MOLECULAR PHARMACOLOGY, 18, 267-273

Lipid–Protein Interactions in the Multiple Forms of Monoamine Oxidases: Lipases as Probes Using Purified Intact Rat Brain Mitochondria

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Received February 28, 1980; Accepted April 21, 1980

SUMMARY

HUANG, R. H., AND R. FAULKNER. Lipid–protein interactions in the multiple forms of monoamine oxidases: Lipases as probes using purified intact rat brain mitochondria. *Mol. Pharmacol.* 18: 267–273 (1980).

It has been suggested that the functional states of the multiple forms of monoamine oxidases (MAO) are regulated by distinctly different lipid–protein interactions *in situ* in an intact rat brain mitochondrial preparation. Treatment of the enzymes with phospholipase A (L.A.) from porcine pancreas hydrolyzed almost all phospholipids and disintegrated the outer mitochondrial membrane with concomitant loss of both MAO-A- and MAO-B-type activities. Digestion with phospholipase C (L.C.) from *Clostridium welchii* hydrolyzed 90% of the phosphatidylcholine, one-third of the phosphatidylethanolamine, and one-half of the sphingomyelin. The specific action of L.C. on the polar zwitterionic head groups of one-half of the total membrane phospholipids resulted in a great reduction of the polar and ionic interactions at the hydrophilic surface which in turn increased the fluidity of the hydrophobic core of the membrane as probed with spin-labeled stearic acid I(12,3) with electron spin resonance (ESR). Treatment with phospholipase D (L.D.) removed one-half of the positively charged choline moiety of phosphatidylcholine. The surface charge of the membrane became more negative, the polarity was reduced, and the microviscosity of the hydrophobic core as probed by I(12,3) was altered. After L.C. or L.D. treatment, the bilayer structure of the outer mitochondrial membrane was retained. In the case of L.D. treatment, MAO-B enzyme was inactivated to a much greater extent (75%) than MAO-A enzyme (25%). In contrast, after L.C. treatment MAO-B enzyme was intact or activated whereas MAO-A was similarly inactivated as in the case of L.D. digestion. Surface charge and polar interactions provided by liposomes made of phosphatidylcholine, phosphatidylethanolamine, cardiolipin, or phosphatidic acid preferentially inhibited MAO-B activity. It was concluded that for MAO-A enzyme the active site was buried in the hydrocarbon core and the functional state was intimately modulated by the fluidity of the hydrophobic region proximal to the polar surface. For MAO-B enzyme the active site was situated closer to or partly in the peripheral hydrophilic region and its functional state was strongly dependent upon the ionic and polar characteristics of the surface layer of the membrane.

INTRODUCTION

The oxidative deamination of biogenic monoamines is believed to be accomplished by at least two prinicpal functionally different forms of monoamine oxidase (MAO,[1] EC 1.4.3.4) (1). In rat brain, serotonin is deaminated by MAO-A enzyme whereas phenylethylamine is deaminated by MAO-B enzyme (2). The multiple MAO enzymes which have been localized in the outer mitochondrial membrane are intrinsic membrane-bound flavoproteins (3). In general, vigorous procedures involving sonication, organic solvent extraction, and/or detergent treatment are required to solubilize the enzyme with concomitant preferential loss of the MAO-A-type characteristics (4). Furthermore, treatment of the MAO-active fractions with chaotropic agents to remove the lipid moiety resulted in the disappearance of the apparent multiplicity both in terms of electrophoretic mobility and substrate–inhibitor specificity (5). MAO-A and MAO-B *in situ* in the outer mitochondrial membrane may be the

This research was supported by Grant NS 14434 from the National Institute of Neurological and Communicative Disorders and Stroke.

[1] Abbreviations used: MAO, monoamine oxidase; L.C. (L.D.), phospholipase C (D).

0026-895X/80/050267-07$02.00/0

same enzyme protein and their specificities may depend upon the different nature of lipid–protein interactions. Recently it has been demonstrated that MAO-A enzyme is strongly modulated by the lipid–protein interactions occurring in the hydrocarbon core region proximal to the membrane hydrophilic surface whereas MAO-B appears to be quite independent of the fluidity of the bulk membrane lipid (6). In order to further investigate the molecular nature of the lipid–protein interactions specific to MAO-A or MAO-B in an intact membrane preparation, phospholipases were used as the probes to achieve chemically specific modifications of the membrane phospholipids.

