Mutagenicity of melanin from human red hair

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Summary. The *Salmonella typhimurium* histidine reversion test of Ames et al. was used to demonstrate that pheomelanin, the red-brown polymeric pigment produced in human skin and hair, becomes mutagenic after exposure to long wavelength UV-light; a finding consistent with the UV-induced somatic mutation hypothesis for the origin of freckles and the high susceptibility of redheads and blonds to sunlight-induced skin cancers.

The color of hair is largely determined by 2 chemically distinct types of melanin, the black and brown eumelanins and the red and yellow pheomelanins[3,4]. Fair-skinned humans exhibit a number of abnormal reactions to sunlight including a high susceptibility to skin cancer[5–8] and in some cases freckling[9]. It has been postulated that freckles, which consist of localized populations of aberrant melanocytes, are clones derived from a somatic mutation arising within a single cell[9,10]. There is also evidence that malignant neoplasms may arise in a similar fashion[11,12]. These, and other UV-light-induced effects, have been attributed to the fact that the skin of these individuals has a poor tanning capacity, sunburns readily and contains little eumelanin. However, there is an increasing amount of evidence that pheomelanin occurs in melanosomes found in various parts of the body, including the skin[10,13–17].

Light of wavelengths between 280 and 380 nm is nondestructive to eumelanin, producing only reversible changes such as free radical formation and pigment darkening that are believed to function as photoprotective mechanisms within the skin[18–23]. Pheomelanin, on the other hand, is readily photodegraded in the presence of oxygen at physiological pHs[24,25], an observation that suggested several mechanisms by which UV-light may deleteriously affect tissue containing this pigment[26]. We present here experimental evidence that photolysis of pheomelanin leads to the formation of a mutagen, and possible carcinogen, and we suggest that this finding could explain the excess of actinic damage seen in the epidermis of fair-skinned humans as a result of chronic exposure to solar radiation.

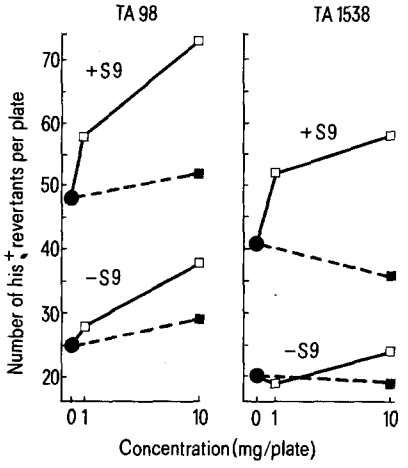

Fig. 1. Frequency of his$^+$ (histidine synthesizing) revertants of *S. typhimurium* strains TA 98 and TA 1538 after treatment with native (PM) and partially photolyzed (PM-P) pheomelanin. Concentrations of test compound are given in mg/plate. Since 0.1 ml test compound was mixed with 0.5 ml S-9 mix or its control buffer, 0.1 ml bacteria and 2.0 ml top agar, the immediate mix thus had an effective concentration which was 3.7% of that shown. The top agar was poured over 15 ml of bottom agar. Each datum point represents the mean of 6–8 plates. ●, Control, no pigment added; ■, PM, □, PM-P.

Materials and methods. Pheomelanin was isolated from red human hair[26], exploiting differences in solubility to insure against contamination with eumelanin, even though we failed to find eumelanin in any of our hair samples, and its insolubility in all known solvents precludes testing it for mutagenicity in microbial systems. The pigment was then dissolved in 0.2 M, pH 7.3 phosphate buffer (final concentration = 3.3 mg/ml) and subjected to illumination from a Hanovia 100-W medium pressure mercury arc fitted into a pyrex photochemical immersion well (Ace Glass). An oxygen bubbler was employed to maintain oxygen-saturation throughout the course of photolysis. Following irradiation the solutions were lyophilized and stored for up to 12 months at room temperature, during which time no change in mutagenicity was noted.

Irradiation of pheomelanin results in the formation of primary photoproducts which, upon subsequent irradiation, are further photolyzed[24,25]. 3 samples, native pheomelanin (PM), partially photolyzed (PM-P), and substantially photolyzed (PM-S) pheomelanin were tested for mutagenicity. The latter were produced by photolyzing pheomelanin for 363 and 1336 min, respectively. These time intervals were chosen to coincide with the spectrophotometric build-up and destruction of the primary photoproducts[24,25].

