Inhibitory Effect of NaCl on Hog Kidney Mitochondrial Membrane-Bound Monoamine Oxidase: pH and Temperature Dependences

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ABSTRACT—For a further understanding of the inhibitory effect of NaCl on hog kidney mitochondrial monoamine oxidase (MAO), the activity for benzylamine as substrate was assayed spectrophotometrically in the absence and presence of NaCl for mitochondrial outer membrane preparations as well as whole mitochondria. The effect of CaCl₂ was also examined for comparison. The inhibition by NaCl but not CaCl₂ was strongly pH dependent. The pH dependence of the inhibitory effect of NaCl in phosphate buffer was parallel to the pH dependence of the MAO activity itself. The point at which the slope of the Arrhenius plot in the absence of NaCl decreases with increasing temperature was to be 32.3 °C at pH 7.0 and 30.4 °C at pH 7.5 in phosphate buffer, while the Arrhenius plot in the presence of NaCl exhibited discontinuities without change in the slope in small temperature ranges, 39.2 °C–40.0 °C and 33.0 °C–34.2 °C. It was estimated that the inhibitory effect of NaCl was due to a pH and temperature sensitive cooperative state change involving MAO protein and boundary lipids, while the effect of CaCl₂ could be induced by specific Ca²⁺ binding to acidic phospholipids.

Keywords: Hog kidney monoamine oxidase, Whole mitochondria, Mitochondrial outer membrane, Inhibitory effect of NaCl, Arrhenius plot

Monoamine oxidase (MAO, EC 1.4.3.4) is known as a mitochondrial outer membrane enzyme, which oxidatively deaminates neurotransmitters such as catecholamines and also exogeneous biogenic amines. At least two types of MAO (MAO-A and MAO-B) have been identified based on the differences in their substrate specificities and inhibitor sensitivities (1, 2) (for a more recent review, see Ref. 3). While there was convincing experimental support for the hypothesis that the two types of MAO were distinct enzyme proteins (4–7), it was suggested by several workers that they might be the same protein in different membrane lipid environments (8–10). Recently, cloning of the cDNAs for MAO-A and MAO-B demonstrated that the two forms of the enzyme share about 70% sequence identity and were encoded by different genes derived from the same ancestral gene. Full length cDNA clones for human (11–13), bovine (14) and rat (15) MAO-A and human (11, 13) and rat (16) MAO-B have been characterized. However, the in vivo presence of membranous MAO-A and MAO-B and the nature of their interaction with lipids are still unsolved biochemical questions (3, 17–22). Further investigations are needed.

In the process of purifying and reconstituting hog kidney mitochondrial MAO to determine if membranous MAO is present in vivo, we found that (NH₄)₂SO₄ and NaCl markedly inhibited the MAO activity at fairly low salt concentrations. We have reported the inhibitory effect of NaCl on MAO in hog kidney whole mitochondria (23). The inhibition was reversible and noncompetitive. The decrease in the inhibition of the Triton X-100 solubilized MAO suggested that the mitochondrial membrane was at least in part responsible for the inhibition: 70% inhibition at a NaCl concentration of 10⁻⁷ M, 85% at 2%, 92% at 3% and 95% at 5% for whole mitochondria in phosphate buffer, pH 7.0 at 38 °C; 53% at 1%, 67% at 2%, 72% at 3%, and 73% at 5% under the same conditions for Triton X-100 solubilized MAO (23).

Therefore, preceding the purification and reconstitution of MAO for the study on the artificial model system, we investigated the mitochondrial MAO from both enzymatic and spectroscopic aspects by using mitochondrial outer membrane preparations to which MAO is tightly bound. These investigations are expected to provide information about the functional state of hog kidney mitochondrial membrane-bound MAO.

