PATTERNS OF ENZYMATIC AND NONENZYMATIC OXIDATION OF DOPA IN VITRO

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The continuous-flow ionization-chamber method has been used for measurements of $^{14}CO_2$ production following administration of ^{14}C-labeled substrates to man (*1*) and animals (*2*) in vivo. This method has achieved widespread use for the measurement of substrate oxidation in isolated tissues (*3,4*). We recently employed the method for continuous measurements of enzyme activities directed toward the development of in vitro radioisotope techniques for biochemical (*5,6*) and clinical investigations (*7,8*), and for early diagnosis of folic acid deficiency (*4*), hyperphenylalaninemia, maple syrup urine disease, and glucose-6-phosphate dehydrogenase deficiency (*9*) in man. In the present study, we demonstrate that this method is a useful tool for continuous measurements of nonenzymatic and enzymatic oxidation of ^{14}C-labeled 3,4-dihydroxyphenylalanine (DOPA), a new drug used now for the treatment of Parkinsonism.

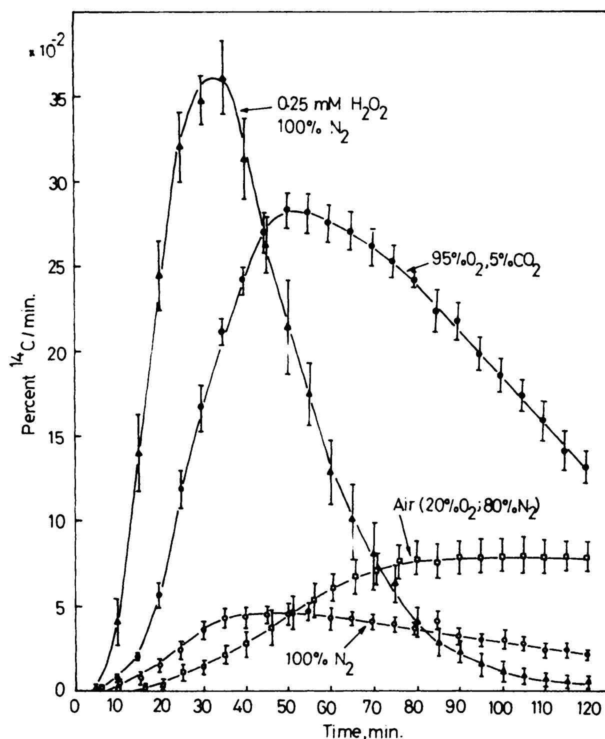

FIG. 1. Composite data of rates of $^{14}CO_2$ production from DOPA-carboxyl-^{14}C incubated with or without H_2O_2 in 0.1 *M* phosphate buffer in presence of 95% O_2 and 5% CO_2, pure N_2, and air atmospheres, respectively. Ordinate represents percent of incubated ^{14}C produced as $^{14}CO_2$/min, and abscissa gives time in minutes after administration of DOPA-carboxyl-^{14}C. Each point represents mean of $^{14}CO_2$ production for each group of four experiments at given time and length of vertical bar through each point represents ±1 standard error of mean.

MATERIALS AND METHODS

Chemicals. Pyridoxal phosphate (PLP) and L-histidine decarboxylase (cl. Welchii) were purchased from Sigma Chemical Co. Hydrogen peroxide (H_2O_2) (50%) was obtained from Fisher Chemical Co. DL-3,4-dihydroxyphenylalanine-carboxyl-^{14}C (DOPA carboxyl-^{14}C) (specific activity : 3.4 mCi/mmole, radiochemical purity >99%) was obtained from New England Nuclear Co. Phosphate buffers (0.1 *M*, pH 7.0) were prepared from distilled water and analytic-grade chemicals and used in all experiments in this study.

$^{14}CO_2$ production study. Details of the apparatus employed have been published previously (*3,4*).

For experimental procedure, 0.12 μCi DOPA-carboxyl-^{14}C was added to phosphate buffer with or without PLP, H_2O_2, and L-histidine decarboxylase, respectively, at 37°C for 120 min. Compressed air or compressed gases with 95% O_2 and 5% CO_2 or pure N_2 were passed through the incubation chamber at constant flow rate (100 cc/min). The flow of gases then passed through an ionization chamber connected to a vibrating-reed electrometer, the output of which was recorded continuously on a chart recorder (*4*).

