Inhibition of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine metabolic activity of porcine FAD-containing monooxygenase by selective monoamine oxidase-B inhibitors

Ru-Feng Wu, Yoshiyuki Ichikawa*

Department of Biochemistry, Kagawa Medical School, Kita-gun, Kagawa 761-07, Japan

Received 25 November 1994; revised version received 13 December 1994

Abstract The MPTP metabolic activity of porcine FAD-containing monooxygenase (FMO) (EC 1.14.13.8) was inhibited considerably by deprenyl and pargyline, selective MAO-B inhibitors, and they showed typical competitive inhibition. Deprenyl and pargyline, amine derivatives were also examined as to whether they are substrates for the FMO. It was found that deprenyl and pargyline are excellent substrates for the FMO. The K_i and K_m values of deprenyl and pargyline for the FMO are 14 μM and 9 μM, and 14.3 μM and 11.6 μM, at pH 8.0 and 25°C, respectively.

Key words: FAD-containing monooxygenase; Monoamine oxidase-B inhibitor; Deprenyl; Pargyline

1. Introduction

It is well known that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity effectively produces Parkinson's disease and Parkinsonism in humans, monkeys and mice [1,2], and it is believed that MPTP is only metabolized by monoamine oxidase-B (MAO-B) [3,4], because deprenyl and pargyline are selective and the only specific MAO-B inhibitors, and MPTP-metabolic activity was inhibited by these inhibitors [3]. However, we have reported that MPTP is also metabolized by FMO [5,6]. In addition, it has been reported that MPTP is metabolized by microsomal cytochromes P-450IA1, P-450IIB1 and P-450IID6 [7].

In this study, we demonstrated that deprenyl and pargyline were not only strong inhibitors of the activity of FMO toward MPTP, but also excellent substrates for FMO. The kinetic parameters for the inhibitors of FMO were determined.

2. Materials and methods

2.1. Materials

Livers from female pigs (Landrace, 1-year-old) were obtained within 1 h of sacrifice from a local slaughterhouse and transported immediately to the laboratory on cracked ice.

2.2. Preparation of liver microsomes and purification of FMO

Fresh and healthy porcine livers were perfused carefully with an ice-cold 0.15 M KCl solution (adjusted to pH 7.4 with 0.5 M Tris) to remove as much blood as possible at 0°C to 4°C. The liver microsomes were prepared by the method of Mitoma et al. [8]. FMO was purified from the porcine liver microsomes by the method of Wu and Ichikawa [6]. The optical spectra of FMO are shown in Fig. 1, and the purified FMO gave a single protein band on SDS-PAGE (Fig. 2).

2.3. Enzyme assay and kinetic parameters

The enzymatic activity was determined optically and aerobically by measuring the decrease in absorbance at 340 nm due to the oxidation of NADPH in the presence of a substrate. The reaction mixture comprised FMO (0.2 nmol), 0.1 M K-phosphate buffer, pH 8.0, at 25°C, and appropriate amounts of NADPH and 10 μM to 600 μM substrate, in a final volume of 1 ml. The reaction mixture was put into a cuvette of 1 cm light path and then the endogenous rate of the absorbance change at 340 nm was recorded for 5 min at 25°C before addition of the substrate. After the addition of the substrate, the rate of the absorbance change was measured for 5 min at 25°C, the endogenous rate being subtracted. For investigation of a reaction with a sequential Bi Bi mechanism, the K_m, V_{max} and K_i values of FMO for deprenyl and pargyline can be determined by means of Lineweaver–Burk plots [9].

2.4. Other analytical methods

Protein was measured by means of the bicinchoninic acid method [10] or the biuret reaction [11], using bovine serum albumin as a standard marker protein. The concentration of FMO was determined using a molar extinction coefficient at 382 nm of 9.8×10^3 $M^{-1} \cdot cm^{-1}$ in 10 mM K-phosphate buffer (pH 7.4) containing 20% (V/V) glycerol, 0.1% (V/V) Triton X-100 and 1 mM EDTA, at 25°C [6]. The albumin content was determined optically using a molar extinction coefficient of 6.67×10^3 $M^{-1} \cdot cm^{-1}$ at 280 nm [12]. The molar extinction coefficient at 340 nm of NADPH was taken to be 6.30×10^3 $M^{-1} \cdot cm^{-1}$ in 0.1 M K-phosphate buffer, pH 7.4 (25°C) [13].

