446

YEAST MUTANT DEFECTIVE IN CDP-DIACYLGLYCEROL SYNTHASE ACTIVITY. M.J. Homann,[1] L.S. Klig,[2] S.A. Henry,[2] and G.M. Carman.[1] [1]Rutgers Univ. New Brunswick, NJ 08903. [2]Albert Einstein College of Medicine, Bronx, NY 10461.

A Saccharomyces cerevisiae mutant was isolated on the basis of inositol overproduction and excretion. This mutant was constitutive for the enzyme inositol-1-phosphate synthase. The addition of inositol to the growth medium of the mutant cells did not result in an increase of cellular phosphatidylinositol as observed in wild type cells. Phosphatidic acid was elevated in the mutant cells compared to wild type cells.

Phospholipid biosynthetic enzyme activities of permeabilized mutant cells on filter paper replica prints were analyzed by colony autoradiography. The mutant cells synthesized reduced levels of CDP-diacylglycerol and phosphatidylinositol. Anaylsis of mutant cell-free extracts revealed that CDP-diacylglycerol synthase activity was reduced 4-fold compared to wild type cells. In addition, CDP-diacylglycerol synthase activity was constitutive. (Supported by NJ AES, PHS grants GM 28140 [to GMC], GM 11301 and GM 19629 [to SAH]).

447

RECONSTITUTION OF PURIFIED YEAST PHOSPHATIDYLSERINE SYNTHASE INTO PHOSPHOLIPID VESICLES. J.M. Hromy and G.M. Carman. Rutgers Univ., New Brunswick, NJ 08903.

Membrane-associated phosphatidylserine synthase from Saccharomyces cerevisiae was purified (Bae-Lee, M., and Carman, G.M. [1984] J. Biol. Chem. 259, 10857-10862) and reconstituted into unilamellar phospholipid vesicles. Reconstitution was performed by removing detergent from an octylglucoside-phospholipid-Triton X-100-enzyme mixed micelle by Sephadex G-50 chromatography. The molar ratios of octylglucoside to Triton X-100 and octylglucoside to phospholipid were 75:1 and 15:1, respectively. The phospholipid composition of the vesicles was phosphatidylcholine-phosphatidylethanolamine-phosphatidylinositol-phosphatidylserine at a molar ratio of 3:2:2:1. 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 $MnCl_2$. Phosphatidylserine synthase activity was regulated by the phospholipid composition of the vesicles. (Supported by NJ AES and PHS grant GM 28140).

448

RATE DETERMINING STEP IN PHOSPHOLIPASE A_2 MECHANISM: ^{18}O ISOTOPE EXCHANGE DETERMINED BY ^{13}C NMR. T. Fanni, D. Lombardo, A. Plückthun and E.A. Dennis. Department of Chemistry, University of California at San Diego, La Jolla, CA 92093.

Pancreatic and venom phospholipase A_2 display a marked preference for micellar substrates and act poorly on monomeric substrates (E.A. Dennis, (1983) The Enzymes 16, 309-353). We have now examined the merit of the proposal that the product-release step is slow, but is accelerated when the enzyme acts on aggregated phospholipids. Measurements of $H_2{}^{18}O$ isotope exchange into specifically-labeled substrate was used to obtain information on the rate-limiting step in the enzyme action. A novel technique of distinguishing ^{18}O incorporation by ^{13}C-^{18}O vs. ^{13}C-^{16}O chemical shift differences at 126 MHz for ^{13}C NMR was employed. The enzymatic hydrolysis of a micellar phosphatidylcholine analogue of platelet activating factor, 1-alkyl, 2 [1-^{13}C]-lauroyl-sn-glycero-3-phosphorylcholine proceeds by O-acyl cleavage of the sn-2 ester bond. The reaction was examined for the possibility of simultaneous ^{18}O 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. (NSF DMB 85-18684 and NIH GM 20,501).

reprinted from:
T. Fanni, D. Lombardo, A. Plückthun and E. A. Dennis, Rate determining step in phospholipase A2 mechanism: 18O Isotope exchange determined by 13C NMR. Fed. Proc. 45, 1557 (1986).