For comparing various $^{14}CO_2$ curves we have used T_{max} and total fraction of incubated substrate expressed as percent ^{14}C or μmole of DOPA converted during 120 min. These two parameters have been defined previously (*4,10*).

RESULTS

Figure 1 represents composite data showing the rates of $^{14}CO_2$ production from DOPA-carboxyl-^{14}C

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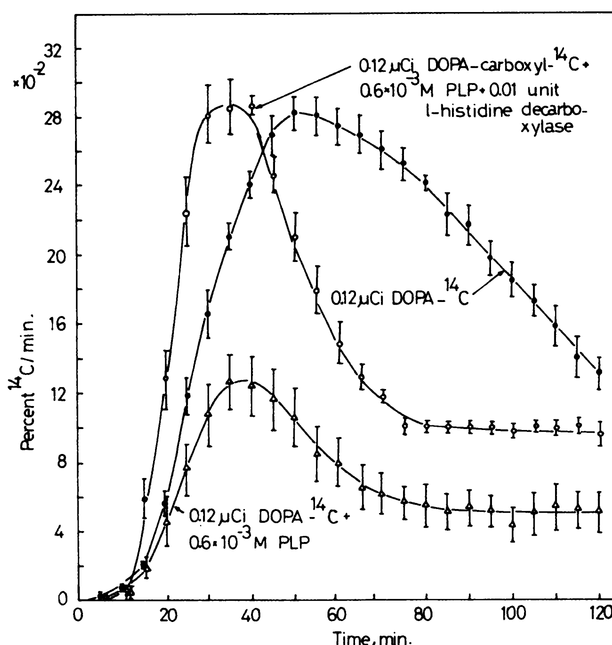

FIG. 2. Composite data of rates of $^{14}CO_2$ production from DOPA-carboxyl-^{14}C incubated with or without PLP, and PLP plus L-histidine decarboxylase in 0.1 *M* phosphate buffer and in 95% O_2 and 5% CO_2. See Fig. 1 for details.

with or without H_2O_2 in N_2, air with 95%, and 5% CO_2, and air atmospheres. It is clearly seen that $^{14}CO_2$ occurred largely when the incubation chamber of the device was gassed with a mixture of 95% O_2 and 5% CO_2. Similar results were noted with H_2O_2 in N_2 atmosphere. A relatively lower rate of oxidation of DOPA was observed in pure N_2 or air with 20% O_2 and 80% N_2. These results were obtained from a nonenzymatic oxidation of DOPA.

Figure 2 represents composite data showing the rates of $^{14}CO_2$ production from DOPA-carboxyl-^{14}C incubated with and without 0.6 m*M* PLP or 0.6 m*M* PLP plus 0.01 unit L-histidine decarboxylase in 95% O_2 and 5% CO_2. An inhibition of this nonenzymatic oxidation of DOPA was noted in the presence of 0.6 m*M* PLP. However, a considerable increase in $^{14}CO_2$ production was found when 0.01 unit L-histidine decarboxylase was added to the incubation chamber containing the same amount of PLP. The latter reaction is enzymatic because of the decarboxylation of DOPA by L-histidine decarboxylase.

Figure 3 represents composite data showing the rates of $^{14}CO_2$ production from DOPA-carboxyl-^{14}C incubated with 0.01 and 0.1 m*M* H_2O_2 in distilled water (pH 6.95) and in N_2 and O_2 atmospheres, respectively. It is clearly seen that the nonenzymatic oxidation of DOPA occurred in the presence of H_2O_2.

Table 1 shows T_{max} and cumulative percentage ^{14}C or micromoles of DOPA converted in 120 min from DOPA-carboxyl-^{14}C incubated with or without H_2O_2, PLP and L-histidine decarboxylase in phosphate buffer or distilled water in the presence of N_2, O_2, and air atmospheres, respectively (the number of experiments in each group is noted in parentheses).

