# One-step Synthesis of (2-Amino-2-carboxyethylthio)dopas (Cys-dopas) from Dopa and Cysteine by Hydrogen Peroxide in the Presence of Iron–EDTA Complex

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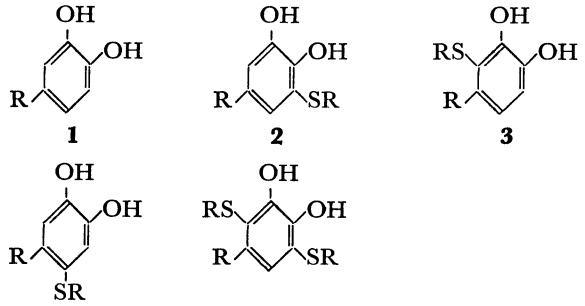
**Synopsis.** 5-(2-Amino-2-carboxyethylthio)dopa, 2-(2-amino-2-carboxyethylthio)dopa, and 2,5-bis(2-amino-2-carboxyethylthio)dopa were synthesized from dopa and cysteine by oxidation in a neutral buffer with $H_2O_2$ in the presence of iron–EDTA complex. $H_2O_2$, iron salt, and EDTA were essential for the reaction. Ultimate oxidizing species seem to be hydroxyl and superoxide radicals.

(2-Amino-2-carboxyethylthio)dopas (represented as Cys-dopas in the following; **2**—**5**) arise in melanocytes by the addition of cysteine to dopaquinone formed by tyrosinase oxidation of dopa (3,4-dihydroxyphenylalanine; **1**).[1] They are precursors of pheomelanin, yellow to reddish-brown pigment of feathers and hair. Increased amounts of these catechols, especially **2**, have been found in the urine of melanoma patients.[2] We have recently shown that **2** is selectively toxic to a variety of human tumor cells *in vitro* and possesses antitumor activity against murine L1210 leukemia and B16 melanoma.[3]




R = $CH_2CH(NH_2)COOH$

Three methods have been reported for the preparation of Cys-dopas (**2**—**5**). 1) Tyrosinase-catalyzed oxidation of dopa in the presence of cysteine gives Cys-dopas (**2**—**5**) in a high total yield;[4] however, this method is not suitable for a large-scale preparation. 2) Multi-step chemical synthesis of **2** and **3** involving nucleophilic addition of cysteine to *N*-acetyldopaquinone ethyl ester requires expensive silver oxide as oxidant.[5,6] 3) Reaction of dopa with cystine in boiling aqueous HBr yields Cys-dopas (**2**—**4**) with the 6-isomer (**4**) being the major product (6—7% yield).[6] This paper presents a more convenient method for a large-scale preparation of **2**, **3**, and **5** by oxidation of a mixture of dopa and cysteine with $H_2O_2$ and $Fe^{2+}$–EDTA complex.

The oxidation, performed on an analytical scale, was followed by TLC on cellulose (in 1-propanol–1 M HCl,† 3:2) and the increase of absorbance at 300 nm where Cys-dopas (**2**—**5**) show strong absorption[6] while **1** does not. In some instances the products were also analyzed by column chromatography. Under the standard conditions (Expt 1 in Table 1), **2**, **3**, and **5** were obtained in 13.9, 3.1, and 4.2%, respectively, while **4** was found only in a trace amount (much less than 1%). The relative yields of the Cys-dopa isomers (**2**—**4**) paralleled those obtained with the tyrosinase oxidation,[4] suggesting that the present reaction also proceeds *via* dopaquinone. Most of the cysteine was consumed as cystine in addition to Cys-dopas. Sum of the yields of **1**—**5** approached nearly 100%, indicating that the reaction did not give pigments or ill-defined products.

Effects of pH, concentrations of $H_2O_2$, iron salts, and EDTA, and addition of radical scavengers were summarized in Table 1. The optimum pH of the reaction was around pH 7 (Expts 2—4). Omitting any one of $H_2O_2$, iron salts, and EDTA resulted in no formation Cys-dopas (Expts 5, 8, 15). Almost identical yields of Cys-dopas were obtained when $FeCl_3$ was used in place of $FeSO_4$ (Expt 13), while $CuSO_4$ had no catalytic activity (Expt 14). Doubling the amount of $H_2O_2$ (and cysteine) did not improve the yield of **2**, but it led to a great increase in the yield of **5** (Expts 21, 22).

