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# FLUOROMETRY OF CATECHOL THIOETHERS

H. Rorsman, A.-M. Rosengren and E. Rosengren

*From the Departments of Dermatology, Biochemistry and Pharmacology, University of Lund, Lund, Sweden*

*Abstract.* Incubation of L-tyrosine, tyramine and of L-tyrosyl-glycyl-glycine with polyphenoloxidase in the presence of cysteine or glutathione resulted in the formation of catechols which by chromatography were found to be different from dopa, dopamine and dopyl-glycyl-glycine. The catechol derivatives formed showed a yellow fluorescence after formaldehyde treatment, whilst oxidation with periodate also resulted in formation of fluorophores. The compounds formed were probably thioethers of dopa, dopamine and the dopa peptide. Oxidation with periodate of cysteinyl dopa or of the cysteinyl dopa peptide (but not of the glutathione catechols) resulted in fluorophores with emission peaks at considerably longer wavelengths than the fluorophores produced by oxidation of dopa or the dopa peptide. The oxidation of cysteinyl dopamine did not give a fluorophore with emission at a longer wavelength. Presence of a carbonyl group in the side chain of the catechol conjugated with cysteine seems to be a prerequisite for the formation of fluorophores with emission at a longer wavelength.

Human skin melanomas treated with formaldehyde fluoresce green or yellow (3, 5). The formaldehyde-induced fluorescence is probably not due to their content of dopa since hamster and mouse melanomas contain equal or larger amounts of this amino acid and yet do not fluoresce after treatment with formaldehyde (4, 6, 7, 8). A catechol compound in human melanomas has been described (6), which is separated from dopa by chromatography and which fluoresces at a longer wavelength than dopa after oxidation. This catechol fluoresced yellow after formaldehyde treatment. It has been assumed that the formaldehyde-induced fluorescence of human melanomas is due to thioethers of dopa, since cysteinyl dopa and a cysteinyl dopa peptide fluoresce yellow when treated with formaldehyde and, like human melanoma extracts, exhibit a green fluorescence after oxidation (11). Further investigations on the characteristics of the fluorescence of thioethers of dopa and of *N*-terminal dopa peptides have now been performed to test the hypothesis. We have synthesized sulphur-containing catechols by incubation of tyrosine, tyramine and an *N*-terminal tyrosine peptide with cysteine and glutathione in the presence of polyhenoloxidase and examined the fluorescence of the compounds formed on oxidation.

## MATERIAL AND METHODS

### *Synthesis of catechol compounds containing cysteine or glutathione*

The incubations were performed with 0.3 mg of polyphenoloxidase (Sigma Chem. Corp.) in 2.3 ml of a 0.1 M phosphate buffer, pH 6.5, at room temperature.

*Dopa–cysteine:* 2.0 mg of L-tyrosine (Sigma) was incubated with 1.2 mg of L-cysteine hydrochloride (E. Merck AG) for 4 hours.

*Dopa–glutathione:* 2.0 mg of L-tyrosine was incubated with 3.1 mg glutathione, reduced form (Sigma) for 4 hours.

*Dopa peptide–cysteine:* 2.0 mg of L-tyrosyl-glycyl-glycine (Fluka AG) was incubated with 1.2 mg of L-cysteine hydrochloride for 2 hours.

*Dopa peptide–glutathione:* 2.0 mg of L-tyrosyl-glycyl-glycine was incubated with 3.1 mg of glutathione, reduced form for 4 hours.

*Dopamine–cysteine:* 2.0 mg of tyramine was incubated with 1.2 mg L-cysteine hydrochloride for 2 hours.

All incubations were stopped by the addition of 0.23 ml of 4 N perchloric acid, and the volume was then adjusted to 20.0 ml with 0.4 N perchloric acid.

### *Fluorometry*

Adsorption of catechols onto $Al_2O_3$ and elution were performed as previously described (11). Fluorometry was carried out with an Aminco-Bowman spectrophotofluorometer, with maximum excitation for quinine sulphate in 0.1 N $H_2SO_4$ at 350 nm and maximum emission at 450 nm. The excitation and emission spectra of oxidized eluates were compared with hose of oxidized L-dopa (Fluka) and dopamine (Fluka).

