Determination of Urinary 5-S-Cysteinyldopamine by High-Performance Liquid Chromatography with Electrochemical Detection

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Key Words
Column liquid chromatography
Electrochemical detection
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Tyrosinase
Autoxidation

Summary
5-S-Cysteinyldopamine, a new metabolite of dopamine, was determined in urine by high-performance liquid chromatography with electrochemical detection. The catechol was detected in 14 of 21 melanoma patients and 7 of 21 normal subjects; the highest values were 657 µg/day for melanoma patients and 44 µg/day for normal subjects. These results suggest that the cysteine conjugate may arise from autoxidation of dopamine but tyrosinase may also participate in the oxidation.

Introduction
We have recently demonstrated that on incubation of rat tissue homogenates with dopamine, the new metabolites 5-S- and 2-S-cysteinyldopamine are formed in a ratio of 7:1 [1]. 5-S-Glutathionyldopamine was also detected in the incubation mixture. It thus appears that the cysteine conjugates are formed by a reaction of dopamine o-quinone with glutathione followed by enzymic hydrolysis. The oxidation of dopamine to dopamine o-quinone may be brought about by a variety of biological oxidation systems such as tyrosinase-O₂, peroxidase-H₂O₂, superoxide radical, hydroxyl radical, and iron-chelates (cf. ref. [1]).

Urinary 5-S-cysteinyldopamine has been widely used as a biochemical marker of melanoma metastasis [2]. It is also known that the level of urinary dopamine may be elevated in certain melanoma patients [3]. Therefore, we have determined the levels of 5-S-cysteinyldopamine in the urine of melanoma patients and healthy subjects.

Experimental
24-h urine samples were collected in bottles containing 50 ml of acetic acid and 1 g of Na₂S₂O₅. The urine samples from melanoma patients were obtained from Dr. K. Jimbow (Sapporo Medical College, Sapporo, Japan).

In a microcentrifuge tube (1.5-ml volume) were placed 50 mg of acid-washed alumina and 1 g of Na₂S₂O₅. To the tube were added 100 µl of a urine sample or 10 µl of a standard solution containing 1 µg each of 5-S- and 2-S-cysteinyldopamine and dopamine in 1 ml of 0.1 M HCl and 10 µl of 1 µg/ml 3,4-dihydroxybenzylamine (DHBA) in 0.1 M HCl as an internal standard. To this was added 0.8 ml of 1.5 M Tris-HCl buffer containing 2 % Na₂EDTA, pH 8.6, and the mixture was immediately shaken for 5 min. The alumina was washed twice with water and catechols were then eluted with 150 µl of 0.4 M HClO₄.

The catechol extracts, usually 50 µl, were analyzed by HPLC with electrochemical detection as follows [1]. A Yanaco Model L-5000 liquid chromatograph (Yanagimoto, Kyoto, Japan) was used with a Yanaco Model VMD-101A electrochemical detector. The detector was set at +600 mV vs. an Ag/AgCl reference electrode and sensitivity was 20 nA f.s.

Separation was achieved on a C₁₈ reversed-phase column (Yanaco ODS-T, particle size 10 µm, 250 x 4 mm, I.D.) at 50 °C. The mobile phase was acetonitrile-0.1 M sodium citrate buffer, pH 5.0, containing 6 mM sodium octanesulfonate and 1 mM Na₂EDTA (60:940, v/v). The flow rate was 1.0 ml/min.

5-S-Cysteinyldopa was determined by our previously reported method [4].

Results
We analyzed 21 urine samples each from melanoma patients and normal subjects. Among them, two melanoma samples contained very high levels of a compound appearing at the position of 5-S-cysteinyldopamine (Fig. 1). The identity was confirmed by (a) the coincidence of retention time
with the standard under HPLC conditions for 5-S-cysteinyl-
dopa determination [4], (b) the close parallelism of volt-
ammograms between the sample and standard in compari-
son with those of dopamine (Fig. 2), and (c) the identical k'
values between the sample and standard in response to a
temperature change (35–60 °C; data not shown).
In the chromatograms of the samples containing high levels
of 5-S-cysteinyldopamine, the 2-S isomer was also detected
at much lower levels, the ratios of 5-S isomer to 2-S isomer
being 5.3 : 1 and 8.7 : 1 in the samples in Fig. 1.
Acid hydrolysis of the above two samples in 0.1 M HCl for
20 min did not increase the 5-S-cysteinyldopamine values,
indicating that the catechol is present mostly in the free
form.
Table I shows the urinary excretion of 5-S-cysteinyldopa-
mine in melanoma patients and normal subjects, in com-
parison with those of 5-S-cysteinyldopa and dopamine. 5-S-
cysteinyldopamine was detected in 14 of 21 melanoma pa-
ients but only 7 of 21 healthy subjects. Four values for
melanoma patients exceeded the highest value for normal
subjects. There were no clear correlations among 5-S-cyste-
inyldopamine, 5-S-cysteinyldopa, and dopamine levels both
in melanoma patients and normal subjects except for one
exception: in normal subjects 5-S-cysteinyldopamine level
(Y) was correlated with 5-S-cysteinyldopa level (X): Y =
0.144X – 15.6, r = 0.756, p < 0.01.

Discussion
The present study demonstrates for the first time the occur-
rence of 5-S-cysteinyldopamine in the urine from normal
subjects as well as from melanoma patients. The occurrence
of the new dopamine metabolite in human brain was re-
cently reported; the 5-S-cysteinyldopamine level was about
5 % of the dopamine level [5]. More recently, the same
group reported the occurrence of 5-S-cysteinylderivatives
of dopamine, dopa, and 3,4-dihydroxyphenylacetic acid in
the brains of eight mammalian species [6]. They have sug-
gested that the 5-S-cysteinylcatechol metabolites are form-
ed by autoxidation of catechols to o-quinones and sub-
sequent coupling to glutathione and the conjugates thus
formed are split to the cysteine conjugates by the action of
peptidases.
The present finding of 5-S-cysteinyldopamine in urine
further supports the presence of such an oxidative process
in vivo. The detection rate and the level were much higher
in melanoma patients than in normal subjects. This seems
to suggest that tyrosinase may also participate in the oxida-
tion of dopamine in melanoma patients. The fact that 5-S-
cysteinyldopamine was not detected in one-third of mela-
noma patients and two-thirds of normal subjects may be
ascribed to the presence of sufficient amounts of o-quinone
scavenger(s) such as ascorbic acid in the tissues [7].

Fig. 1
Chromatograms of urine samples from melanoma patients. (A) sub-
ject 2 in Table I. (B) subject 1 in Table I. Peaks: 1 = norepinephrine
(5.6 min), 2 = 2-S-cysteinyldopamine (6.7 min), 3 = DHBA (8.8 min),
4 = 5-S-Cysteinyldopamine (11.0 min), 5 = dopamine (12.8 min).

Fig. 2
Voltammograms of 5-S-cysteinyldopamine and dopamine in sample
(Subject 1 in Table I) and standard. o, 5-S-cysteinyldopamine in
sample; , 5-S-cysteinyldopamine in standard; a, dopamine in
sample; A, dopamine in standard. 50 μl of the urine sample after
alumina extraction or 10 μl of a standard solution containing 1 μg
each of the catechols per ml was injected into the HPLC.