To investigate the effect of NaCl on the spectral
characteristics of the mitochondrial outer membranes, we studied the effect of NaCl on the near infrared excited Fourier transform Raman spectra of hog kidney mitochondrial outer membrane preparations. We observed a spectral change in the phospholipid acyl chain CC stretching region, indicating that the presence of NaCl decreases the gauche character of the phospholipid acyl chains (24). The observed spectral change was different from that in an aqueous dispersion of phosphatidylcholine, for which it has been reported that monovalent ions such as Na⁺ do not appreciably affect the $I_{trans}/I_{gauche}$ ratio (25, 26). It was suggested that the presence of membrane proteins was required for this membrane condensation effect by NaCl (24). It was also estimated that the presence of NaCl might increase the amount of immobilized boundary lipid (24).

In the present study, to elucidate the cause of the inhibitory effect of NaCl on hog kidney mitochondrial MAO, measurements of the inhibitory effect of NaCl on mitochondrial outer membrane preparations were performed at various concentrations of NaCl and pH values. Furthermore, the inhibitory effects of 0.1 M NaCl were measured in phosphate buffer, pH 6.0, 6.5, 7.0 and 7.5 at 29°C and 8.0 at 38°C, for mitochondrial outer membrane preparations and whole mitochondria. To evaluate the thermal properties of the effect of NaCl, I measured the MAO activity in hog kidney mitochondrial outer membrane preparations for oxidative deamination of benzylamine as a substrate at various temperatures in the absence and presence of NaCl, and Arrhenius plots were made from the data. The results are reported and discussed in this article in terms of a cooperative change of state involving membrane proteins as well as phospholipid acyl chains.

**MATERIALS AND METHODS**

**Preparation of mitochondrial outer membranes**

Hog kidney mitochondrial outer membranes were prepared as described in our previous paper (24). Briefly, 50 g of hog kidney cortex was cut into small pieces and homogenized with a teflon homogenizer in 450 ml of 10 mM Na-K phosphate buffer, pH 7.5 containing 0.25 M sucrose (1 : 9 w/v). The homogenate was centrifuged at 600 × g for 10 min at 4°C, and the supernatant was recentrifuged at 5,000 × g for 10 min. The resulting precipitate (crude mitochondrial fraction) was suspended in 200 ml of the same buffer (1 : 4 w/v) and centrifuged at 5,000 × g for 10 min (mitochondrial fraction, 9–10 ml). One portion of the mitochondrial fraction was diluted to 1/100 in concentration by 0.25 M sucrose, and this was used to determine the protein concentration. The mitochondrial preparation was immediately used for the procedure described below.

The subfractionation of mitochondria by digitonin treatment was carried out essentially according to the method of Schnaitman et al. and Okamoto et al. (27–29). A 2% digitonin solution was prepared just prior to use by adding digitonin (biochemical use; Wako Chemicals Ltd., Osaka) to the warm 0.25 M sucrose solution in a boiling water bath. The cold 2% digitonin solution was added to the mitochondrial fraction in the ice bath with continuous stirring for 15 min so that the relative weight of digitonin to 10 mg mitochondrial protein was 1.2–1.5 mg. The suspension was diluted by 34 ml of 0.25 M sucrose and was further stirred for 1–2 min at 0°C. The diluted suspension was centrifuged at 9,500 × g for 10 min at 4°C. The first 9,500 × g pellet (inner membrane and matrix fractions) was suspended in 34 ml of 0.25 M sucrose, and the suspension was recentrifuged at 9,500 × g for 10 min. The resulting pale yellow supernatant was combined with the first 9,500 × g supernatant. The combined supernatant was centrifuged at 105,000 × g for 90 min at 4°C by a Beckman L5-65 ultracentrifuge with a Type 65 rotor (Beckman, Palo Alto, CA, USA).

The 105,000 × g pellet is the outer membrane fraction. The activity of MAO, which is a marker enzyme of the mitochondrial outer membrane, was assayed for the 105,000 × g pellet at 29°C in phosphate buffer, pH 7.5. The pellet was stored at −20°C just until used.

**Determination of protein concentration**

The concentration of mitochondrial protein was rapidly determined spectrophotometrically by the Coomassie blue G dye-binding assay (30) preceding the procedure of digitonin treatment. Bovine serum albumin was used for the standard protein.