449

PURIFICATION AND PROPERTIES OF CTP: PHOSPHORYLCHOLINE CYTIDYLYLTRANSFERASE FROM RAT LIVER. P. Weinhold, M. Rounsifer and D. Feldman, VA Med.Ctr., U of M Med.Sch., Ann Arbor, MI. 48105

CTP: phosphorylcholine cytidylyltransferase (CT) is a major rate-determining enzyme in the biosynthesis of phosphatidylcholine (PC). We purified CT from rat liver. A pH 5 precipitate from cytosol was extracted with 20 mM octylglucoside. The octylglucoside extract was fractionated by column chromatography on DEAE agarose and hydroxyapetite (HTP). Purified CT was eluted from HTP with 0.2M potassium phosphate - 0.03% Triton X100. The purified CT had a specific 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 contained two nonidentical subunits with Mr of 39,000 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 PC-oleic acid vesicles for maximal activity. Phosphatidylglycerol gave about half the maximal activity. CT was stable for several months at -70°C in 0.2M phosphate and Triton X100. The pH optimum of CT was 7.0. The true Km values for CTP and phosphorylcholine were 0.29 mM and 0.14 mM, respectively. CT was inactivated by the sulfhydryl reagents DTNB, PCMP, and NEM. CTP protected CT from inactivation by NEM; phosphorylcholine gave partial protection. CT binds to liver microsomes. The apparent binding was increased 3X by oleic acid and was saturable (apparent Kd 4.6nM; Bmax 0.32 nmol/mg microsomal protein). Supported by VA and by NICHHD.

450

PROPERTIES OF ACYL-CoA:1-ACYL-sn-GLYCERO-3-PHOSPHOCHOLINE ACYLTRANSFERASE FROM BOVINE HEART MUSCLE MICROSOMES. R. MacQuarrie, G.Y. Sun and M. Sanjanwala. School of Basic Life Sciences and Dept. of Chemistry, Univ. of Missouri-KC, Kansas City, MO 64110 and Dept. of Biochemistry, Univ. of Missouri, Columbia, MO 65211.

The enzyme acyl-CoA:1-acyl-sn-glycero-3-phosphocholine acyltransferase (EC 2.3.1.23) was purified approximately 3300-fold from bovine heart muscle microsomes using previously established procedures of detergent extraction and chromatography. The molecular weight of the enzyme in the presence of dodecyl sulfate was determined to be 64,000 by gel electrophoresis. The substrate specificity of the enzyme was studied by using various lysophospholipids as acyl acceptors and acyl-CoA derivatives as acyl donors. The enzyme displayed little or no catalytic activity with lysophosphatidylethanolamine, lysophosphatidylinositol or lysophosphatidylserine but high activity with lysophosphatidylcholine. The highest activity was obtained with 1-palmitoyl-sn-glycero-3-phosphocholine. The enzyme showed wide specificity for the acyl donor with oleoyl-CoA, arachidonoyl-CoA, and palmitoyl-CoA having high substrate activity. The 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 BSN-8419063.)

451

REGULATION OF CHOLINEPHOSPHOTRANSFERASE ACTIVITY AND SUBSTRATE SPECIFICITY BY SUBSTRATE MIXTURE COMPOSITION, Joseph C. Miller and Paul A. Weinhold, Natl Inst Aging, Bethesda, MD 20892, and VA Med Ctr/Univ Michigan, Ann Arbor, MI 48105.

We demonstrated previously (JBC 256, 12662-5, 1981) that cholinephosphotransferase (CPTase) activity in vitro increased when the diacylglycerol (DG) substrate was sonicated at elevated temperatures in the presence of phosphatidylglycerol (PG). The presence of PG was critical particularly with dipalmitoyl DG (DPDG). Further study demonstrates that the increased activity depends on the non-substrate lipid species, the acyl composition of the DG and the non-substrate lipid, substrate mixture concentration, and the relative molar ratio of the components in the substrate mixture. Other acidic phospholipids (card, PI, PS, PA) also increased CPTase activity with dioleoyl DG (DODG) but they were progressively less effective with DPDG. Mixtures with PC, PE, and sphingomyelin were essentially inactive. Lower molar ratios of lysophospholipids (LPG, LPC, LPE) produced active preparations of DODG but not DPDG. Fatty acids (16:0, 18:1, 18:2) increased utilization of both DPDG and DODG although to differing degrees. Both the lysophospholipids and fatty acids produced increasing then decreasing activity as their concentration increased, however the pattern differed between DG species. Increased CPTase activity with DPDG required PG containing unsaturated fatty acids but activity with DODG 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)