Phospholipid Synthesis in Mammary Tissue. Choline and Ethanolamine Kinases: Kinetic Evidence for Two Discrete Active Sites

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ABSTRACT

Choline and ethanolamine kinases are located in the high speed supernatant of lactating bovine mammary gland. Maximum activities of choline and ethanolamine kinases were observed at pH 9.2 and 8.0, respectively, with the rate of ethanolamine phosphorylation being 1/15 that of choline phosphorylation. Activation energies of 29 joules (Q_{10} - 1.5) and 31 joules (Q_{10} = 1.5) were calculated between 3.4 and 31.3 C for choline kinase and ethanolamine kinase, respectively. Above 31.3 C, the Arrhenius plot deviated from linearity for both enzymes, suggesting that denaturation was occurring. An apparent Km of 0.25 mM for choline was obtained for choline kinase activity. The apparent Km of ethanolamine kinase for ethanolamine was unusually high (17 mM), and activity was not linear with increasing protein concentration. Activity was tripled and the Km decreased to 2.5 mM when the enzyme preparation was washed with butanol: benzene mixture, suggesting the presence of an endogenous competitive inhibitor(s), with respect to ethanolamine. Choline kinase was not affected by the solvent wash. Substrate competition studies revealed that choline kinase was slightly inhibited competitively by ethanolamine (apparent Ki = 19-21 mM), whereas choline was a potent competitive inhibitor of ethanolamine kinase (apparent Ki = 0.33-0.50 mM). The results indicated that these two kinase activities were mediated by two distinct active sites, possibly on a single protein. The significance of choline in the regulation of phosphatidylethanolamine synthesis is discussed.

INTRODUCTION

ATP:choline phosphoryltransferase (choline kinase, EC.2.7.1.32) and ATP:ethanolamine phosphotransferase (ethanolamine kinase) are the initiating enzymes in the cytidine or Kennedy pathway for the de novo synthesis of 3-sn-phosphatidylcholine (PC) and 3-sn-phosphatidylethanolamine (PE), respectively. Because these enzymes may catalyze the controlling steps in the synthesis of PC and PE and since mammary cells display an apparent preferential synthesis of PC compared to PE (1), we studied the relative activities of these enzymes in lactating mammary tissue.

Ethanolamine kinase activity has been demonstrated in crude and partially purified choline kinase preparations (2-4). A highly purified choline kinase from rabbit brain has also been shown to have ethanolamine kinase activity (5). Weinhold and Rethy (6) resolved the ethanolamine kinase from rat liver into two fractions by DEAE-cellulose chromatography. One of the fractions (ethanolamine kinase II) copurified with choline kinase. The ratio of these two activities remained constant during DEAE-cellulose and Sephadex G-200 chromatography, and both activities showed similar stabilities (6). The copurification of ethanolamine and choline kinase activities from rat liver has recently been confirmed by Brophy and Vance (7) by affinity chromatography. These studies suggest that both kinase activities are associated with the same protein in rabbit brain and rat liver. However, Sung and Johnstone (3) reported indirect evidence that two distinct proteins are responsible for these two kinase activities in Ehrlich ascites cells. Broad and Dawson (8) partially separated these two kinases from the rumen protozoan *Entodinium caudatum* by gel filtration.

If, indeed, choline and ethanolamine kinases are associated with the same protein, a question that must be answered is whether the same active site is responsible for these two kinase activities, or if two or more specific sites are involved. We studied the properties of these enzymes from bovine mammary tissue, compared their activities under different assay conditions, and used the kinetic technique of substract competition to answer this question. Results indicate that the choline and ethanolamine kinase activities are mediated by at least two distinct active sites.

MATERIALS AND METHODS

Materials

(Me-^{14}C) choline chloride (61 μCi/μmol,

diluted tenfold for enzyme assays) was purchased from Amersham/Searle Corp. (Arlington Heights, IL). (1,2-^{14}C) Ethanolamine-HCl (30 μCi/μmol, diluted fourfold for enzyme assays) was purchased from New England Nuclear (Boston, MA). Choline bromide, monoethanolamine-HCl, and phosphatidylethanolamine (dipalmityl, synthetic) were purchased from Calbiochem (San Diego, CA). Lecithin (bovine) was purchased from P.L. Biochemical Inc. (Milwaukee, WI). Egg lysolecithin was purchased from Pierce Chemicals (Rockford, IL). Tween-20 and adenosine-5′-triphosphate (disodium crystalline, 99-100%, low calcium content) were purchased from Sigma Chemical Co. (St. Louis, MO).

All common chemicals were of reagent grade or of the highest purity of commercially available materials. Glass redistilled and deionized water was used in all solutions and buffers.

Methods

Enzyme preparation: Mammary tissue was excised from lactating Holstein cows immediately after slaughter and stored in an ice chest (4 C). