Acta Dermatovener (Stockholm) 52: 357–360, 1972

CYSTEINYL DOPA IN HUMAN MALIGNANT MELANOMA

A. Björklund, B. Falck, S. Jacobsson, H. Rorsman, A.-M. Rosengren and E. Rosengren

From the Institute of Anatomy and Histology, Departments of Plastic Surgery, Dermatology, Biochemistry and Pharmacology, University of Lund, Lund, Sweden

Abstract. Melanoma metastases in a man with red hair contained a catechol with characteristics identical with those of cysteinyl dopa.

Human malignant melanomas contain substantial amounts of dopa (2). Recently, another catechol, which also forms a fluorophore with formaldehyde, has been isolated from melanoma extracts by chromatography. This fluorophore has other spectral characteristics than the formaldehyde-induced fluorophore of dopa. Furthermore, this catechol fluoresces at a longer wavelength than dopa after oxidation with periodate (3). Model experiments with enzymatically synthetized thioethers of dopa appear to confirm that the previously described catechol in melanoma is a thioeter catechol (6). This paper reports studies on a thioeter of dopa which was found in large amounts in metastases of a human skin melanoma.

MATERIAL AND METHODS

Melanoma tissue was obtained from a 51-year-old male who had red hair and freckles. In January 1971, he had noticed that a nevus on the left shoulder had grown and bled occasionally. He was admitted to hospital in March. At operation, the tumour was 20 mm in diameter and clinically a malignant melanoma. It was excised with a margin of about 10 cm and the defect covered with split skin graft. In July 1971, there was a recurrence at the border of the graft. A wide excision, including the trapezious muscle, was performed in September 1971, but was not radical. In December 1971, a new skin metastasis was excised. Extraction and purification of the catechol was performed according to previously described methods (6). The following methods were used for identification of the catechols in the eluates from Al_2O_3. Dopa ad cysteinyl dopa were studied as reference substances.

Ascending chromatography in butanol/acetic acid/water (60 : 15 : 25). Electrophoresis in pyridine/acetic acid/water (2 : 1 : 47), pH 5.1 for 90 minutes. 10 V per cm.

Fluorimetry after oxidation was performed as previously described (6). Fluorimetry of chromatograms after formaldehyde treatment was performed as follows: After drying, the chromatograms were exposed to formaldehyde vapours at + 80°C for 1 hr in 1 l glass vessels containing 5 g paraformaldehyde at standardized humidity (cf. 1, 4). Fluorescent spots were localized under UV-light (365 nm). Fluorescence excitation and emission spectra were recorded *in situ* with a modified Leitz microspectrofluorimeter as described elsewhere (1). The chromatogram was placed on the microscope stage in the microspectrofluorimeter, and the exciting light was focused with a quartz brightfield condenser onto the paper. A quartz optical system for the exciting light was used to allow registration down to 300 nm. Areas above or below the fluorescent spots were used to obtain blank values. All spectra were corrected for instrumental errors according to the procedures previously described.

Acidification and alkalinization of the chromatogram papers were obtained by exposing the paper to the vapours of concentrated hydrochloric acid or ammonia in a closed vessel for 5 min at room temperature.

Spectrometry was performed after washing the Al_2O_3 eluate with ether. Photometry of eluates heated to 100°C was also performed in 2 N HCl and in 0.1 N NaOH.

RESULTS

Chromatography of eluates from Al_2O_3 of melanoma perchloric acid extract showed a catechol with Rf value of 0.09. This catechol fluoresced yellow after paraformaldehyde treatment and became red after oxidation with potassium ferricyanide.

Electrophoresis of the eluate from Al_2O_3 showed a catechol with the same moving characteristics as cysteinyl dopa.

Fluorimetry after oxidation of the Al_2O_3 eluate showed a compound with fluorescence emission

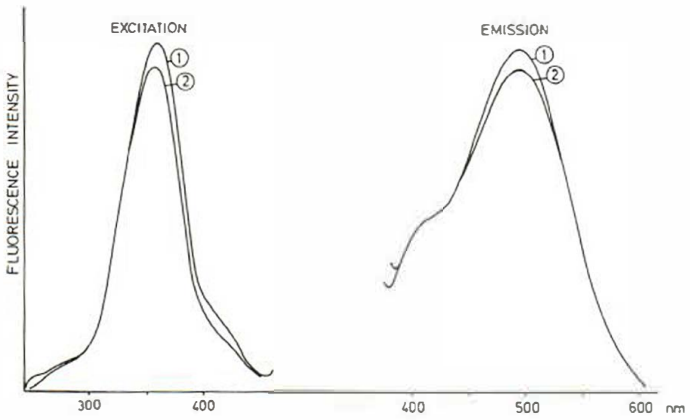

Fig. 1. Excitation and emission spectra of oxidized cysteinyl dopa (1) and of oxidized materials from melanoma (2).

