Monoamine Oxidase-Catalyzed Amine Oxidation in Organic Solvents

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Introduction

Over approximately the past 10 years, particularly as a result of the work of Klibanov and co-workers,[1-5] it has been found that enzymes can function in anhydrous and slightly aqueous organic media. Although catalytic rates are usually slower in organic solvents than in aqueous media, there are several advantages to carrying out enzymatic reactions in organic media. Among these advantages are (1) ease of recovery of enzyme and product from the organic solvent, (2) increased stability of enzymes in an organic solvent, and (3) high solubility of organic substrates in organic media. The use of enzymes as catalysts in organic solvents has wide applicability in organic synthesis.[6] Generally, enzymes that catalyze hydrolytic reactions have been studied in organic solvents; few redox enzymes have been used in organic solvents.[2-6]

Monoamine oxidase B (MAO B, EC 1.4.3.4), a promiscuous flavoenzyme from beef liver that catalyzes the oxidation of a variety of primary, secondary, and tertiary amines to the corresponding aldehydes in aqueous media, was found recently to catalyze the oxidation of *N*-methylbenzylamine to the corresponding imine in benzene containing 1% water.[7] This provided the first direct nonspectroscopic evidence that the product of MAO B-catalyzed oxidation of amines is the corresponding imine and that hydrolysis, therefore, occurs after the imine is released from the enzyme. Here we demonstrate generally that MAO B is catalytically active in a variety of organic solvents containing low concentrations of water.

Results and Discussion

N-Methylbenzylamine was oxidized by MAO B to *N*-benzylidenemethylamine in various organic solvents as summarized in Table 1. MAO B was catalytically active in ether, carbon tetrachloride, *n*-octane, benzene, and cyclohexane, but was inactive in polar water-soluble organic solvents such as acetonitrile, DMSO, DMF, ethanol, acetone, and pyridine. In moderately polar water-insoluble solvents such as methylene chloride, chloroform, and ethyl acetate, small amounts of the imine were detected after extended incubation times. None of the reactions in organic solvents had k_{cat}/K_m ratios comparable to that in buffer, but the k_{cat} values in *n*-octane and benzene were greater than that in buffer; the K_m in carbon tetrachloride was only a factor of 3–4 higher than that in buffer.

Table 1. Kinetic Constants[a] for the Oxidation of *N*-Methylbenzylamine in Various Organic Solvents Containing 1 v/v % Water

solvent	K_m (mM)	k_{cat} (min^{-1})	k_{cat}/K_m (mM^{-1} min^{-1})
50 mM NaP_i, pH 7.2	0.152 ± 0.001	117 ± 1	770 ± 4
Et_2O	0.76 ± 0.07	66 ± 4	87 ± 10
CCl_4	0.54 ± 0.01	44 ± 1	81 ± 1
n-octane	2.1 ± 0.1	149 ± 5	71 ± 5
benzene	2.3 ± 0.1	124 ± 4	54 ± 4
cyclohexane	5.1 ± 0.2	21 ± 2	4 ± 1

[a] Kinetic constants were obtained at various substrate concentrations with the use of Lineweaver–Burk plots (1/V vs 1/[S])[11] and a nonlinear regression analysis. See the Experimental Section for details of rate measurement determination.

Various other compounds were subjected to MAO B oxidation in slightly aqueous media (Table 2). With all of the amines except cyclohexylamine, the resulting products were the corresponding condensation products of the amine with either the corresponding aldehyde or aldimine. In the case of *N*-methylphenethylamine, it appears that the product imine undergoes reaction with the substrate, which breaks down, presumably with loss of methylamine, to give the product. *N*-Methylbenzylamine does not condense with the starting amine, presumably, because of the stability of the conjugated system generated. In aqueous medium only the aldehyde products are observed. This suggests that if MAO B is to be used as a synthetic catalyst in organic solvents for the formation of imines, and complete consumption of starting materials is important, either tertiary amines should be used or the products have to be stabilized imines so that condensation with the substrate amine does not occur.

When either (*R*)- or (*S*)-α-methylbenzylamine, both very poor substrates for MAO B in buffer ($k_{cat} = 6.73 \times 10^{-4}$ min^{-1}),[8] was employed in cyclohexane, no activity was observed. *N*-Methylaniline, which is not a substrate for MAO B in buffer, also failed to undergo oxidation in *n*-octane.

The effect of water concentration on the oxidation of benzylamine by MAO B in *n*-octane was determined and is shown in Table 3. It appears that some water is needed to effect reasonable catalytic activity, perhaps by bringing the enzyme into its active conformation. Near maximum oxidation rates in *n*-octane occurred with as little as 0.1% water. Water addition (up to 20%) to aqueous-miscible solvents did not result in any enzyme activity. To induce ligand activation[9] of the enzyme, so that added water would not be required, incubation of MAO B with α-methylbenzylamine prior to lyophilization was attempted; however, this approach failed to have any effect on the reaction rate.

