APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 1979, p. 565-569
0099-2240/79/10-0565/05$02.00/0
Vol. 38, No. 4

# Distribution of Membrane-Bound Monoamine Oxidase in Bacteria

YOSHIKATSU MUROOKA,* NOBUYUKI DOI, AND TOKUYA HARADA

*The Institute of Scientific and Industrial Research, Osaka University, Yamadakami, Suita, Osaka (565), Japan*

Received for publication 22 March 1979

The distribution of membrane-bound monoamine oxidase in 30 strains of various bacteria was studied. Monoamine oxidase was determined by using an ammonia-selective electrode; analyses were sensitive and easy to perform. The enzyme was found in some strains of the family *Enterobacteriaceae*, such as *Klebsiella*, *Enterobacter*, *Escherichia*, *Salmonella*, *Serratia*, and *Proteus*. Among strains of other families of bacteria tested, only *Pseudomonas aeruginosa* IFO 3901, *Micrococcus luteus* IFO 12708, and *Brevibacterium ammoniagenes* IAM 1641 had monoamine oxidase activity. In all of these bacteria except *B. ammoniagenes*, monoamine oxidase was induced by tyramine and was highly specific for tyramine, octopamine, dopamine, and norepinephrine. The enzyme in two strains oxidized histamine or benzylamine. Correlations between the distributions of membrane-bound monoamine oxidase and arylsulfatase synthesized in the presence of tyramine were discussed.

Monoamine oxidase catalyzes the oxidative deamination of monoamines by the following reaction: $R\text{-}CH_2NH_2 + O_2 + H_2O \rightarrow R\text{-}CHO + NH_3 + H_2O_2$.

The enzyme usually has a broad substrate specificity in animals and plays a major role in the metabolism of biogenic amines (10). The enzyme is also found in microorganisms when they are grown with amine compounds as the nitrogen source (16). Amine oxidases in microorganisms are important in biological inactivation of naturally occurring or synthetic amine compounds. The monoamine oxidases in *Micrococcus luteus* (*Sarcina lutea*) and *Klebsiella aerogenes* have been named tyramine oxidases (11, 17) because they are highly specific for tyramine and related compounds. Synthesis of tyramine oxidase in *K. aerogenes* is induced specifically by these compounds (11). Studies by manometric and radiometric methods showed that the enzyme was membrane bound (11) and was involved in the regulation of arylsulfatase synthesis (7, 9, 12). However, enzyme assay by manometric measurement of oxygen uptake lacks sensitivity and specificity, especially with crude enzyme preparations. Furthermore, although the radiometric procedure is sensitive, it requires various radioactive substrates for tests of substrate specificity (14). It also has the inherent problems of nonspecific binding of radioactive metabolites to denatured protein and differences in the extraction coefficients of the products, labeled aldehydes or acids. Since monoamine oxidase had broad substrate specificity, the enzyme in bacteria can be assayed conveniently by potentiometric measurement of ammonia formation with an ammonia-selective electrode by the method used for the brain enzyme by Meyerson et al. (6).

This paper reports studies on the distribution of membrane-bound monoamine oxidase in bacteria by the method of potentiometric assay. From the results, we propose that most bacterial monoamine oxidases should be classified as tyramine oxidases.

## MATERIALS AND METHODS

**Strains.** Most of the bacterial strains used in this study were obtained from the Institute for Fermentation, Osaka. *Brevibacterium ammoniagenes* IAM 1641 was obtained from the Institute of Applied Microbiology, Tokyo. *S. lutea* IAM 1099, which is now named *M. luteus* (2), was obtained from the Institute for Fermentation, Osaka, as stock no. IFO 12708. *K. aerogenes* W70, *Escherichia coli* K-12, *Salmonella typhimurium* LT2, and *Corynebacterium acetophilum* A51 are kept as stock cultures in our laboratory.

