JOURNAL OF BACTERIOLOGY, Feb. 1985, p. 772–774
0021-9193/85/020772-03$02.00/0

Vol. 161, No. 2

Enzymatic Detection of Phospholipid Biosynthetic Enzymes After Electroblotting†

MARGARET A. POOLE, ANTHONY S. FISCHL, AND GEORGE M. CARMAN*

Department of Food Science, Cook College, New Jersey Agricultural Experiment Station, Rutgers, The State University, New Brunswick, New Jersey 08903

Received 24 August 1984/Accepted 22 October 1984

The membrane-associated enzymes phosphatidylinositol synthase (CDPdiacylglycerol:*myo*-inositol 3-phosphatidyltransferase; EC 2.7.8.11) and phosphatidylserine synthase (CDPdiacylglycerol:L-serine *O*-phosphatidyltransferase; EC 2.7.8.8) from *Saccharomyces cerevisiae* were detected enzymatically after sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electroblotting. Enzyme activities were measured on nitrocellulose blots by using pure enzyme preparations as well as Triton X-100-solubilized membranes. Phosphatidylinositol synthase activity migrated to M_r 34,000, and phosphatidylserine synthase activity migrated to M_r 23,000.

Phosphatidylinositol (3, 8, 9) and phosphatidylserine (1) are membrane phospholipids important to the growth of *Saccharomyces cerevisiae*. Phosphatidylinositol synthase (CDPdiacylglycerol:*myo*-inositol 3-phosphatidyltransferase; EC 2.7.8.11) is the enzyme responsible for the formation of phosphatidylinositol (12). Phosphatidylserine synthase (CDPdiacylglycerol:L-serine *O*-phosphatidyltransferase; EC 2.7.8.8) catalyzes the formation of phosphatidylserine (11). Since phosphatidylinositol synthase and phosphatidylserine synthase catalyze reactions involving the common substrate CDPdiacylglycerol, their activities may regulate the relative proportion of their phospholipid products in the membrane. Both of these enzymes are tightly associated with the membrane fraction of *S. cerevisiae* (5, 6, 13). We have recently purified phosphatidylinositol synthase (7) and phosphatidylserine synthase (2) from the microsomal fraction of *S. cerevisiae*. The purification procedures developed for phosphatidylinositol synthase (7) and phosphatidylserine synthase (2) resulted in the isolation of nearly homogeneous protein preparations of M_r 34,000 and 23,000, respectively, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. However, we were unable to measure the activities of phosphatidylinositol synthase and phosphatidylserine synthase in polyacrylamide gels.

A number of integral membrane proteins have been renatured after sodium dodecyl sulfate denaturation (for references, see 10). Hjertén (10) has recently shown that the membrane-associated *p*-nitrophenylphosphatase from *Acholeplasma laidlawii* could be renatured in gels, with restoration of activity after sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The transfer of proteins to nitrocellulose paper after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (4, 13) makes proteins more accessible for analysis. In this communication we show that the activity of phosphatidylinositol synthase and phosphatidylserine synthase can be restored after sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electroblotting.