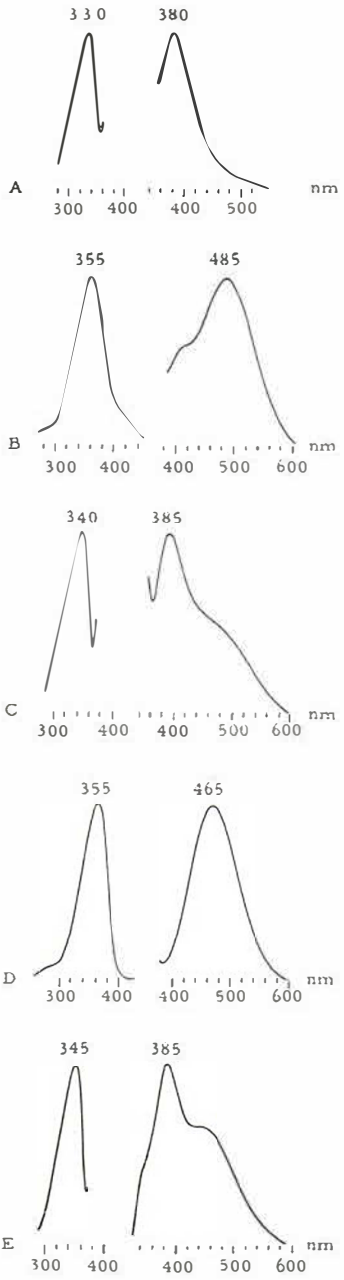


*Fig. 1.* Excitation and emission spectra for fluorophores formed at oxidation of dopa (*A*) and of catechols formed at incubation with polyphenoloxidase of tyrosine + cysteine (*B*), tyrosine + glutathione (*C*), tyrosyl-glycyl-glycine + cysteine (*D*), or tyrosyl-glycyl-glycine + glutathione (*E*).

### *Chromatography*

Samples of eluates and of L-dopa and dopamine were developed in ascending chromatograms on Whatman paper No. 1 in *n*-butanol/acetic acid/water (60 : 15 : 25). Chromatograms were placed in a jar containing paraformaldehyde and heated at 80°C for 1 hour. The chromatograms were then examined in UV-light at wavelength of 350 nm. They were afterwards sprayed with a solution of 0.4% potassium ferricyanide, heated at 80°C for 5 min and examined in visible light and in UV-light at 350 nm.

## RESULTS

### *Fluorometry of oxidized $Al_2O_3$ eluates*

The presence of cysteine or glutathione at the incubation of tyrosine or a tyrosine peptide with polyphenoloxidase gave rise to the formation of substances different from dopa and a dopa peptide. The excitation and emission curves for oxidized 0.1 N HCl eluates from $Al_2O_3$ are given in Fig. 1. Excitation maxima of oxidation products for compounds formed in the presence of cysteine or glutathione were invariably at slightly longer wavelengths than those for dopa. The emissions of the oxidized eluates differed widely. The product formed by incubation of tyrosine and cysteine gave a peak at 485 nm with a shoulder a 420 nm. Incubation of tyrosine and glutathione gave a compound with a maximum excitation at 340 nm and an emission with a maximum at 385 nm and a slope at 460–480 nm.

The incubation of the tyrosine peptide with cysteine resulted in a compound which, after oxidation, showed excitation at 355 nm and emission at 465 nm. Oxidation of the tyrosine peptide in the presence of glutathione resulted in the formation of a compound having an excitation peak at 345 nm. The emission was at 385 nm with a shoulder at 440 nm. Oxidation of the compound formed by incubation of tyramine and cysteine gave a product having an excitation maximum at 320 nm and an emission maximum at 370 nm and with the same fluorescence characteristics as dopamine (1). However, spectrophotometry showed formation of a compound with the same absorption as cysteinyl dopa (9).

