THE LIPID DEPENDENCE OF GLUCOSE-6-PHOSPHATE PHOSPHOHYDROLASE

RICHARD C. CRAIN
Biochemistry and Biophysics Section, The University of Connecticut, Storrs, Connecticut 06268 U.S.A.

DONALD B. ZILVERSMIT
Division of Nutritional Sciences and Section of Biochemistry, Molecular and Cell Biology, Division of Biological Sciences, Cornell University, Ithaca, New York 14853 U.S.A.

The functional dependence of membrane enzyme activities on the composition of the lipid bilayers with which they are associated has been studied by many techniques (1). Unfortunately, many of these techniques may alter the structure of the membrane-bound enzymes or, in the case of chemical modifications of membrane lipids or phospholipase treatment, the introduction of by-products may affect enzyme activities. Recent advances in the isolation and application of phospholipid transfer proteins permit the manipulation of membrane lipid composition in vitro under mild conditions that would not be expected to alter the structure of functional membrane proteins. One of the many "lipid-dependent" membrane-bound enzymes that has been studied extensively is glucose-6-phosphatase, a microsomal complex composed of a glucose-6-phosphate (Glc-6-P) carrier and of a phosphohydrolase located on the luminal membrane surface (2). In this communication we present evidence that Glc-6-P phosphohydrolase activity is altered by some modifications of the microsomal membrane lipid composition but not by others.

RESULTS AND DISCUSSION

Modification of membrane phospholipid composition was accomplished by incubation of small unilamellar vesicles (SUV) of varied phospholipid composition with microsomes and either phosphatidylcholine-specific transfer protein (PC-TP) or nonspecific lipid transfer protein (NS-TP). Incubations of microsomes with PC-vesicles and PC-TP altered the distribution of fatty acids in membrane PC without altering the phospholipid head group composition of the microsomal membrane (Table I). When 43% of the PC was replaced by dipalmitoyl PC, Glc-6-P phosphohydrolase activity was inhibited by 10%, which suggests that a substantial decrease in PC fatty acid unsaturation is not crucial for enzyme activity. In contrast, incubation of microsomes with PC-vesicles and NS-TP altered the phospholipid head group composition of the membranes as well as the fatty acid distribution of the membrane PC. Incubation of microsomes with SUV containing principally dipalmitoyl PC resulted in a small increase in membrane PC and a substantial decrease in phosphatidylethanolamine (PE), and phosphatidylinositol (PI) content as well as enzyme activity. Incubations of SUV containing principally rat liver PC caused a large increase in membrane PC with decreases in PE and PI content (Table I). Accompanying these changes in phospholipid composition was a 26–33% inhibition of Glc-6-P phosphohydrolase activity (Table I). The failure of control microsomes incubated with NS-TP or SUV alone to show either an altered lipid composition or enzyme inhibition suggests that the alterations in enzyme activity result from the altered lipid compositions. Glc-6-P phosphohydrolase activity in the modified microsomes was inhibited to the same extent when assayed in the presence and in the absence of taurocholate (data not shown). The latter removes the microsomal permeability barrier to Glc-6-P. This finding demonstrates that phospholipid replacement affects the

TABLE I
EFFECT OF THE CHANGE IN MICROSOMAL PHOSPHOLIPID COMPOSITION ON GLUCOSE-6-PHOSPHATE PHOSPHOHYDROLASE ACTIVITY

PL-TP	SUV	Microsomal phospholipid composition (% total phospholipid) DPPC	Total PC	PE	PI	Glc-6-Pase (% of control)
—	—	—	58.4	24.2	12.0	—
—	A	0.5	57.8	24.2	12.5	100
PC-TP	A	25.0	57.1	24.9	12.5	90 ± 6
NS-TP	A	19.1	65.2	20.4	8.9	67 ± 6
—	B	—	58.8	24.0	11.7	100
PC-TP	B	—	59.1	23.8	11.6	97 ± 5
NS-TP	B	—	71.5	15.2	7.8	74 ± 5
—	C	—	58.0	24.8	11.7	100
PC-TP	C	—	58.0	25.1	11.3	103 ± 7
NS-TP	C	—	61.4	25.1	8.1	95 ± 5

Microsomes (5 mg protein) were incubated for 60 min at 37°C with 0 or 1.25 units transfer protein (3) and 0 or 5000 nmol phospholipid in the form of SUV composed of: (A) DPPC/ rat liver PC/ diPG/ cholesterol (7.5/2.5/1/1.5; mol/mol), (B) rat liver PC/ diPG/ cholesterol (10/1/1; mol/mol), (C) rat liver PC/ egg PE/ diPG/ cholesterol (7.5/2.5/1/1; mol/mol). Microsomes were sedimented and resuspended in sucrose buffer. Phospholipid compositions were determined after lipid extraction, correcting for "sticking" of SUV (4). PI may be contaminated with PS. Glc-6-P phosphohydrolase activities were measured at 30°C and pH 6.5 as previously described (5). Activities are expressed as percent of control microsomes incubated with SUV in the absence of transfer protein and are the mean and standard deviation of 3 experiments. Abbreviations: PL-TP, phospholipid transfer protein; diPG, diphosphatidylglycerol; DPPC, dipalmitoyl PC.

Biophys. J. © Biophysical Society · 0006-3495/82/01/46/02 $1.00
Volume 37 January 1982 46–47

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.

Support was provided in part by United States Public Health Services research grant HL 10940. Dr. Zilversmit is a Career Investigator of the American Heart Association. Dr. Crain was recipient of United States Public Health Services Postdoctoral Fellowship HL 05730.

Received for publication 27 April 1981.

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

ROSA H. HUANG
Department of Biochemistry, University of South Alabama, Mobile, Alabama 36688 U.S.A.

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