Phosphatidylinositol synthase (7) and phosphatidylserine synthase (2) were purified from *S. cerevisiae* S288C, as previously described. Samples for electrophoresis were dialyzed for 3 h at 8°C against 62.5 mM Tris-hydrochloride (pH 6.8)–2% sodium dodecyl sulfate–50% glycerol–5% 2-mercaptoethanol. 2 μl of 1% methyl green was added to samples as a transfer indicator, and sodium dodecyl sulfate-polyacrylamide slab-gel electrophoresis was performed with 10% gels, as previously described (7). After electrophoresis, proteins were transferred electrophoretically to unmodified nitrocellulose (4, 13) in 25 mM Tris-hydrochloride (pH 8.3)–0.192 M glycine–20% methanol–0.02% sodium dodecyl sulfate by using a Hoefer TE 52 Transphor unit. The transfer was run for 45 min at 100 V. After transfer, the nitrocellulose sheet was cut in half. One half was stained with Coomassie blue (7), and the other half was soaked in renaturation buffer (50 mM Tris-hydrochloride [pH 8.0], 30 mM $MgCl_2$, 1 mM CDPdiacylglycerol, 0.5% Triton X-100, 10 mM 2-mercaptoethanol, 20% glycerol, 3% bovine serum albumin) for 1 h at 8°C. The renaturation buffer for phosphatidylserine synthase also contained 0.6 mM $MnCl_2$. The presence of CDPdiacylglycerol and cofactors in the renaturation buffers stabilizes phosphatidylinositol synthase and phosphatidylserine synthase activities. Each lane was cut into 0.5-cm strips and used for enzyme assays. All assays were conducted at 30°C for 60 min. Phosphatidylinositol synthase activity was measured by following the incorporation of 0.5 mM *myo*-[2-^{3}H]inositol (12,000 cpm/nmol) into chloroform-soluble product in the presence of 50 mM Tris-hydrochloride buffer (pH 8.0), 2 mM $MnCl_2$, 0.2 mM CDPdiacylglycerol, 1.6 mM Triton X-100, and a 0.5-cm strip of nitrocellulose in a total of 0.2 ml as previously described (5). Phosphatidylserine synthase activity was assayed in a similar manner (5), with 0.5 mM L-[3-^{3}H]serine (13,000 cpm/nmol), 50 mM Tris-hydrochloride buffer (pH 8.0), 0.6 mM $MnCl_2$, 0.2 mM CDPdiacylglycerol, 4 mM Triton X-100, and a 0.5-cm strip of nitrocellulose in a total of 0.2 ml.

As previously described (7), the purification of phosphatidylinositol synthase resulted in the isolation of a major peptide with an M_r of 34,000 (Fig. 1). Phosphatidylinositol synthase activity was found in the nitrocellulose fractions with the same M_r as that in the stained peptide band (Fig. 1). The recovery of activity after electroblotting was 0.7%. The purification of phosphatidylserine synthase resulted in the isolation of a major peptide with an M_r of 23,000 (Fig. 2), as previously described (2). Phosphatidylserine synthase activity was found in the nitrocellulose fractions corresponding to

* Corresponding author.

† Publication no. D-10531-1-84 of the New Jersey Agricultural Experiment Station.

the stained peptide band (Fig. 2). The recovery of activity after electroblotting was 10%. These results are further that the proteins with minimum subunit molecular weights of 34,000 and 23,000 are phosphatidylinositol synthase and phosphatidylserine synthase, respectively.

Phosphatidylinositol synthase activity (7) and phosphatidylserine synthase activity (2) were extracted from *S. cerevisiae* microsomes with 1% Triton X-100, as previously described. Samples of the Triton X-100-solubilized phosphatidylinositol synthase (0.8 nmol/min) and phosphatidylserine synthase (1.3 nmol/min) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to nitrocellulose paper, incubated in renaturation buffer for 1 h at 8°C, cut into 0.5-cm strips, and assayed for activity at 30°C, as described above for the purified enzymes. Phosphatidylinositol synthase activity migrated to M_r 34,000, and phosphatidylserine synthase activity migrated to M_r 23,000 (data not shown). The activity profiles for each enzyme were the same as that shown in Fig. 1 and 2 for phosphatidylinositol synthase and phosphatidylserine synthase, respectively. The recovery of phosphatidylinositol synthase activity and phosphatidylserine synthase activity after electroblotting was 0.3 and 0.8% respectively.

The recovery of phosphatidylinositol synthase and phosphatidylserine synthase after the electroblotting procedure was very low. This was not unexpected, considering the treatment to which both enzymes were exposed. The method is not suited to the purification of enzymes; however, it did allow us to confirm the subunit molecular weights of phosphatidylinositol synthase and phosphatidylserine synthase by using pure enzyme preparations or Triton X-100 extracts. The enzymatic detection of proteins on electroblots should prove useful for other enzymes of phospholipid metabolism.