EXPERIMENTAL PROCEDURES

Chemicals. The chemicals used in this work were obtained as follows: spin-labeled stearic acids I(12,3) [2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxyl] from SYVA Corporation, Palo Alto, California; β-[1-^{14}C]phenylethylamine hydrochloride and β5-hydroxy[^{14}C]tryptamine binoxalate from New England Nuclear; phospholipase A_2 (porcine pancreas), phospholipase C (*Clostridium welchii* type 1), and phospholipase D type 1 (cabbage) from Sigma; purified phospholipids (cardiolipin and phosphatidylcholine from bovine heart, and phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, and sphingomyelin from bovine brain) from Avanti; other chemicals were obtained from commercial sources.

Enzyme preparation. Pure intact rat brain mitochondria were prepared by purifying crude mitochondrial fractions from rat brain (male, Sprague–Dawley, weight 120–180 g) utilizing a discontinuous Ficoll density gradient (3 and 6%) procedure (7). The preparation was determined by electron microscopy to be intact and contained at least 90% mitochondria with minute synaptosomal contamination (6). The integrity of the outer mitochondrial membrane after lipase treatment was also monitored by electron microscopy.

Treatment of mitochondria with phospholipase A. Three volumes of intact rat brain mitochondrial suspension in an isotonic medium (0.25 M sucrose, 10 mM Tris, pH 7.4) was added to 1 vol of phospholipase A solution (porcine pancreas) at a ratio of 30 mg of mitochondrial protein/mg of phospholipase A protein. $CaCl_2$ was added to give a final concentration of 10 mM. The mixture was incubated for the desired time at 37°C. The reaction was stopped by the addition of 0.09 M EDTA to a final concentration of 18 mM. The phospholipase A-treated mitochondria were centrifuged at 40,000*g*. The pellet was then washed four times with a 1% solution of bovine serum albumin (fatty acid free) in 30 mM Tris, pH 7.4, containing 5 mM dithiothreitol, 1 mM EDTA, and 0.25 M sucrose. The pellet was then washed twice again with a 30 mM Tris solution, pH 7.4, containing 5 mM dithiothreitol, 1 mM EDTA, and 0.25 M sucrose. After washing, the pellet was divided into aliquots and stored at −80°C for subsequent monoamine oxidase assay and phospholipid analysis.

Treatment of mitochondria with phospholipase C. The mitochondria were incubated in the isotonic medium containing 10 mM $CaCl_2$ and phospholipase C from *Cl. welchii* at a concentration equivalent to one-tenth that of the mitochondrial protein. The mixture was incubated at 37°C for the desired time. The reaction was stopped by the addition of 10 mM EDTA. The phospholipase C-treated mitochondria were centrifuged at 40,000*g* and washed five times in 50 mM Tris buffer, pH 7.2, containing 5 mM dithiothreitol, 5 mM EDTA, and 0.25 M sucrose. The washed pellet was then divided into small aliquots and stored at −80°C.

Treatment of mitochondria with phospholipase D. Intact rat brain mitochondria suspended in an isotonic solution of 0.1 M acetate, pH 5.6, containing 0.25 M sucrose and 100 mM $CaCl_2$ were incubated with phospholipase D from cabbage at a concentration equivalent to 1/40th that of the mitochondrial protein. The incubation was carried out at room temperature for the desired time. The reaction was terminated by the addition of an equimolar amount of EDTA. The phospholipase D-treated pellet was similarly washed and stored as in the case of the phospholipase C-treated sample. In either phospholipase C or phospholipase D treatment, no significant loss of mitochondrial protein occurred.

In order to test for intrinsic proteolytic activities of the phospholipases A, C, and D, a 2.5% bovine serum albumin solution in the appropriate medium as described above was prepared. In all cases phospholipase was added at a concentration equivalent to one-tenth that of the albumin. The solution was allowed to incubate at 37°C for 15, 30, and 60 min. The reaction was stopped by the addition of trichloroacetic acid (final concentration 5%) and then centrifuged at 3000*g*. The optical density of the supernatant was measured at 280 nm. A control digest was also incubated, except phospholipase was added after the addition of trichloroacetic acid. No change was observed in the optical density of the digest incubated for 1 h. Therefore it was assumed that the phospholipases A, C, and D did not contain any proteolytic activity.

