
A Saccharomyces cerevisiae mutant was isolated on the basis of itsositol over-prodiction and excretion. This mutant was constitutively producing a 150 kDa phosphatidylglycerol (PG) synthase. The addition of inositol to the growth medium of the mutant cells did not reverse the phenotype. The enzyme was purified from liver microsomes using previously established procedures of detergent extraction and acyltransferase chromatography. The molecular weight of the enzyme in the presence of dipalmitoyl DG and the non-substrate lipid, PG, was determined by Sephadryl S-1000 chromatography. Protease treatment of intact vesicles indicated that over 60% of the enzyme was the active site facing outward. Activity was linear with time and vesicle concentration. Maximum activity was obtained at pH 8.0 with 5 mM MnCl2. Phosphatidylglycerol synthase activity was reproducible by the phospholipid composition of the vesicles (Supported by NJ AES and PHS grant GM 28140).

RATE DETERMINING STEP IN PHOSPHOLIPASE A2 MECHANISM: 180 ISOTOPE EXCHANGE DETERMINED BY 12C NMR. T. Fanni, D. Lombardo, A. Plickthun and E.A. Dennis. Department of Chemistry, University of California at San Diego, La Jolla, CA 92039.

Pancreatitis is a major cause of human disease. The enzyme hydrolys a marked preference for micellar substrates and acts poorly on monomeric substrates (E.A. Dennis, (1983) The Enzymes 16, 309-353). We have now examined the mer of the proposal that the product-release step is slow, but is accelerated when the enzyme acts on aggregated phospholipids. Measurements of H-14C isotope exchange into specifically-labeled substrate was "used to test for specificity of the enzyme. A novel technique of distinguishing 180 incorporation by 12C-18C vs. 12C-14C chemical shift differences at 126 MHz for PC and PI using the steric hindrance of a 12C, 14C-labeled PC and PI in a 1:1 mixture as substrate. The reaction was examined for the possibility of simultaneous 180 incorporation into the substrate. No exchange was found suggesting that the catalytic step is not followed by a higher energy transition state and that it or a step before it appears to be rate-limiting.


PROPERTIES OF ACYL-CoA:ACLY-GROSER-3-PHOSPHOCHOLINE ACYLTRANSFERASE FROM BOVINE HEART MUSCLE MITOCHONDRIA. R. MacQuarrie, G.Y. Sun and M. Sanatanlula. School of Basic Life Sciences and Dept. of Chemistry, Univ. of Missouri-KC, Kansas City, MO 64110 and Dept. of Biochemistry, Rush Univ., Chicago, IL 60612.

The enzyme acyl-CoA:acyl-glycerol-3-phosphocholine acyltransferase (EC 2.3.1.23) was purified approximately 3300-fold from bovine heart muscle mitochondria using previously established procedures of detergent extraction and chromatography. The enzyme was purified to homogeneity in the presence of 1 mM sulfite was determined to be 64,000 by gel electrophoresis. The substrate specificity of the enzyme was studied by using various phospholipids as acceptor and acyl-CoA derivatives as donors. The enzyme displays little or no activity with catalytic activity with lysophosphatidylthanolamine, lysophosphatidylglycerol or lysophosphatidylinositol but high activity with lysophosphatidylethanolamine. The enzyme was obtained with l-palmitoyl-sn-glycerol-3-phosphocholine. The enzyme showed wide specificity for the acyl donor with oleoyl-CoA, arachidonyl-CoA, and stearoyl-CoA. The acyltransferase activity dependence of catalytic activity on the concentration of either substrate did not correspond to the Michaelis-Menten equation. This enzyme is similar to but distinct from an acyltransferase isolated from brain tissue by the same procedures. (Supported in part by NSF grant BNS-8419063.)