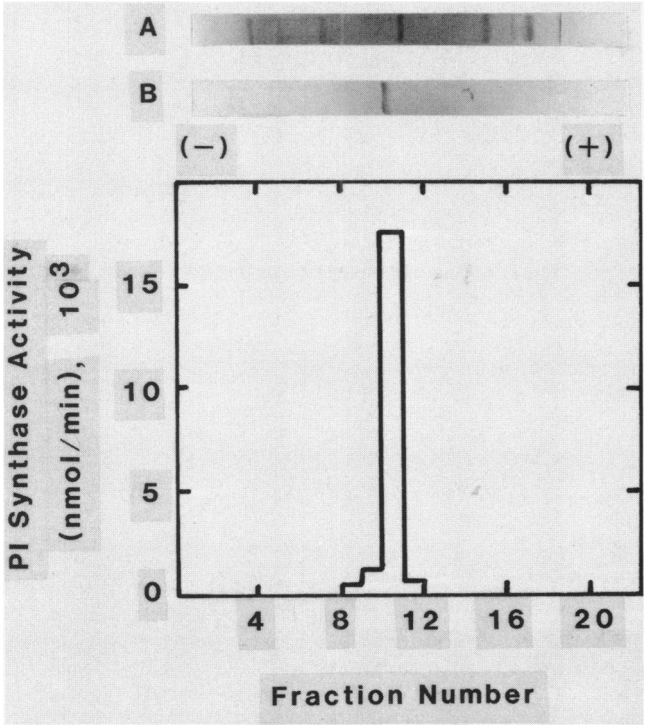

FIG. 1. Detection of phosphatidylinositol synthase activity after electroblotting. A purified sample of phosphatidylinositol (PI) synthase (4 nmol/min) was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to nitrocellulose paper, incubated in renaturation buffer for 1 h at 8°C, cut into 0.5-cm strips, and assayed for activity at 30°C, as described in the text. The counts associated with [2-^{3}H]phosphatidylinositol in the peak nitrocellulose fraction were 9,500 cpm above background (80 cpm). Lane A contained 12 μg of standards (phosphorylase B [M_r, 92,500], bovine serum albumin [M_r, 66,200], ovalbumin [M_r, 45,000], carbonic anhydrase [M_r, 21,500], and lysozyme [M_r, 14,400]), and lane B contained 5 μg of purified phosphatidylinositol synthase. Lanes A and B were stained with Coomassie blue directly after transfer to the nitrocellulose sheet.