2.5. Instruments

Optical determinations were performed with a Hitachi double beam spectrophotometer, Model 557, equipped with a thermostatically controlled cell holder at 25°C.

2.6. Chemicals

R(−)-Deprenyl HCl was obtained from Research Biochemicals Incorporated (USA), and pargyline HCl and MPTP were purchased from Sigma Chemical Co. (St. Louis, MO). NADPH was purchased from Oriental Yeast Co. (Tokyo, Japan). The reagent solutions were prepared just before use to prevent decomposition. All other chemicals were of the highest grade commercially available.

3. Results

We purified FMO from porcine liver microsomes, and it gave a single protein band, and thus was very pure, on SDS-PAGE. The FMO was used for the experiments. The specific activity toward MPTP of the FMO was 6.2 nmol/min/nmol FMO at pH 8.0 and 25°C.

The effects of deprenyl and pargyline on the MPTP-oxidation activity of the FMO were examined kinetically by means of Lineweaver–Burk plots.

Figs. 3 and 4 show plots of the reciprocals of the MPTP concentrations against the reciprocals of the reaction velocities in the absence and presence of deprenyl or pargyline. The

*Corresponding author. Fax: (81) (878) 98 7109.

Abbreviations: FMO, FAD-containing monooxygenase; MAO, monoamine oxidase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

SSDI 0014-5793(94)01412-4

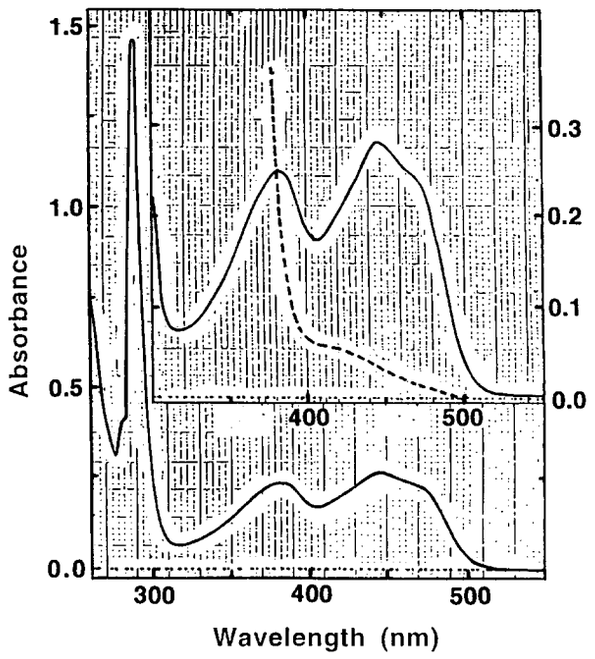

Fig. 1. Absolute optical absorption spectra of the FMO in the oxidized and sodium dithionite-reduced forms. The cuvette contained 1.38 mg protein/ml in 10 mM K-phosphate buffer (pH 7.4) containing 20% (v/v) glycerol, 0.1% (v/v) Triton X-100 and 1 mM EDTA, at 25° C. The magnified spectra in the visible region were recorded at 2.5-fold greater sensitivity. Solid lines, oxidized form; broken line, sodium dithionite-reduced form; dotted lines, baseline.

figures show typical competitive inhibition, and the K_i values are summarized in Table 1.

Deprenyl and pargyline are amine derivatives, and they are excellent substrates for the FMO.

The K_m and V_{max} values for deprenyl and pargyline of the FMO were determined from reciprocal plots of the initial oxidative activities toward the substrates with various concentrations, from 25 to 100 μM, of NADPH against a series of fixed concentrations of deprenyl or pargyline. Figs. 5 and 6 show double reciprocal plots of the initial velocity of the FMO reaction with various concentrations of deprenyl or pargyline

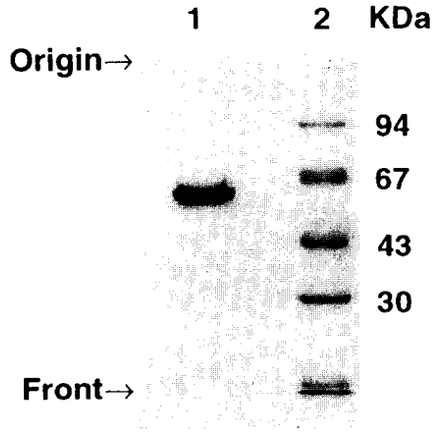

Fig. 2. Electrophoretic pattern of the FMO on SDS-PAGE. 3.5 μg of FMO protein was subjected to SDS-PAGE using 10% (w/v) acrylamide. (1) FMO, (2) standard proteins.