Reaction of $H_2O_2$ with $Fe^{2+}$–EDTA complex produces hydroxyl radical (·OH) (Reaction 1) which is a powerful oxidizing agent.[7] The resulting $Fe^{3+}$–EDTA complex may be reduced back by $H_2O_2$ with the formation of superoxide radical ($O_2^{\cdot-}$) (Reaction 2).[7]

$$Fe^{2+}\text{-EDTA} + H_2O_2 \longrightarrow Fe^{3+}\text{-EDTA} + \cdot OH + OH^- \quad (1)$$

$$Fe^{3+}\text{-EDTA} + H_2O_2 \longrightarrow Fe^{2+}\text{-EDTA} + O_2^{\cdot-} + 2H^+ \quad (2)$$

Scavengers of hydroxyl radical, D-mannitol and formate,[8] inhibited the formation of Cys-dopas by 20 and 17%, respectively (Expts 18, 19). Addition of superoxide dismutase also caused a 21% inhibition of the reaction (Expt 20). These results suggest that not only ·OH (Reaction 1) but also $O_2^{\cdot-}$ (Reaction 2) participated in the iron–EDTA-catalyzed oxidation of dopa with $H_2O_2$. We have recently shown that superoxide radical generated in hypoxanthine–xanthine oxidase system can mediate the formation of Cys-dopas from dopa and cysteine.[9]

When **2** was used in place of **1**, a 6.3% yield of **5** was obtained with a 93.6% recovery of **2**.

Based on these results, the following conditions were established for the preparation of **2**, **3**, and **5** on a preparative scale. The reaction mixture consisted of dopa, cysteine (added in 5 portions), $FeSO_4$, EDTA·2Na, and $H_2O_2$ (added in 5 portions) at a molar ratio

† 1 M = 1 mol $dm^{-3}$.

Table 1. Effects of pH, concentrations of $H_2O_2$, iron salts, and EDTA, and radical scavengers on the formation of Cys-dopas from DOPA and cysteine[a)]

| Expt No. | Conditions | $A_{300}-A_{340}$[b)] | Yield/% | | | |
|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 5 |
| 1 | Complete system (pH 7.0)[a)] | 0.117 | 77.0 | 13.9 | 3.1 | 4.2 |
| 2 | pH 5.0 | 0.036 | | | | |
| 3 | pH 6.0 | 0.096 | | | | |
| 4 | pH 8.0 | 0.064 | | | | |
| 5 | $H_2O_2$ omitted ($Fe^{2+}$ or $Fe^{3+}$) | 0.000 | | | | |
| 6 | $H_2O_2$ (1 mmol; in 50 portions) | 0.054 | | | | |
| 7 | $H_2O_2$ (1 mmol; in one portion) | 0.091 | | | | |
| 8 | $Fe^{2+}$ omitted | 0.000 | | | | |
| 9 | $Fe^{2+}$ (0.01 mmol) | 0.048 | | | | |
| 10 | $Fe^{2+}$ (0.05 mmol) | 0.083 | | | | |
| 11 | $Fe^{2+}$ (0.2 mmol)[c)] | 0.162 | 68.4 | 16.5 | 3.5 | 4.1 |
| 12 | $Fe^{2+}$ (1.0 mmol) | 0.109 | | | | |
| 13 | $Fe^{3+}$ (0.1 mmol) | 0.127 | 74.8 | 13.9 | 3.0 | 4.0 |
| 14 | $Cu^{2+}$ (0.1 mmol)[d)] | 0.000 | | | | |
| 15 | EDTA omitted | 0.000 | | | | |
| 16 | EDTA (0.2 mmol) | 0.116 | 76.0 | 16.0 | 3.0 | 2.8 |
| 17 | EDTA (5.0 mmol) | 0.069 | | | | |
| 18 | L-Mannitol (10 mmol) added | 0.079 | ND[e)] | 12.4 | 2.2 | 2.3 |
| 19 | HCOONa (10 mmol) added | 0.092 | ND | 11.7 | 2.2 | 3.7 |
| 20 | SOD (5 mg) added | 0.083 | 82.3 | 12.3 | 2.1 | 2.3 |
| 21 | Complete system[f)] | 0.144 | 70.5 | 18.6 | 3.4 | 7.4 |
| 22 | Complete system[g)] | 0.265 | 54.6 | 17.9 | 5.5 | 13.1 |