**Culture.** Bacteria for enzyme assay were grown with aeration on a rotary shaker at 30°C in medium (100 ml) composed of 0.1 M potassium phosphate buffer (pH 7.2); 0.1% $NH_4Cl$; 0.1% yeast extract; 0.01% $MgCl_2 \cdot 6H_2O$; 0.001% each of NaCl, $MnCl_2 \cdot 4H_2O$, and $FeCl_3 \cdot 6H_2O$; 1 mM $Na_2SO_4$; and 0.5% carbon compounds. Unless otherwise mentioned, xylose and 3 mM tyramine were used as the carbon source and inducer, respectively. Growth was followed in a Klett-Summerson colorimeter (590 to 660 nm).

**Preparation of membrane-bound enzymes.**

Cells grown for 1 to 2 days in 200-ml cultures (to about 100 to 200 Klett units) were harvested by centrifugation. They were then washed twice with 0.05 M potassium phosphate buffer (pH 7.4), suspended in the same buffer (6 ml), and disrupted by sonication at 20 kc for 5 min. The cell debris was removed by centrifugation at 5,000 × *g* for 10 min, and the supernatant was centrifuged at 105,000 × *g* for 60 min. The supernatant was saved as periplasmic and cytoplasmic fractions. The precipitate was washed with 5 ml of 0.05 M potassium phosphate buffer (pH 7.4) with recentrifugation and homogenized by a Potter blender with 5 ml of the same buffer.

**Assay of monamine oxidase.** Bacterial monoamine oxidase was assayed by using an ammonia-selective electrode (6). Ammonia in the sample diffuses through a gas-permeable membrane until the $NH_3$-N partial pressure is in equilibrium, and the resulting potential (in millivolts) is measured with a millivolt/pH meter. The enzyme activity in the bacterial cell membrane was assayed in a reaction mixture consisting of the following components: 0.05 M sodium phosphate buffer (pH 7.4), 0.1 ml of homogenized membrane fraction (0.5 to 3 mg of protein), 10 mM freshly prepared substrate, and distilled water to a final volume of 2.0 ml. After incubation for 30 min with shaking in a water bath at 30°C, the reaction was terminated by adding 4 ml of phosphate-sodium hydroxide buffer (Titrisol buffer), pH 12.0. Substrates were added to control tubes after termination of the reaction. Standard solutions of ammonia were prepared by serial dilution of 0.1 M ammonium chloride with distilled water, and 0.2-ml samples of each dilution ($10^{-2}$ to $10^{-5}$ M) were added to reaction mixtures in the presence of bacterial cell membranes (2 mg of protein). Readings in millivolts were plotted against the ammonia concentration. The standard curve was affected by the source of the bacterial membrane preparation and thus had to be made for each bacterial sample. Moreover, crude extracts of sonicated cells, but not membrane fractions, had to be dialyzed against distilled water to remove the internal ammonium ions before assay. The electrode response time (equilibrium potential) was about 10 min at the lowest ammonia concentration and about 3 min at the highest concentration. Control and test ammonia concentrations were calculated from the standard curve, and the difference between the two values was taken as the amount of ammonia formed enzymatically.

**Determination of protein.** Membrane-bound proteins were assayed by sodium deoxycholate-trichloroacetic acid protein precipitation by using the modification by G. L. Peterson (13) of Lowry's method. Membrane proteins were recovered quantitatively by this method, and the standard curve plotted on a log-log scale was linear with 5 to 100 μg of bovine serum albumin.

**Apparatus and reagents.** A Hitachi-Horiba millivolt/pH meter (model F7), an ammonia electrode (Horiba model 5002-05T, Kyoto, Japan), and a research pH electrode (Horiba model 6028-10T) were used. Titrisol buffer was purchased from E. Merck Japan Ltd. The other compounds employed were standard commercial preparations.