Analysis of phospholipids. Phospholipids were extracted from the mitochondrial pellet by the procedure of Folch *et al.* (8), five extractions of lower phase with theoretical Folch upper phase. The lower phase was evaporated to dryness in Evapomix. The lipid residue was dissolved in a minimum volume of 1:1 chloroform–methanol and spotted on silica gel G thin-layer chromatography plates. Purified phospholipids obtained from Avanti were used as standards.

Phospholipid class was separated by use of nonactivated Brinkman silica gel G plates without fluorescent indicator. The plates were developed according to the two-dimensional procedure of Horrocks (9) in solvent system 1, (chloroform–methanol–ammonia, 65:25:4) and solvent system 2 (chloroform–methanol–acetone–glacial acetic acid–H_2O, 75:15:30:15:7.5). After development plates were removed, allowed to dry, sprayed with 50% H_2SO_4, and charred at 180°C. Good separation was achieved for phosphatidylcholine, phosphatidylethanolamine, cardiolipin, phosphatidylinositol, phosphatidylserine, and sphingomyelin. The phospholipid spots were scraped from the plate and their phosphorus content was determined according to the procedure of Bartlett (10).

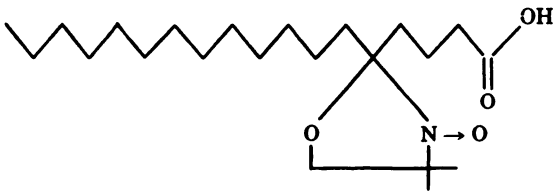
Preparation of liposome. A thin film of phospholipid in the bottom of a large test tube was formed by drying

the solution of purified phospholipid (Avanti) in 9:1 hexane–ethanol with a stream of N_2 gas. The liposome suspension was made to a final concentration of 2000 μg/ml by addition of N_2 gas-saturated 0.07 M phosphate buffer, pH 7.4, and subsequent sonication with a microprobe (Bronson 185 sonifier) for about 3 min at a setting of 3–6 in an ice-H_2O bath.

Assay of monoamine oxidase. The rat brain mitochondrial pellet obtained from two rat brains was suspended in a final volume of 5.0 ml at 0°C containing 0.25 M sucrose, 10 mM Tris, 0.5 mM Na^+-EDTA at pH 7.4. The protein concentration of the suspension varied from 1 to 1.5 mg/ml. The monoamine oxidase activity was determined in triplicate by incubating 0.10-ml aliquots of the mitochondrial suspension in 1 ml of 0.07 M potassium phosphate buffer, pH 7.4, with ^{14}C-labeled serotonin for 20 min and ^{14}C-labeled phenylethylamine for 10 min at 37°C. The final concentrations used for serotonin and phenylethylamine were 200 and 30 μM, respectively. After incubation, the reaction was stopped by rapid cooling in an ice bath and the product was separated by passing the reaction mixture through an Amberlite column (0.5 × 4 cm) according to the method of Robinson *et al.* (11). The product was collected and counted in 15 ml of Aquasol.

Protein measurement. Protein concentrations used in the calculations of specific activities were measured by the method of Lowry *et al.* (12) using bovine serum albumin as standard.

ESR measurement. The spin label I(12,3) was incorporated into the outer



I(12,3)

mitochondrial membrane by the procedure described previously (6). The sample after labeling was transformed immediately to an aqueous solution sample cell. Spectra were recorded using a Bruker ER 200 tt EPR spectrometer.

RESULTS

Effects of phospholipase A. Both MAO-A-type activity using serotonin as substrate and MAO-B-type activity using phenylethylamine as substrate are lost in parallel when the enzyme is incubated with phospholipase A at a concentration equivalent to 1/40th that of the mitochondrial protein. Inactivation of both enzymes to 75% is achieved after 10 min under the experimental conditions (Fig. 1). The loss of enzymic activities is parallel to the digestion of 90% of phosphatidylcholine and phosphatidylethanolamine and almost 70% of cardiolipin and phosphatidylserine (Table 1). The outer mitochondrial membrane is partially disintegrated as suggested by electron microscopy.