**Standard assay of MAO activity**

The MAO activity was assayed spectrophotometrically on the basis of the method of Tabor et al. (31) by recording the absorbance at 250 nm due to benzaldehyde at 29°C in a standard assay system containing 0.1 ml of 0.1 M benzylamine hydrochloride as substrate, 1.0 ml of 0.2 M Na-K phosphate buffer adjusted to the appropriate pH by mixing 0.2 M Na₂HPO₄ and KH₂PO₄ solution at the same concentration and 0.5 ml of enzyme solution for the 3.0-ml final volume of reaction mixture in the quartz cell of 10-mm path length. The final concentrations of phosphate buffer and substrate were 0.067 M and 3.33 × 10⁻³ M, respectively. A blank cell was set up similarly in the reference side except for omission of substrate, i.e., 0.1 ml of H₂O was added instead of the same volume of substrate solution. The initial velocity $\Delta A_{250}$/min was regarded as the MAO activity.
Measurements of inhibitory effects of salts on MAO activity

Concentration and pH dependences: The MAO activity measurements were made by the procedure described above in the absence and the presence of NaCl in phosphate buffer at pH 6.5, 7.0 and 7.5 and in Tris-HCl buffer at pH 7.5 and 8.0. Similar experiments were performed with CaCl₂ for comparison, except that only Tris-HCl buffer was used for the calcium salt. The effect of MgCl₂ on whole mitochondria was also investigated for comparison to that of CaCl₂.

The percent relative MAO activity at each pH in the presence of salt to that in the absence of salt was determined at each concentration of 0-0.6 M salt at 29°C. In the assay system, the same concentration of salt as in the sample cell was contained in the reference cell.

pH dependence of inhibitory effect of NaCl at 29°C and 38°C: The percent relative MAO activity in phosphate buffer in the presence of 0.1 M NaCl to that in the absence of NaCl was determined at pH 6.0, 6.5, 7.0, 7.5 and 8.0 at 29°C and 38°C.

Temperature dependence of inhibitory effect of NaCl on MAO activity

The inhibitory effect of 0.1 M NaCl on hog kidney mitochondrial MAO activity was measured at various temperatures by the procedure described above. The percent relative value of the MAO activity in the presence of NaCl to that in the absence of NaCl was determined at each temperature from 28°C to 44°C in phosphate buffer, pH 7.0. Here, the percent inhibition means 100% minus the percent relative activity. The temperature of the reaction mixture in the reaction cuvette was controlled to be constant during recording of the absorbance at 250 nm. The temperature of the sample solution was monitored by a Chromel-Alumel thermocouple inserted into the cuvette in the cell holder thermostatically controlled by circulating water at a constant temperature.

Temperature dependence of MAO activity in the absence and presence of NaCl

The MAO activities in phosphate buffer at pH 7.0 and pH 7.5, respectively, were measured for mitochondrial outer membranes in the absence of NaCl at various temperatures; and Arrhenius plots were made by plotting the value of $-\log \Delta A_{250}/\text{min}$ against $1/T$. Here, $T$ is the absolute temperature. The same enzyme solution was used for one set of Arrhenius plots. The same kind of measurements were performed for the reaction mixture in phosphate buffer, pH 7.0 in the presence of 0.1 M NaCl, and an Arrhenius plot was made.

RESULTS

Concentration dependence of inhibitory effects of NaCl and CaCl₂ on MAO

Figure 1 shows the concentration dependence of the inhibitory effect of NaCl on the activity of hog kidney mitochondrial MAO for whole mitochondria and mitochondrial outer membranes. Figure 2 shows the results of the similar experiments for CaCl₂ in Tris-HCl buffer, pH 7.5 and pH 8.0. Stronger inhibition than in the case of NaCl was found. Since similar features of the inhibitions by salts were found for both whole mitochondria and outer membranes, it is estimated that incomplete vesicles are formed in the outer membrane preparations.