As shown in the table, approximately 5.9% and 21.6% ^{14}C, and more than 120 and 54.5 min of T_{max}, were obtained from a nonenzymatic oxidation of DOPA-carboxyl-^{14}C in air atmosphere with 20% O_2 and 80% N_2 with 95% O_2 and 5% CO_2, respectively. An inhibition of $^{14}CO_2$ production ($p < 0.001$) and a shortened T_{max} ($p < 0.001$) from this nonenzymatic oxidation of ^{14}C-labeled DOPA were noted when incubated with 0.6 m*M* PLP as compared to results obtained without PLP. However, an increase in $^{14}CO_2$ production ($p < 0.001$) and an unchanged T_{max} ($p > 0.05$) were found when L-histidine decarboxylase was added to the incubation chamber containing the same amount of PLP. An increased $^{14}CO_2$ production ($p < 0.001$) and a shortened T_{max} ($p < 0.001$) were also noted from a nonenzymatic oxidation of ^{14}C-labeled DOPA with H_2O_2 in N_2 as compared to values obtained without H_2O_2. No $^{14}CO_2$ production from DOPA-carboxyl-^{14}C was found in distilled water and in N_2 and O_2 atmospheres, respectively. A considerable production of $^{14}CO_2$ occurred when 0.01–0.10 m*M* H_2O_2 were added to distilled water containing ^{14}C-labeled DOPA in O_2 atmosphere.

DISCUSSION

It is known that DOPA is decarboxylated to CO_2 and dopamine in the presence of PLP by DOPA decarboxylase (Fig. 4) in animal (*11*) and human (*12*) tissues, and by L-phenylalanine decarboxylase

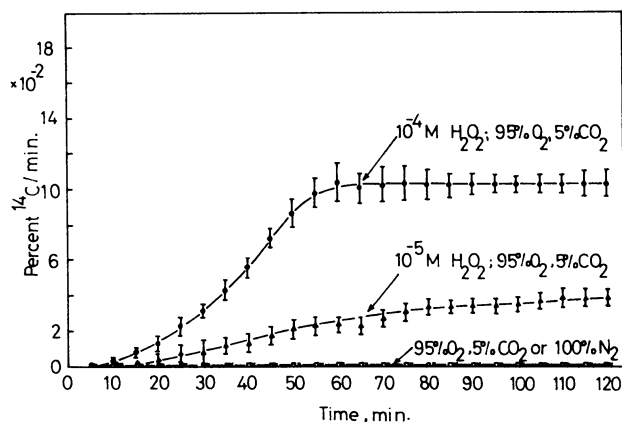

FIG. 3. Composite data of rates of $^{14}CO_2$ production from DOPA-carboxyl-^{14}C incubated with or without H_2O_2 in distilled water in presence of O_2 and N_2, respectively. See Fig. 1 for details.

TABLE 1. T_{max} AND CUMULATIVE DOPA CONVERTED OR $^{14}CO_2$ PRODUCTION FROM DOPA-CARBOXYL-^{14}C

Experimental conditions	Conc of H_2O_2 (m*M*)	Conc of PLP (m*M*)	T_{max} (min ± s.e.)	^{14}C production in 120 min (% ± s.e.)	DOPA converted in 120 min (μmoles ± s.e.) $\times 10^{-3}$
Nitrogen and phosphate buffer:					
0.12 μCi-DOPA-carboxyl-^{14}C (*4*)	0	0	>120	3.794 ± 0.470	1.100 ± 0.136
0.12 μCi-DOPA-carboxyl-^{14}C (*4*)	0.25	0	29.87 ± 1.51 ($p < 0.001$)	15.011 ± 0.970 ($p < 0.001$)	4.353 ± 0.281 ($p < 0.001$)
Nitrogen and distilled water:					
0.12 μCi-DOPA-carboxyl-^{14}C (*4*)	0	0	—	0	0
Air and phosphate buffer:					
0.12 μCi-DOPA-carboxyl-^{14}C (*4*)	0	0	>120	5.926 ± 1.066	1.742 ± 0.313
Oxygen and distilled water:					
0.12 μCi-DOPA-carboxyl-^{14}C (*4*)	0	0	—	0	0
0.12 μCi-DOPA-carboxyl-^{14}C (*4*)	0.01	0	>120	2.785 ± 0.160	0.817 ± 0.046
0.12 μCi-DOPA-carboxyl-^{14}C (*4*)	0.10	0	>120	10.640 ± 0.510 ($p < 0.001$)	2.916 ± 0.014 ($p < 0.001$)
Oxygen and phosphate buffer:					
0.12 μCi-DOPA-carboxyl-^{14}C (*4*)	0	0	54.50 ± 0.60	21.618 ± 1.183	6.174 ± 0.347
0.12 μCi-DOPA-carboxyl-^{14}C (*4*)	0	0.6	37.62 ± 1.74 ($p < 0.001$)	7.627 ± 1.617 ($p < 0.001$)	2.204 ± 0.464 ($p < 0.001$)
0.12 μCi-DOPA-carboxyl-^{14}C (*4*) +0.01 unit L-histidine decarboxylase	0	0.6	35.00 ± 1.24 ($p > 0.05$)	16.512 ± 0.485 ($p < 0.001$)	4.855 ± 0.013 ($p < 0.001$)

