ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 10 (2000) 291–294

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

www.elsevier.com/locate/molcatb

Xanthone derivatives as inhibitors for monoamine oxidase

Nobuko Ohishi *, Takehiko Suzuki, Tomio Ogasawara, Kunio Yagi

Institute of Applied Biochemistry, Yagi Memorial Park, Mitake, Gifu 505-0116, Japan

Received 1 November 1999; accepted 22 November 1999

Abstract

Various xanthone derivatives were tested for in vitro inhibition of monoamine oxidase (MAO) in the mitochondrial fraction from rat brain and mouse liver. 1,6-Dihydroxyxanthone and 1,3,6-trihydroxyxanthone exhibited much potent inhibitory activity toward rat brain mitochondrial MAO (type A). On the other hand, 1,3,7-trihydroxyxanthone showed great potency for inhibition of mouse liver mitochondrial MAO (type B). These results indicate that 1,3,6-trihydroxyxanthone and 1,3,7-trihydroxyxanthone were rather specific inhibitors of type A MAO and type B MAO, respectively. 1,3-Dihydroxy-6-alkoxyxanthones containing alkoxy residues with carbon number 1 to 8 were synthesized and compared for their inhibitory activity toward rat brain mitochondrial MAO. Among them, 6-ethoxy-, 6-propyloxy-, and 6-butyloxy-derivatives were potent inhibitors, and especially, 1,3-dihydroxy-6-propyloxyxanthone was the most inhibitory. Lineweaver–Burk plot analysis demonstrates that these xanthone derivatives inhibited MAO in a competitive and reversible manner. Spectroscopic examinations suggest that complex formation between the flavin moiety and a xanthone derivative could be involved at least partly in their inhibitory action.

Keywords: Xanthones; 1,3-Dihydroxy-6-alkoxyxanthones; MAO inhibitor; Reversible inhibitor; Charge transfer complex

1. Introduction

It is well known that monoamine oxidase(s) (MAO) [monoamine:O_2 oxidoreductase (deaminating) (flavin containing) EC 1.4.3.4] contain a covalently bound flavin adenine dinucleotide as a coenzyme, localize in the outer membrane of mitochondria, catalyze the oxidative deamination of biogenic amines as well as xenobiotic monoamines, and are classified into two forms, A and B, on the basis of their specificity to the substrates, susceptibility to specific inhibitors, tissue and cell distribution, and immunological nature.

MAO plays an important role in the metabolism of monoamines in the central nervous system and its inhibitors are expected to be useful in the therapy of psychosis, depression, schizophrenia, and so on. Up to now, many compounds inhibitory toward it have been isolated from natural substances or synthesized for development of medicine.

Xanthones (Fig. 1) are yellow pigments in plants such as *Gentianaceae*. Some xanthones were reported to act as inhibitors of MAO [1–3]. Suzuki et al. [3] reported that the xanthones bearing 1,3-dihydroxy groups exhibited potent

* Corresponding author. Tel.: +81-574-67-5500; fax: +81-574-67-5310.

E-mail address: yagiab@po.sphere.ne.jp (N. Ohishi).

1381-1177/00/$ - see front matter
PII: S1381-1177(00)00112-0

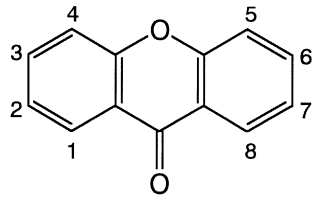

Fig. 1. Structure of xanthones.

inhibitory activity against both types of MAO A and B. We also examined the inhibitory activities of various new xanthone derivatives toward MAO in rat brain mitochondria and mouse liver mitochondria. Further, the inhibitory mechanism of these substances was examined by analyzing the change in absorption spectrum of riboflavin caused by a xanthone derivative. In this paper, we describe the inhibitory activity against MAO of newly synthesized xanthone derivatives and suggest that complex formation between them and MAO can account for their inhibitory action.

2. Materials and methods

2.1. Materials

Various hydroxyxanthone derivatives were synthesized by the reaction of salicylic acid replaced with some residue at position 4 and phloroglucinol in the presence of phosphorus oxychloride and zinc chloride according to the ordinary method [4] and purified by silica gel column chromatography and recrystallization. In the case of 1,3-dihydroxy-6-alkoxyxanthones, 4-*n*-alkoxysalicylic acid containing alkoxy residues with carbon number 1 to 8 was used as the starting material. Other xanthone derivatives were obtained by the similar method. Kynuramine, clorgyline, deprenyl, and other reagent grade chemicals were obtained from Nacalai tesque, Kyoto. Rat brain or mouse liver was homogenized with nine volumes of 0.25 M sucrose and the mitochondrial fraction washed three times with the sucrose solution was used as the enzyme preparation.

