

# The Red Pigmentary System and Its Relation to Black Melanogenesis\*

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**Synopsis**—Identification of the chromophores of PIGMENTS of red hair and feathers as derived from two molecules each of cysteine and dopa explains many unique properties of the PHEOMELANOPROTEINS: their easy separation into protein and nonprotein parts; the loose, patterned electron microscopic appearance of the “red melanosomes”; and the tyrosinase- and dopa-positivity of the “red melanocytes.” As the initial step in the synthesis of black and red pigments (tyrosine → dopa) is the same, it is postulated that pigment formation may be switched from black to red or red to black. An experimental switch mechanism of this type may be produced by converting red rabbit hair to black by local irritation from rubbing; physiologic switches occur in agouti animals (hamster, rat, mouse). Further evidence is presented in support of the theory that iron is an essential part of red pheomelanoproteins and their structure is probably: (cysteinyl-dopa)<sub>2</sub>-derivative-Fe(III)-protein.

## INTRODUCTION

The past three years have greatly expanded our knowledge of the red pigments of hair and feathers. At present, their chemical composition is better known than that of the black melanins and great advances have been made in clarifying the pathways leading to their formation. This publication summarizes these recent developments and discusses the interrelation between black and red pigmentary biosynthetic processes.

## CHEMISTRY

Because of their amphoteric nature and reactivity with hot acids, the red pigments may be isolated in two forms: (a) With weak acids or alkalis at room temperature we may obtain the orange-colored proto-pigments (PP) which have no characteristic absorption bands in the visible part of the spectrum and do not display indicator properties; (b) Hot acids extract the characteristic purplish-pink compounds (trichosiderin or feather siderin) with absorption bands in an acid medium—maximum

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at about  $535 \text{ m}\mu$  (1). The siderins have indicator properties (red to yellow at pH 2). The siderins also may be obtained by heating PP with acids (2), which causes decarboxylation of the pigment portion of the molecule (3).

Like all melanocyte-produced pigments, the red pigments consist of a protein attached to a nonprotein pigment molecule (chromophore or pyrrotrichol) (4). However, in contrast to the black melanoproteins, the link between pigment and protein is loose and hence the chromophores can be separated by splitting the crude siderins with iron-binding anions (e.g., thiocyanate) followed by dialysis and gel filtration (2), or by a combination of gel filtration with passage through ion-exchange resins. Using the latter method, several chemists (3, 5) isolated a relatively pure chromophore that contained sulfur. They suggested that the chromophore may result from a combination of dopa with cysteine (6). When they reacted dopa with cysteine in the presence of an oxidizing agent, a mixture was obtained from which a compound could be isolated which had the same IRS as the chromophore. It was concluded that cysteinyl-dopa was a precursor of the red pigments (7). After preliminary studies with a model system, Prota *et al.* fully elucidated the reactions that take place when cysteine combines with dopa (8), and identified the red pigment chromophore as derived from two molecules each of cysteine and dopa (3) (Fig. 1).

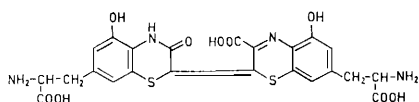


Figure 1. Structure of chromophore of red pigment (3)

This structure does not account for a number of closely related chromophore derivatives which can be extracted from hair and feathers (4, 6). Nevertheless, clarification of the basic chemistry of the red pigments has thrown light on many aspects of red pigmentation which will be discussed below.

Less is known about the two other components of red pigments: protein and ferric ion. Proteins isolated from different species had a remarkably similar amino acid composition. A minimum molecular weight of 6700 to 8300 was suggested (2). In contrast to the black melanoproteins (9, 10), neither the proteins isolated by us nor the proteins analyzed by others (11) contained cysteine or dopa. These amino

acids may be absent, because cysteine and dopa are required for the synthesis of the chromophore. The presence of iron as an essential component of the red pigment-protein complex has been challenged (3) on the ground that the chromophore itself contained no iron. A similar argument advanced in the case of hemoglobin would deny its iron-protein nature, because the porphyrin nucleus is free of iron. Nevertheless, the possibility that iron may be a contaminant, chelated by the chromophore in the course of the purification of the pigment, must be considered.

Three types of experiments were performed to test this possibility:

1. The pigments were isolated under mildest possible conditions with careful avoidance of external contamination which might lead to iron uptake (splitting of PP and siderins with KSCN, followed by dialysis without gel filtration). Such fractions still contained traces of protein (3–5%) with the enrichment of iron (to about 1.5%) observed earlier by ourselves (2) and Boldt (5).

2. We found that PP and siderins alike could be split with a variety of iron-binding anions (thiocyanate, ferrocyanide, cyanide, azide,  $\beta$ -hydroxamate) to a nondialyzable and dialyzable fraction. The latter displayed the typical properties of the chromophore unless a secondary reaction occurred, as with cyanide.