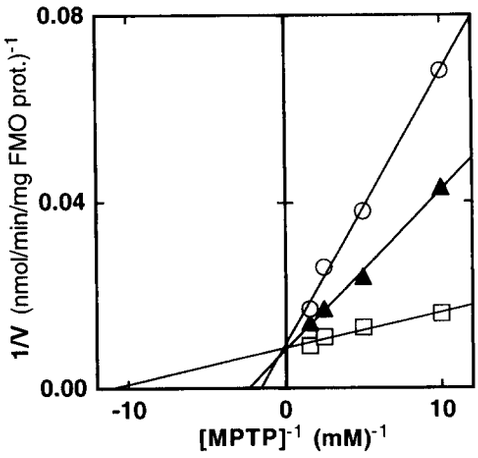

Fig. 3. Effect of deprenyl on the activity of FMO toward MPTP. Double reciprocal plots of the initial reaction velocity of FMO against MPTP concentrations with a series of fixed concentrations of deprenyl. The enzymatic assays performed as described in section 2. Open circles, 80 μM deprenyl; closed triangles, 40 μM deprenyl; open squares, without deprenyl.

against a series of fixed concentrations of NADPH, respectively. The K_m and V_{max} values for deprenyl or pargyline of the FMO are also summarized in Table 1.

4. Discussion

Parkinson's disease and Parkinsonism are neurodegenerative disorders characterized clinically by rigidity and tremor, and pathologically by neuronal cell death in the substantia nigra of the brain [14]. The cause of the dopaminergic cell death that underlies Parkinson's disease remains obscure. However, the discovery that MPTP can induce Parkinson's disease and Parkinsonism in humans has provided an important insight into the causes of idiopathic Parkinson's disease and Parkinsonism [2]. It has been believed that the toxicity of MPTP toward dopaminegic neurons is dependent on the metabolism of MPTP by only glial MAO-B [15], because selective inhibitors, deprenyl and pargyline, of MAO-B completely inhibited the metabolism of MPTP, and these inhibitors did not inhibit other enzymes [3,4].

In this study, we demonstrated that deprenyl and pargyline, which are recognized to be selective MAO-B inhibitors, competitively inhibited the MPTP metabolic activity of FMO, as well as MAO-B activity [3,4]. In addition to their inhibition of FMO, deprenyl and pargyline are excellent substrates for FMO.

Table 1
Kinetic parameters for the MAO-B inhibitors of FMO

Inhibitors or substrates	K_m (μM)	V_{max} (nmol/min/mg protein of FMO)	K_i[a] (μM)	References
Deprenyl	14.3	313	14.0	This study
Pargyline	11.6	385	9.0	This study
MPTP	38.0	495		[6]

The kinetic parameters were determined as described in section 2.
[a]The values for deprenyl or pargyline comprise the oxidative activity of FMO toward MPTP at pH 8.0 and 25°C.

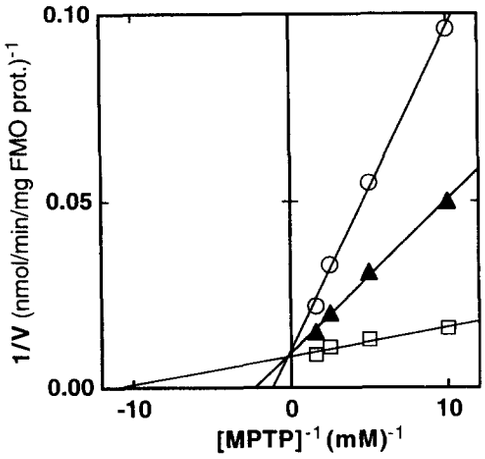

Fig. 4. Effect of pargyline on the activity of FMO toward MPTP. The enzymatic reaction conditions were measured as described in section 2. The method of plotting was the same as described in the legend to Fig. 3, except that deprenyl was replaced with pargyline. Open circles, 60 μM pargyline; closed triangles, 30 μM pargyline; open squares, without pargyline.

