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Properties of catechol-*O*-methyl transferase in soluble and particulate preparations from rat red blood cells

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Catechol-*O*-methyl transferase (COMT) (EC 2.1.1.6) inactivates catecholamines by *O*-methylation [1]. Red blood cells incubated with norepinephrine form the *O*-methylated product normetanephrine [2]. Initially, COMT was thought to exist exclusively in the cytoplasm [3, 4], but recent reports have indicated that *O*-methyl transferase activity has also been detected in the particulate fraction of several tissues including brain, red blood cells and liver [5–7]. In addition, recent studies have indicated that at least two forms of COMT exist in rat red blood cells [5]. In the present study, we compared the K_m and pH optima of COMT in preparations from the particulate and soluble fractions of rat red blood cells.

Preparation of soluble and particulate enzymes. Heparinized blood was centrifuged at 1000 *g* at 4° for 10 min. The plasma was removed by aspiration and discarded. The red blood cells were lysed by hypo-osmotic shock, i.e. by diluting 10-fold with ice-cold 5×10^{-4} M dithiothreitol (DTT). After standing on ice for 10 min with intermittent shaking, the lysed red cells were centrifuged for 20 min at 2000 *g*. The supernatant was used as the soluble enzyme preparation. The pellet was then washed four times by resuspension in a volume of DTT (5×10^{-4} M) equivalent to that used to lyse the red cells. (No measurable enzyme activity was present after the third wash.) The resuspended pellet was then shaken vigorously and centrifuged at 2500 *g* for 10 min. After discarding the supernatant of the final wash, the pellet was resuspended in 0·002 M phosphate buffer, pH 7·8, containing 0·1% Triton X-100 and 5×10^{-4} DTT in a volume equivalent to the original volume of red cells. The resuspended pellet was then vigorously shaken on a vortex mixer for 3 min and centrifuged at 2000 *g* for 10 min. The resulting supernatant was used as the particulate enzyme preparation.

Enzyme assay. The incubation mixture expressed as final concentrations contained 4×10^{-2} M potassium phosphate buffer (pH 7·8), 8×10^{-4} M magnesium chloride, 2×10^{-5} M [^{14}C]methyl-*S*-adenosyl methionine (sp. act. 2·5 mCi/m-mole obtained from ICN Nuclear, Cleveland, Ohio), 1×10^{-3} M 3,4-dihydroxybenzoic acid (DBA) and 0·2 ml enzyme preparation in a total volume of 0·5 ml. After incubation at 37° for 20 min, the reaction was stopped with 0·1 ml of 3 N hydrochloric acid. Blanks to correct for methanol-forming enzyme activity contained all reagents except DBA. The ^{14}C-methylated products were extracted into 2·5 ml toluene isoamyl alcohol (7:3, v/v). After shaking for 5 min on a horizontal mechanical shaker, the tubes were centrifuged at 600 *g* for 5 min and the ^{14}C-methylated products were determined by liquid scintillation spectrometry. The reaction of DBA with either the soluble or particulate enzyme preparation was linear for at least 80 min at all concentrations of DBA.

In order to control for nonspecific effects of the detergent on the physical or chemical properties of the enzyme preparation from the particulate fraction, in experiments measuring substrate kinetics, the enzyme preparation from the soluble fraction was diluted to approximately equal activity as the particulate enzyme preparation using the same Triton-containing buffer as was used to solubilize the particulate enzyme preparation.

Statistics. Student's *t*-test (two-tailed) was used to compare differences between soluble and particulate enzyme activity.

In order to determine the optimal pH for each enzyme preparation, the pH was varied from 6·7 to 8·7. Data presented in Fig. 1 indicate that the activity of the soluble enzyme preparation was optimal within the range of pH 7·9 to 8·3, while the activity of the particulate enzyme preparation was optimal within the range of pH 7·9 to 8·1. A striking feature of the pH curves is the relative stability of the particulate enzyme preparation within the lower pH range. For instance, at pH 7·1, the activity of the particulate enzyme preparation declined from the maximal activity by only 25 per cent. At a similar pH, the activity of the soluble enzyme preparation declined from maximal activity by 60 per cent. Thus, while the enzyme activity of both preparations appears to have similar pH optima, the enzyme activity of the particulate preparation was less sensitive to alterations in pH than was the enzyme activity of the soluble preparation.

In contrast to the results reported here, a difference in pH optima was observed by Assicot and Bohuon [5]. However, the discrepancies between the results of Assicot and Bohuon and those reported here are restricted to the pH optimum of the particulate enzyme preparation. While several factors may explain this discrepancy, such as differences in the concentrations of detergent or other differences in the preparation of membrane-bound enzyme, it should be pointed out that at the optimal pH (7·0) reported by Assicot and Bohuon, we have observed 75 per cent of maximal activity, while at the optimal pH reported here (7·9 to 8·1), Assicot and Bohuon report approximately 80 per cent of maximal activity. Thus, both studies seem to indicate that the particulate enzyme preparation is relatively stable over a broad pH range.

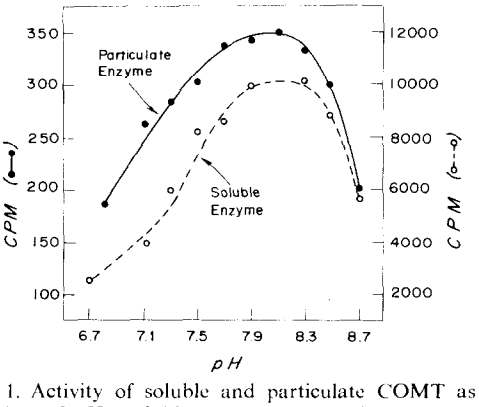

Fig. 1. Activity of soluble and particulate COMT as a function of pH. Soluble enzyme preparations (○—○), equivalent to 8 μl red blood cells, and particulate enzyme preparations (●—●), equivalent to 80 μl red blood cells, were incubated with 3,4-dihydroxybenzoic acid (1×10^{-3} M) for 20 min as described in Methods. Results are expressed as cpm/enzyme preparation/20 min. Each point represents the mean of six determinations.

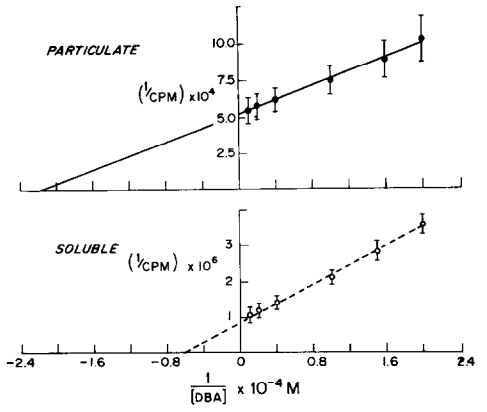

Fig. 2. Lineweaver–Burk plots of soluble and particulate COMT activity and substrate concentration. The concentration of substrate, 3,4-dihydroxybenzoic acid, was varied from 5×10^{-5} M to 1×10^{-3} M. Particulate enzyme preparations, equivalent to 80 μl red blood cells, and soluble enzyme preparations, equivalent to 0·5 μl red blood cells, were incubated with appropriate concentrations of substrate for 10 min as described in Methods. Velocity is defined as cpm/ml of RBC/10 min. Each point represents the mean ± standard error of four determinations.

In order to determine the apparent Michaelis constants for the two enzyme preparations using DBA as a substrate, double reciprocal Lineweaver–Burk plots were drawn. As shown in Fig. 2, the apparent K_m for the particulate enzyme preparation ($4{\cdot}7 \pm 0{\cdot}70 \times 10^{-5}$ M) was significantly lower ($P < 0{\cdot}005$) than the apparent K_m of the soluble enzyme preparation ($1{\cdot}7 \pm 0{\cdot}20 \times 10^{-4}$ M), indicating that the particulate enzyme preparation had more than three times the affinity for the substrate (DBA) than did the soluble enzyme preparation. These results confirm earlier studies of Assicot and Bohuon [5].

These investigators also found that the enzyme activity in the particulate enzyme preparation was more heat labile than was the activity in the soluble enzyme preparation [5]. Similar results have been obtained in our studies [8].

While several differences between the two enzyme preparations have been noted, some similarities have also been observed. As described above, the two enzyme preparations have similar pH optima (Fig. 1), and previous studies have indicated that the enzyme preparations have similar substrate specificities as well as similar Michaelis constants for lower concentrations of magnesium [8].

After partial purification, soluble COMT has been shown to exist in two forms [9]. Thus, it is possible that the activity obtained from the particulate enzyme preparation represented one of the soluble forms that may have attached to membranes or to fragments of membranes. On the other hand, it is possible that a distinct particulate enzyme may exist in red blood cells. Support for the latter possibility comes from the findings of Assicot and Bohuon [5] who demonstrated that antibodies to partially purified hepatic soluble COMT almost completely inhibited COMT activity from the soluble fraction of red blood cells while only barely affecting the enzyme of the particulate fraction. However, additional studies involving purification will be necessary to resolve this question.

A synaptosomal enzyme has also been demonstrated in the central nervous system [6]. However, further studies will be required to determine whether the physical and chemical properties of the particulate enzyme in the red blood cell resemble those of the synaptosomal enzyme. Since there are indications that at least some COMT activity occurs postsynaptically [10, 11] and that the enzyme may be associated with adrenergic receptors [12], if a distinct membrane-bound enzyme does exist it may be expected that such an enzyme would be more closely related to this receptor than would a soluble enzyme.

Several investigators have examined soluble red blood cell COMT activity in various psychiatric disorders [13–15]. One study observed a reduction in soluble red blood cell COMT activity in female patients with primary affective disorders [13], while another study noted that the K_m (for DBA) of the soluble COMT was higher in patients with paranoid schizophrenia than in patients with other psychiatric disorders or controls [15]. Further studies will be required to determine if a membrane-bound enzyme can be identified in human red blood cells, since measures of the activity or kinetic properties of this enzyme may be of further interest in biochemical studies of patients with various psychiatric and addictive disorders.

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