2.2. Assay of MAO activity

The activity of MAO was assayed fluorometrically with kynuramine as a substrate by the method of Kraml [5] with a slight modification. Xanthone derivatives were dissolved in dimethyl sulfoxide (DMSO)-methanol (1:1, v/v). The final concentration of each solvent (0.5%) had been confirmed not to affect MAO activity by preliminary experiment. Mitochondrial fraction from rat brain was used as a type A MAO preparation and that from mouse liver, as a type B MAO preparation, respectively. The reaction mixture containing an inhibitor and enzyme preparation in 0.1 M phosphate buffer (pH 8.2) was preincubated at 37°C for 10 min. Then the substrate, kynuramine, was added, and the reaction mixture with a total volume of 1 ml was further incubated for 30 min. The enzyme reaction was stopped by the addition of $HClO_4$ and heated at 95°C for 3 min. After centrifugation, 0.5 ml of the supernatant was mixed with 1 ml of 1 N NaOH, and the fluorescence was measured at 380 nm emission with excitation at 315 nm. As a blank, the value obtained when the substrate was added after the stopping of the reaction with $HClO_4$ was used. MAO activities were measured in the presence and absence of an inhibitor, and the value of IC_{50} was determined from the dose–response curve.

To confirm the specificity of the inhibitor for type A or type B of MAO, the enzyme reaction was carried out after the enzyme preparation had been treated with an irreversible inhibitor, clorgyline (0.1 μM) for type A or deprenyl (0.1 μM) for type B at 37°C for 30 min: after centrifugation of the reaction mixture at 18,000 g for 10 min, the pellet was washed with 0.1 M phosphate buffer (pH 7.4) and resuspended in the same buffer. The activity of MAO using these preparations was measured as described above.

2.3. Spectroscopic analysis of the reaction of riboflavin with a xanthone

Riboflavin (0.1 mM) and 1,3,6-trihydroxyxanthone (1, 5, or 10 mM) were mixed in 25 mM phosphate buffer (pH 7.2)–25% DMSO and the spectral change was recorded with a spectrophotometer (UV-250, Shimadzu, Kyoto).

3. Results and discussion

3.1. Inhibitory action of various hydroxyxanthone derivatives

In Table 1, the IC_{50} values of various hydroxyxanthone derivatives are shown. Among them, 1,6-dihydroxyxanthone, 1,3,6-trihydroxyxanthone, and 1,3,7-trihydroxyxanthone were shown to be potent inhibitors. Especially, the former two inhibited preferentially type A MAO, as shown by the low value of IC_{50} for the rat brain mitochondrial preparation. 1,3,7-Trihydroxyxanthone inhibited preferentially type B MAO as revealed by the low IC_{50} value for the mouse liver mitochondrial enzyme. These results seem to be confirmed by the value of the ratio of IC_{50} obtained with clorgyline-pretreated preparation to that with deprenyl-pretreated preparation: The high value indicates that the inhibitor examined is specific against type A MAO and the low value, specific against type B MAO. Consequently, 1,3,6-trihydroxyxanthone exhibiting a high value of the ratio is specific for type A MAO and 1,3,7-trihydroxyxanthone having a low value is specific for type B MAO (Table 1).

Table 1
Inhibitory effects of xanthones on MAO activity of rat brain mitochondria and mouse liver mitochondria

Xanthones	IC_{50} (μM)		Clorgyline-treated/deprenyl-treated[a]
	Rat brain	Mouse liver	
Dihydroxy-			
1,3-	0.470	–	3.50
1,6-	0.304	64.3	69.8
1,8-	0.924	>100	15.3
Trihydroxy-			
1,3,5-	0.805	>100	348
1,3,6-	0.282	95.1	304
1,3,7-	1.34	0.37	0.318
1,3,8-	1.23	0.54	0.986
Tetrahydroxy-			
1,3,5,6-	>10	1.54	7.49
1,3,5,8-	7.30	6.42	23.4
1,3,6,8-	1.98	3.48	24.8

[a] Ratio of IC_{50} toward MAO activity of rat brain mitochondrial preparation treated with clorgyline to that with deprenyl.

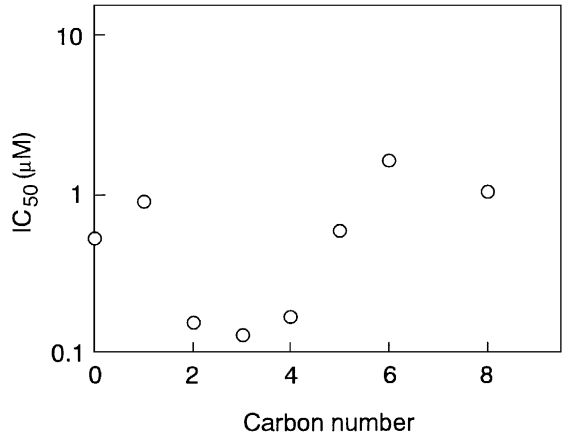

Fig. 2. Effect of substitution by an alkoxy group at position 6 of 1,3-dihydroxyxanthone on the inhibition of MAO. IC_{50} values obtained from dose–response curve were plotted against carbon number 0–8 of alkoxy group introduced. Enzyme, rat brain mitochondrial preparation (type A MAO). 0: 1,3-dihydroxyxanthone.

Lineweaver–Burk plot analysis clearly demonstrated that the inhibition of MAO activity with these xanthone derivatives was competitive and reversible (data not shown).