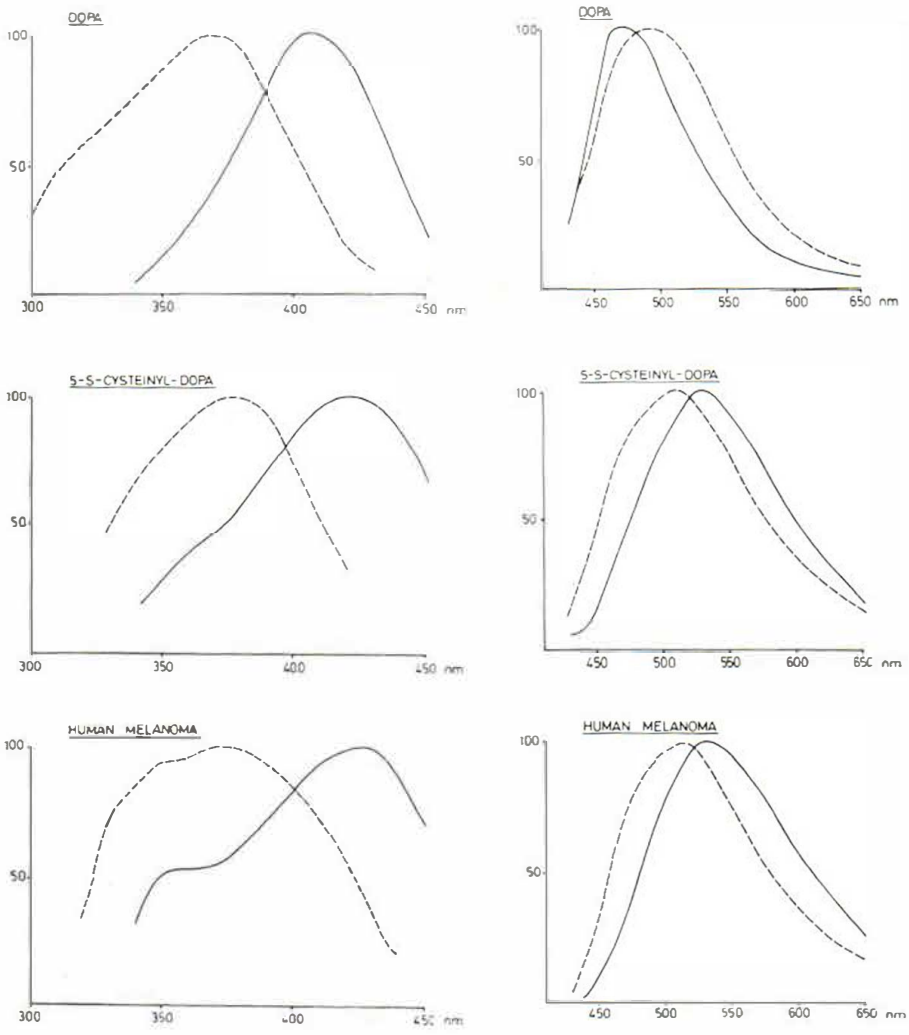

Fig. 2. Excitation and emission spectra of dopa, cysteinyl dopa, and materials from melanoma after formaldehyde treatment. ——, fluorescence in alkaline medium; - - - - -, fluorescence in acid medium.

maximum at 485 nm and excitation maximum at 355 nm corresponding to the maxima of cysteinyl dopa (7).

Fluorimetry after formaldehyde treatment of the chromatographed catechol from melanoma showed an excitation maximum at 420 nm after alkaline treatment and at 375 nm after acid treatment. The corresponding emission maxima were at 530 nm and 510 nm. All maxima of the melanoma catechol corresponded to those of cysteinyl dopa.

Spectrophotometry of the Al_2O_3 eluates of the melanoma extracts showed absorption maxima at 255 nm and 292 nm and minimum at 275 nm. The maxima and the minimum corresponded to those of cysteinyl dopa (5) or of a cysteinyl dopa peptide (6). The amount of cysteinyl dopa was calculated to be about 50 μg per g wet weight melanoma tissue using the molar extinction coefficient of cysteinyl dopa at 292 nm as given by Prota et al. (5).

Photometry of heated Al_2O_3 eluate in 2 N HCl showed that the apparent violet colour had absorption maxima at 587 and 330 nm and a slope at 550 nm. After alkalinization, the solution turned yellow-brown, and the absorption maximum was at 482 nm. This colour is characteristic of the reaction product of cysteinyl dopa (Nicolaus & Prota, personal communication). Glutathione dopa in acid medium did not turn violet after heating.

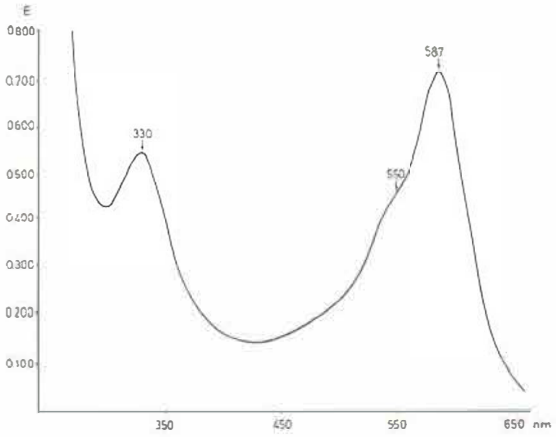

Fig. 4. Absorption spectrum of melanoma material heated in 2 N HCl.

