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Tyrosine Decarboxylase

Spectrophotometric Assay and Application in Determining Pyridoxal-5′-Phosphate

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Abstract

We have developed a highly sensitive and rapid spectrophotometric assay for tyrosine decarboxylase that can be applied to determining pyridoxal- 5′-phosphate. In the assay, tyramine, a product of tyrosine decarboxylation, reacts with 2,4,6-trinitrobenzenesulfonic acid to give a product soluble in toluene whereas tyrosine does not. We determined the amount of tyramine produced enzymatically by reading the absorbance at 340 nm of a toluene extract of the reaction mixture. This method is capable of detecting as low as 2.9 μg/mL of the enzyme. Using this method, we find the K_m for tyrosine decarboxylase from *Streptococcus faecalis* to be $3.55 \times 10^{-4} M$. We have also developed a specific and extremely sensitive method for determining pyridoxal-5′-phosphate, a cofactor of the enzyme, by using this spectrophotometric assay with apotyrosine decarboxylase.

Index Entries: Tyrosine decarboxylase; decarboxylase, tyrosine; pryidoxal-5′-phosphate, assay for; spectrophotometric assay, for pyridoxal- 5′-phosphate

Introduction

Tyrosine decarboxylase (L-tyrosine carboxylase, E.C.4.1.1.25), which catalyzes the decarboxylation of tyrosine to form tyramine and CO_2, has been measured

†Abbreviations used: TNBS, 2,4,6-trinitrobenzenesulfonic acid; TNP, *N*, *N*′-trinitrophenyl; PLP, pyridoxal 5′-phosphate.

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manometrically (*1–5*) and by radiochemical methods (*6–7*). The former method lacks sensitivity. The radiochemical ones, which use ^{14}C-labeled substrate, are sensitive, but are time-consuming.

We describe a spectrophotometric assay for tyrosine decarboxylase based on the principle we used for developing a similar assay for lysine decarboxylase (*9*). In brief, tyrosine, the substrate, reacts with trinitrobenzenesulfonic acid (TBNS), forming a water-soluble yellow product, whereas the enzymatic product, tyramine, reacts with TNBS to form a water-insoluble yellow product extractable into an organic solvent. We quantify the amount of tyramine produced spectrophotometrically by measuring the absorption of TNP–tyramine extracted into the organic phase. We have also applied this assay using apotyrosine decarboxylase for selectively measuring trace amounts of pyridoxal- 5′-phosphate (PLP).

Materials and Methods

Reagents

Toluene (J. T. Baker Co.); tyrosine decarboxylase apoenzyme (1.1 U/mg) from *Streptococcus faecalis*, L-tyrosine, PLP, pyridoxine monohydrochloride, pyridoxamine dihydrochloride, pyridoxal hydrochloride, 4-pyridoxic acid, pryidoxal ethyl acetyl hydrochloride, picrylsulfonic acid (2,4,6-trinitrobenzenesulfonic acid), and tyramine (Sigma Chemical Co.).