### *Chromatography of eluates from $Al_2O_3$*

Chromatography of the 0.1 N HCl eluates from $Al_2O_3$ and subsequent treatment with formaldehyde demonstrated fluorescent compounds with the Rf-values given in Table I. All the fluorescent compounds formed from incubates in the presence of cysteine and glutathione were easily separable from dopa, dopamine and dopyl-glycyl-glycine, respectively. The spots turned red after treat-

Table I. *Rf values of catechols formed by incubation of tyrosine, tyramine or tyrosyl-glycyl-glycine with polyphenoloxidase and cysteine or glutathione (n-butanol/acetic acid/water, 60 : 15 : 25, ascending chromatography)*

Dopa, dopamine and the dopa peptide fluoresced green, and the other compounds yellow after formaldehyde treatment

| Substances incubated | Rf value |
|---|---|
| Dopa (standard) | 0.30 |
| Dopamine (standard) | 0.32 |
| Tyrosyl-glycyl-glycine | 0.18 |
| Tyrosine + cysteine | 0.10 |
| Tyrosine + glutathione | 0.09 |
| Tyrosyl-glycyl-glycine + cysteine | 0.08 |
| Tyrosyl-glycyl-glycine + glutathione | 0.09 |
| Tyramine + cysteine | 0.18 |
| Tyramine + glutathione | 0.07 |

ment with potassium ferricyanide. After this treatment the formaldehyde-induced fluorescence colours were no longer detectable in UV-light, but the spots became white.

## DISCUSSION

The presence of cysteine or of glutathione in the incubation fluid on enzymatic oxidation of tyrosine, tyramine or af an *N*-terminal tyrosine peptide induced formation of catechols which produced yellow fluorophores with formaldehyde. The catechols formed in the presence of cysteine or glutathione showed certain differences from dopa, dopamine and the dopa peptide. The fluorescence emitted after oxidation of the products formed from tyrosine and tyrosyl-glycyl-glycine in the presence of cysteine was at longer wavelengths and seems to represent the sulphur-containing oxidized catechols.

Oxidation of tyrosyl-glycyl-glycine in the presence of cysteine gave a compound with maximum excitation at 355 nm and an emission peak at 465 nm. The excitation maximum of cysteinyl-dihydroxyphenylalanyl-glycyl-glycine has previously been given at a shorter, and the emission at a longer, wavelength (11). The present values were obtained with a fluorometer with maximum excitation for quinine sulphate at 350 nm and maximum emission for this compound at 450 nm. Previously reported maxima were obtained with a fluorometer not calibrated with quinine sulphate but with an excitation maximum for the oxidized dopa fluorophore at 335 nm and emission at 380 nm.

Oxidation of tyrosine in the presence of cysteine can be expected to give 5-*S*-cysteinyl and 2-*S*-cysteinyl dopa (10). Oxidation of the enzymatically formed cysteinyl dopa gave a main emission peak at 485 nm. Chemically synthetized 5-*S*-cysteinyl dopa (9) also gives rise to two emission peaks at fluorometry (11).

Comparison of the emission spectra obtained after oxidation of melanoma extracts and of sulphur-containing catechols formed *in vitro* will probably be most helpful in the definition of the catechols present in melanomas. We have recently studied a melanoma which contained a catechol which, after oxidation, showed fluorescence with excitation at 355 nm and a definite emission at 485 nm, corresponding to the excitation and emission of cysteinyl dopa (2).

The enzymatic oxidation of tyramine in the presence of cysteine gave a product which was probably cysteinyl dopamine, as judged from UV-absorption. But the oxidation product of this compound in contrast to cysteinyl dopa and the cysteinyl dopa peptide did not fluoresce at a longer wavelength. Thus, the presence of a carbonyl group in the side chain of the catechol conjugated with cysteine seems to be of importance for the formation of fluorophores with emission at longer wavelengths.

The presence of glutathione in the incubation fluid led to formation of catechols which could be distinguished from dopa, dopamine and the dopa peptide, respectively, by chromatography, but the fluorophores formed at oxidation of these catechols did not have a distinct emission at longer wave-lengths. Thus, the oxidation method used seems to be suitable for the determination of certain cysteine catechols but not for glutathione catechols.

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