## RESULTS

**Assay of tyramine oxidase with an ammonia-selective electrode.** Ammonia production in the tyramine oxidase reaction was linear for the first 40 min of incubation, with tyramine (10 mM) as substrate and membrane fractions of *K. aerogenes* W70 as enzyme source (Fig. 1). The tyramine oxidase of this strain specifically oxidizes tyramine, dopamine, norepinephrine, and octopamine as shown previously in manometric experiments (11); it did not oxidize any other monoamines, diamines, polyamines, or amino acids. When control and test incubations were run with norepinephrine, which is unstable in air and in light, potentiometric readings were lower than those with tyramine. The dialyzed supernatant fluid obtained by ultracentrifugation contained slight tyramine oxidase activity, as reported previously (9, 11). The apparent $K_m$ and $V_{max}$ values for tyramine of the enzyme in *K. aerogenes* W70 were $1.4 \times 10^{-4}$ M and 9.1 nmol of ammonia formed per min per mg of protein and were $2.6 \times 10^{-3}$ and 2.9 nmol of ammonia formed per min per mg of protein for octopamine.

Thus, results obtained by using the ammonia-selective electrode were consistent with previous results (11) obtained by manometric and radiometric procedures. The method was more rapid and convenient for estimation of bacterial monoamine oxidase.

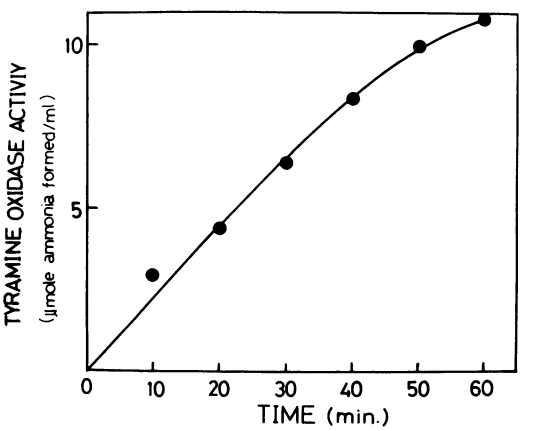


FIG. 1. *Time course of tyramine oxidase activity. Membrane fractions of K. aerogenes W70 (15 mg of protein) and 10 mM tyramine were incubated in 50 mM potassium phosphate buffer (pH 7.4) at 30°C in a total volume of 20 ml. Two-milliliter volumes were pipetted out from the reaction mixture at the times indicated. The concentration of ammonia formed at each time interval was determined as described in the text.*

**Distribution of monoamine oxidase in various bacteria.** Thirty strains from various genera of bacteria were tested for membrane-bound monoamine oxidase. Fifteen strains possessed monoamine oxidase activity (Table 1). In the family *Enterobacteriaceae*, *K. pneumoniae* strains IFO 3317, IFO 3319, and IFO 3321 and *Proteus inconstans* IFO 12930 showed high specific activity toward tyramine, octopamine, dopamine, and norepinephrine, whereas *Enterobacter cloacae* IFO 3320 and *Escherichia coli* K-12 showed low activities on these substrates. *K. aerogenes* W70 also showed high specific activity toward tyramine, octopamine, and dopamine, but very weak specific activity for norepinephrine. *Salmonella typhimurium* LT2 and *Serratia marcescens* IFO 3046 oxidized all these substrates except norepinephrine. The relative activities of the enzymes in these bacterial strains for the different substrates varied; for instance, the enzyme from *S. typhimurium* LT2 showed much higher activity for dopamine than for tyramine or octopamine. Besides the above substrates, the enzymes of *K. pneumoniae* IFO 3318 and *E. aerogenes* IFO 12010 also oxidized benzylamine and histamine, respectively. The monoamine oxidases in all bacteria tested had no activity towards β-phenethylamine, tryptamine, or *n*-amylamine. No monoamine oxidase activity was detected in *Citrobacter freundii* IFO 12681, *P. morganii* IFO 3168, *P. mirabilis* IFO 3849, *P. vulgaris* strains IFO 3045, IFO 3167, IFO 3851, and IFO 3988, *Erwinia amylovora* IFO 12687, or *Erwinia carotovora* IFO 3380.