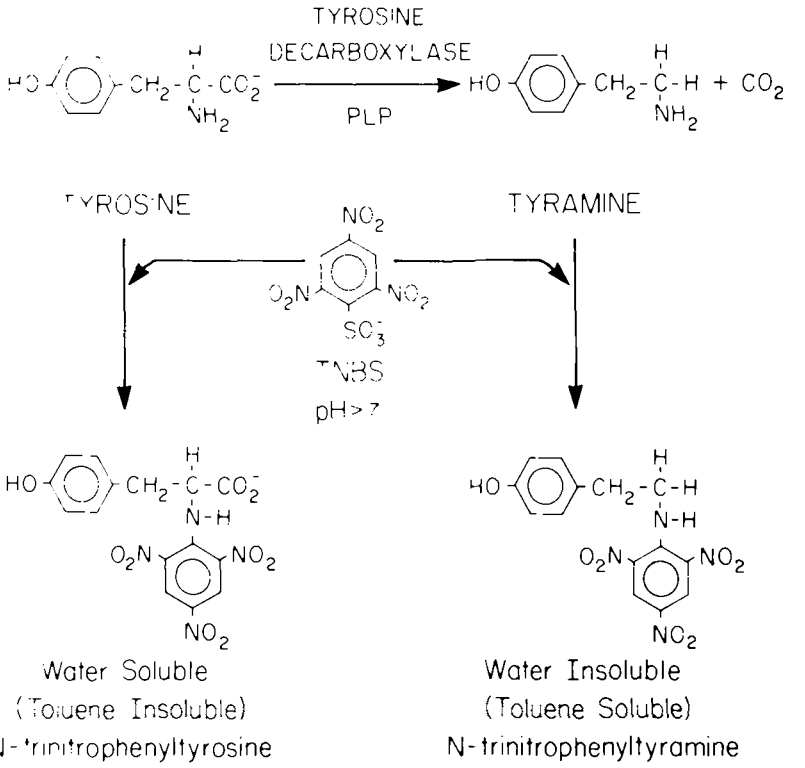
L-tyrosine, 8 m*M*, was prepared by dissolving 0.11491 g in 100 mL of 0.5*M* sodium acetate buffer. PLP stock solution, $10^{-4}M$, was prepared by dissolving 2.47 mg in 100 mL of deionized water. L-Tyrosine decarboxylase apoenzyme, 0.05 g, was dissolved in 20 mL of 0.5*M* acetate buffer, pH 5.5. The enzyme solutions were centrifuged at 2000 rpm for 30 min and the supernatant was used for assay. Protein was determined by its absorbance at 280 nm. All optical measurements were made using a Beckman Acta C-III Spectrophotometer.

Formation of Tyrosine and Tyramine Adducts of TNBS, and Extraction of Chromophore

Tyrosine, tyramine, and the components of the enzyme reaction mixture for tyrosine decarboxylase, except for the enzyme, were dissolved in 1.0 mL of 0.5*M* acetate buffer, pH 5.5 To each sample, 1 mL of 1.0*M* K_2CO_3 and 1 mL of $1.02 \times 10^{-2}M$ TNBS were added. Each sample was immediately mixed with 2 mL of toluene using a Vortex mixer run at top speed for 20 s. The toluene and aqueous layers were separated by centrifuging the tubes at about 2000 rpm for 5 min.

Results and Discussion

The reactions involved in the spectrophotometric assay for tyrosine decarboxylase is outlined in Scheme I. The assay for PLP involves the same reactions except that instead of using holotyrosine decarboxylase, we use its apoenzyme.



Scheme I

Absorption Spectra

The spectra from toluene extracts of TNP–tyramine and the reaction of TNBS with the components and products of tyrosine decarboxylase reaction (Fig. 1, solid and dashed lines, respectively) show similar absorption patterns consisting of a small, broad peak with maximum absorbance at 420 nm and a major peak with maximum absorbance at 340 nm. The absorbance at 340 nm of a toluene extract of a high concentration of tyrosine (8 m*M*) reacted with 2.04 m*M* TNBS (Fig. 1, dotted line) was negligible, indicating that TNP-tyrosine is not significantly soluble in toluene.

Standard Curve and Molar Extinction Coefficient for Tyramine

A range of concentrations of tyramine, assayed as under Materials and Methods, gave a standard curve as shown in Fig. 2. The curve was linear in the range of 3.37 to 84 μM with a correlation coefficient of 0.9992. The average operational molar extinction coefficient was $0.93 \times 10^4\ M^{-1}\ cm^{-1}$. Satake et al. (*10*) reported the molar extinction coefficient of TNP-tyramine derivative obtained under different conditions to be $1.14 \times 10^4\ M^{-1}\ cm^{-1}$.

Assay of Tyrosine Decarboxylase

Each reaction was initiated by adding 0.5 mL of a solution of L-tyrosine decarboxylase enzyme or apoenzyme in 0.5*M* acetate buffer, pH 5.5, to 0.5 mL

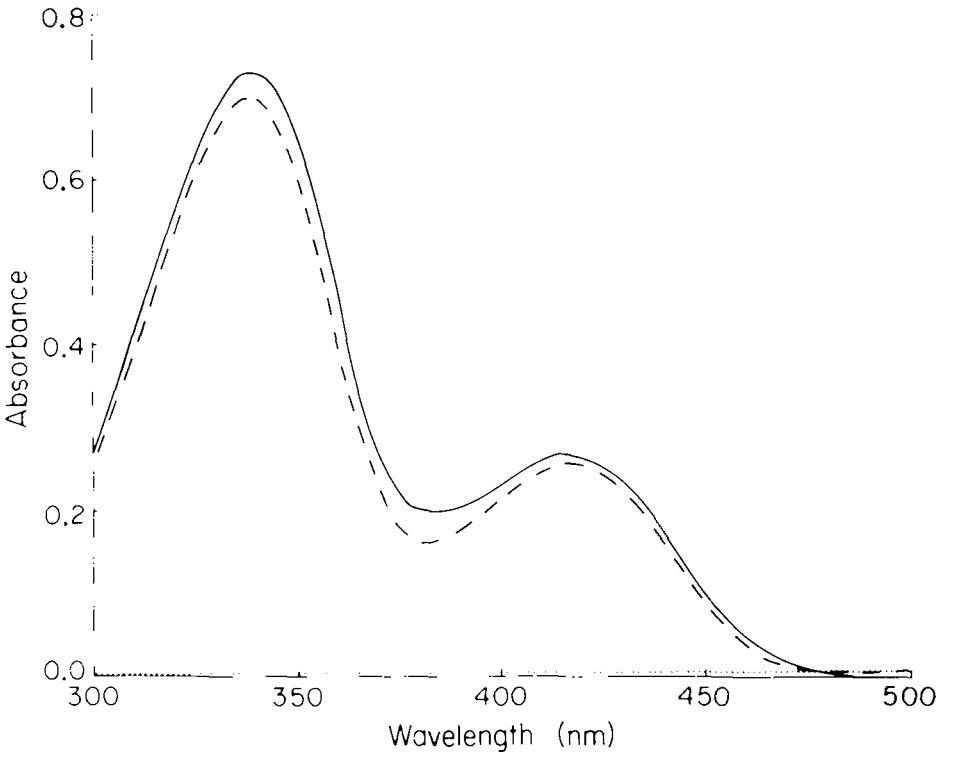


Fig. 1. Absorption spectra obtained from toluene extracts of TNP–tyramine (solid line), TNP derivative of product of tyrosine decarboxylase action (dashed line), and TNP–tyrosine (dotted line).

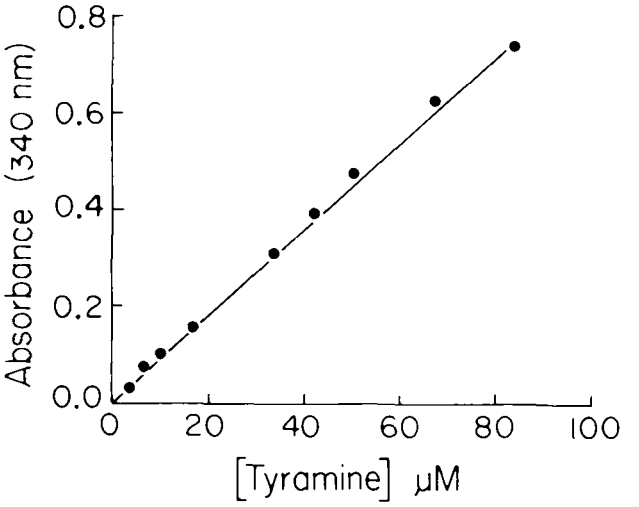


Fig. 2. Standard curve for tyramine determination. Experimental details described in Materials and Methods section.