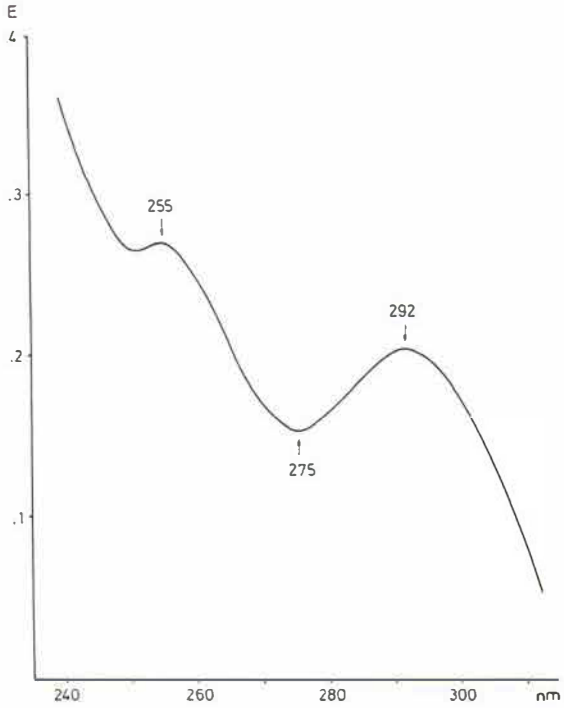

Fig. 3. UV-spectrum of melanoma material.

DISCUSSION

All characteristics of the catechol extracted from the melanoma metastases were identical with those of cysteinyl dopa. Furthermore, the combined findings of chromatography, absorption spectrum after heating and fluorimetry after oxidation and after formaldehyde treatment excluded the presence of a reaction product of glutathione and dopa in our case (6, 7). We obtained no evidence of the presence of significant amounts of dopa peptides. Preliminary experiments with differential centrifugation showed that at least some of the cysteinyl dopa in the melanoma is particle-bound (unpublished observations). Cysteinyl dopa is considered to be the precursor of the pigment in red hair (5). The fact that the melanoma studied was obtained from a patient with red hair might suggest that the presence of cysteinyl dopa in a melanoma reflects the original pigment type of the individual. But it is also possible that the presence of cysteinyl dopa is due to an aberration of melanin synthesis in some melanomas, independent of the original pigmentation of the patient. Current studies at our laboratories will

show whether the presence of cysteinyl dopa and/or other thiocatechols in melanomas are related to the pigmentation type of the carrier or to the qualities of the melanomas per se.

ACKNOWLEDGEMENTS

This investigation has been supported by grants from The Swedish Medical Research Council and from the Swedish Cancer Society.

REFERENCES

1. Björklund, A., Falck, B. & Owman, Ch.: Fluorescence microscopic and microspectrofluorometric techniques for the cellular localization and characterization of biogenic amines. *In* Methods in Investigative and Diagnostic Endocrinology (ed. J. E. Rall & I. J. Kopin), vol. 1: The Thyroid and Catecholamines. North-Holland, Amsterdam, 1972.
2. Falck, B., Jacobsson, S., Olivecrona, H., Olsen, G., Rorsman, H. & Rosengren, E.: Determination of catecholamines, 5-hydroxytryptamine and 3,4-dihydroxyphenylalanine (dopa) in human malignant melanomas. Acta Dermatovener (Stockholm) *46:* 65, 1966.
3. Falck, B., Jacobsson, S., Olivecrona, H., Rorsman, H., Rosengren, A. M. & Rosengren, E.: On the occurrence of catecholderivatives in malignant melanomas. Communications from the Department of Anatomy, University of Lund, Sweden, No. 5, 1966.
4. Hamberger, B., Malmfors, T. & Sachs, C.: Standardization of paraformaldehyde and of certain procedures for the histochemical demonstration of catecholamines. J Histochem Cytochem *13:* 147, 1965.
5. Prota, G., Scherillo, G. & Nicolaus, R. A.: Struttura e biogenesi delle feomelanine. Nota IV. Sintesi e proprietá della 5-S-cisteinildopa. Gazz Chim Ital *98:* 495, 1968.
6. Rorsman, H., Rosengren, A. M. & Rosengren, E.: Fluorometry of a dopa peptide and its thioether. Acta Dermatovener (Stockholm) *51:* 179, 1971.
7. — Fluorometry of catecholthioethers. Acta Dermatovener (Stockholm). In press.

Received April 17, 1972

H. Rorsman, M.D.
Department of Dermatology
University Hospital
S-221 85 Lund
Sweden