Of the bacteria tested in families other than *Enterobacteriaceae*, only *Pseudomonas aeruginosa* IFO 3901, *M. luteus* IFO 12708 (IAM 1099), and *B. ammoniagenes* IAM 1641 possessed monoamine oxidase activity (Table 1). Like the monoamine oxidase in *K. aerogenes* W70, more than 90% of the activity of the enzyme in all these strains, except *M. luteus*, was recovered in the cell membrane fraction. In *M. luteus* IFO 12708, as much activity was found in the supernatant as in the membrane fraction (Table 2). When the cells were grown in the absence of tyramine, no monoamine oxidase activity for any monoamine compound was detected in any strain, except *M. luteus* IFO 12708 and *B. ammoniagenes* IAM 1641. The level of enzyme in the latter was not significantly affected by addition of tyramine to the medium, but the level in *M. luteus* IFO 12708 was increased about three to five times by addition of tyramine during cell growth. These results suggest that the monoamine oxidases in all these bacteria except *B. ammoniagenes* were induced by tyramine, and probably also by related compounds as shown with *K. aerogenes* W70 (11). Thus, the enzymes should be classified as tyramine oxidases, like those of *K. aerogenes* W70 and *M. luteus* IAM 1099 (11, 18). No membrane-bound monoamine oxidase activity was detected in *P. aeruginosa* IFO 3456, *Achromobacter liquidum* IFO 3084, *Bacillus subtilis* strains IFO 3021 and IFO 3022, *Corynebacterium acetophilum* A51, or *Mycobacterium smegmatis* IFO 3083. Strong diamine oxidase activities toward putrescine (224 nmol of ammonia formed/min per mg of protein) and cadaverine (18 nmol of ammonia formed/min per mg of protein) were found in *M.*

TABLE 1. *Distribution of membrane-bound monoamine oxidase in bacteria*[a]

| Strain | Sp act (nmol of ammonia formed/min per mg of protein) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Tyramine | Octopamine | Dopamine | Norepinephrine | Histamine | Tryptamine | Benzylamine |
| *K. aerogenes* W70 | 9.7 | 7.9 | 10 | 0.61 | 0 | 0 | 0 |
| *K. pneumoniae* IFO 3317 | 7.9 | 9.6 | 23 | 7.9 | 0 | 0 | 0 |
| *K. pneumoniae* IFO 3318 | 5.2 | 2.8 | 7.6 | 0.43 | 0 | 0 | 0.29 |
| *K. pneumoniae* IFO 3319 | 5.6 | 5.6 | 10 | 5.1 | 0 | 0 | 0 |
| *K. pneumoniae* IFO 3321 | 2.3 | 4.4 | 8.5 | 4.9 | 0 | 0 | 0 |
| *E. cloacae* IFO 3320 | 0.06 | 0 | 0.08 | 0.08 | 0 | 0 | 0 |
| *E. aerogenes* IFO 12010 | 11 | 19 | 29 | 4.9 | 8.4 | 0 | 0 |
| *E. coli* K-12 | 1.3 | 0.22 | 0.22 | 0.22 | 0 | 0 | 0 |
| *S. typhimurium* LT2 | 1.1 | 1.1 | 9.4 | 0 | 0 | 0 | 0 |
| *S. marcescens* IFO 3046 | 5.4 | 10 | 9.4 | 0 | 0 | 0 | 0 |
| *P. inconstans* IFO 12930[b] | 11 | 7.9 | 9.0 | 6.7 | 0 | 0 | 0 |
| *P. rettgeri* IFO 13501[b] | 3.3 | 6.0 | 6.7 | 6.4 | 0 | 0 | 0 |
| *P. aeruginosa* IFO 3901 | 17 | 0.55 | 8.9 | 0.25 | 0 | 0 | 0 |
| *B. ammoniagenes* IAM 1641[b] | 16 (17) | 7.2 (3.1) | 14 (22) | 10 (12) | 0 (0) | 27 (21) | 0 (0) |
| *M. luteus* IFO 12708 | 67 (21) | 0 (0) | 88 (16) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |

[a] Membrane fractions were prepared from cells grown in xylose-$NH_4Cl$ medium in the presence of tyramine as an inducer. Values in parentheses represent monoamine oxidase activities of cells grown in the absence of tyramine. Monoamine oxidase was assayed using an ammonia selective electrode.