However, it is possible that the inactivation of the enzymes is not dependent upon the degradation of phospholipids, but rather upon the production of the end products, fatty acids and lysophosphatides, both of which may be inhibitors of the enzymes. Extensive washing of the treated mitochondria with bovine serum albumin (fatty acid free) removed the liberated fatty acids. The effect of lysolecithin on the specific activities of monoamine oxidase is summarized in Table 2. Lysolecithin at the concentration of 400 μg/mg of mitochondrial protein inhibits MAO-A by 7% and MAO-B by 25%. If it is assumed that all of the phospholipids bound to mitochondrial membrane are converted to their respective lyso derivatives and these lyso derivatives are not removed from the protein, then approximately less than 5 μg of lysophosphatides/mg of protein would be produced. From Table 1 it may be seen that an equivalent amount of lysolecithin will not produce the effects observed in Fig. 1.

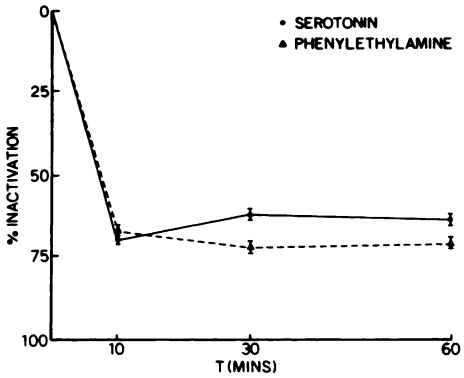


FIG. 1. *Time course of digestion of intact rat brain mitochondria with phospholipase A*

The values represent the average and standard deviations of three determinations. Intact rat brain mitochondria were incubated in phospholipase A medium at the ratio of 40:1 (40 mg of mitochondrial protein/mg of phospholipase A) at 37°C for the indicated times. The digested mitochondria were washed and assayed for enzymatic activity as described under Experimental Procedures. The specific activities of the control samples were 155.30 ± 6.02 and 52.60 ± 1.57 units using serotonin and phenylethylamine as substrates for monoamine oxidase, respectively.

TABLE 1

Digestion of intact rat brain mitochondria with phospholipase A

Intact rat brain mitochondria were treated with phospholipase A at a 20:1 ratio for 1 h and washed as described under Experimental Procedures. The values are based on phosphorus analysis of the lipids after Folch extraction and separation by thin-layer chromatography as described under Experimental Procedures and represent the average and standard deviations of two independent determinations.

	Content (μg mg^{-1})		Percentage digestion[a]
	Control	Treated	
Phospholipids	9.02 ± 0.50	2.15 ± 0.59	76.2
Phosphatidylcholine	3.73 ± 0.27	0.32 ± 0.11	91.4
Phosphatidylethanolamine	2.75 ± 0.29	0.37 ± 0.21	86.6
Cardiolipin	1.28 ± 0.09	0.39 ± 0.17	69.5
Phosphatidylinositol	0.47 ± 0.03	0.49 ± 0.18	—
Phosphatidylserine	0.51 ± 0.04	0.16 ± 0.05	68.6
Sphingomyelin	0.28 ± 0.05	0.41 ± 0.21	—

[a] The values of this column are calculated based on the average control and treated values.

TABLE 2

Effect of lysolecithin on specific activities of monoamine oxidase

Intact rat brain mitochondria preincubated with lysolecithin (Avanti) for 30 min at 37°C prior to assay for activity as described under Experimental Procedures. Percentage of inhibition is calculated based on the average of six independent determinations. The specific activities of the control samples are 111.87 ± 3.70 units using serotonin as the substrate and 19.07 ± 0.22 units using phenylethylamine as the substrate. Each unit represents 1 nmol of substrate oxidized/mg of protein per 20 min.

Lysolecithin (μg mg of protein^{-1})	Inhibition (%)	
	Serotonin	Phenylethylamine
100	6.32	9.37
400	6.73	24.83

Effects of phospholipase C. The time course of inactivation during incubation with phospholipase C is unlike that described for phospholipase A. In Fig. 2 and Table 3 it is shown that (a) MAO-A enzyme is inactivated within 30 min of digestion by 25%, no further inactivation occurs even if the treatment is prolonged to 90 min; (b) under the same experimental condition, MAO-B enzyme remains intact or slightly activated above the control value; (c) phospholipase C does not hydrolyze cardiolipin, phosphatidylinositol, or phosphatidylserine, whereas 90% of the phosphatidylcholine, one-third of the phosphatidylethanolamine, and one-half of the sphingomyelin are digested within 1 h. The specific action of phospholipase C on the zwitterionic phospholipids, which constitute 80% of the mitochondrial membrane phospholipids, results in a 50% reduction of the polar and ionic interactions at the hydrophilic surface of the membrane. The electron micrograph of the lipase C-treated mitochondria indicates that the gross bilayer morphology of the outer membrane is retained. ESR spectra using spin label I(12,3) as a structural probe for the membrane indicate changes in the parameters $T_\parallel$ and $T_\perp$. The fluidity in the hydrocarbon core proximal to the hydrophilic surface (three $-CH_2$ groups away from the polar head group) is increased due to the lipase C digestion (Fig. 3).

