

GLUTATHIONEDOPA IN MALIGNANT MELANOMA

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Abstract. A human melanoma metastasis was found to contain a substance identical with synthetic glutathionedopa when examined by means of an automatic amino acid analyser, chromatography on a Dowex-50 column, thin-layer electrophoresis, and by fluorimetry. The presence of glutathionedopa in melanoma tissue and of enzymes capable of hydrolysing the glutathione moiety of the molecule, together with the absence of glutathionedopa in the urine, suggest that glutathionedopa is an intermediate substance in the formation of 5-S-cysteinyl-dopa.

Key words: Glutathionedopa; Melanoma; 5-S-cysteinyl-dopa

It has recently been shown that 5-S-cysteinyl-dopa, an amino acid that plays a key role in pigment cell metabolism, can be formed by enzymatic degradation of glutathionedopa (1).

5-S-cysteinyl-dopa may thus be formed either by direct conjugation of cysteine to dopaquinone or by conjugation of glutathione to the quinone with subsequent hydrolysis of the peptide by γ -glutamyl transferase and a peptidase (Fig. 1). These enzymes are present in melanoma tissue and in large amounts in the kidneys and in the liver.

The aim of the present study was to prove the existence of a glutathionedopa pathway for 5-S-cysteinyl-dopa formation. We therefore investigated human melanoma for the presence of glutathionedopa.

We have previously examined urine from melanoma patients for the presence of glutathionedopa, but without success. This is not surprising, since the enzymes mentioned can split any glutathionedopa formed in the melanoma. However, it ought to be possible to detect glutathionedopa at the site of its formation.

MATERIAL AND METHODS

Glutathionedopa was synthesized by NFR Syntestjänst, Lund. Another sample was prepared enzymatically by incubation of a mixture of glutathione, tyrosine, and

tyrosinase, and purified by a technique previously described (6). Glutathionedopa isolated from melanoma tissue was identified by three different methods.

A. *Amino acid analyser* (LKB 3201) using a 9×119 mm column containing Durum DC-6A cation exchange resin, equilibrated with a sodium-citrate buffer, pH 3.82. Glutathionedopa was eluted by means of 0.10 N sodium-citrate buffer, pH 3.82, the flow being 104 ml/h. The glutathionedopa peak appeared after 44 minutes.

B. A *Dowex-50 column* (5×54 mm) in H⁺ form. The glutathionedopa, dissolved in 0.1 N HCl, was put on the column and eluted first by passing 40 ml 0.5 N HCl. This eluate was discarded. The subsequent elution was performed with N HCl. The effluent was collected in 5 ml fractions. The glutathionedopa peak appeared in the fourth fraction thus obtained.

C. *Thin-layer electrophoresis* using Kisel-Gel of 0.25 mm thickness. The buffer was pyridine-acetic acid, pH 4 (12 ml pyridine, 40 ml glacial acetic acid, and water to 1000 ml). The voltage was 25 V cm⁻¹. The glutathionedopa moved at a rate of 0.68 cm h⁻¹ towards the anode. It was visualized by spraying with diazotized *p*-nitroaniline (10).

Some 25–50 g of melanoma tissue obtained at necropsy from a liver metastasis of a red-haired man was homogenized in 0.4 N perchloric acid and centrifuged for 10 minutes at 17 000 r.p.m. The catechols of the supernatant were adsorbed on Al₂O₃ at pH 8.6, and eluted with 0.1 N HCl. The eluate was then evaporated to dryness.

For the automatic amino acid analyser the residue was dissolved in water, and the pH adjusted to 2.5. The solution was filtered through a Millipore filter (GSWP 01300), and 1 ml of the solution was injected into the automatic amino acid analyser.

For chromatography on the Dowex column another melanoma extract was prepared as above. After evaporation the Al₂O₃ eluate to dryness, the residue was dissolved in 0.1 N HCl.

The 4th 5 ml fraction from the Dowex column was used for thin-layer electrophoresis and fluorimetry (6). Thin-layer electrophoresis of the peak appearing after 44 minutes in the automatic amino acid analyser was also performed.