Fig. 1. Concentration dependence of inhibitory effect of NaCl on hog kidney MAO activity at 29°C for whole mitochondria (A) and outer membranes (B). All curves are from independent experiments. Each point was obtained from one set of measurement. a: Tris-HCl buffer, pH 8.0; b: Tris-HCl buffer, pH 7.5; c: phosphate buffer, pH 7.5; d: phosphate buffer, pH 7.0; e: phosphate buffer, pH 6.5. The enzyme solution was prepared by diluting whole mitochondria to 1/50 or 1/100 in concentration (v/v) with 0.25 M sucrose for panel A and by vigorously agitating the ultracentrifuge pellet with 10 ml of 0.25 M sucrose in the ultracentrifugation tube using a vortex mixer for panel B.
as shown in the electron microscopic photograph (Fig. 9 in Ref. 29). However, in the case of NaCl, the inhibitions are rather stronger for outer membranes than for whole mitochondria (Fig. 1), while such a difference between whole mitochondria and outer membranes is not observed in the case of CaCl₂ (Fig. 2). The inhibition by NaCl is strongly pH dependent over the entire concentration range. Increasing the pH decreases the inhibition in both phosphate and Tris-HCl buffer (Fig. 1), while that by CaCl₂ is almost pH independent (Fig. 2).

The inhibitory effect of MgCl₂ showed an unexpected pH dependency as shown in Fig. 3. The inhibitory effect of MgCl₂ is rather more similar to that of NaCl in Tris-HCl buffer, pH 7.5 and pH 8.0, than that of CaCl₂. The inhibition by MgCl₂ appears to be about twice as strong

Fig. 2. Concentration dependence of inhibitory effect of CaCl₂ on hog kidney MAO activity at 29°C for whole mitochondria (A) and outer membranes (B). All curves are from independent experiments. Each point was obtained from one set of measurement. a: Tris-HCl buffer, pH 8.0; b: Tris-HCl buffer, pH 7.5. The enzyme solution was prepared by diluting whole mitochondria to 1/100 in concentration (v/v) with 0.25 M sucrose for panel A and by vigorously agitating the ultracentrifuge pellet with 10 ml of 0.25 M sucrose in the ultracentrifugation tube using a vortex mixer for panel B.

Fig. 3. Concentration dependence of inhibitory effect of MgCl₂ on hog kidney MAO activity for whole mitochondria at 29°C. The enzyme solution was prepared by diluting whole mitochondria to 1/50 concentration (v/v) by 0.25 M sucrose. a: Tris-HCl buffer, pH 8.0; b: Tris-HCl buffer, pH 7.5. Each point was obtained from one set of measurement.

Fig. 4. Influence of the kind of phosphate buffer on inhibitory effects of NaCl (A) and KCl (B) on hog kidney mitochondrial MAO. ●: Na-K phosphate buffer, ○: Na phosphate buffer, ×: K phosphate buffer. Each point was obtained from one set of measurement.
as that by NaCl at the same concentration. As shown in Fig. 1, the inhibition by NaCl at pH 7.5 in phosphate buffer is stronger than that at the same pH in Tris-HCl buffer.

Figure 4A shows the concentration dependence of the inhibitory effects of NaCl on the MAO activity for the whole mitochondrial preparation in Na-K (identical to the curve d in Fig. 1A), K and Na phosphate buffers, pH 7.0. The results are independent of the kind of phosphate buffer. Figure 4B shows the results of the similar experiments for the inhibitory effects of KCl. The inhibitory effects of KCl were observed to be identical to those of NaCl in all types of phosphate buffer.

**pH dependence of inhibition by NaCl at 29°C and 38°C**

Figure 5A shows the pH dependence of the inhibition of mitochondrial MAO activity by 0.1 M NaCl in phosphate buffer for both whole mitochondria and outer membranes at 29°C and 38°C. These curves (a, b, c and d) are each the sigmoid type, and their midpoints are between 7.0 and 7.5. There is no difference in inhibition between whole mitochondria and outer membranes at 29°C. However, the decrease in the inhibition with increasing temperature exhibits the largest value, 20%, at pH 7.0 for the outer membranes. At pH 8.0, the relative activity increase by temperature elevation is negligible in both whole mitochondria and outer membranes.