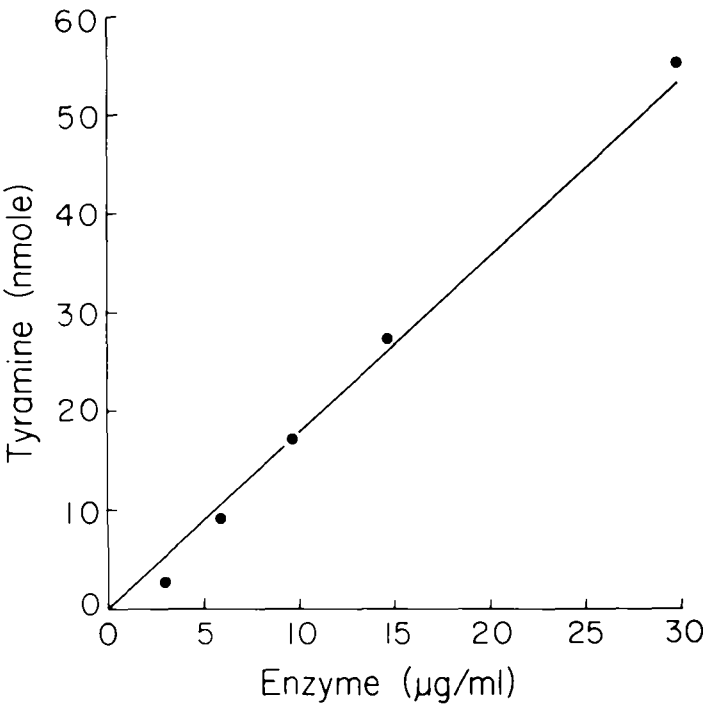


Fig. 3. Relationship between tyramine produced and enzyme concentration at a fixed reaction time of 20 min.

tyrosine (from 0.27 to 1.34 m*M*) acetate buffer, pH 5.5, and containing a total of 3 μ*M* PLP. After the reaction mixture was incubated at 37°C for 20 min, it was stopped by adding 1 mL of 1*M* K_2CO_3. Next TNBS was added to each sample, and the apolar products were extracted and separated as described under Materials and Methods. The absorbance of the toluene extracts was read at 340 nm against a toluene extract of the same reaction and color development mixtures differing in that the K_2CO_3 was added to the tyrosine solution before the enzyme was. Under these conditions, we easily detected amounts of tyrosine decarboxylase as low as 2.92 μg/mL. A linear relationship with a correlation coefficient of 0.9996 was obtained between amount of tyramine and enzyme concentration in the range of 2.9–29 μg/mL (Fig. 3).

Kinetics of Tyrosine Decarboxylase

The effect of substrate concentration on the initial rate of tyrosine decarboxylase is shown in Fig. 4. From a least-square fit of a Lineweaver-Burk plot, we obtained a K_m of $3.54 \times 10^{-4}M$ and a V_{max} of 1.30 nmol tyramine/min/ng enzyme. This K_m is comparable to that of $5 \times 10^{-4}M$ obtained potentiometrically by Hassan and Rechnitz (*11*).

Assay of PLP

Various concentrations of PLP in 0.1 mL aliquots, and 0.4 mL of the apoenzyme (10 mg) in 0.5*M* acetate buffer, pH 5.5, were incubated at room temperature for 5

