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FORMATION OF CYSTEINYLDOPA FROM GLUTATHIONEDOPA IN MELANOMA

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Abstract. Glutathionedopa injected intravenously into mice is metabolized and excreted in the urine as a compound with the fluorescence characteristics of cysteinyldopa. Glutathionedopa incubated with a guinea-pig kidney homogenate is metabolized to a compound with the fluorescence characteristics of cysteinyldopa. Boiling of the kidney homogenate prevents the metabolism of glutathionedopa.

Incubation of glutathionedopa with a homogenate of a melanoma metastasis led to the formation of a compound with the fluorescence characteristics of cysteinyldopa. Boiling of the melanoma homogenate prevented the metabolism of glutathionedopa. Large amounts of glutathione added to the incubate inhibited the reaction. Lung tissue and blood plasma had no detectable ability to metabolize glutathionedopa.

The results show that human melanoma contains one or several enzymes capable of metabolizing glutathionedopa to a smaller dopathioether, probably cysteinyldopa. Such enzymes seem to be normally present in mice and guinea-pigs and have been demonstrated in the guinea-pig kidney.

Key words: Melanoma; Cysteinyldopa; Glutathione; Amino acids, sulfur; Amino acids, diamino; Amino acids, dicarboxylic

The presence of 5-S-cysteinyldopa in melanomas of Africans and Caucasians (2, 6) and in the urine of patients with pigmented melanomas has recently been reported (3). This amino acid has also been detected in the urine of normal subjects (1). Cysteine condenses with dopaquinone and the product obtained is reduced to form 5-S-cysteinyldopa. This reaction proceeds quite spontaneously after dopaquinone has been formed by the action of the enzyme tyrosinase. Dopaquinone and reduced glutathione should be present in the melanocyte and we therefore expected that 5-S-glutathionedopa would be formed in melanin-producing cells, since the cysteinyl part of the glutathione peptide reacts just as well with dopaquinone as does cysteine (Fig. 1).

Thus far, however, we have been unable to detect any glutathionedopa in pigment-forming cells despite extensive work. This led us to conclude that any glutathionedopa formed in these cells is rapidly hydrolysed to cysteinyldopa by the action of a γ-glutamyl transpeptidase and a peptidase hydrolysing the cysteine-glycine linkage. Experiments to be described below have demonstrated that such enzymes are present in laboratory animals and that they also occur in human malignant melanomas.

MATERIAL AND METHODS

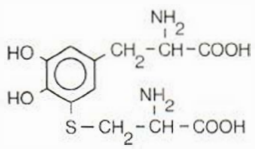
Glutathionedopa was prepared as previously described (4). It was purified by adsorption to Al_2O_3, eluted with 0.1 N HCl and then passed through a column (15 × 300 mm) containing Sephadex G-25. Glutathionedopa was eluted with water and appeared in the effluent between the 35th and 45th ml. This portion was freeze-dried. Finally, glutathionedopa was crystallized in 95% ethanol. Glutathionedopa was injected intravenously into 2 mice in the same metabolic cage (80 μg/animal) and the urine collected between 0 and 3 and 3 to 6 hours was analysed for the presence of glutathionedopa and cysteinyldopa.

A homogenate of guinea-pig kidney was prepared by addition of 3 ml 0.1 M phosphate buffer, pH 7.4, for each gram kidney tissue and the homogenate was dialysed against water overnight. Kidney homogenate was incubated with glutathionedopa for 2 hours at room temperature in the presence of 3 ml 5% sodium sulphite solution and glutamine. The effect of reduced glutathione was also investigated and control experiments were performed with boiled kidney homogenate. For details see Table I.

10 g human melanoma tissue from a lung metastasis was homogenized in 30 ml 0.1 M phosphate buffer, pH 7.4, and dialysed against water overnight. 7 ml homogenate was incubated with glutathionedopa in the presence of 4.5 ml 5% sodium sulphite and glutamine for 2 hours at room temperature. Incubation was also performed with the addition of glutathione. As a control, incubations were also carried out with boiled homogenate (Table II).

Incubation of lung tissue obtained at autopsy from a patient without melanoma was also performed. Incubation of plasma from healthy persons with glutathionedopa, sodium sulphite, and glutamine, provided further control

5-S-CYSTEINYLDOPA



5-S GLUTATHIONEDOPA

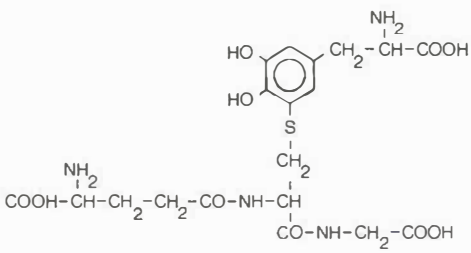


Fig. 1

experiments. After 2 hours' incubation, 4 N perchloric acid was added to stop the enzymatic reactions. After centrifugation and filtration, glutathionedopa and cysteinyldopa were determined as previously described (4, 5).

RESULTS

The mice injected intravenously with glutathionedopa passed no detectable glutathionedopa in the urine. In contrast, large amounts of cysteinyldopa were excreted. Thus, the urine collected 0–3 and 3–6 hours after the injection contained 26.4 and 0.85 μg cysteinyldopa respectively. Cysteinyldopa could not be detected in the urine of control mice with the method used.