[b] Mannitol was used in place of xylose as the sole source of carbon, because these strains were unable to use xylose.

TABLE 2. *Distribution of tyramine oxidase in cell fractions*[a]

| Strain | Fraction | Total protein (mg) | Tyramine oxidase activity (total U)[b] |
|---|---|---|---|
| *K. aerogenes* W70 | Supernatant | 94 | 12 |
| | Membrane | 17 | 165 |
| *M. luteus* IFO 12708 | Supernatant | 73 | 867 |
| | Membrane | 15 | 998 |

[a] Cells grown for 20 h in 200-ml culture in the presence of tyramine were harvested at 5°C. Cell fraction procedures are described in the text.

[b] One unit is defined as the amount utilizing 1 nmol of tyramine per min at 30°C.

*luteus* IFO 12708, but these diamine oxidases were synthesized in basal medium without tyramine. Polyamines such as spermine and spermidine were not oxidized by any bacteria tested.

## DISCUSSION

There are reports of the presence of monoamine oxidase in *M. luteus* (*S. lutea*) (18), *P. aeruginosa* (5), and *K. aerogenes* (12). Tyramine oxidase in *M. luteus* was purified from the supernatant of sonically disrupted cells grown on nutrient broth containing glucose (19). However, we showed that synthesis of tyramine oxidase in *M. luteus* was induced by tyramine and that about half the total activity was recovered in the cell membrane fraction. Crystalline preparations of the enzyme from *M. luteus* oxidized tyramine and dopamine (19). Tetrahydropapaveroline, which is a potent hypotensive agent, was produced from dopamine by using *M. luteus* tyramine oxidase (4). Monoamine oxidases from *P. aeruginosa* and *K. aerogenes* were synthesized in the presence of tyramine and found in a membrane-bound form (5, 11). More than 90% of the monoamine oxidases from both organisms was precipitated from crude extracts by ultracentrifugation at 105,000 × *g*, and attempts to solubilize them by treatments with various detergents were unsuccessful (5, 11). Since monoamine oxidase of *P. aeruginosa* is specific for tyramine and related compounds, this enzyme should be named tyramine oxidase, like the enzymes in *M. luteus* and *K. aerogenes*. Tyramine oxidase in *K. aerogenes* was subjected to catabolite repression by glucose in the presence of ammonium salts, and the repression was relieved when the cells were grown under conditions of nitrogen limitation (11). The enzyme is involved in regulation of arylsulfatase synthesis in *K. aerogenes* (12); arylsulfatase is regulated by sulfur compounds, and tyramine oxidase is induced by tyramine and related compounds (7). Immunological and electrophoretic techniques showed the presence of active and inactive forms of arylsulfatase in some strains of *Klebsiella*, *Enterobacter*, *Escherichia*, *Citrobacter*, *Salmonella*, *Serratia*, and *Proteus* (19). It has also been found that the formation of arylsulfatase protein, irrespective of whether it is in the active or inactive form, was regulated by sulfur compounds and tyramine (19). The present work shows that, except for the enzyme in *C. freundii* IFO 12681, the distribution of membrane-bound monoamine oxidase coincided with the distribution of arylsulfatase synthesized in the presence of tyramine (derepressing condition) in enteric bacteria reported previously (19). This finding supports the conclusion (8, 19) that arylsulfatase synthesis under conditions of derepression by tyramine is controlled by membrane-bound monoamine oxidase in some strains of enteric bacteria.

Further studies are required to determine whether the monoamine oxidases in *K. pneumoniae* IFO 3318 and *E. aerogenes* IFO 12010, which are induced by tyramine but which also oxidize benzylamine and histamine, are single entities. Tyramine is formed from tyrosine by tyrosine decarboxylase in various animal tissues and microorganisms and accumulates in putrefied animal tissues and ripe foods (3, 15). Thus, besides being of evolutionary interest, the distribution of monoamine oxidase in bacteria is important in relation to the decomposition of tyramine and related compounds.

