luminal phosphohydrolase activity and not the Glc-6-P carrier To investigate whether the enzyme responds to the decrease of membrane PE or of PI, microsomes were incubated with NS-TP and SUV composed of PC and PE (3:1 mol/mol). In contrast to the above studies, this resulted in an increased PC content and decreased PI content, but no change in membrane PE (Table I). Very little Glc-6-P phosphohydrolase inhibition (5%) was produced by these incubations. The role of PI was further investigated with PI-specific phospholipase C. Incubation of microsomes with phospholipase C caused hydrolysis of over 55% of the PI, no change in other membrane lipids, and no change in phosphohydrolase activity.

We conclude that microsomal Glc-6-P phosphohydrolase activity is decreased by 26–33% when PE is depleted by 24–28%. Alterations in PI and PC contents of the membrane or in PC fatty acid unsaturation produce little or no change in enzyme activity.

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REFERENCES

1. Sandermann, H., Jr. 1978. Regulation of membrane enzymes by lipids. *Biochim. Biophys. Acta.* 515:209–237.
2. Nilsson, O. S., W. J. Arion, J. W. DePierre, G. Dallner, and L. Ernster. 1978. Evidence for the involvement of a glucose-6-phosphate carrier in microsomal glucose-6-phosphatase activity. *Eur. J. Biochem.* 82:627–634.
3. Zilversmit, D. B., and M. E. Hughes. 1976. Phospholipid exchange between membranes. *In* Methods in Membrane Biology. E. D . Korn, editor. Plenum Publishing Corp., New York. 7:211–259.
4. Crain, R. C., and D. B. Zilversmit. 1980. Net transfer of phospholipid by the nonspecific phospholipid transfer protein from bovine liver. *Biochim. Biophys. Acta.* 620:37–48.
5. Zakim, D., and D. A. Vessey. 1973. Techniques for the characterization of UDP-glucuronyltransferase, glucose-6-phosphatase, and other tightly-bound microsomal enzymes. *In* Methods of Biochemical Analysis. D. Glick, editor. John Wiley & Sons, New York. 21:1–37.

LIPID-PROTEIN INTERACTIONS IN THE RAT BRAIN MITOCHONDRIAL MULTIPLE MONOAMINE OXIDASE

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The oxidative deamination of biogenic monoamines is accomplished by two functionally different forms of membranous monoamine oxidase (MAO), EC1.4.3.4) (1): MAO-*A* type preferentially deaminates serotonin and is more sensitive to inhibition by clorgyline. MAO-*B* type deaminates phenylethylamine and is more sensitive to inhibition by deprenyl. Biochemical and immunological studies strongly suggest that the *A*- and *B*-type activities which depend upon the different natures of lipid-protein interactions *in situ* in the membrane may reside within the same enzymic protein (2, 3).

RESULTS AND DISCUSSION

The phospholipid requirement and the lipid-protein interactions for the multiple forms of MAO were investigated by rebinding the purified phospholipid to a lipid-depleted brain mitochondrial preparation. It was found that phosphatidylinositol uniquely stimulated the *A*-type activity to 80% over that in the original intact mitochondria (Fig. 1). Other negatively charged phospholipids, although not as potent, could fully or partially reactivate the *A*- or *B*-type activity. Phosphatidylcholine, a zwitterionic phospholipid, reconstituted 70% of the *A*-type activity but did not influence the *B*-type. Phosphatidylethanolamine had no effect on either type. More importantly, efficiency-gradient analyses indicated a distinct nature in the molecular mechanism of lipid-protein interactions for the negatively charged and the zwitterionic phospholipids (Fig. 2). The potencies of the negatively charged phospholipid, phosphatidylserine, cardiolipin, or phosphatidylinositol decreased sharply with increasing lipid molecules. No further stimulation could be detected when the lipid:protein ratio reached ~ 30 mol of negatively charged phospholipid/100,000 daltons of membrane protein. The negatively charged phospholipid appeared to bind directly to the monoamine oxidase protein boundary with a high affinity. In contrast, the potency of activating MAO-*A* remained constant up to the first 150 mol of phosphatidylcholine. Phosphatidylcholine which interacted with the enzyme with a lower affinity and higher capacity might reassociate as the membrane fluid bilayer.