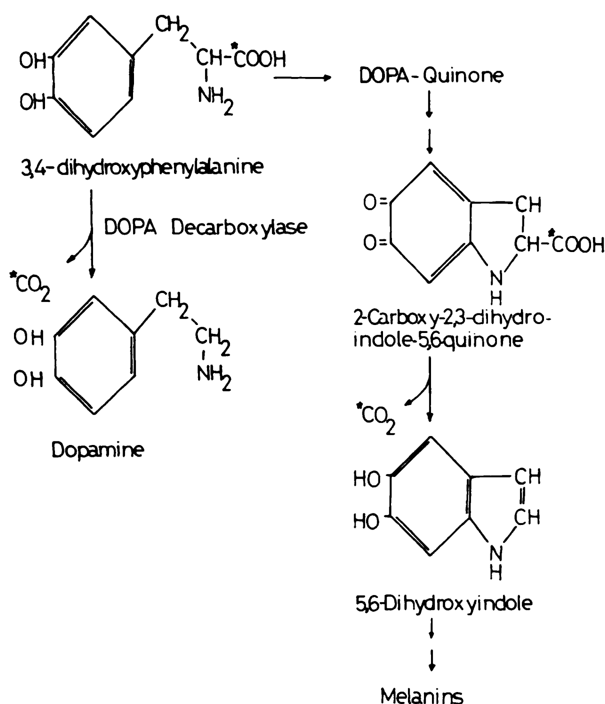

FIG. 4. Metabolic fate of No. 1 carbon atom of 3,4-dihydroxyphenylalanine.

and L-tyrosine decarboxylase of streptoccocus faecalis (*6*). We demonstrate here that DOPA can be decarboxylated by L-histidine decarboxylase (cl. Welchii). Interestingly, L-DOPA decarboxylase of the hog kidney was shown recently to decarboxylate L-histidine in vitro (*13*).

It is also known that DOPA is oxidized nonenzymatically (Fig. 4) to CO_2 and DOPA melanin via DOPA quinones (*14,15*). The results obtained in this study confirm that the nonenzymatic oxidation of DOPA is possible in the presence of either molecular O_2 (*15*) or ions HO_2^- and O_2^{2-} of H_2O_2 in both distilled water and phosphate buffer. This evidence is supported further by the observed formation of black DOPA melanin from 1–10 $\times 10^{-3}$ m*M* nonradioactive DOPA incubated with O_2 or H_2O_2 during 10 hr in our unpublished experiments. Such a nonenzymatic oxidation of DOPA was inhibited significantly by a relatively large dose of PLP (*16*), possibly due to the formation of DOPA-PLP complex (*17*).

Our overall results suggest that the continuous-flow ionization-chamber method should be a useful tool for measurements of both nonenzymatic and enzymatic oxidation of DOPA in vitro.

SUMMARY

The present study confirms that both enzymatic and nonenzymatic oxidation of DOPA is possible in vitro. The continuous-flow ionization-chamber method provided a useful tool for measurements of nonenzymatic and enzymatic oxidation of DOPA under various experimental conditions.

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