RESULTS

Synthetic glutathionedopa appeared in the 4th 5 ml elution fraction from the Dowex column. The

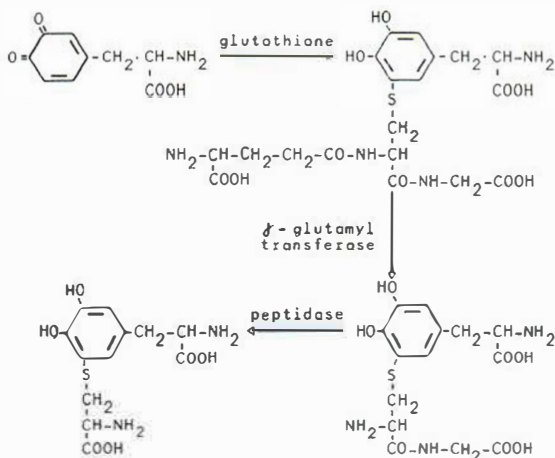


Fig. 1. Formation of 5-S-cysteinyltyrosine from dopaquinone and glutathione.

melanoma tissue extract contained a substance that also appeared in this fraction. Oxidation of the catechols in this fraction as described by Anton & Sayre yielded fluorophores identical with those of glutathionedopa (6).

Chromatography of the melanoma extract in the automatic amino acid analyser showed a peak after 44 minutes, which was also the elution time for synthetic glutathionedopa. By comparison with authentic glutathionedopa the amount in the melanoma was calculated to be 2 $\mu\text{g/g}$.

Thin-layer electrophoresis of the substance isolated from the melanoma extract by chromatography on the Dowex column or by chromatography in the automatic amino acid analyser disclosed a compound moving towards the anode at the same rate as authentic glutathionedopa and showing the same colour reaction with diazotized *p*-nitroaniline.

DISCUSSION

The presence of glutathionedopa in melanoma tissue now reported is direct evidence of the role of glutathione in the regulation of pigment formation. It was originally thought that the SH-group of glutathione acted by inhibiting tyrosinase (3, 8). The inhibitory effect of glutathione on melanin synthesis may however prove to be due to the formation of thiol-derived reaction products by the action of tyrosinase (2, 4, 7, 9); such thioethers are apparently less inclined to form stable polymers.

Several structures have been proposed for the glutathione reaction products (5). Our studies on the hydrolysis of the compounds formed by incubation of tyrosine with tyrosinase in the presence of glutathione suggest that the 1,6-addition of the thiol group to dopaquinone is an important reaction, since 5-S-cysteinyltyrosine is formed by the action of γ -glutamyl transferase and peptidase on the reaction product (1).

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REFERENCES

1. Agrup, G., Falck, B., Kennedy, B.-M., Rorsman, H., Rosengren, A.-M. & Rosengren, E.: Formation of cysteinyltyrosine from glutathionedopa in melanoma. *Acta Dermatovener (Stockholm)* 55: 1, 1975.
2. Bouchilloux, S. & Kodja, A.: Combinaison des thiols avec les quinones se formant au cours de la mélanogénèse. *Bull Soc Chim Biol* 42: 1045, 1960.
3. Flesch, P.: Inhibitory action of extracts of mammalian skin on pigment formation. *Proc Soc Exp Biol Med* 70: 136, 1949.
4. Mason, H. S. & Peterson, E. W.: Melanoproteins. I. Reactions between enzyme-generated quinones and amino acids. *Biochim Biophys Acta* 111: 134, 1965.
5. Nicolaus, R. A.: Melanins, p. 67. Hermann, Paris 1968.
6. Rorsman, H., Rosengren, A.-M. & Rosengren, E.: Fluorometry of catechol thioethers. *Acta Dermatovener (Stockholm)* 52: 353, 1972.
7. Roston, S.: Reaction of the sulfhydryl group with an oxidation product of β -3,4-dihydroxyphenyl-L-alanine. *J Biol Chem* 235: 1002, 1960.
8. Rothman, S., Krysa, H. E. & Smiljanic, A. M.: Inhibitory action of human epidermis on melanin formation. *Proc Soc Exp Biol Med* 62: 208, 1946.
9. Seiji, M., Yoshida, T., Itakura, H. & Irimajiri, T.: Inhibition of melanin formation by sulfhydryl compounds. *J Invest Dermatol* 52: 280, 1969.
10. Studnitz, W. v.: Methodische und klinische Untersuchungen über die Ausscheidung der 3-methoxy-4-hydroxy Mandelsäure im Urin. *Scand J Clin Lab Invest* 12: Suppl. 48, 1960.

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