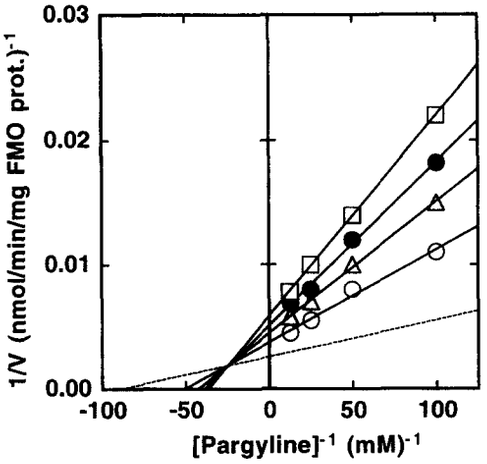

Fig. 6. Kinetic analysis for pargyline of FMO. The enzymatic reaction conditions were measured as described in section 2. The method of plotting was the same as described in the legend to Fig. 5, except that deprenyl was replaced with pargyline.

The MPTP metabolic activity of FMO was strongly inhibited by deprenyl and pargyline. This indicates that the FMO may also contribute to the neuroprotective effect.

This new finding suggests that, through inhibition of the activities of FMO and MAO-B, deprenyl may protect dopaminergic nigral neurons of the substantia nigra from MPP^+-induced neurotoxicity by induction of neurotrophic factors in astroglial cells [16], up-regulation of antioxidant defense enzymes in the striatum [17], and/or suppression of the generation of cytotoxic OH and semiquinone radicals in iron-rich basal ganglia.

The mammalian FMOs comprise a family of xenobiotic-metabolizing enzymes and they are found in most tissues of all mammalian species [18]. FMO catalyzes the oxygenation of nucleophilic nitrogen, sulfur, phosphorous, and selenium atoms in a multitude of structurally diverse compounds [19]. In this study, deprenyl and pargyline were found to be typical tertiary amines, and they were metabolized strongly by porcine liver FMO, being converted quantitatively to *N*-oxides [20]. Judging from this and previous studies [3–7, 21], MPTP is metabolized by FMO [5,6], MAO-B [3], and cytochromes *P*-450 (IA1, IIB1 and IID6). The metabolic pathways and enzymes related to MPTP are indicated in Fig. 7. This figure suggests that the metabolites of MPTP with FMO, MAO-B and P-450 suppress the activities of NADH-CoQ reductase (Complex 1) and related enzymes, and ATP synthase ($F_1 \cdot F_0$) in the mito-

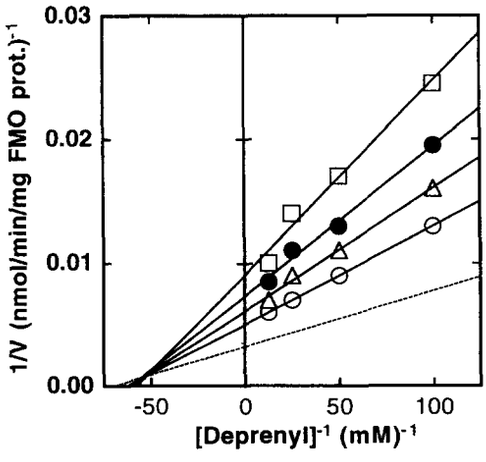

Fig. 5. Kinetic analysis for deprenyl of FMO. Double reciprocal plots of the initial velocity of the FMO reaction against various concentrations of deprenyl with a series of fixed concentrations of NADPH. The broken line shows the values extrapolated to an infinite concentration of deprenyl. The enzymatic assays were carried out as described in section 2. Open squares, 25 μM NADPH; closed circles, 50 μM NADPH; open triangles, 75 μM NADPH; open circles, 100 μM NADPH.

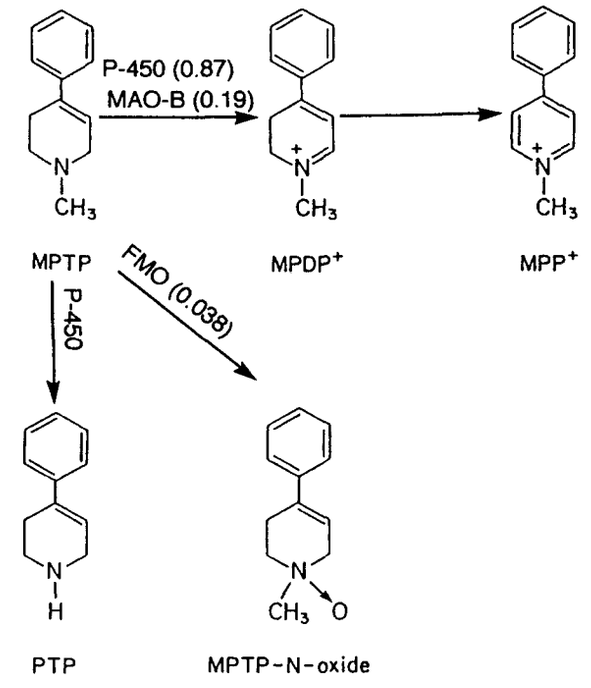

Fig. 7. Metabolic pathways, and the enzymes for MPTP and its metabolites. The values in parentheses indicate K_m (μM). The K_m value for MPTP of cytochrome *P*-450 IA1 was 0.87 μM.