In addition, the inhibitory activity was examined for the 1,3-dihydroxyxanthone derivatives replaced at position 6 with various other residues such as halogens. Among those examined, 6-ethoxy- (IC_{50}, 0.16 μM) and 6-iodo- (IC_{50}, 0.17 μM) derivatives were very potent inhibitors against type A MAO.

3.2. Inhibitory action of 1,3-dihydroxy-6-alkoxyxanthones

Since 1,3-dihydroxy-6-ethoxyxanthone had potent inhibitory activity as described above and

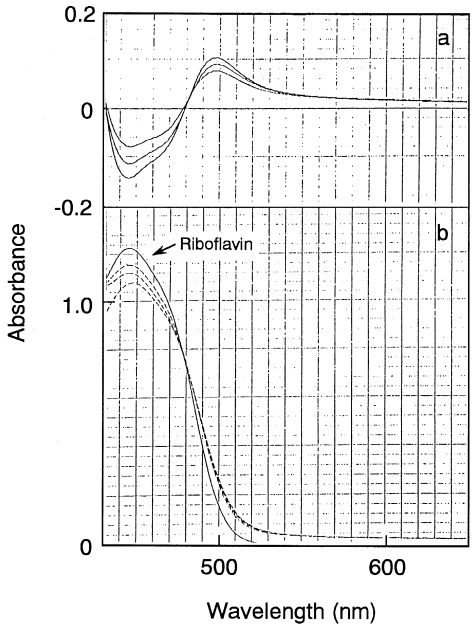

Fig. 3. Interaction between riboflavin and 1,3,6-trihydroxyxanthone. Absorption spectrum was measured at 25°C in 25 mM phosphate buffer (pH 7.2)/25% DMSO. Concentration: riboflavin, 0.1 mM; 1,3,6-trihydroxyxanthone, 1, 5, and 10 mM. (a) Difference absorption spectrum between riboflavin and 1,3,6-trihydroxyxanthone additives; (b) absorption spectrum. Dotted lines, after addition of 1,3,6-trihydroxyxanthone. By the addition of the xanthone, the absorbance at 445 nm decreased and that in the longer wavelength it increased indicating the formation of a charge transfer complex.

more powerful type A MAO inhibitors to penetrate into the brain are desired, we synthesized the 6-alkoxy derivatives of 1,3-dihydroxyxanthone with different lengths of carbon chain from 1 to 8. Among them, the compounds with a carbon chain number of 2–4 inhibited type A MAO at the same degree of potency as 6-iodo-derivatives: especially, 1,3-dihydroxy-6-propyloxyxanthone was most effective, and its IC_{50} value was estimated to be 0.13 μM (Fig. 2). When the number of carbons in the chain was increased over 4, the potency of inhibition decreased, as shown in Fig. 2.

The inhibition of MAO activity with these xanthone derivatives was also of competitive and reversible nature (data not shown) as the derivatives mentioned above.

3.3. Complex formation between riboflavin and a xanthone derivative

Changes in the absorption spectrum of riboflavin by the addition of 1,3,6-trihydroxyxanthone showed the existence of a molecular interaction between these two compounds, as shown in Fig. 3. Moureau et al. [6] suggested by conducting the electron absorption spectroscopy and ab initio calculation of frontier orbitals that the physical association between R-Toloxatone, an MAO inhibitor, and flavin, a cofactor of MAO, is a possible mechanism of its reversible inhibition: the existence of a charge transfer complex between R-Toloxatone and riboflavin and favourable overlap of complementary electronic zones of R-Toloxatone and a lumiflavin derivative. In our case also, this might explain the mechanism of the reversible inhibitory action of xanthone derivatives on MAO: xanthone derivatives may exert their inhibitory action by means of reversible interaction with FAD linked covalently to the enzyme apoprotein through the charge-transfer complex formation.

From these results, 1,3-dihydroxyxanthone derivatives having a substituent at position 6, especially 6-ethoxy, propyloxy, and butyloxy derivatives, are considered to be very desirable drugs on the basis of the reversible, competitive, and specific nature of their inhibition.

References

[1] O. Suzuki, Y. Katsumata, M. Oya, V.M. Chari, R. Klapfenberger, H. Wagner, K. Hostettmann, Biochem. Pharmacol. 27 (1978) 2075.
[2] O. Suzuki, Y. Katsumata, M. Oya, V.M. Chari, R. Klapfenberger, H. Wagner, K. Hostettmann, Planta Med. 39 (1980) 19.
[3] O. Suzuki, Y. Katsumata, M. Oya, V.M. Chari, B. Vermes, H. Wagner, K. Hostettmann, Planta Med. 42 (1981) 17.
[4] T.R. Govindachari, B.R. Pai, P.S. Subramanian, U.R. Rao, N. Muthukumaraswamy, Tetrahedron 23 (1967) 243.
[5] M. Kraml, Biochem. Pharmacol. 14 (1965) 1684.
[6] F. Moureau, J. Wouters, D.P. Vercauteren, S. Collin, G. Evlard, F. Durant, F. Ducrey, J.J. Koenig, F.X. Jarreau, Eur. J. Med. Chem. 29 (1994) 269.