

SHORT REPORTS

5-S-Cysteinyl dopa and Trichochromes in Red Feathers

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Abstract. Red cock feathers (Rhode Island) were found to contain dopa and cysteinyl dopa, trichochromes B and C, and two unidentified trichochromes. Trichochromes E and F were not found. Previous findings of trichochromes E and F may be explained as artifacts.

Key words: Cysteinyl dopa; Dopa; Trichochromes; Feathers

Trichochromes are the simplest phaeomelanin pigments (5). Until recently they had been found only in certain red hair and feathers, but they have now also been demonstrated in human melanoma tissue and in the urine of some patients with melanoma metastasis (2, 3, 5, 6). Four different trichochromes (B, C, E and F) have been found in red feathers (5), but only two (B and C) in human hair, in melanoma tissue, and in melanoma urine (1, 2, 3, 5, 6).

It has been demonstrated that certain previously used analytical procedures for trichochromes induce the formation of trichochromes as artifacts if cysteinyl dopas are present in the tissue examined (6). If cysteinyl dopas were present in red feathers, all or some of their trichochromes might be explained as artifacts. We have therefore examined red feathers for the presence of 5-S-cysteinyl dopa and trichochromes. Analysis for trichochromes was done by a method proved not to give rise to artificial trichochromes from cysteinyl dopas present.

MATERIAL AND METHODS

Feathers were obtained from Rhode Island cocks. For determination of dopa and 5-S-cysteinyl dopa, 3.6 g of red feathers were extracted with 80 ml of 1 M HCl. Purifica-

tion and fluorimetry were performed by previously described methods (4, 8). For trichochrome determination, 24 g of red feathers were extracted with 0.1 M NaOH for 16 hours under oxygen. The pH was adjusted to 1.0 with 6 M HCl, and the fluid was centrifuged for 10 min at 17 000 r.p.m.

The supernatant was added to a Dowex 50W-X4 column, 2×10 cm, which was washed with 140 ml of 1 M HCl and then with distilled water to pH 5, and eluted with 0.5 M NaOH. The brownish-yellow eluate was acidified to pH 1 with HCl and refluxed for 15 min. A red tinge appeared. The material was adsorbed onto a Dowex 50W-X4 column, H⁺-form, 2×5 cm, which was eluted with 100 ml 1 M HCl and H₂O to pH 5, and then washed with 0.2 M NaAc to neutral pH.

Subsequent elution with 0.1 M NaOH gave a brownish-yellow solution which was acidified to pH 1 with 6 M HCl. After evaporation to dryness the material was dissolved in methanol-2 M HCl, 95:5, and chromatographed twice on a Sephadex LH-20, 20×1.5 cm column.

The fractions containing the coloured material were evaporated to dryness and dissolved in 2 M HCl for spectrophotometry to determine the total yield of pigments. The solution was then analysed by cellulose thin-layer chromatography (TLC), (isopropanol-HCOOH-concentrated HCl; 30:70:1 v/v).

For identification of trichochromes from feathers, trichochromes B, C, E, and F were prepared from cysteinyl dopas and purified as previously described (6).

RESULTS AND DISCUSSION

Red feathers contained dopa and cysteinyl dopa in substantial amounts: that of dopa was 1.0 µg/g feathers, and that of 5-S-cysteinyl dopa 2.7 µg/g.

The trichochrome content of the red feathers was calculated on the basis of molecular absorption of trichochrome C. Spectrophotometry gave 0.9 mg/g.

TLC of the trichochromes revealed trichochromes B and C but no trace of trichochromes E and F. Two other trichochromes, red at acid pH, were observed, one moving faster than trichochrome C and one close to trichochrome B. These trichochromes showed the same colour change to yellow at alkaline pH as trichochromes B and C.

Our results confirm previous findings on trichochromes B and C in red feathers. Trichochromes E and F, which have been reported in red feathers (5) were not found, however. The cysteinyl dopa in feathers now observed may explain the earlier reports on trichochromes E and F, since these com-

pounds are formed artificially if cysteinyl-dopas present are not oxidized before the analysis for trichochromes.

Dopa and 5-S-cysteinyl-dopa have previously been observed in guinea-pig hairs (9). The finding of these catechol amino acids in hair and feathers indicates that a third path of distribution of these compounds from the melanocytes exists. Firstly, they may form pigments, which are transferred to keratinocytes or phagocytes. Secondly, they may be excreted as such into the connective tissue and into the circulation. Thirdly, they may be directly ingested by keratinocytes, where they remain non-oxidized.

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Free and Bound 5-S-Cysteinyl-dopa and Dopa in Human Malignant Melanomas

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Abstract. Free dopa and 5-S-cysteinyl-dopa were extracted from two human melanomas. Subsequent hydrolysis of carefully washed melanoma tissue released dopa and 5-S-cysteinyl-dopa, indicating the presence of these catechol amino acids in proteins. Cysteinyl-dopa-containing proteins may represent the antigens previously demonstrated in human melanomas.

Key words: Melanoma; Cysteinyl-dopa; Dopa; Protein

Studies on patients with malignant melanoma have produced evidence of tumour-directed serum antibodies and also of cell-mediated allergy to melanoma cells (3, 6). Antibodies to cytoplasmic components of the tumour cells have been reported, but no tumour-specific antigens have so far been defined.

Melanomas contain dopa and cysteinyl-dopas, amino acids which are precursors of melanin, but which may also leave the cell, enter the circulation, and be excreted in the urine.

Tyrosine in a protein may be oxidized to dopa and we assume that this dopa can also be oxidized, with resultant formation of immobilized dopa-quinone. By nucleophilic addition of cysteine to this dopaquinone, cysteinyl-dopa in a protein may be produced. Cysteinyl-dopa as part of a protein may also be formed by the addition of free dopaquinone to a sulfhydryl group of cysteine in a protein.

If dopa or cysteinyl-dopas are formed in a protein this may have immunological implications. It seems quite possible that such a protein when exposed to immunocompetent cells, is registered as "non-self". An immune reaction against the melanocytes may result.

The present study was performed with the object of demonstrating dopa and cysteinyl-dopas as components of peptides or proteins in human melanomas.