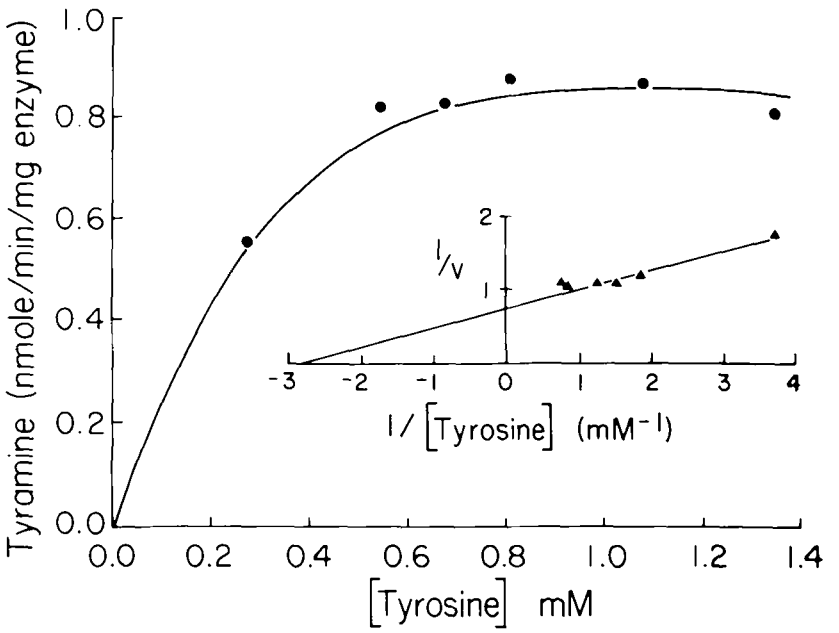


Fig. 4. Effect of tyrosine concentration on rate of tyrosine decarboxylation. All points were average of three determinations.

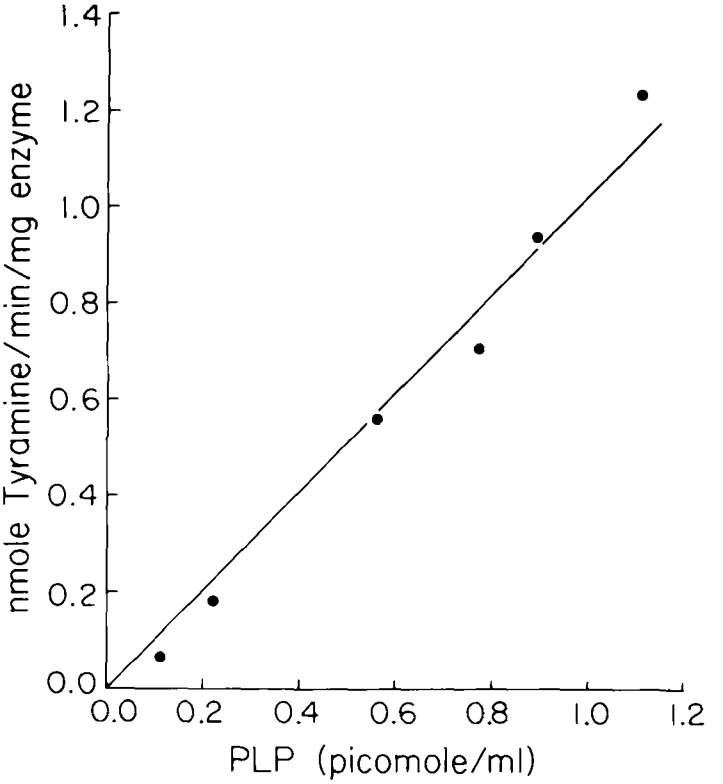


Fig. 5. Standard curve for pyridoxal-5'-phosphate determination using spectrophotometric assay for tyrosine decarboxylase described in this paper. (For experimental details, see Assay of PLP.)

min. The reaction was initiated by adding 0.5 mL of 1.34 m*M* tyrosine in 0.5*M* acetate buffer, pH 5.5. After the reaction mixture was incubated at 37°C for 20 min, it was stopped by adding 1 mL of 1*M* K_2CO_3. Next, TNBS was added to each sample and each was extracted with a 2 mL aliquot of toluene.

The blank solutions for this assay were prepared by adding 1 mL of 1*M* K_2CO_3 to a mixture of 0.5 mL tyrosine and 0.5 mL of tyrosine decarboxylase apoenzyme after they were incubated for 20 min at 37°C.

Under these conditions, we easily detected amounts of PLP as low as 0.11 n*M*. A linear relationship with a correlation coefficient of 0.994 was obtained between absorbance at 340 nm and PLP level in the range of 0.11–1.11 n*M* (Fig. 5). This assay is 10 times more sensitive than the potentiometric method of Hassan and Rechnitz (*11*).

Other members of the vitamin B_6 group and their phosphate derivatives, including pyridoxine monohydrochloride, pyridoxamine dichloride, pyridoxal hydrochloride, 4-pyridoxic acid, and pyridoxal ethyl acetal hydrochloride, do not interfere with this assay for PLP.

Other methods for measuring PLP, based on reactions with semicarbamide (*12*), phenylhydrazine (*13*), or cyanide (*14*), lack sensitivity and convenience.

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