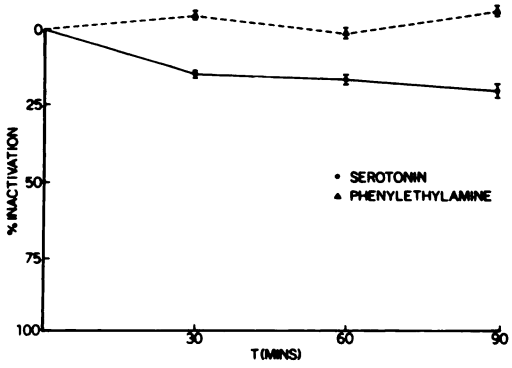


FIG. 2. *Time course of digestion of intact rat brain mitochondria with phospholipase C*

The values represent the average and standard deviations of these determinations. Intact rat brain mitochondria were incubated in phospholipase C medium at the ratio of 10:1 (10 mg of mitochondrial protein/mg of phospholipase C) at 37°C for the indicated times. The digested mitochondria were washed and assayed for enzymatic activity as described under Experimental Procedures. The specific activities of the control samples were 87.3 ± 3.1 and 58.9 ± 1.1 units using serotonin and phenylethylamine as substrates for monoamine oxidase, respectively.

TABLE 3

Digestion of intact rat brain mitochondria with phospholipase C

Intact rat brain mitochondria were treated with phospholipase C at a 10:1 ratio for 1 h and washed as described under Experimental Procedures. The values are based on phosphorus analysis of the lipids after Folch extraction and separation by thin-layer chromatography as described under Experimental Procedures and represent the average and standard deviations of two independent determinations.

	Content (μg mg^{-1})		Percentage digestion[a]
	Control	Treated	
Phospholipids	10.13 ± 0.35	5.84 ± 0.26	42.35
Phosphatidylcholine	4.24 ± 0.19	0.44 ± 0.08	89.62
Phosphatidylethanolamine	3.34 ± 0.06	2.18 ± 0.02	34.73
Cardiolipin	1.14 ± 0.07	1.70 ± 0.06	—
Phosphatidylinositol	0.47 ± 0.06	0.56 ± 0.05	—
Phosphatidylserine	0.56 ± 0.08	0.76 ± 0.06	—
Sphingomyelin	0.39 ± 0.007	0.21 ± 0.02	46.15

[a] The values of this column are calculated based on the average control and treated values.

Effects of phospholipase D. In Fig. 4 and Table 4, it is indicated that (a) the time course of inactivation during incubation with phospholipase D is different from that of phospholipase A; (b) MAO-A enzyme is inactivated to a maximum of 25% after 30 min digestion similarly to that observed in the case of phospholipase C treatment; (c) phospholipase D does not hydrolyze cardiolipin, phosphatidylinositol, phosphatidylserine, or sphingomyelin, whereas one-half of the phosphatidylcholine and 8% of the phosphatidylethanolamine are digested within 1 h. More than one-half of the membrane surface is covered with negatively charged phospholipids due to the removal of the positively charged choline or ethanolamine moieties as the consequence of the phospholipase D treatment. The effect on the physical structure of the outer mitochondrial membrane as judged by electron microscopy or ESR is similar to that described for the phospholipase C treatment.

Effect of charge and dipolar interactions. Charge and dipolar interactions at the membrane surface are introduced by incubating the intact rat brain mitochondria with varying amounts of liposomes made of purified phosphatidylcholine, phosphatidylethanolamine, cardiolipin, or phosphatidic acid for half an hour at 37°C before the MAO assay (Fig. 5). Under the experimental conditions, the liposomal phospholipids do not incorporate into the intact mitochondria membrane. However, appreciable incorporation does occur if the mitochondria preparation is in a lipid-depleted state (manuscript in preparation). In Fig. 5, it is shown that (a) the surface charge and dipolar perturbations on the MAO-membrane system have little or no effect on the activity of MAO-A enzyme, (b) at a concentration of 2.5 mg of liposomal phospholipid/mg of mitochondrial protein, MAO-B activity is inhibited by 30% in the presence of liposomes made of phosphatidylcholine, phosphatidylethanolamine, or cardiolipin whereas the same level of phosphatidic acid inactivates MAO-B to half of its original value.

