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KINETIC PROPERTIES OF TYROSINE HYDROXYLASE WITH NATURAL TETRAHYDROBIOPTERIN AS COFACTOR

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A reproducible purification procedure of native tyrosine hydroxylase (L-tyrosine, tetrahydropteridine : oxygen oxidoreductase (3-hydroxylating), EC 1.14.16.2) from the soluble fraction of the bovine adrenal medulla has been established. This procedure accomplished a 90-fold purification with a recovery of 30% of the activity. This purified enzyme served for studying the kinetic properties of tyrosine hydroxylase using (6*R*)-L-*erythro*-1′,2′-dihydroxypropyltetrahydropterin [(6*R*)-L-*erythro*-tetrahydrobiopterin] as cofactor, which is supposed to be a natural cofactor. Two different K_m values for tyrosine, oxygen and natural (6*R*)-L-*erythro*-tetrahydrobiopterin itself were obtained depending on the concentration of the tetrahydrobiopterin cofactor. In contrast, when unnatural (6*S*)-L-*erythro*-tetrahydrobiopterin was used as cofactor, a single K_m value for each tyrosine, oxygen and the cofactor was obtained independent of the cofactor concentration. The lower K_m value for (6*R*)-L-*erythro*-tetrahydrobiopterin was close to the tetrahydrobiopterin concentration in tissue, indicating a high affinity of the enzyme to the natural cofactor under the in vivo conditions. Tyrosine was inhibitory at 100 μM with (6*R*)-L-*erythro*-tetrahydrobiopterin as cofactor, and the inhibition by tyrosine was dependent on the concentrations of both pterin cofactor and oxygen. Oxygen at concentrations higher than 4.8% was also inhibitory with (6*R*)-L-*erythro*-tetrahydrobiopterin as cofactor.

Introduction

Tyrosine hydroxylase (L-tyrosine, tetrahydropteridine : oxygen oxidoreductase (3-hydroxylating), EC 1.14.16.2) is a monooxygenase which catalyzes the formation of L-3,4-dihydroxyphenylalanine (L-dopa) from L-tyrosine in peripheral and central catecholaminergic neurons and chromaffin cells of the adrenal medulla [1]. It requires a tetrahydropterin as cofactor [1,2].

We reported that kinetic behavior of tyrosine hydroxylase is dependent on the stereochemical structures of the side chain at position 6 of the tetrahydropterin cofactors [3–5]. The K_m value for tyrosine is controlled to a fair extent by configuration, especially by the C-1′ configuration of the side chain and the L-configuration at C-1′ similar to that of L-*erythro*-tetrahydrobiopterin is preferable for the cofactor activity [4]. On the other hand, the K_m value for tetrahydropterin itself is influenced by the structure of the side chain [5]. The chemical reduction of a 6-substituted pterin to the 5,6,7,8-tetrahydro derivative introduces another center of asymmetry at position 6 resulting in the formation of a mixture of enantiomers or diastereoisomers. The absolute configuration at C-6 chiral center of natural tetrahydrobiopterin was determined to be L, as in (6*R*)-L-*erythro*-tetrahydrobiopterin [6]. In a preliminary study we found that the natural (6*R*), and unnatural (6*S*), tetrahydrobiopterins (Fig. 1) have

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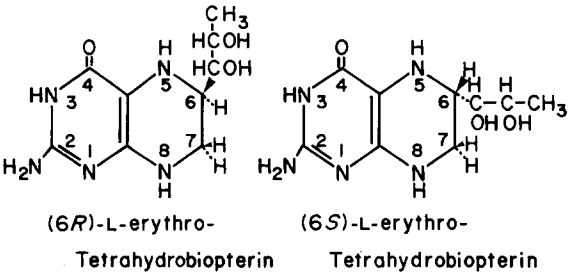

Fig. 1. Structures of two 6-diastereoisomers of L-*erythro*-1',2'-dihydroxypropyl-5,6,7,8-tetrahydrobiopterin (L-*erythro*-tetrahydrobiopterin).

different cofactors characterisitcs with tyrosine hydroxylase [7]. Some of the kinetic differences observed between natural and unnatural tetrahydrobiopterin were also observed with phenylalanine hydroxylase by Bailey and Ayling [8].

Since oxygen at 20.9% is inhibitory with L-*erythro*-tetrahydrobiopterin as cofactor [4], we have made detailed kinetic studies on tyrosine hydroxylase using the natural cofactor, (6*R*)-L-*erythro*-tetrahydrobiopterin, at varying concentrations of both tyrosine and oxygen. Such kinetic studies could be important both in elucidating the mechanism of enzyme reaction and in understanding the mechanism of in vivo short-term regulation of the enzyme accompanying the changes in affinity of the pterin cofactor [9].