Figure 5B shows the pH dependence of the MAO activity for mitochondrial outer membrane preparations in the absence and presence of NaCl at 29°C. Figure 5 A and B shows that the pH dependence of the MAO activity in the presence of 0.1 M NaCl is parallel to that in the absence of NaCl and that the dependence of the inhibitory effect of 0.1 M NaCl on MAO activity is also parallel to that of the MAO activity. Similar results were also obtained at 38°C and for whole mitochondria (data are not shown).

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**Fig. 5.** pH dependence of hog kidney MAO activity in the absence and presence of NaCl. A: pH dependence of the inhibitory effect of 0.1 M NaCl on hog kidney mitochondrial MAO activity in phosphate buffer for whole mitochondria and outer membranes. a (○) and b (●): whole mitochondria at 29°C and 38°C, respectively; c (▲) and d (▲): mitochondrial outer membranes at 29°C and 38°C, respectively. Curves a, b, c and d come from independent experiments. Each point was obtained from one set of measurement. Enzyme solution was prepared by diluting whole mitochondria to 1/100 concentration (v/v) by 0.25 M sucrose (a, b) and by vigorously agitating the ultracentrifuged pellet with 11 ml of 0.25 M sucrose using a vortex mixer (c, d). B: pH dependence of MAO activity in the absence (c) and presence (f) of 0.1 M NaCl at 29°C. They are the original data points of curve c (▲).

**Fig. 6.** Temperature dependence of inhibitory effect of 0.1 M NaCl on the MAO activity for hog kidney mitochondrial outer membranes in phosphate buffer, pH 7.0. The relative activity in % of the MAO activity in the presence of NaCl to that in the absence of NaCl is plotted as a function of temperature in °C. Points were obtained with two separate membrane preparations. Enzyme solution was prepared in a similar way as described in the captions for Figs. 1, 2 and 5 (8 ml of 0.25 M sucrose for the pellet).
Temperature dependence of inhibitory effect of NaCl on MAO

Figure 6 shows the temperature dependence of the inhibitory effect of 0.1 M NaCl on the MAO activity for hog kidney mitochondrial outer membranes in phosphate buffer, pH 7.0. The inhibition does not change from 28°C to 32°C. However, between 32°C to 38°C, the inhibition decreases by about 20%, and it increases again slightly in the range from 38°C to 44°C.

Arrhenius plot in the absence of NaCl

Figure 7 shows the Arrhenius plots in the absence of NaCl in phosphate buffer, pH 7.0 and pH 7.5. In both cases, as temperature is increased, the point at which the slope of the Arrhenius plot decreases is found at 32.3°C and 30.4°C at pH 7.0 and 7.5, respectively.

The breaks in the Arrhenius plots at 32.3°C (pH 7.0) and 30.4°C (pH 7.5) (Fig. 7) do not necessarily correspond to the thermotropic phase transition temperature in hog kidney mitochondrial outer membranes; i.e., two independent straight lines of the Arrhenius plot do not necessarily intersect at the phase transition temperature. In fact, the usual phase transition temperature of mammalian membranes is much lower than the physiological temperature and membranes have a fluid nature (32, 33). It appears that the distribution of lipids in the membranes is heterogeneous.

Arrhenius plot in the presence of NaCl

Figure 8 shows the Arrhenius plot in the presence of 0.1 M NaCl in phosphate buffer, pH 7.0. The presence of NaCl not only inhibited the MAO activity but also produced large changes in the features of the Arrhenius plot. As temperature is increased, a discontinuous decrease of MAO activity without any change in the slope is detected between 39.2°C and 40.0°C. The point at which the slope decreases as temperature increases is found at 43.6°C. The discontinuous decrease at 40°C with increas-
ing temperature in the Arrhenius plot in the presence of NaCl appears to be an abrupt decrease in the frequency factor in the equation for the rate constant. This feature appears to be similar to that observed in the sugar transport in Escherichia coli (34). The other discontinuity is detected between 33.0°C and 34.2°C. This discrepancy between the two parallel straight lines is somewhat small.