It can be speculated that the properties of the negatively-charged phospholipid associated with the enzymic protein at the lipid-protein interface may regulate, rapidly, the conformation of the active site, which in turn influences the nature of substrate and inhibitor specificity

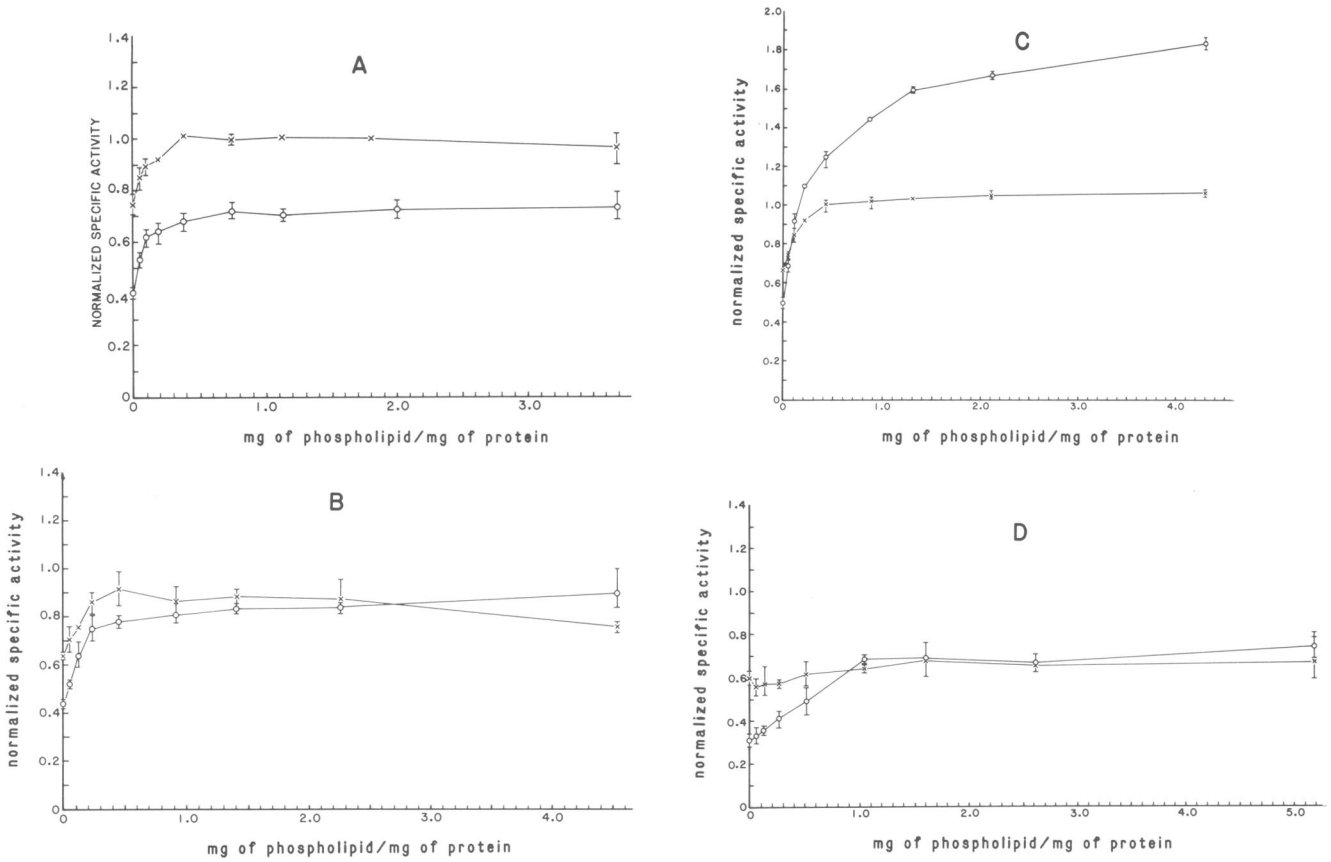

FIGURE 1 Effect of reconstitution using purified phospholipids on the activities of the delipidated monoamine oxidase. ○, serotonin as substrate; ×, phenylethylamine as substrate. *A*, phosphatidylserine from bovine brain; *B*, cardiolipin from bovine heart; *C*, phosphatidylinositol from bovine liver; *D*, phosphatidylcholine from bovine heart. Highly purified intact rat brain mitochondria (IBM) were prepared as described previously (4). IBM (20 mg protein) was digested with phospholipase A_2 from porcine pancreas (1 mg protein, Sigma Chemical Co., St. Louis, MO) in an isotonic medium (0.25 M sucrose, 10 mM Tris, pH 7.4, 10 mM $CaCl_2$) for one h at 37°C and followed by extensive washings using medium containing fatty-acid-free bovine serum albumin as described previously (5). The delipidated IBM retained ~30% of the phospholipids and 50% of the MAO-*A* and the MAO-*B* activities. The method of reconstitution described by Eytan et al. (6) was employed using liposomes made of purified phospholipids (Avanti Biochemicals Inc., Birmingham, AL). Varying amounts of liposomes were added to the delipidated IBM (0.5-1.0 mg, 0.07 M phosphate buffer, pH 