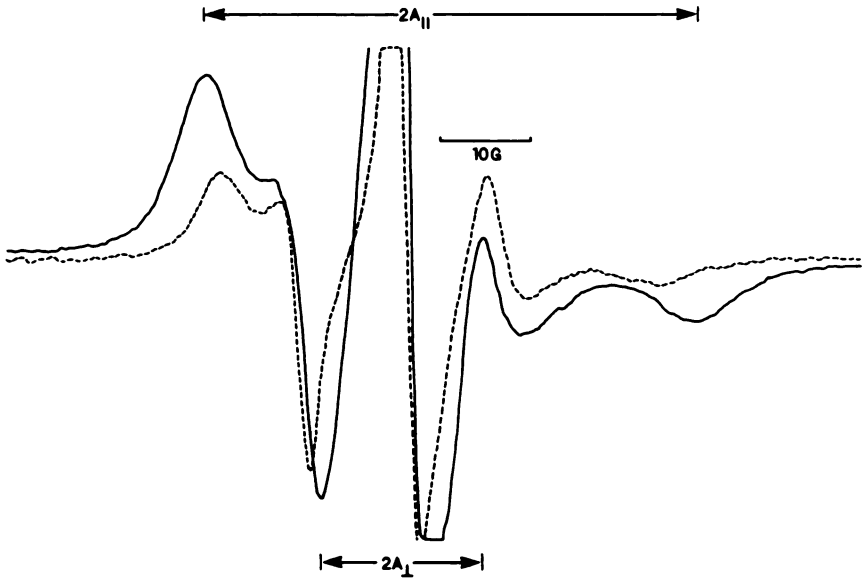


FIG. 3. *Spectra of I(12,3) incorporated into mitochondrial membranes*
Solid line: intact rat brain mitochondria. Dotted line: intact rat brain mitochondria treated with either phospholipase C or phospholipase D.

DISCUSSION

The multiple forms of monoamine oxidase may play an important role in the regulation of serotonin and catecholamines in the brain. Ample pharmacological and biochemical evidence has led to the hypothesis that lipid–protein interactions are crucial to the characteristic substrate–inhibitor sensitivities of the multiple enzymes (5). Recently, studies of the functional state of the membrane-bound MAO-A and MAO-B and the physical properties of the membrane lipids using ESR spin labels demonstrate that the fluidity at the hydrophobic region, three methylene groups proximal to the surface, effectively modulates the activity of MAO-A enzyme but not that of MAO-B (6).

Our present study used phospholipases as the probes to chemically modify the membrane phospholipids, which in turn, perturbed the physical state of the lipid-bilayer domain in a specific and definitive manner.

In the case of phospholipase A treatment, enzymic activities characterizing MAO-A and MAO-B were lost, in parallel to the partial disintegration of the bilayer membrane structure (Fig. 1 and Table 1). It was also shown that the end products, fatty acids and lyso derivatives, were not responsible for the inactivation of the MAO enzymes (Table 2). These data indicated both MAO-A and MAO-B were integral membrane proteins that required, to some degree, an intact gross bilayer structure for the enzymic function.

Phospholipase C or phospholipase D hydrolyzed mainly phosphatidylcholine and phosphatidylethanolamine but little or none of the acidic phospholipids (Tables 3, 4). Phosphatidylcholine and phosphatidylethanolamine were found to constitute about 80% of the phospho-

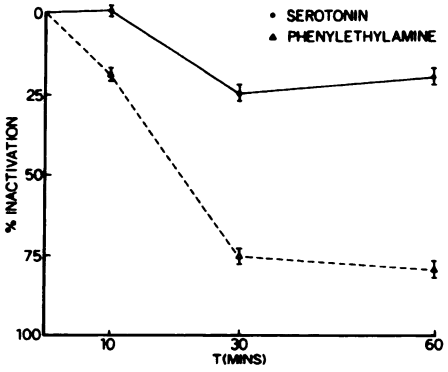


FIG. 4. *Time course of digestion of intact rat brain mitochondria with phospholipase D*
The values represent the average and standard deviations of three determinations. Intact rat brain mitochondria were incubated in phospholipase D medium at the ratio of 40:1 (40 mg of mitochondrial protein/mg of phospholipase D) at 22°C for the indicated times. The digested mitochondria were washed and assayed for enzymatic activity as described under Experimental Procedures. The specific activities of the control samples were 83.80 ± 1.36 and 22.10 ± 0.26 units using serotonin and phenylethylamine as substrates for monoamine oxidase, respectively.