Membrane-associated phosphatidylserine synthase from Saccharomyces cerevisiae was purified (Bae-Lee, N., and Carman, G.M. (1984) J. Biol. Chem. 259, 10857-10862) and reconstituted into unilamellar phospholipid vesicles or detergents were performed by removing detergent from an octylglucoside-phosphatidyl-Triton X-100-enzyme mixed mixed micelle by dialysis. The purified yeast enzyme required both phosphatidylinositol and octylglucoside to Triton X-100 and octylglucose to phospholipase to be 75:1 and 15:1, respectively. The presence of this enzyme catalyzed conversion of phosphatidylycholine-phosphatidylethanolamine-phosphatidylglycerol to phosphatidylserine at a molar ratio of 3:1:2:1. The average diameter of the vesicles was 90 nm as determined by Sephacryl S-1000 chromatography. Protease treatment of intact vesicles indicated that over 60% of the enzyme had its active site facing outward. Activity was linear with time and vesicle concentration. Maximum activity was obtained at pH 8.0 with 5 mM MnCl2. Phosphatidylserine synthase activity was reproducible by the phospholipid composition of the vesicles. (Supported by NJ AES and PHS grant GM 28140.)


CTP:phosphocholine cytidyltransferase (CT) is a major rate-controlling enzyme in phosphatidylcholine biosynthesis. The enzyme displayed little or no activity with acyl donor with oleoylchloroform as acyl donors. The enzyme displayed little or no activity with acyl donor with oleoylchloroform. The enzyme was purified from rat liver. A pH 5 precipitate from cytosol was extracted with 20% octylglucoside. The enzyme was released by SDS-PAGE chromatography on DEAE agarose and hydroxyapatite (HAP). Purified CT was eluted from HAP with 0.2M potassium phosphate - 0.1M Tris pH 10.0. The purified enzyme was a single activity of 12,250 nmol/min/mg protein, a 2180 fold purification. Non-denaturing PAGE of the CT showed a single protein band which coincided with enzyme activity. SDS-PAGE analysis indicated that CT consists of two polypeptide chains of Mr 120,000 and 48,000. Chromatography on Biogel A 1.5m suggested that native CT consisted of two 39,000 and two 48,000 subunits. Purified CT required Mg2+ for activity and was maximal for activity. Phosphatidylcholine gave about half the maximal activity. CT was stable for several months at -70°C in 0.2M phosphate and Tris pH 10. The pH optimum of CT was 7.0. The true Kms for CTP and phosphocholine were 0.29 mM and 0.14 mM, respectively. CT was inactivated by the sulphydryl reagents DTT, PMSF and MEM. CTP protected CT from inactivation by MEM; phosphocholine gave partial protection. CT binds to liver microsomes. The apparent binding was increased 3X by oleic acid and was saturable (apparent Kd 0.32 mM/mg microsomal protein). Supported by VA and by NICHD.


We have recently reported (Kontos et al., Science 266-25, 1981) that cholinephosphotransferase (CTPase) activity in vitro increased when the diglyceride (DG) substrate was saturated at elevated temperatures in the presence of phosphatidylglycerol (PG). The presence of PG was critical particularly with dipalmitoyl DG (DPPG). Further study demonstrates that the increased activity of CT with DG depended on the acyl composition of the DG and the non-substrate lipid, substrate mixture concentration, and the relative molar ratio of DG and PG. Substrates containing the Other acidic phospholipids (card, PI, PS, PA) also increased CTPase activity with dioleoyl DG (DOOG) but they were progressively less effective with DPPG. Mixtures with PG, PS, and sphingomyelin were also more effective than PG alone. Biosynthesis of phospholipids (LPC, LPG, LPE) produced active preparations of DOOG but not DPPG. Fatty acids (16:0, 18:1, 18:2) increased CTPase activity with DOOG although to differing degrees. Both the phosphatidylglycerols and fatty acids produced increasing their decreasing activity as their concentration increased, however the pattern differed between DG species. Increased CTPase activity with DPPG required PG containing unsaturated fatty acids but activity with DOOG was independent of the PG's acyl composition. These data suggest that membrane lipid composition may influence enzyme utilization of specific substrate species. (Supported by NICHD and VA)