7.4). The mixture was incubated at 37°C for 20 min prior to the monoamine oxidase assay (in triplicate) using ^{14}C-labeled serotonin and ^{14}C-labeled phenylethylamine (New England Nuclear, Boston, MA) as the substrate for the MAO-*A* and MAO-*B*, respectively (5). In the presence of the negatively-charged phospholipids, $CaCl_2$ (0.1 M) was added to the reaction-mixture after incubation to prevent interference of product separation using an Amberlite column during the MAO assaying procedure. Protein concentrations were determined by the methods of Lowry et al. (7). The normalized specific activity represents the ratio of MAO activity of the reconstituted to that of the intact mitochondria.

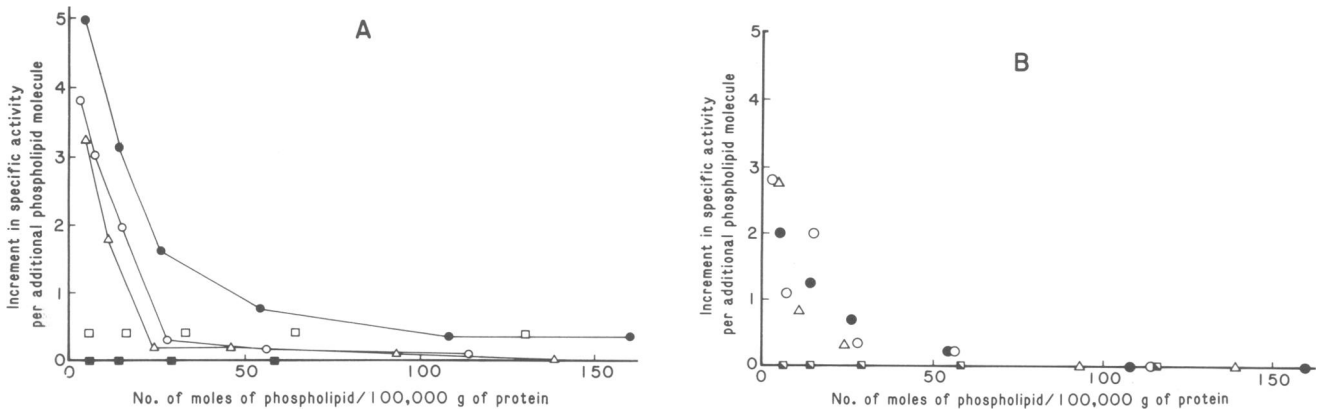

FIGURE 2 Efficiency gradient of phospholipids in the reconstitution of monoamine oxidase activity. Increments in the specific activity per additional phospholipid molecules vs. the total moles of phospholipids present per 100,000 daltons of mitochondrial membrane protein. The values are calculated based on the data shown in Fig. 1. The curves in Fig. 2 represent the first derivatives of those in Fig. 1. ●, phosphatidylinositol; ○, cardiolipin; Δ, phosphatidylserine; □, phosphatidylcholine; ■, phosphatidylethanolamine. *A*, serotonin as substrate; *B*, phenylethylamine as substrate.

of MAO. In addition, the dynamic redistribution of phosphatidylcholine might further affect the functional level of the MAO-*A* type activity.