**DISCUSSION**

**Inhibitory effects of salts on hog kidney mitochondrial MAO**

It has been reported that the presence of negatively charged acidic phospholipids such as cardiolipin, phosphatidylinositol and phosphatidylserine is an important factor in the rebinding of solubilized MAO to the mitochondrial residues and for determining the properties of the enzyme (8, 19–22). Most biomembranes are negatively charged under physiological conditions due to the presence of acidic lipids. The resultant electrostatic surface potential concentrates counter ions and/or H⁺ on the membrane surface. The electrostatic surface phenomenon would play a role in the effects of NaCl and temperature.

However, the experimental result shown in Fig. 5 can not be interpreted in terms of electrostatic membrane surface properties such as membrane surface potential, surface charges and surface pK or pH shifts from bulk pK or pH values or induced membrane fluidity change. Since the pK value of natural acidic phospholipids is lower than 2 and it is lowered by the presence of NaCl, dissociable phosphate groups on the membrane surface are fully deprotonated and negatively charged even at pH 6.0 (35). Therefore, the observed effect of NaCl in Fig. 5 may be due to such a cooperative pH sensitive state transition, involving the MAO protein as well as membrane phospholipids as reported in the Raman spectroscopic study of erythrocyte membranes (36) rather than due to a pH dependence of membrane fluidity in the dissociation process of acidic phospholipid with increases in pH; i.e.,

**Fig. 8.** Arrhenius plots of the MAO activity for mitochondrial outer membranes in the presence of 0.1 M NaCl in phosphate buffer, pH 7.0. ○: Enzyme solution was prepared in a similar way to that described in the captions for Figs. 1, 2 and 5 (8 ml of 0.25 M sucrose for the pellet). ●: Separate set of experiments from that expressed by ○. The membrane pellet used was the preparation ultracentrifuged at the same time as that for the data points ○. Each point was obtained from one measurement of activity.
charge neutralization process with decreasing pH (35).

As shown in Fig. 5B, the overall behavior in the pH dependence of the MAO activity appears to be independent of the presence of NaCl, although NaCl exhibits MAO inhibition. The pH dependence of the inhibitory effect of NaCl itself (Fig. 5A) and that of the absolute MAO activity in the absence of NaCl (curve e) may be attributable to the same mechanism. The effect of NaCl may be explained by the following proposal: First, NaCl directly influenced the MAO conformation itself including the active site and then the conformational change in the MAO protein induced a concerted conformational change of the phospholipid acyl chains from gauche to trans (24) and in turn, the membrane fluidity change affected the MAO conformation and/or substrate diffusion into the membranes.

The cause of the inhibitory effect of CaCl₂ is apparently different from that of NaCl. It has been reported that divalent cations decreased the membrane fluidity by specific interactions with membrane acidic phospholipid (37, 38). However, the distinct difference in the features of the inhibitory effect on MAO between CaCl₂ and MgCl₂ can not be interpreted in terms of the difference in a simple membrane fluidity change. The effect specific to CaCl₂ observed in this study should be interpreted differently from the effect of MgCl₂; for example, in terms of an isothermal phase separation induced by specific binding of Ca²⁺ to acidic phospholipid, because it is known that Mg²⁺ does not induce an isothermal phase separation (38). The direct influence of pH and Ca²⁺ ions on the MAO conformation might play a role in the inhibitory effect. However, different from the case of NaCl inhibition, the specific interaction of Ca²⁺ with membrane phospholipid may contribute more predominantly to the inhibitory effect.