chondria of dopaminergic cells of the substantia nigra, and thus are the underlying cause of the ATP deficiency and subsequent dopaminegic cell death, and thereby produce Parkinson's disease and Parkinsonism [22].

Acknowledgements: We wish to thank Drs. T. Ohnishi, S. Tomita, and H. Yoneyama, Department of Biochemistry, Kagawa Medical School, for the discussions. We are also grateful to Mr. A. Miyatake, Research Center of Medical Science, Kagawa Medical School, for his assistance.

References

[1] Kloss, R.E., Cavagnaro, J., Rosen, G.M. and Rauckman, E.J. (1982) Toxicol. Appl. Pharm. 64, 88–93.
[2] Langston, J.W., Ballard, P., Tetrud, J.W. and Irwin, I. (1983) Science 219, 979–980.
[3] Chiba K., Trevor A. and Castagnoli Jr., N. (1984) Biochem. Biophys. Res. Commun. 120, 579–585.
[4] Singer, T.P. (1991) in: Chemistry and Biochemistry of Flavoenzymes (F.R. Muller, Ed.) Chapter 17, pp. 437–470, CRC Press, Bocaraton, Anchor, Boston, London.
[5] Wu, R.F., Miura, S. and Ichikawa, Y. (1992) Biochem. Pharmacol. 44, 2079–2081.
[6] Wu, R.F. and Ichikawa, Y. (1994) Biochem. Biophys. Acta 1208, 204–210.
[7] Ottoboni, S., Carlson, T. J., Trager, W. F., Castagnoli, K. and Castagnoli Jr., N. (1990) Chem. Res. Toxicol. 3, 423–427.
[8] Mitoma, C., Posner, H.S., Reitz, H.C. and Udenfriend, S. (1956) Arch. Biochem. Biophys. 61, 431–441.
[9] Dixon, M. and Webb, E.C. (1979) in: Enzymes (3rd edn.), Chemical IV, pp. 47–206, Academic Press, New York.
[10] Smith, P.K., Krohn, I.R., Hermanson, G.T., Mallis, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J. and Klenk, D.C. (1985) Anal. Biochem. 150, 76–85.
[11] Gornall, A.G., Bardwill, G.J. and David, M.M. (1949) J. Biol. Chem. 177, 751–760.
[12] Ryan, M.T. and Chopra, R.K. (1976) Biochim. Biophys. Acta 427, 337–340.
[13] Ziegenhorn, J., Senn, M. and Bucher, T. (1976) Clin. Chem. 22, 151–160.
[14] Davis, G.C., Williams, A.C., Markey, S.P., Ehert, M.A., Calne, C.D., Riechert, C.M. and Kopin, I.J. (1979) Psychiatry Res. 1, 249–254.
[15] Salach, J.I., Singer, T.P., Castagnoli Jr., N. and Trevor, A. (1984) Biochem. Biophys. Res. Commun. 125, 831–835.
[16] Date, I., Notter, M.F.D., Felten, S.Y. and Felten, D.L. (1990) Brain Res. 526, 156–160.
[17] Thiffault, C., Quirion, R., Aumont, N. and Poirier, J. (1992) Soc. Neurosci. Abstr. 18,1443–1448.
[18] Poulsen, L.L. (1981) Rec. Biochem. Toxic. 3, 33–49.
[19] Hajjar, N.P. and Hodgson. E. (1980) Science 209, 1134–1136.
[20] Baker, J.R. and Chaykin, S. (1963) J. Biol. Chem. 273, 1309–1313.
[21] Monte, D.D., Shinka, T., Sandy, M.S., Castagnoli Jr., N. and Smith, A.M. (1988) Drug Metab. Disposi. 16, 250–255.
[22] Schapira, A.H., Cooper, J.M., Dexter, D., Jenner, P., Clark, J.B. and Marsden, C.D. (1989) Lancet 1269.