The *Salmonella typhimurium* histidine reversion test (strains TA 1535, TA 1538, TA 100, and TA 98) of Ames et al.[27] was used to test the hypothesis that one or more of the pheomelanin photoproducts is a mutagen. Each concentration of test compound was examined in at least 3 separate experiments, employing 6–8 plates per datum point.

Results. In no experiment did the mean number of revertants arising in the plates containing PM vary from control plates by more than 10%. PM-S was tested at concentrations of 7, 14, and 28 mg/plate, and again, there was no significant deviation from the control or the PM plates. Treatment with PM-P, however, resulted in a slightly less than 2-fold, but reproducible increase in the number of revertants (table).

Because frameshift mutations are known to be produced by metabolic activation by microbial enzymes, pheomelanin

Mean number (6–8 plates) of colonies of histidine synthesizing revertants of *S. typhimurium* after treatment with partially photolyzed pheomelanin (PM-P)

Strain	Control	Concentration PM-P/plate	
		2.4 mg	6.0 mg
TA1535	18	31	56
TA1538	30	NT	73
TA100	261	309	434
TA98	58	NT	71

NT = not tested.

and its photoproducts were examined, after activation, in strains TA 98, and TA 1538. The microsomal S-9 fractions (Bionetics, Lot No. 51) used for activation were tested for activity in each experiment with benzo-(a)-pyrene. A concentration of 10 mg/100 ml gave a mean value of 61 reversions with activation, 21 without, and 20 for controls. Concentrations of PM and PM-S from 1 to 10 mg/plate again showed a frequency of reversion which did not differ significantly from control values. PM-P, however, caused an increase in the frequency of revertants both with and without activation with the S-9 fraction. There was no overlap in the distribution of the number of colonies arising in the plates receiving 10 mg of PM-P and those receiving none (figure 1). Although the addition of the S-9 fraction increased the overall frequency of both the control and treated colonies, the increments were approximately equal. It was concluded that S-9 treatment does not substantially increase the mutagenicity of PM-P.

To establish firmly the mutagenicity of PM-P, a 3-point dose response curve was constructed at somewhat higher concentrations in an additional experiment (figure 2). It can be seen that PM-P demonstrates a dose response for the concentrations 6.8, 13.7, and 27.5 mg/plate, whereas PM-S again shows no mutagenicity even at the highest concentrations.

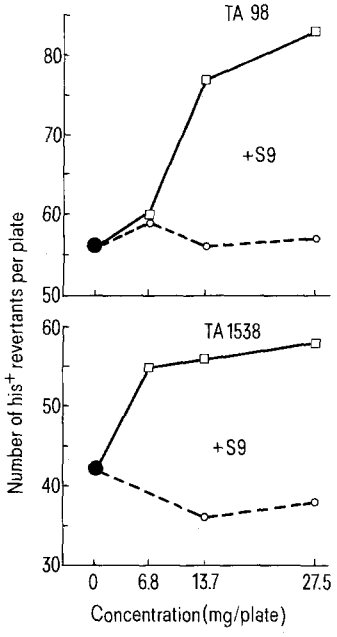

Fig. 2. Frequency of his$^+$ (histidine synthesizing) revertants of *S. typhimurium* strains TA 98 and TA 1538 after treatment with partially photolyzed (PM-P) and substantially photolyzed (PM-S) pheomelanin. Refer to figure 1 legend for details concerning concentrations. ●, Control, no pigment added; □, PM-P; ○, PM-S.

Discussion. It is clear that PM treated with UV-light is an active mutagen. The activity, which was shown by an approximately 2-fold increase in the frequency of revertants, was low but dose related and consistently reproducible, criteria workers in this field consider significant[26]. We would have been surprised if pheomelanin itself had proved to be highly mutagenic. Indeed, a natural compound with a rather wide distribution in animals and human beings would not be expected to be a potent mutagen.

McCann et al.[29] found that most frameshift mutagens are also carcinogens, and thus the activity of PM-P in the frameshift strains, TA 98 and TA 1538, suggest that there may be a carcinogen present in the PM-P. Finally, the fact that PM is not mutagenic but becomes so upon exposure to UV-light, is consistent with the observations that freckles are produced by UV-light, and the incidence of skin cancer in humans, particularly in redheads and blonds, is augmented by exposure to sunlight.

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2 Acknowledgments. *S. typhimurium* strains were kindly provided by Dr Bruce N. Ames. Z. Harzanyi was supported by NIEHS grant 00675-03 and an Andrew W. Mellon Teacher-Scientist Award. M. Chedekel thanks The Ohio State University Graduate School for financial assistance.
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