Incubation of glutathionedopa with guinea-pig kidney homogenate in the presence of sodium sulphite for 2 hours led to the formation of cysteinyldopa. The amount of cysteinyldopa formed was not increased by the addition of 7×10^{-4} M glutamine to the incubate. Boiling of the kidney homogenate prevented the formation of cysteinyldopa from glutathionedopa added to the homogenate. Addition of 3×10^{-2} M glutathione caused complete inhibition of cysteinyldopa formation. Incubation of the human melanoma homogenate with glutathionedopa in the presence of sulphite and glutamine led to formation of cysteinyldopa. Boiling of the melanoma homogenate before incubation with glutathionedopa prevented the formation of cysteinyldopa. Addition of large amounts of glutathione (3×10^{-2} M) to the incubate containing dialysed melanoma homogenate, sulphite, glutamine and glutathionedopa resulted in complete inhibition of cysteinyldopa formation.

Incubation of human lung tissue homogenate, from a patient who had died of cardiac failure, with glutathionedopa in the presence of sodium sulphite and glutamine did not result in the formation of cysteinyldopa. Similarly, cysteinyldopa was not formed by the incubation of dialysed plasma from a normal subject with glutathionedopa.

DISCUSSION

We have considered cysteinyldopa to be the substance formed by the metabolism of glutathionedopa. This statement is in all cases based on fluorimetric determination of a substance which, when oxidized according to a standardized procedure for the determination of cysteinyldopa (5), gives a fluorophore having the same fluorescence characteristics as cysteinyldopa. It is not out of the question, however, that dopa linked by a thioether bond to a glutamylcysteine peptide or to a cysteinylglycine peptide could give an identical fluorophore under the given

Table I. *Formation of cysteinyldopa from glutathionedopa in homogenate of guinea-pig kidney*

3 ml 5% sodium sulphite was added to each incubate

No. of exp.		Addition to the incubate mixture (M)			Cysteinyldopa formed μg/2 hrs
		Glutathionedopa	Glutamine	Glutathione	
1	Dialysed homogenate, 6 ml	5×10^{-6}	7×10^{-4}	—	22.2
2	Dialysed homogenate, 6 ml	5×10^{-6}	7×10^{-4}	—	22.2
3	Boiled, dialysed homogenate, 6 ml	5×10^{-6}	7×10^{-4}	—	0.0
4	Dialysed homogenate, 6 ml	5×10^{-6}	—	—	20.4
5	Dialysed homogenate, 6 ml	5×10^{-6}	—	3×10^{-2}	0.0
6	Dialysed homogenate, 6 ml	—	7×10^{-4}	—	0.0

Table II. *Formation of cysteinyldopa from glutathionedopa in human malignant melanoma homogenate*

3.5 ml 5% sodium sulphite was added to each incubate

No. of exp.		Addition to the incubate mixture (M)			Cysteinyldopa (μg) found after 2 h
		Glutathionedopa	Glutamine	Glutathione	
1	Dialysed homogenate, 7 ml	4×10^{-6}	6×10^{-4}	—	14.1
2	Dialysed homogenate, 7 ml	4×10^{-6}	6×10^{-4}	—	15.8
3	Boiled, dialysed homogenate, 7 ml	4×10^{-6}	6×10^{-4}	—	2.6
4	Dialysed homogenate, 7 ml	4×10^{-6}	—	—	14.5
5	Dialysed homogenate, 7 ml	4×10^{-6}	—	3×10^{-2}	5.5
6	Dialysed homogenate, 7 ml	—	6×10^{-4}	—	6.0

conditions. Synthesis of these compounds is in progress and fluorimetry of their oxidation fluorophores will give a more definite answer as to the final product of the metabolism of glutathionedopa.

The experiments reported have shown that glutathionedopa, a substance which may be formed in pigment-producing cells, is effectively catabolized in the organism. The metabolism in vivo was studied in mice and in these animals glutathionedopa injected intravenously could not be detected in the urine, whereas substantial amounts of cysteinyldopa were excreted after the glutathionedopa injection. Thus, the mice have an effective mechanism for splitting the glutathione moiety of glutathionedopa.

The in vitro studies on the metabolism of glutathionedopa, incubated with guinea-pig kidney homogenate, demonstrated one or several enzymes capable of metabolizing glutathionedopa. Attempts to define the enzymes have not yet been made.

Our working hypothesis for studies in progress is that the enzymes responsible for the splitting of glutathionedopa are the same as those that metabolize glutathione. γ-glutamyl transpeptidase active on S-glutathionedopa and an enzyme active on the peptide bond of cysteinylglycinedopa would result in cysteinyldopa.

Incubation of the melanoma homogenate with glutathionedopa demonstrated that the melanoma, like the guinea-pig kidney, contains enzymes capable of metabolizing glutathionedopa to a compound with the same fluorescence as cysteinyldopa when oxidized. In the light of this finding our inability to demonstrate glutathionedopa in a long series of unreported experiments is not surprising. Any glutathionedopa formed in the melanocyte may be rapidly metabolized to cysteinyldopa. Future experiments will show to what extent the cysteinyldopa formed in the melanocytes is produced by metabolism of glutathionedopa or by direct conjugation of dopaquinone to cysteine.

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