## LITERATURE CITED

1. **Adachi, O., H. Yamada, and K. Ogata.** 1966. Purification and properties of putrescine oxidase of *Micrococcus rebens*. Agric. Biol. Chem. **30**:1202–1210.
2. **Baird-Parker, A. C.** 1974. Gram-positive cocci, p. 478–528. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. Williams & Wilkins Co., Baltimore.
3. **Boeker, E. A., and E. E. Snell.** 1972. Aminoacid decarboxylase, p. 217–253. *In* P. D. Boyer (ed.), The enzymes, vol. 6. Academic Press Inc., New York.
4. **Kumagai, H., H. Matsui, K. Ogata, H. Yamada, and H. Fukami.** 1968. Oxidation of dopamine by crystalline tyramine oxidase from *Sarcina lutea*. Food Sci. Kyoto Univ. **29**:69–71.
5. **Maki, Y., Y. Itsuno, M. Takeshita, S. Miyata, and S. Tanaka.** 1964. Studies on bacterial monoamine oxidase. Kumamoto Med. J. **17**:90–100.
6. **Meyerson, L. R., K. D. McMurtrey, and V. E. Davis.** 1978. A rapid and sensitive potentiometric assay for monoamine oxidase using an ammonia-selective electrode. Anal. Biochem. **86**:287–297.
7. **Murooka, Y., T. Adachi, H. Okamura, and T. Harada.** 1977. Genetic control of arylsulfatase synthesis in *Klebsiella aerogenes*. J. Bacteriol. **130**:74–81.
8. **Murooka, Y., T. Higashiura, and T. Harada.** 1978. Genetic mapping of tyramine oxidase and arylsulfatase genes and their regulation in intergeneric hybrids of enteric bacteria. J. Bacteriol. **136**:714–722.

9. **Murooka, Y., T. Yamada, S. Tanabe, and T. Harada.** 1977. Immunological study of the regulation of cellular arylsulfatase synthesis in *Klebsiella aerogenes*. J. Bacteriol. **132**:247-253.
10. **Nagatsu, T.** 1973. Biochemistry of catecholamines—the biochemical method. University of Tokyo Press, Tokyo.
11. **Okamura, H., Y. Murooka, and T. Harada.** 1976. Regulation of tyramine oxidase synthesis in *Klebsiella aerogenes*. J. Bacteriol. **127**:24-31.
12. **Okamura, H., Y. Murooka, and T. Harada.** 1977. Tyramine oxidase and regulation of arylsulfatase synthesis in *Klebsiella aerogenes*. J. Bacteriol. **129**:59-65.
13. **Peterson, G. L.** 1977. A simplification of the protein assay method of Lowry *et al.* which is more generally applicable. Anal. Biochem. **83**:346-356.
14. **Snyder, S. H., and E. D. Hendley.** 1971. Sensitive fluorometric and radiometric assays for monoamine oxidase and diamine oxidase. Methods Enzymol. **17B**:741-746.
15. **Spector, S., K. Melmou, W. Lovenberg, and A. Sjoerdsma.** 1963. The presence and distribution of tyramine in mammalian tissues. J. Pharmacol. Exp. Ther. **140**:229-235.
16. **Yamada, H., O. Adachi, and K. Ogata.** 1965. Amine oxidases of microorganisms. I. Formation of amine oxidase by fungi. Agric. Biol. Chem. **29**:117-123.
17. **Yamada, H., H. Kumagai, and T. Uwajima.** 1971. Tyramine oxidase (*Sarcina lutea*). Methods Enzymol. **17B**:722-726.
18. **Yamada, H., T. Uwajima, H. Kumagai, M. Watanabe, and K. Ogata.** 1967. Bacterial monoamine oxidases. I. Purification and crystallization of tyramine oxidase of *Sarcina lutea*. Agric. Biol. Chem. **31**:890-896.
19. **Yamada, T., Y. Murooka, and T. Harada.** 1978. Comparative immunological studies on arylsulfatase in bacteria of the family *Enterobacteriaceae*: occurrence of latent arylsulfatase protein regulated by sulfur compounds and tyramine. J. Bacteriol. **133**:536-541.