TABLE 4

Digestion of intact rat brain mitochondria with phospholipase D

Intact rat brain mitochondria were treated with phospholipase D at a 40:1 ratio for 1 h and washed as described under Experimental Procedures. The values are based on phosphorus analysis of the lipids after Folch extraction and separation by thin-layer chromatography as described under Experimental Procedures and represent the average and standard deviations of four independent determinations.

	Content (μg mg^{-1})		Percentage digestion[a]
	Control	Treated	
Phospholipids	7.92 ± 0.07	6.53 ± 0.21	17.55
Phosphatidylcholine	3.23 ± 0.05	1.53 ± 0.007	52.63
Phosphatidylethanolamine	2.16 ± 0.04	1.98 ± 0.12	8.33
Cardiolipin	1.43 ± 0.08	1.81 ± 0.04	—
Phosphatidylinositol	0.48 ± 0.00	0.52 ± 0.04	—
Phosphatidylserine	0.46 ± 0.007	0.48 ± 0.007	—
Sphingomyelin	0.16 ± 0.007	0.21 ± 0.007	—

[a] The values of this column are calculated based on the average control and treated values.

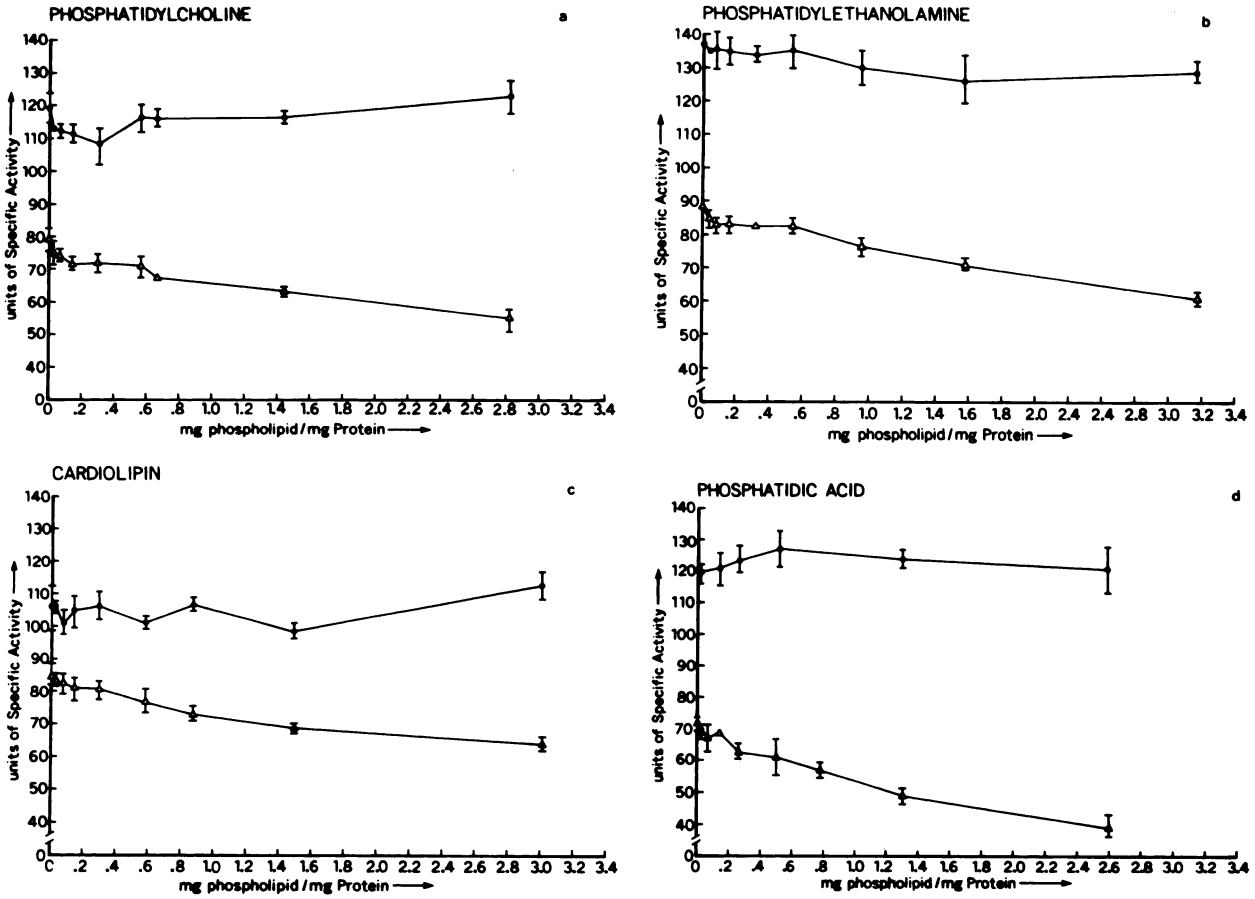


FIG. 5. *Effect of the surface polar and ionic interactions provided by liposomes on the specific activities of monoamine oxidase A and monoamine oxidase B in situ*

(a) In the presence of liposomes made of purified phosphatidylcholine. (b) In the presence of liposomes made of purified phosphatidylethanolamine. (c) In the presence of liposomes made of purified cardiolipin. (d) In the presence of liposomes made of purified phosphatidic acid.

lipids in the purified rat brain mitochondria (Tables 1, 3, 4). The gross bilayer structure of the outer mitochondrial membrane was retained after L.C. or L.D. digestion. After the L.C. treatment, the polar and ionic interactions in the hydrophilic surface region were reduced greatly. In the case of L.D. digestion, the polar interactions decreased but the negatively charged ionic interactions were significantly enhanced. MAO-B enzyme was inactivated to a great extent (75%) after L.D. treatment; in contrast to the case of L.C. treatment, MAO-B enzyme was intact or slightly activated.