3. We observed that ferric ion, when added to the chromophore and chromophore peptide fractions of the siderins, abolished the color and absorption spectra of these compounds. After addition of ferric ions this change could be reversed with acids, as in other ferric-phenolic complexes (higher concentrations of iron irreversibly altered the chromophore). Thus, it is unlikely that combination of the chromophore with iron would occur in the course of purification.

All these observations give added support to the structure: protein-Fe(III)-(cysteinyl-dopa)<sub>2</sub>-derivative. By analyzing the peptides still attached to the chromophore in the incompletely purified iron-rich fractions, information may be obtained about the mode of attachment of iron to the protein molecule. Preliminary data suggest a marked enrichment in glycine.

#### IMPLICATIONS OF THE CHROMOPHORE STRUCTURE

Chemical identification of the chromophore accounts for many unique properties of the red pigments. These are summarized briefly.

1. As cysteine is incorporated in the chromophore, the sulfur cannot form the thioether bridge which in the black melanins binds them to the

protein part of the melanoprotein molecule. The protein part of the red pigment is very loosely attached and can be removed by column chromatography, gel filtration, or splitting with iron-complexing ions. This easy operation opens up new avenues for studies of malignant melanomas.

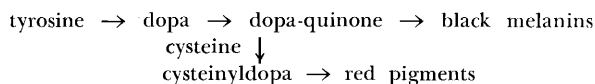
2. The chromophore of the red pigment has a much smaller molecular weight (561) than has been estimated for the black melanins (in the tens of thousands). It is less polymerized and forms a less firm molecular aggregate with its protein portion. Electron microscopic pictures of "red melanosomes" (which should be called erythrosomes) reflect this circumstance. They are not as fully "melanized" as their black counterparts, but instead form a loose, regularly patterned network of pigment. Their ovoid rather than round shape has been attributed to the lesser stress exerted on their membranes by the incompletely polymerized red pigment (12).

3. The first step in pigment formation, the conversion of tyrosine to dopa, is the same in red and black pigments. Therefore, both red and black melanocytes give a histochemical and chemical tyrosinase reaction.

4. A major consequence of this common first step is the potentiality of red pigment cells to switch to black melanin production; conversely, rerouting of black melanogenesis toward the synthesis of red pigments also may occur. The possible interconversion of these two pathways will be discussed below.

#### INTERCONVERSION BETWEEN RED AND BLACK PIGMENT FORMATION

A simplified scheme of a possible switch mechanism between the red and black pigmentary systems is as follows (6):



It is possible to divert dopa toward the black pathway by decreasing the "side-tracking" effect of cysteine; conversely, the black system may be forced to produce red pigments through an "overdose" of cysteine. Conversions of black pigment forming systems to red pigment forming systems (and *vice versa*) are known to occur under experimental or physiologic conditions.

Starvation in certain black fowls leads to the production of red feathers. It has been postulated that during starvation the decreased growth rate of feathers prolongs the exposure of the feather matrix to a

“red-orienting” (presumably cysteine-rich) milieu and thus prevents dopa from forming black melanin. Conversely, increased feather growth from thyroxine can convert red feathers to black (13), perhaps by allowing them to escape “way-laying” by cysteine.

A similar process may account for the red discoloration of the hair of starving black children as in Biafra.\* However, the nature of the red pigment in kwashiorkor is still unknown and therefore this mechanism is speculative.

The change from red to black may be induced *in vitro* by overwhelming the cells with dopa (dopa-positivity) or *in vivo* by purely local means. By daily prolonged rubbing of New Zealand red rabbits with a neutral ointment we caused increased hair growth. During these periods of irritation the rabbits produce black hair on areas where normally only red hair grows (Fig. 2).

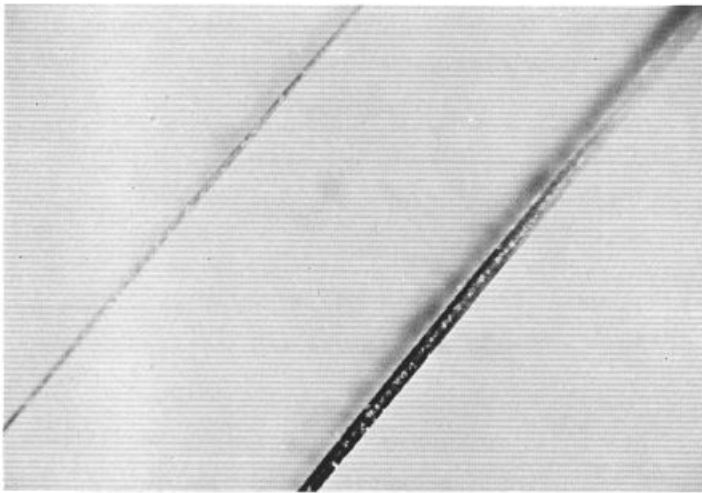


Figure 2. Right: Black band formed in hair of New Zealand red rabbit during period of local irritation by rubbing. Left: Control hair

The switch between black and red pigment production is the physiologic way in which agouti color arises in some black and orange animals. Accordingly, we were able to obtain typical siderin-like indicator pigments from the orange portion of the hair of golden hamsters; no red

\* The question may be raised as to how a protein-deficient diet could provide the cysteine required for this mechanism. It should be remembered that hair has an enormous avidity for sulfur-containing amino acids, a circumstance which may explain the normal cysteine of the hair of children suffering from kwashiorkor.

pigments could be extracted from the black sections. A similar sequence of events occurs in the Norwegian rat.