a) L-Dopa (1 mmol), L-cysteine (2 mmol), and $FeSO_4$ (0.1 mmol) were dissolved in water (100 ml), pH being adjusted to 7.0 with $Na_2HPO_4$. Then, $H_2O_2$ (1 mmol) was added in 5 portions every 10 min and the mixture was vigorously stirred at room temperature (23—26 °C). The reaction was terminated 10 min after the final addition of $H_2O_2$ by adding 1 ml of 6 M HCl. The mixture was applied on a column (1.0 cm×7 cm) of Dowex 50W-X2 and the products eluted with 3 M HCl after washing with 0.5 M HCl (100 ml). The products were separated by rechromatography on Dowex 50W-X2 (2.0 cm×24 cm; equilibrated with 2 M HCl) as described in Ref. 4. b) Difference in absorbances at 300 and 340 nm. Cys-dopas (**2**—**5**) show strong absorptions at 300 nm where **1** shows no absorption. Aliquots of the reaction mixture were diluted 50-fold with 1 M HCl and the UV spectra recorded. c) Some formation of purple pigment was noted. d) The formation of cystine was greatly accelerated. e) Not determined. f) L-Cysteine (2 mmol) was added in 5 portions 1 min prior to the additions of $H_2O_2$. g) L-Cysteine (4 mmol) and $H_2O_2$ (2 mmol) were added in 10 portions every 10 min.

of 1:2:0.1:0.2:1. Starting from 9.9 g of dopa and 12.1 g of cysteine, the reaction gave, in addition to 7.5 g (76%) of dopa recovered, 2.4 g (14%) of **2**, 0.5 g (3%) of **3**, and 0.9 g (4%) of **5** after chromatographic separation and crystallization. Thus, the present method appears to be the most convenient one for the large-scale preparation of **2**, **3**, and **5**.

Udenfriend and co-workers[10)] have described a system, consisting of L-ascorbic acid, iron-EDTA complex, and either $O_2$ or $H_2O_2$, and a buffer around neutral pH, which can hydroxylate various aromatic compounds. Our present system offered another example of chemical oxidation using $H_2O_2$ and iron–EDTA complex which mimics enzymic oxidation.

## Experimental

*Synthesis of Cys-dopas on a Preparative Scale.* A solution of 9.86 g (50 mmol) of L-dopa, 3.72 g (10 mmol) of EDTA·2Na, and 1.39 g (5 mmol) of $FeSO_4 \cdot 7H_2O$ in 5 l of water was adjusted to pH 7.0 with crystals of $Na_2HPO_4 \cdot 12H_2O$. To the vigorously stirred solution were added at 23 °C 2.42 g (20 mmol) of L-cysteine and 1 min later 860 μl (10 mmol) of 11.6 M $H_2O_2$. The additions of the same amounts of L-cysteine and $H_2O_2$ were repeated 5 times at 10-min intervals. The reaction was terminated 10 min after the final addition of $H_2O_2$ by adding 50 ml of 6 M HCl and 5 ml of mercaptoacetic acid. The mixture was evaporated *in vacuo* at 60 °C to *ca.* 300 ml and stored overnight in a refrigerator. The resulting precipitate of cystine and EDTA was filtered off and the filtrate was applied on a column (3.6 cm×25 cm) of Dowex 50W-X2 ($H^+$ form, 200—400 mesh; equilibrated with water). Elution with 0.5 M HCl gave dopa (frs. 48—144; 20 ml per fraction) which was recovered as 7.48 g (75.9%) of crystals after evaporation and neutralization. Further elution with 3 M HCl afforded a mixture of **2**—**5** (frs. 20—140; 20 ml per fraction). The mixture was rechromatographed on a column (3.6 cm×25 cm) of Dowex 50W-X2 (equilibrated and eluted with 3 M HCl) and fractions of 20 ml were collected and analyzed by UV. Fractions 104—128, 132—220, and 229—325 contained **3**, **2**, and **5**, respectively, which were evaporated to give HCl salts of the amino acids. They were crystallized from aqueous 1% $Na_2S_2O_5$ adjusted to pH 6 with $CH_3COONa$ to afford 2420 mg (14.4%) of **2**, 520 mg (2.8%) of **3**, and 932 mg (4.0%) of **5**. These catechols were identified with the authentic samples[6)] by comparison of chromatographic behaviors and UV spectra.

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