In the hydrophobic core region, the microviscosity as probed by spin label I(12,3) increased to the same extent whereas MAO-A enzyme was also similarly inactivated (25%) in both L.C. and L.D. cases (Figs. 2–4). We found, previously, that the substrate and inhibitor sensitivities of MAO-A enzyme might be regulated in part by the fluidity in this particular region as reported by I(12,3) (6).

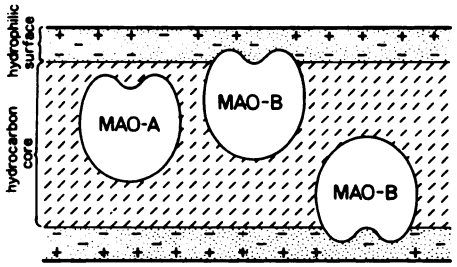


FIG. 6. *The hypothetical gross organization of monoamine oxidase A and monoamine oxidase B in the outer mitochondrial membrane*

When the charge and dipolar interactions were introduced to the membrane surface using liposomes made of purified phosphatidylcholine, phosphatidylethanolamine, cardiolipin, or phosphatidic acid, only MAO-B enzyme was selectively inhibited in all cases (Fig. 5).

These data strongly suggest that uniquely different lipid–protein interactions which may be the root of the functional differentiation do exist for the MAO-A and MAO-B enzymes. MAO-A is influenced intimately by the fluidity in the hydrocarbon core whereas MAO-B is extremely sensitive to the charge and ionic interactions in the hydrophilic surface of the membrane bilayer. Immunochemical studies by Russell *et al.* suggested an asymmetric localization of MAO-A and MAO-B enzymes in the membrane (13). It has been known for many other membrane enzyme systems, that an intimate relationship exists between the function and topology (14). We conclude that the active site of the MAO-A enzyme may be more deeply buried in the hydrocarbon core and the functional state is directly modulated by the fluidity of the hydrophobic region proximal to the polar surface.

For MAO-B enzyme, the active site may be situated close to or inside the peripheral hydrophilic region, and its functional state is strongly dependent upon the ionic and polar characteristics of the surface polar head layer of the membrane (Fig. 6). Our present data demonstrate a distinct nature of topology and lipid–protein interacting forces in the MAO-A and MAO-B *in situ*. Studies on the chemical nature of the phospholipid requirement specific for the MAO-A and MAO-B are currently underway.

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