Several purification procedures of the native form or protease-digested form of the enzyme have been tried: the protease-digested form from bovine adrenal medulla [10,11]; and the native form from bovine adrenal medulla [12], from rat pheochromocytoma [13,14], from guinea pig adrenals [15], from rabbit adrenal [16] and from rat caudate nuclei [17]. However, it is still difficult to purify native tyrosine hydroxylase extensively from large tissue sources such as bovine adrenal medulla. In order to estimate the in vivo regulating mechanism of the enzyme, use of the native form of the enzyme appears to be preferable for the kinetic studies. In this study we have developed a reproducible purification procedure to get sufficiently purified enzyme with a high specific activity from bovine adrenal medulla, and the purified enzyme was used for the kinetic studies.

Experimental procedures

Materials. Sepharose 6B, Sephadex G-200, and Heparin-Sepharose CL-6B were purchased from Pharmacia, Bio-Gel A-1.5 m from Bio-Rad, DEAE-cellulose from Whatman and 6-methyltetrahydropterin from Calbiochem. Standard oxygen gas at various concentrations balanced with nitrogen gas were obtained from Seitetsu Kagaku Co. (Chiba, Japan). L-*erythro*-Biopterin was synthesized by the method of Sugimoto and Matsuura [18]. The corresponding tetrahydrobiopterin was prepared by catalytic hydrogenation in 0.1 M HCl using platinum oxide as catalyst [19]. The 6-diastereoisomers of tetrahydrobiopterin were isolated by the method of Bailey and Ayling [8] using HPLC on a Whatman Partisil 10 SCX column. The first peak eluted corresponded to the natural (6*R*) isomer and the second peak to the unnatural (6*S*) isomer; each fraction was collected, purified on a CM-Sephadex column [6], lyophilized and stored at −80°C until assay. Tetrahydrobiopterin was estimated by diminished absorbance of 2,6-dichlorophenolindophenol solution [20] on the extinction coefficient of 18 500 $M^{-1} \cdot cm^{-1}$ at 603 nm, at final pH of 6.99, 30 s after the addition of tetrahydrobiopterin in 0.1 M HCl.

Enzyme assay. Tyrosine hydroxylase activity was measured based on the assay of L-dopa from L-tyrosine by HPLC with electrochemical detection [21] with slight modifications. For the routine assay during the purification of the enzyme, the incubation mixture contained 0.2 M sodium acetate buffer (pH 6.0)/0.2 mM $FeSO_4$/0.2 mM L-tyrosine/1 mM 6-methyltetrahydropterin/0.1 M mercaptoethanol/enzyme/water to make up a total volume of 100 μl. For the blank incubation, D-tyrosine plus 100 μM 3-iodotyrosine (a tyrosine hydroxylase inhibitor) was used as substrate instead of L-tyrosine. Incubation was started by the addition of tyrosine and was carried out at 37°C for 10 min. The reaction was stopped by adding 300 μl 0.4 M perchloric acid (containing 1 nmol α-methyldopa as internal standard)/20 μl 0.2 M EDTA. After 10 min, 100 μl 0.8 M potassium carbonate and 1 ml 1.0 M Tris-HCl buffer (pH 8.5) were added, and the mixture was centrifuged at 1 600 × *g* for 10 min. The resulting supernatant was applied on an aluminium oxide column (0.4 cm internal diameter) containing 100 mg aluminium

oxide at room temperature, and the column was washed with 2 ml 0.05 M Tris-HCl buffer (pH 8.5), 5 ml water three times, and 100 μl 0.5 M HCl, and then dopa and α-methyldopa were eluted with 200 μl 0.5 M HCl. The eluate from the alumina column was injected into an HPLC (Yanako L-2000) with an Yanaco VMD-101 electrochemical detector (Yanagimoto Manufacturing Co., Kyoto, Japan) and a column (25 × 0.4 cm internal diameter) packed with Whatman Partisil 10 ODS. The mobile phase was 0.1 M sodium phosphate buffer (pH 2.0) with a flow rate of 1.0 ml/min. The detector potential was 0.7 V against Ag/AgCl electrode. When 20 μg catalase were used instead of $FeSO_4$, the sensitivity of this HPLC with electrochemical detection assay was about 0.5 pmol dopa formed enzymatically, because both the L- and D-tyrosine preparations contained about 0.4 pmol contaminated dopa in 20 nmol tyrosine, which contributed to the blank value. The D-tyrosine also contained contaminated L-tyrosine, and the activity due to the contaminated L-tyrosine was inhibited by the addition of 3-iodotyrosine. In order to get the highest sensitivity in the assay using crude enzyme preparations, a double column procedure (the top Amberlite CG-50 column and the bottom alumina column) [21] is needed, since the former column can remove endogenous catecholamines which interfere with the dopa peak under high sensitivity.

Kinetic studies. For kinetic study, the following reaction mixture was used; 0.2 M sodium acetate buffer (to obtained the final pH 6.0)/1 mM $FeSO_4$/ 0.1 M mercaptoethanol/various concentrations of L-tyrosine in 0.01 M HCl/various concentrations of tetrahydrobiopterin in 0.1 M HCl/10 μl enzyme solution/water to make up a total volume of 100 μl. For control incubation mixture, D-tyrosine was added instead of L-tyrosine. After the addition of tyrosine, standard oxygen gas at a regulated concentration was delivered and bubbled into the incubation mixture for 5 min in an ice bath prior to incubation, and the reaction was carried out at 30°C for 5 min. The amount of L-dopa formed by the reaction was completely proportional to the reaction time under these conditions. The K_m values and maximal velocity (V) were determined from Lineweaver-Burk plots [22] using Wilkinson's program [23].

Purification of enzyme. A new procedure for purification of tyrosine hydroxylase from bovine adrenal medulla was established. Bovine adrenal glands were freshly obtained at a slaughterhouse. All subsequent procedures were performed at 4°C. In a typical experiment, 38 g adrenal medulla were dissected from the bovine adrenal glands and homogenized in 5 vol. of 50 mM potassium phosphate buffer (pH 7.3)/0.32 M sucrose. The homogenate was centrifuged at 100000 × g for 60 min, and the supernatant was applied to a DEAE-cellulose column (29.5 × 2.6 cm internal diameter), which had been equilibrated with 20 mM potassium phosphate buffer (pH 7.3)/ 8% sucrose/1 mM dithiothreitol. This buffer was used throughout the following purification procedure. The column was washed with the same buffer containing 0.05 M KCl and eluted with a linear gradient of 0.05–0.5 M KCl. The active fraction was collected and brought to 40% saturation with solid $(NH_4)_2SO_4$. The precipitate was collected by centrifugation at 25000 × g for 15 min and dissolved in a minimum volume of the buffer. The resulting solution was passed through a Sepharose 6B (83 × 2.6 cm internal diameter) equilibrated with the same buffer and elution was made with the buffer. The active fraction was collected and put onto a heparin-Sepharose CL-6B column (5.3 × 2.3 cm internal diameter), as described by Yamauchi and Fujisawa [12] except for the change of buffer used. The active fraction was collected and brought to 50% saturation with solid $(NH_4)_2SO_4$, and after centrifugation at 25000 × g for 15 min, the resulting precipitate was dissolved in the buffer and the solution passed through a Sephadex G-200 column (90 × 1.6 cm internal diameter) equilibrated with the same buffer. The active fraction was collected, stored at −80°C and used as enzyme for kinetic studies.

The approximate molecular weight was determined by gel filtration on Bio-Gel A 1.5 M according to the method of Whitaker [24]. The molecular weights of aldolase, catalase and apoferritin were taken as 158000, 240000 and 480000, respectively. Disc electrophoresis was carried out as described by Davis [25].

The protein was measured by the method of Lowry et al. [26] using bovine serum albumin as standard.

Results

Purification of tyrosine hydroxylase from bovine adrenal medulla

An example of purification of tyrosine hydroxylase from bovine adrenal medulla is shown in Table I. This purification procedure was found to be highly reproducible. The chromatography of tyrosine hydroxylase on a column of heparin-Sepharose CL-6B is shown in Fig. 2. This column produced about 7-fold purification with a high recovery. The purity of the final preparation was judged to be approx. 40% pure, based on disc electrophoresis. The purified enzyme was thought to be native, since the approximate M_r estimated by gel filtration in the soluble supernatant of the adrenal medulla or the purified enzyme was identical and 280 000.

Initial velocity studies using natural (6R)-L-erythro-tetrahydrobiopterin as cofactor

The Lineweaver-Burk plots of velocity against tyrosine concentration in the presence of (6*R*)-L-*erythro*-tetrahydrobiopterin at varying concentrations and air (20.9% (217 μM) oxygen) are shown in Fig. 3A, and parallel lines were obtained. Tyrosine at 100 μM was inhibitory regardless of the concentration of tetrahydrobiopterin. Inhibition by tyrosine, however, was dependent on the concentration of tetrahydrobiopterin; 50 μM tyrosine was not inhibitory at concentrations of tetrahydrobiopterin higher than 220 μM, but was inhibitory at cofactor concentrations lower than 220 μM. Fig. 3B shows a plot of the apparent V as a function of tetrahydrobiopterin concentration. The apparent V values were determined by extrapolating the data of Fig. 3A to infinite concentration of tyrosine. Two different K_m values were obtained depending on whether the concentrations of tetrahydrobiopterin were lower or higher than 220 μM (Table II). Fig. 3C is the re-plot of the data of Fig. 3A as the Lineweaver-Burk plots of velocity against (6*R*)-L-*erythro*-tetrahydrobiopterin concentration. Data by re-plotting the data of Fig. 3C are shown in Fig. 3D. The apparent V values were also determined from Lineweaver-Burk plots of velocity against (6*R*)-L-*erythro*-tetrahydrobiopterin concentration in the presence of different concentrations of tyrosine by extrapolating to infinite concentration to tetrahydrobiopterin. Two different K_m values for tyrosine were also obtained depending on the concentration of tetrahydrobiopterin cofactor, as for the K_m values for tetrahydrobiopterin.

Lineweaver-Burk plots of velocity against concentration of (6*R*)-L-*erythro*-tetrahydrobiopterin in the presence of oxygen at different concentrations and 20 μM tyrosine are shown in Fig. 4, and the intersecting lines are obtained. The K_m value for oxygen or tetrahydrobiopterin itself was estimated from the data by re-plotting vertical intercepts of Fig. 4, and is shown in Table II. Two K_m values for oxygen, 2.8 and 4.4 μM, were obtained depending on whether the concentration of tetrahydrobiopterin is lower or higher than 56 μM. Also, two K_m values for tetrahydrobiopterin itself were obtained to be about 60 and about 18 μM. Oxygen was inhibitory at 93.9 μM (about 9.0%) and this inhibition by oxygen was dependent on the concentration of tetrahydrobiopterin. The inhibition at low concentrations of

TABLE I

PURIFICATION OF TYROSINE HYDROXYLASE FROM BOVINE ADRENAL MEDULLA

38 g wet weight of bovine adrenal medulla was used for purification.

Fraction	Total protein (mg)	Total activity (nmol/min)	Specific activity (nmol/min per mg protein)	Recovery (%)
100 000 × *g* supernatant	1 864	4 473	2.4	100
DEAE-cellulose	681	5 781	8.5	129
Sepharose 6B	172	3 671	21.3	82
Heparin-Sepharose CL-6B	14.7	2 269	154	51
Sephadex G-200	6.0	1 419	210	32

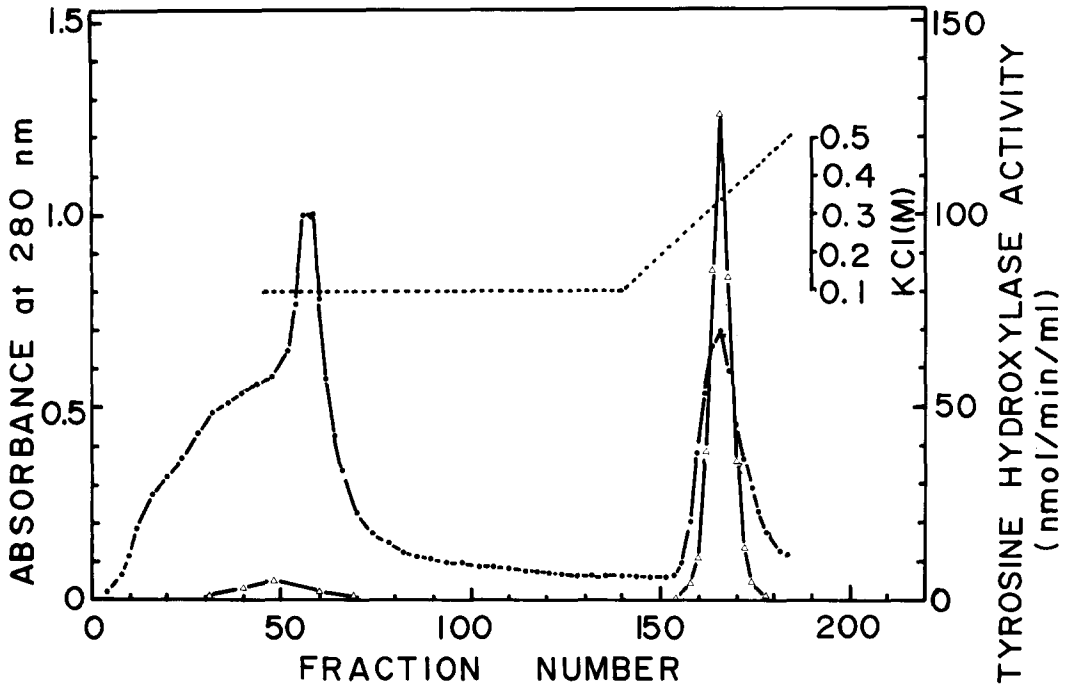

Fig. 2. Chromatography of tyrosine hydroxylase from bovine adrenal medulla on a column of heparin-Sepharose CL-6B. The active fraction from Sepharose 6B column was applied to a heparin-Sepharose column (5.3 × 2.6 cm internal diameter). The fraction size was 2.8 ml. △——△, Tyrosine hydroxylase activity; ●——●, absorbance.

TABLE II

K_m VALUES FOR (6*R*)-L-*ERYTHRO*-TETRAHYDROBIOPTERIN (BPH_4), TYROSINE AND OXYGEN OF TYROSINE HYDROXYLASE FROM BOVINE ADRENAL MEDULLA

Each K_m value and V value was estimated from the re-plots of vertical intercepts using Wilkinson's program [19], and expressed as means ± S.E.

Constant substrate	(6*R*)-L-*erythro*-tetrahydrobiopterin K_m(μM)	Tyrosine K_m (μM)	Oxygen K_m(μM)	Maximal velocity (nmol/min per mg protein)
217 μM oxygen				
[BPH_4] > 220 μM	556 ± 20	22 ± 7		143 ± 2
[BPH_4] < 220 μM	74 ± 11	7.4 ± 2.2		37 ± 2
20 μM tyrosine				
[BPH_4] > 56 μM	64 ± 4		4.4 ± 0.1	47.3 ± 0.3
[BPH_4] < 56 μM	18		2.8 ± 0.4	29.2 ± 0.5
104 μM (6*R*)-L-*erythro*-tetrahydrobiopterin		14 ± 4	8.5 ± 1.4	31.6 ± 1.6
49.3 μM oxygen				
[BPH_4] > 64 μM	53 ± 12	11 ± 1		43.9 ± 2.0
[BPH_4] < 64 μM	7.5	7.7 ± 2.5		33.4 ± 2.8

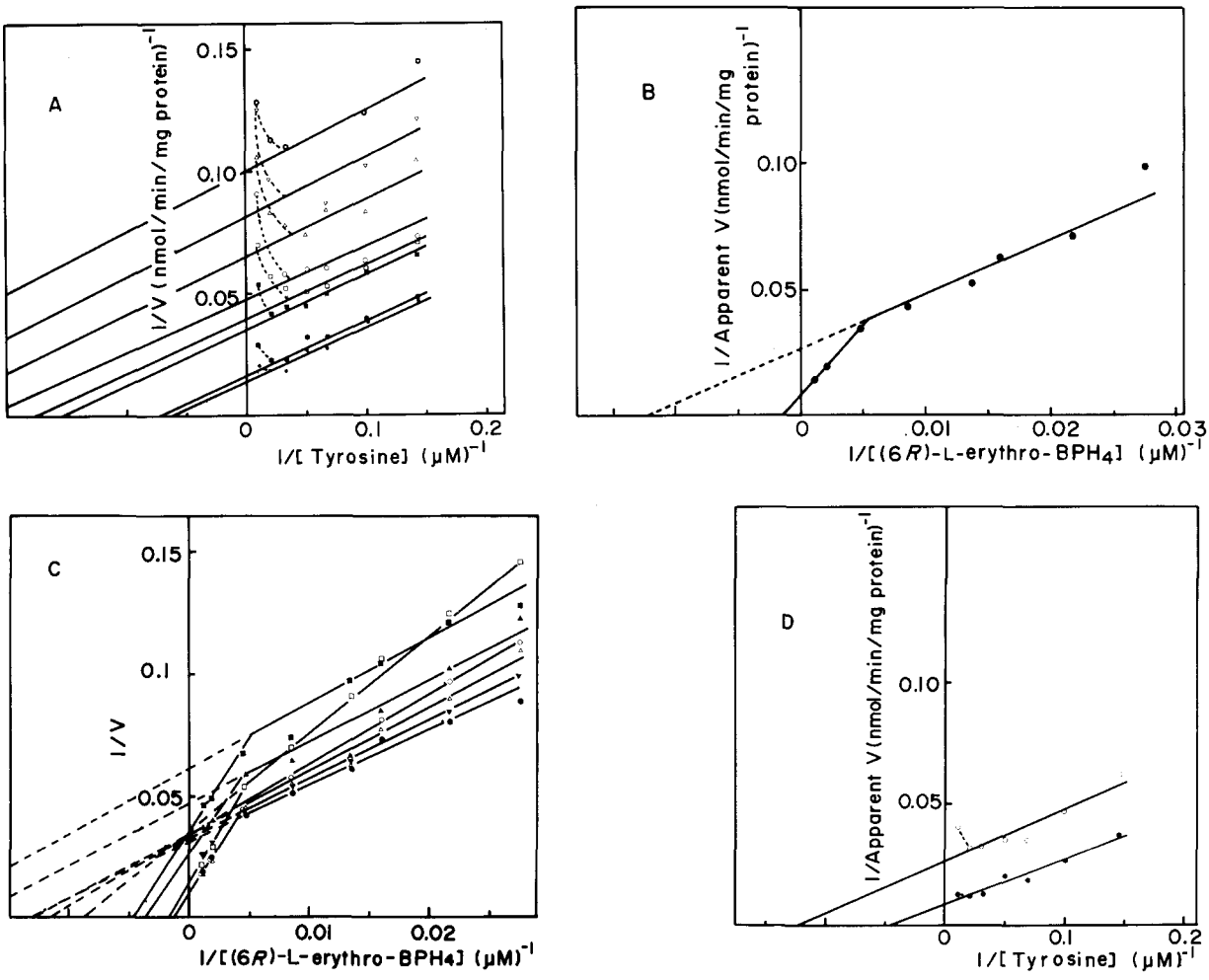

Fig. 3A. Lineweaver-Burk plots of velocity against tyrosine concentration at varying concentrations of (6*R*)-L-*erythro*-tetrahydrobiopterin. The concentration of tetrahydrobiopterin was: 36.5 μM (○), 46.5 μM (▽), 63.1 μM (△), 73.8 μM (○), 118.5 μM (□), 221.7 μM (■), 516.2 μM (●) and 952 μM (●). B. Re-plots of the vertical intercepts from Fig. 3A. C. Lineweaver-Burk plots of velocity against (6*R*)-L-*erythro*-tetrahydrobiopterin at varying concentrations of tyrosine. The concentration of tyrosine was: 7 μM (■), 10 μM (▲), 15 μM (▼), 20 μM (●), 30 μM (△), 50 μM (○) and 100 μM (□). D. Re-plots of the vertical intercepts from Fig. 3C. The concentration of tetrahydrobiopterin was: above 220 μM (●), below 220 μM (○).

tetrahydrobiopterin was more significant than at high concentrations of the pterin cofactor.

Lineweaver-Burk plots of velocity against tyrosine concentration in the presence of oxygen at different concentrations and 104 μM (6*R*)-L-*erythro*-tetrahydrobiopterin are shown in Fig. 5. K_m values for oxygen and tyrosine were estimated by re-plotting the data of Fig. 5, and are presented in Table II. Only the higher K_m value of the two K_m values was obtained, because as illustrated in Fig. 4, only the higher K_m value can be estimated in the presence of tetrahydrobiopterin higher than 60 μM. Tyrosine was inhibitory at 100 μM, however, the inhibition by tyrosine was decreased by lowering the oxygen concentration.

Lineweaver-Burk plots of velocity against (6*R*)-L-*erythro*-tetrahydrobiopterin concentration in the presence of tyrosine at varying concentrations at 49.3 μM oxygen showed parallel lines (Fig. 6). K_m values for tyrosine and tetrahydrobiopterin are presented in

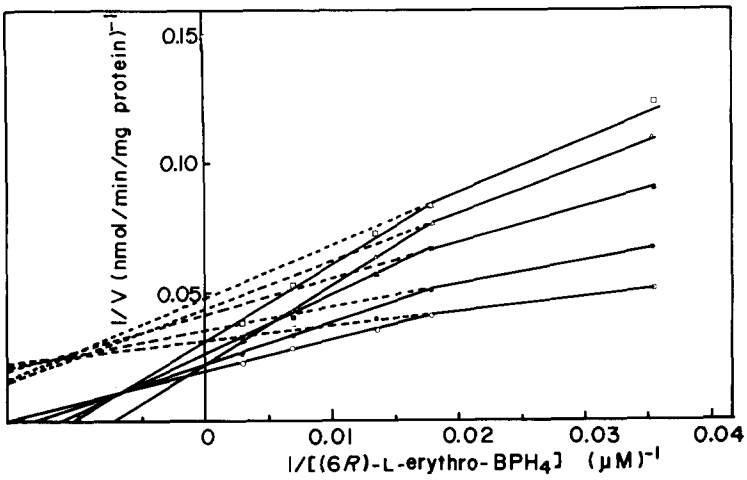

Fig. 4. Lineweaver-Burk plots of velocity against (6*R*)-L-*erythro*-tetrahydrobiopterin at varying concentrations of oxygen. The concentration of oxygen was : 10.5 μM (□), 19.7 μM (■), 49.3 μM (●), 93.9 μM (△) and vertical intercepts from Lineweaver-Burk plots of velocity against oxygen concentration at varying concentrations of (6*R*)-L-*erythro*-tetrahydrobiopterin (○). The incubations were carried out in the presence of 20 μM tyrosine. Different concentrations of oxygen were delivered and bubbled for 5 min prior to the incubation in an ice bath. Each point is a mean value of duplicate experiments.

Table II. Two K_m values were obtained depending on whether the concentration of (6*R*)-L-*erythro*-tetrahydrobiopterin is higher or lower than 64 μM. The lower K_m values for tetrahydrobiopterin and tyrosine were 7.5 and 7.7 μM, respectively.

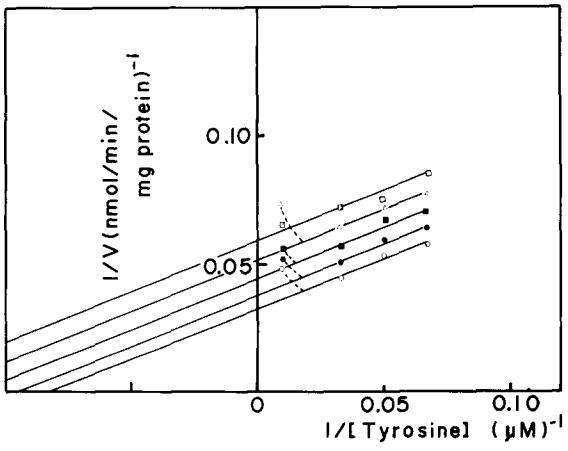

Fig. 5. Lineweaver-Burk plots of velocity against concentration of tyrosine at varying concentrations of oxygen. The concentration of oxygen was: 10.5 μM (□), 19.7 μM (■), 49.3 μM (●), 93.9 μM (△) and vertical intercepts from Lineweaver-Burk plots of velocity against oxygen concentration at varying concentrations of tyrosine (○). The incubations were carried out in the presence of 104 μM (6*R*)-L-*erythro*-tetrahydrobiopterin. Each value is a mean of duplicate experiments.

Initial velocity studies using unnatural (6S)-L-erythro-tetrahydrobiopterin as cofactor

To estimate K_m values for unnatural (6*S*)-L-*erythro*-tetrahydrobiopterin, which is 6-diastereoisomer of natural (6*R*)-L-*erythro*-tetrahydrobiopterin, initial velocities in the presence of tyrosine and (6*S*)-L-*erythro*-tetrahydrobiopterin at various concentrations and of oxygen at 20.9% (217 μM) were investigated. Lineweaver-Burk plots of velocity against tyrosine concentration in the presence of different concentrations of tetrahydrobiopterin gave intersecting lines. Tyrosine was not inhibitory even at 100 μM and only one K_m value for tetrahydrobiopterin was obtained regardless of concentration of tetrahydrobiopterin. K_m values for (6*S*)-L-*erythro*-tetrahydrobiopterin and tyrosine (522 ± 54 and 20 ± 4 μM, respectively) were almost same as the higher K_m values using (6*R*)-L-*erythro*-tetrahydrobiopterin as cofactor. Maximal velocity (V, 120 ± 6 nmol/min per mg protein) was not significantly different from that using (6*R*)-isomer. The K_m value for oxygen was 11.3 ± 0.6 μM in the presence of 107 μM (6*S*)-L-*erythro*-tetrahydrobiopterin. No substrate inhibition by oxygen was observed in the presence of the unnatural cofactor.

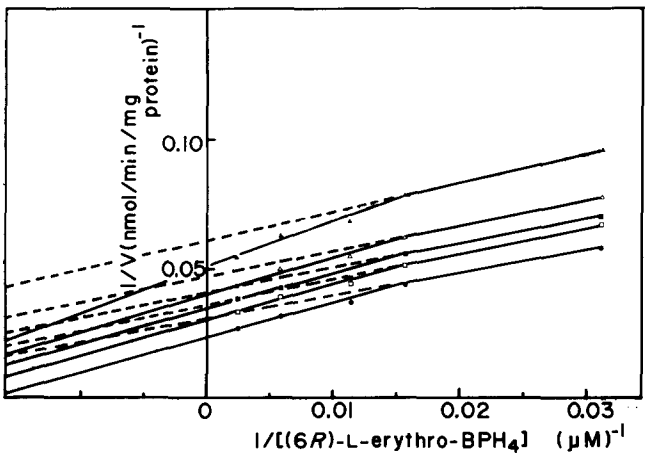

Fig. 6. Lineweaver-Burk plots of velocity against concentration of (6*R*)-L-*erythro*-tetrahydrobiopterin at varying concentrations of tyrosine. The concentration of tyrosine was: 10 μM (▲), 15 μM (△), 20 μM (■), 40 μM (□) and infinite tyrosine (●), which was estimated from vertical intercepts of Lineweaver-Burk plots of velocity against tyrosine at varying concentrations of (6*R*)-L-*erythro*-tetrahydrobiopterin. The incubations were carried out in the presence of 49.3 μM oxygen. Each value is a mean of duplicate experiments.

Discussion

When natural (6*R*)-L-*erythro*-tetrahydrobiopterin was used as cofactor, tyrosine hydroxylase showed characteristic kinetic properties: first, two distinct K_m values for (6*R*)-L-*erythro*-tetrahydrobiopterin, oxygen and tyrosine were obtained depending on the concentration of the tetrahydrobiopterin cofactor; second, tyrosine was inhibitory, however, the inhibition by tyrosine was dependent on the concentrations of tetrahydrobiopterin and oxygen; third, oxygen at concentrations higher than 43.9 μM (about 4.8%) was inhibitory, and the inhibition was dependent on the concentration of tetrahydrobiopterin. These results suggest that substrate inhibition was decreased by increasing the concentration of tetrahydrobiopterin.

On the other hand, unnatural (6*S*)-L-*erythro*-tetrahydrobiopterin showed monophasic Lineweaver-Burk plots, and tyrosine or oxygen was not inhibitory even at 100 μM tyrosine or 20.9% oxygen, respectively. K_m values for tyrosine and (6*S*)-L-*erythro*-tetrahydrobiopterin and the maximal velocity with unnatural cofactor were similar to the higher K_m values and the maximal velocity observed using natural (6*R*)-L-*erythro*-tetrahydrobiopterin as cofactor.

When a mixture of (6*R*)- and (6*S*)-tetrahydrobiopterin obtained by catalytic hydrogenation of biopterin, was used as cofactor, substrate inhibition on tyrosine hydroxylase and alteration of K_m values of tyrosine and the cofactor, depending on the cofactor concentration, were also observed by Fisher and Kaufman [27] and Numata et al. [4]. The present study revealed that such properties as substrate inhibition and alteration of K_m values are attributed only to (6*R*)-L-*erythro*-tetrahydrobiopterin and not to the (6*S*)-isomer. Some of the kinetic differences observed between natural and unnatural tetrahydrobiopterin with tyrosine hydroxylase were also observed with phenylalanine hydroxylase by Bailey and Ayling [8]; the K_m values for the cofactors and for phenylalanine are identical, but the natural cofactor gives faster V values and also triggers a substrate inhibition not elicited by unnatural tetrahydrobiopterin.

It should be noted that the lower K_m value for natural cofactor itself, 7.5 μM under 4.8% oxygen, was comparable to the in vivo biopterin concentration in rat adrenals reported by Fukushima et al. [28]. However, it is conceivable that tyrosine hydroxylase is not saturated with tetrahydrobiopterin in vivo, and the tetrahydrobiopterin level may influence tyrosine hydroxylase activity in vivo [29].

The biphasic curves obtained in Lineweaver-Burk plots with natural (6*R*)-L-*erythro*-tetrahydrobiopterin as cofactor may be explained either by the presence of two enzyme forms or two different conformations. Weiner et al. [30] proposed the presence of two different forms of the enzyme; one is phosphorylated active form with high affinity for pterin cofactor, the other is a less active form with low affinity for cofactor. However, this kinetic behavior is changeable depending on pH, ion strength and the presence of polyanion, as has been reported [31,32]. In the present work, a turning point of the biphasic lines in Lineweaver-Burk plots of natural (6*R*)-L-*erythro*-tetrahydrobiopterin was changed by oxygen concentration. These results suggest that tyrosine hydroxylase may be present in an equilibrium state of two forms, which can be interconverted by alteration of environment around the enzyme molecule, and tyrosine hydroxylase may be regulated by these characteristics in vivo.

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References

1 Nagatsu, T., Levitt, M. and Udenfriend, S. (1964) J. Biol. Chem. 239, 2910–2917
2 Brenneman, A.R. and Kaufman, S. (1964) Biochem. Biophys. Res. Commun. 17, 177–183
3 Numata (Sudo), Y., Ikuta, K., Kato, T., Nagatsu, T., Sugimoto, T. and Matsuura, S. (1975) Biochem. Pharmacol. 24, 1998–2000
4 Numata (Sudo), Y., Kato, T., Nagatsu, T., Sugimoto, T. and Matsuura, S. (1977) Biochim. Biophys. Acta 480, 104–112
5 Kato, T., Oka, K., Nagatsu, T., Sugimoto, T. and Matsuura, S. (1980) Biochim. Biophys. Acta 612, 226–232
6 Matsuura, S., Sugimoto, T., Hasegawa, H., Imaizumi, S. and Ichiyama, A. (1980) J. Biochem. 87, 951–957
7 Hasegawa, H., Imaizumi, S., Ichiyama, A., Sugimoto, T., Matsuura, S., Oka, T., Kato, T., Nagatsu, T. and Akino, M. (1979) in Chemistry and Biology of Pteridines (Kisliuk, R.L. and Brown, G.M., eds.), pp. 183–188, Elsevier/North-Holland, Amsterdam
8 Bailey, S.W. and Ayling, J.E. (1978) J. Biol. Chem. 253, 1598–1605
9 Nagatsu, T. (1979) in Catecholamines: Basic and Clinical Frontiers (Usdin, E., Kopin, I.J. and Barchas, J., eds.), pp. 34–39, Pergamon Press, New York
10 Shiman, R., Akino, M. and Kaufman, S. (1971) J. Biol. Chem. 246, 1330–1340
11 Hoeldtke, R. and Kaufman, S. (1977) J. Biol. Chem. 252, 3160–3167
12 Yamauchi, T. and Fujisawa, H. (1979) J. Biol. Chem. 254, 503–507
13 Markey, M.K., Kondo, S., Shenkman, L. and Goldstein, M. (1980) Mol. Pharmacol. 17 79–85
14 Vulliet, P.R., Langan, T.A. and Weiner, N. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 92–96
15 Nakashima, Y., Suzue, R., Sanada, H. and Kawada, S. (1972) Arch. Biochem. Biophys. 152, 515–520
16 Lloyd, T. (1979) J. Biol. Chem. 254, 7247–7254
17 Joh, T.H., Park, D.H. and Reis, D.J. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 4744–4748
18 Sugimoto, T. and Matsuura, S. (1975) Bull. Chem. Soc. Jap. 48, 3767–3768
19 Kaufman, S. (1967) J. Biol. Chem. 242, 3934–3943
20 Kaufman, S. (1959) J. Biol. Chem. 234, 2677–2682
21 Nagatsu, T., Oka, K. and Kato, T. (1979) J. Chromatogr. 163, 247–252
22 Lineweaver, H. and Burk, D.L. (1934) J. Am. Chem. Soc. 56, 658–666
23 Wilkinson, G.N. (1961) Biochem. J. 80, 324–332
24 Whitaker, J.R. (1963) Anal. Chem. 35, 1950–1953
25 Davis, B.J. (1964) Ann. N.Y. Acad. Sci. 121, 404–427
26 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
27 Fisher, D.B. and Kaufman, S. (1972) J. Neurochem. 19, 1359–1365
28 Fukushima, T. and Nixon, J.C. (1980) Anal. Biochem. 102, 176–188
29 Mandell, A.J., Bullard, W.P., Yellin, J.B. and Russo, P.V. (1980) J. Pharmacol. Exp. Ther. 213, 569–574
30 Weiner, N., Lee, F., Dreyer, E. and Barnes, E. (1978) Life Sci. 22, 1197–1216
31 Nagatsu, T., Numata (Sudo), Y., Kato, T., Sugiyama, K. and Akino, M. (1978) Biochim. Biophys. Acta 523, 47–52
32 Bustos, G., Simon, J. and Roth, R.H. (1980) J. Neurochem. 35, 47–57