Conclusion

These results indicate that MAO B is capable of catalysis in organic solvents containing low concentrations of water. Because this is a heterogeneous mixture,

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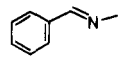
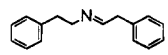
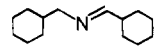
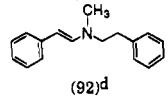
Table 2. Oxidation of Amines by MAO B in Various Solvents at 1 v/v % Water

Compound (conc. used)	Product (%)[a]	Time (h)	Solvent
N-methylbenzylamine (7.7 mM)	 (99)	1.5	*n*-octane
phenethylamine (6.6 mM)	 (99)[b]	1.5	*n*-octane
cyclohexane-methylamine (6.1 mM)	 (69)[c]	8.5	*n*-octane
N-methyl-phenethylamine (6.7 mM)	 (92)[d]	4	*n*-octane
cyclohexylamine (8.7 mM)	 (14)	24	*n*-octane

[a] Percent product was based on the relative ratio of the integrated areas of the product and starting material peaks in the GC trace to the internal standard compound *n*-dodecane. [b] Percent product was based on the relative ratio of the integrated areas in the GC of the starting material peak to the internal standard compound (*n*-dodecane) at $t = 0$ versus $t = 1.5$ h. A product peak could not be detected probably because of polymerization or decomposition. In a nonenzymatic control reaction in which phenylacetaldehyde and phenethylamine were mixed, the same phenomenon was observed, namely that while the peaks corresponding to the two starting materials decreased dramatically after a short period, the peak corresponding to the condensed product could not be observed. Therefore, the structure shown as the product is the hypothesized initial product. [c] This compound was synthesized by the method of Enholm et al.[12] A solution containing equimolar amounts of cyclohexylmethylamine and cyclohexanecarboxaldehyde in benzene were mixed; then the benzene was removed by rotary evaporation. Additional benzene was added, and the solution was rotary evaporated; this process was repeated two more times. The resulting aldimine was obtained in a quantitative yield after solvent evaporation. The product could not be purified satisfactorily by distillation (bp 120 °C (4 mmHg)) or by chromatography because of its ease of hydrolysis: HRMS calcd for $C_{14}H_{25}N$ 207.1987, found 207.1995; 300 MHz ^{1}H NMR ($CDCl_3$) δ 0.82–0.95 (m, 2 H), 1.1–1.4 (m, 8 H), 1.6–1.8 (m, 11 H), 2.18 (m, 1 H), 3.18 (d, 2 H), 7.42 (d, 1 H); ^{13}C NMR (75.2 MHz) δ 25.50, 26.04, 26.11, 26.66, 29.88, 31.34, 38.53, 43.52, 68.46, 169.04. [d] This compound was synthesized as described above using *N*-methylphenethylamine and phenylacetaldehyde: mp 55–56 °C; 300 MHz ^{1}H NMR ($CDCl_3$) δ 2.79 (s, 3 H), 2.83 (t, 2 H), 3.36 (t, 2 H), 5.18 (d,1 H), 6.78 (d, 1 H), 7.3 (m, 10 H); ^{13}C NMR ($CDCl_3$) (75.2 MHz) d 139.85, 139.42, 138.92, 128.86, 128.79, 128.5, 126.34, 123.49, 123.19, 97.42 57.39, 37.13, 34.79; MS *m*/*z* 237 (M^+), 146, 131, 105, 91, 77. Anal. Calcd for $C_{16}H_{19}N$: C, 86.02 H, 8.07, N, 5.90. Found: C, 85.72, H, 7.78, N, 5.82.

it is not clear if catalysis is occurring in aqueous globules or dispersed in the organic solvent, but a mild method of hydrophobic amine oxidation has been demonstrated.

Table 3. Effect of Water Concentration on MAO B-Catalyzed Oxidation of Benzylamine in *n*-Octane

v/v % H_2O	time (min)	% benzylidenebenzylamine[a]
0.5	120	quantitative
0.3	120	quantitative
0.2	120	95.5
0.1	120	92.4
0.05	120	20
0.0	120	13

[a] Percent benzylidenebenzylamine was based on the relative ratio of the integrated areas of the benzylidenebenzylamine and benzylamine GC peaks to the peak of the internal standard compound *n*-dodecane.

Experimental Section

General. All chemicals, including substrates and products, were obtained from Aldrich Chemical Co. and were used without further purification, except as noted below and in the tables. α-Methylbenzylamine and *N*-methylbenzylamine were converted into their hydrochloride salts by bubbling gaseous HCl through an ethereal solution of each compound. The hydrochloride salts were then recrystallized from ethanol. Benzaldehyde was purchased from MCB Manufacturing Chemists, Inc., distilled, and stored under argon.

Enzyme and Assay. Beef liver MAO B was isolated as described previously[10] and stored as a concentrated solution (15–25 mg/mL) in sodium phosphate buffer (50 mM, pH 7.2) at 4 °C. The specific activity varied among preparations, ranging from 3.5 to 7 units per mg, where a unit of activity is the conversion of 1 μmol of benzylamine to benzaldehyde per min in Tris-HCl buffer, pH 9.0 and 30 °C.

General Procedure in Organic Solvents. Stock MAO B (30 μL) was pipetted into a half-dram vial, frozen in a −78 °C dry ice/acetone bath, and lyophilized. The substrate solution (3 μL) was syringed into the vial containing the dried enzyme; then 3 μL of a solution of the internal standard, *n*-dodecane (final concentration 4.4 mM), in the same solvent was added. Water was then pipetted into the mixture to give the desired water content. The mixture was sonicated for 10 s in an ultrasonic cleaning bath. For analyses, 1 μL aliquots were periodically removed and analyzed by capillary gas chromatography (Hewlett-Packard 5730A flame ionization gas chromatograph equipped with an Alltech 30 m, 32 mm i.d. SE-30 capillary column). The temperature of the incubation mixture was kept at 25 °C employing a Lauda K-2/RD constant temperature bath. In cases where ligand activation was attempted, MAO B was preincubated with 1.2 mM α-methylbenzylamine, lyophilized, and used as described above with no addition of water.

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