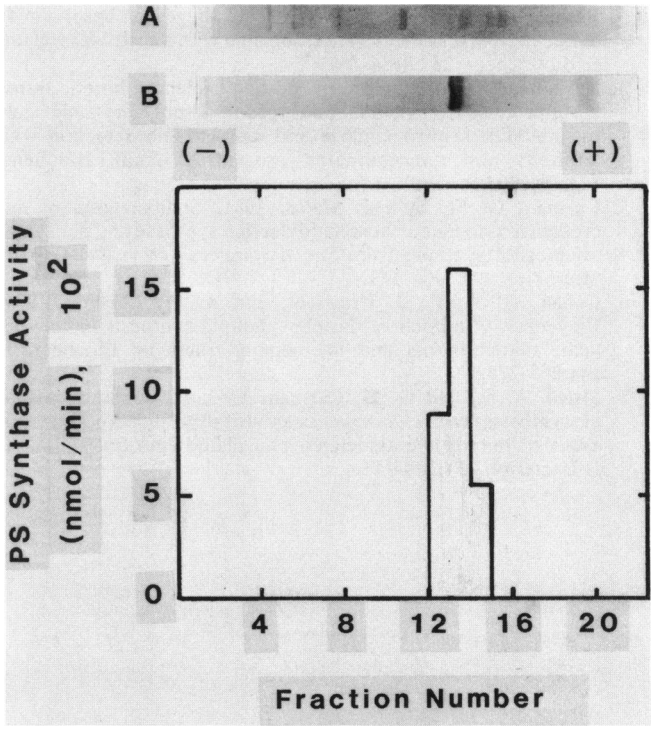

FIG. 2. Detection of phosphatidylserine synthase activity after electroblotting. A purified sample of phosphatidylserine (PS) synthase (3 nmol/min) was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to nitrocellulose paper, incubated in renaturation buffer for 1 h at 8°C, cut into 0.5-cm strips, and assayed for activity at 30°C, as described in the text. The counts associated with [3-^{3}H]phosphatidylserine in the peak nitrocellulose fractions were 21,000-62,000 cpm above background (400 cpm). Lane A contained 12 μg of standards (phosphorylase B [M_r, 92,500], bovine serum albumin [M_r, 66,200], ovalbumin [M_r, 45,000], carbonic anhydrase [M_r, 21,500], and lysozyme [M_r, 14,400]), and lane B contained 9 μg of purified phosphatidylserine synthase. Lanes A and B were stained with Coomassie blue directly after transfer to the nitrocellulose sheet.

This work was supported by state funds, the provisions of the Hatch Act of 1887, Public Health Service grant GM-28140 from the National Institutes of Health, the Charles and Johanna Busch Memorial Fund.

We thank William Dowhan for his helpful discussions and Rose Ann Ullrich for typing the manuscript.

LITERATURE CITED

1. **Atkinson, K. D., B. Jensen, A. I. Kolat, E. M. Storm, S. A. Henry, and S. Fogel.** 1980. Yeast mutants auxotrophic for choline or ethanolamine. J. Bacteriol. **141**:558–564.
2. **Bae-Lee, M. S., and G. M. Carman.** 1984. Phosphatidylserine synthesis in *Saccharomyces cerevisiae*. Purification and characterization of membrane-associated phosphatidylserine synthase. J. Biol. Chem. **259**:10857–10862.

3. **Becker, G. W., and R. L. Lester.** 1977. Changes in phospholipids of *Saccharomyces cerevisiae* associated with inositol-less death. J. Biol. Chem. **252**:8684–8691.
4. **Burnette, W.** 1981. "Western Blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified, nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal. Biochem. **112**:195–203.
5. **Carman, G. M., and J. Matas.** 1981. Solubilization of microsomal-associated phosphatidylserine synthase and phosphatidylinositol synthase from *Saccharomyces cerevisiae*. Can. J. Microbiol. **27**:1140–1149.
6. **Cobon, G. S., P. D. Crowfoot, and A. W. Linnane.** 1974. Biogenesis of mitochondria: phospholipid synthesis *in vitro* by yeast mitochondrial and microsomal fractions. Biochem. J. **144**:265–275.
7. **Fischl, A. S., and G. M. Carman.** 1983. Phosphatidylinositol biosynthesis in *Saccharomyces cerevisiae*: purification and properties of microsome-associated phosphatidylinositol synthase. J. Bacteriol. **154**:304–311.
8. **Hanson, B. A., and R. L. Lester.** 1980. Effects of inositol starvation on phospholipid and glycan synthesis in *Saccharomyces cerevisiae*. J. Bacteriol. **142**:79–89.
9. **Henry, S. A., K. D. Atkinson, A. I. Kolat, and M. R. Culbertson.** 1977. Growth and metabolism of inositol-starved *Saccharomyces cerevisiae*. J. Bacteriol. **130**:472–484.
10. **Hjertén, S.** 1983. A study on the renaturation of membrane proteins after solubilization in SDS or following polyacrylamide gel electrophoresis in SDS, with special reference to a phosphatase from *Acholeplasma laidlawii*. Biochim. Biophys. Acta **736**:130–136.
11. **Kanfer, J., and E. P. Kennedy.** 1964. Metabolism and function of bacterial lipids. II. Biosynthesis of phospholipids in *Escherichia coli*. J. Biol. Chem. **239**:1720–1726.
12. **Paulus, H., and E. P. Kennedy.** 1960. The enzymatic synthesis of inositol monophosphatide. J. Biol. Chem. **235**:1303–1311.
13. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. U.S.A. **76**:4350–4354.