according to the Ames test with *S. typhimurium* TA100, TA104, different species of shrimps from Penaeidae family indicated similar antimutagenic effects in which the skin of tested shrimps that are regarded as waste materials, showed stronger antimutagenic effects in comparison with shrimps' skin (Fig. 2).

DISCUSSION

Active oxygen and free radicals are related to various physiological and pathological events, such as inflammation, immunization, aging, mutagenicity and carcinogenicity indicated that active oxygen scavengers reduce mutation induced by various mutagens. It has been suggested that compounds, which possess antioxidant activity, can inhibit mutation and cancer because they can scavenge free radicals or induce antioxidant enzymes (Mikula and Ikova, 2003).

TLC analysis showed that astaxanthin and its isomers seemed to be the dominant carotenoid in shrimp skin and flesh. In fact increase of body astaxanthin content through dietary supplementation in Penaeid shrimps could enhance their antioxidant defense capability and resistance to thermal and osmotic stress (Negro and Garrido-Fernandeg, 2000). Also, astaxanthin seemed to help prolong the life of the post larvae to acute environmental stress (Armenta-Lopez *et al*., 2002).

Since crustacean waste generated from the fishing industry represents approx 70% of the total landings, this abundant waste may pose an environmental hazard due to the ease of deterioration of the by-products in the landfill sites. So, they can be achieved at considerable cost to the industry by extracting useful components such as pigments-Astaxanthin-and high-unsaturated fatty acids (HUFA) and incorporating them into desirable seafood products. Epidemiological studies indicate that there is a close relationship between diet, life style and human cancer (Nair and Risch, 2000).

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