Of the agouti animals, the mouse has been the subject of thorough studies by Cleffmann. Both yellow and black melanocytes of this species are converted to black-producing cells in tissue culture. Glutathione restores the yellow pigment production; the amounts required are governed by genetic factors (14). During the yellow phase more sulfhydryl was taken up by the pigment cells; in the black stage the incorporation of tyrosine and dopa predominated (15). From the hair of this species we were able to isolate a trichosiderin-like indicator pigment.

Drugs and aging also may bring about red-black conversions; these have been discussed in previous publications (2, 15).

#### CONCLUSIONS

The red pigmentary system is widely distributed in nature. So far we isolated indicator-like red pigments ("siderins") from seven species: man, dog, rabbit, hamster, rat, mouse, and chicken. As they provide a biologically less advanced method of protection against ultraviolet irradiation, red pigments may be phylogenetically older than black melanins. The interconversion between red and black pigmentary pathways lends support to such an evolutionary hypothesis.

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#### REFERENCES

- (1) Flesch, P., Esoda, E. J., and Katz, S., The iron pigment of red hair and feathers, *J. Invest. Dermatol.*, **47**, 595-7 (1967).
- (2) Flesch, P., The epidermal iron pigments of red species, *Ibid.*, **51**, 337-43 (1968).
- (3) Protta, G., Scherillo, G., and Nicolaus, R. A., On the structure of "trichosiderins," *Rend. Accad. Sci. Fis. Mat. (Soc. Naz. Sci., Napoli)*, **35**, 1-4 (May, 1968).
- (4) Boldt, P., and Hermstedt, E., Pyrrotrichole, eine Gruppe farbiger Verbindungen aus rotem Menschenhaar, *Z. Naturforsch.*, **22b**, 718-22 (1964).
- (5) Boldt, J., Zur Kenntnis des Trichosiderins, eines Pigments aus roten Haaren, *Naturwissenschaften*, **51**, 265 (1964).
- (6) Protta, G., and Nicolaus, R. A., Struttura e biogenesi delle feomelanine. Nota I. Isolamento e proprietà dei pigmenti delle piume, *Gazz. Chim. Ital.*, **97**, 665-84 (1967).
- (7) Protta, G., Scherillo, G., Napolano, E., and Nicolaus, R. A., Struttura e biogenesi delle feomelanine. Nota II. Sulla reazione tra o. chinoni e cisteina, *Ibid.*, **97**, 1451-78 (1967).

- (8) Prota, G., Scherillo, G., and Nicolaus, R. A., Struttura e biogenesi delle feomelanine. Nota IV. Sintesi e proprietà della 5-S-cisteinildopa, *Ibid.*, **98**, 495-510 (1968).
- (9) Takahashi, H., and Fitzpatrick, T. B., Large amounts of dioxyphenylalanine in the hydrolysate of melanoma from Harding-Passey mouse melanoma, *Nature*, **209**, 888-90 (1966).
- (10) Duchon, J., Hori, Y., Seiler, H., and Fitzpatrick, T. B., The amino acid composition of melanosomes from Harding-Passey, B-16, and Cloudman S-19 mouse melanomas, *Clin. Res.*, **16**, 255 (1968).
- (11) Minalc, L., Fattorusso, E., Cimino, G., De Stefano, S., and Nicolaus, R. A., Struttura e biogenesi delle feomelanine. Nota III. Prodotti di degradazione, *Gazz. Chim. Ital.*, **97**, 1636-63 (1967).
- (12) Brumbaugh, J. A., Ultrastructural differences between forming eumelanin and pheomelanin, as revealed by the pink-eye mutation in the fowl, *Develop. Biol.*, **18**, 375-90 (1968).
- (13) Brumbaugh, J. A., Differentiation of black-red melanin in the fowl. Interaction of pattern genes and feather follicle milieu, *J. Exptl. Zool.*, **166**, 11-23 (October, 1967).
- (14) Cleffmann, G., Agouti pigment cells *in situ* and *in vitro*, *Ann. N. Y. Acad. Sci.*, **100**, 749-61 (1963).
- (15) Cleffmann, G., Function-specific changes in the metabolism of agouti pigment cells, *Exptl. Cell Res.*, **35**, 590-600 (1964).
- (16) Flesch, P., Esoda, E. J., and Katz, S. A., The color of red hair, *J. Soc. Cosmetic Chemists*, **18**, 777-84 (1967).