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REFERENCES

1. Jain, M. 1977. Examination of multiple forms. *Life. Sci.* 20:1925–1933.
2. Dennick, R. G., and R. J. Mayer. 1977. Purification and immunochemical characterization of monoamine oxidase from rat and human liver. *Biochem. J.* 161:167–174.
3. Houslay, M. D., and K. F. Tipton. 1973. The nature of the electrophoretically separable multiple forms of rat liver monoamine oxidase. *Biochem. J.* 135:173–186.
4. Huang, R. H., and S. Eiduson. 1977. Significance of multiple forms of brain monamine oxidase *in situ* as probed by electron spin resonance. *J. Biol. Chem.* 252:284–290.
5. Huang, R. H., and R. Faulkner. 1980. Lipid-protein interactions in the multiple forms of monoamine oxidase: lipases as probes using purified intact rat brain mitochondria. *Molec. Pharmacol.* 18:267–273.
6. Eytan, G. D., M. J. Matheson, and E. Racker. 1976. Incorporation of mitochondrial membrane proteins into liposomes containing acidic phospholipids. *J. Biol. Chem.* 251:6831–6837.
7. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265–275.

^{31}P NMR STUDIES OF ORIENTED MULTILAYERS FORMED FROM ISOLATED SARCOPLASMIC RETICULUM AND RECONSTITUTED SARCOPLASMIC RETICULUM

EVIDENCE THAT "BOUNDARY-LAYER" PHOSPHOLIPID IS NOT IMMOBILIZED

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Sarcoplasmic reticulum is one of the most intensively studied membrane systems. As isolated in highly purified form, it is capable of energized calcium uptake and has a relatively simple composition. The major protein constituent (>90%) is the calcium pump protein (1, 2), which has been dissociated from the sarcoplasmic reticulum membrane and reconstituted to form functional membrane vesicles (3, 4). Such membranes of defined lipid content make possible detailed studies aimed at correlating membrane composition with structure and structure with function (4–7).

^{31}P NMR has been used previously to study the motion of the polar head group region of model phospholipid membranes (8–10). Oriented multilamellar systems have proved particularly useful for this purpose (8, 9, 11). The angular dependence of the position of the ^{31}P NMR signal from oriented membranes can be used to calculate the phosphorus chemical shift anistropy and the direction of the symmetry axis for the motion of the phosphate group. The angular dependence of the width of the ^{31}P NMR signal can be used to calculate the dipolar interaction between the phosphorus nucleus and the protons on the two adjacent methylene groups, and the direction of the symmetry axis for the motion of these two groups.

RESULTS AND DISCUSSION

Fig. 1 shows the angular dependence of the ^{31}P NMR signal from oriented sarcoplasmic reticulum membranes. Similar spectra were obtained from oriented reconstituted sarcoplasmic reticulum membranes with lipid-to-protein ratios ranging from 42:1 to 110:1 and from oriented bilayer membranes formed from sarcoplasmic reticulum phospholipids (12). We used the dependence of the ^{31}P NMR spectra on the alignment of the membranes with respect to the magnetic field to draw two conclusions about the motion of the phospholipid molecules that contribute to the observed spectra. First, the phosphate group and the two adjacent methylene groups are able to rotate rapidly (i.e., faster than 10^{-5} s) around the normal to the plane of the membrane. Second, the restricted internal motion of the phosphate group and the glycerol CH_2OP group is very similar to that found in liposomes formed from sarcoplasmic reticulum phospholipids. Calibration experiments showed that all (100 ± 7%) of the