Functional state of hog kidney mitochondrial membrane-bound MAO which metabolizes benzylamine as substrate

It is now clarified that the substrate specificity of MAO isozymes depends on the concentration, the affinity and turnover rate of the substrate. It has been reported that 2-phenylethylamine, which is classified as a substrate of MAO-B, loses its substrate specificity for MAO-B in rat brain mitochondria at 125 and 1250 μM concentrations, while benzylamine (substrate of MAO-B) and serotonin (substrate of MAO-A) do not (39). Similar results for rat brain have been reported (40, 41).

The ratio of the content of MAO-A to that of MAO-B in hog kidney mitochondria is roughly estimated to be 2:3 from the height of the plateau in the double-sigmoidal inhibition curve for deprenyl (MAO-B inhibitor) using tyramine as a substrate (42). At this time, we have no experimental data on the benzylamine concentration dependence of substrate specificity. While we leave this subject for hog kidney mitochondrial MAO open to further investigation, it would be adequate in this article to discuss the experimental results on the basis of the hypothesis that benzylamine at 3.33 × 10⁻³ M concentration used in the present experiments acts as a MAO-B substrate. Considering the previous experimental results reported in Refs. 39 and 41 and the fairly high kₘ value of benzylamine in comparison to that of 2-phenylethylamine (in the case of hog kidney mitochondrial MAO, the kₘ value for benzylamine is estimated to be 290 μM at pH 7.4 (42); in the case of rat kidney homogenate, the kₘ value is 10 μM for 2-phenylethylamine and 260 μM for benzylamine at pH 8.0 (43)).

Taken together, the data from the present and previous studies (23, 24), suggest that the functional state of MAO-B in hog kidney mitochondria which metabolizes benzylamine as substrate is constituted by the MAO protein that binds tightly to the specific acidic phospholipids such as cardiolipine, phosphatidylserine and phosphatidylinositol (19, 20) to form a separate phase and that the discontinuity in the Arrhenius plot in the absence of NaCl may reflect a change of state in this phase rather than a phase transition in the bulk lipid bilayer. Presently, it is not clear whether the break in the Arrhenius plot is attributable to a cooperative conformation change of MAO-B protein through a boundary lipid-protein interaction or an intrinsic conformational change of the enzyme protein.

The present conclusion is in agreement with that derived from the investigation of the rebinding effect of the purified phospholipid to the lipid-depleted rat brain mitochondrial MAO (20). Huang and Faulkner estimated in their study (20) that phosphatidylcholine, a zwitterionic phospholipid, was responsible for the fluidity of the bulk membrane bilayer which in turn modulated the activity of MAO-A, not that of MAO-B. It has been reported that Triton X-100-sodium cholate solubilization of hog kidney mitochondrial MAO did not decrease appreciably the MAO activity (only a 9% decrease) (42). This result also suggests that the detergent treatment depleted little boundary lipid and that the benzylamine oxidation by MAO-B in hog kidney mitochondria was regulated only by boundary lipids and not by the bulk bilayer.

The experimental result of the Arrhenius plot in the presence of NaCl (Fig. 8) does not give us any direct information about the cause of the inhibitory effect of NaCl itself but it gives information about the cause of the temperature dependence of the inhibitory effect. Such a decrease in the activation energy at the transition temperature as observed in the absence of NaCl is not detected in
the presence of NaCl. This observation is consistent with the decrease in the inhibition with increasing temperature in the range of 32°C – 38°C (Fig. 6).

It appears that the present membrane system in the presence of NaCl probably underwent a temperature-induced phase separation characterized by two temperatures, which correspond to the observed discontinuities in the Arrhenius plot at 39.2°C - 40.0°C and at 33.0°C – 34.2°C (Fig. 8). The state of thermotropic phase separations, where lipids are partially in fluid and partially in solid states, may enhance the lateral compressibility of membrane lipids and facilitate the perpendicular penetration into membranes of the unprotonated form of benzylamine, which has been considered to be the main species that binds to the enzyme (44).

Further investigations will be needed using purified MAO-A and MAO-B reconstituted into the liposomes of specific phospholipids from both the enzymatic and spectroscopic points of view. Raman spectroscopic investigation could be applied to this study as predicted in our previous paper (24).

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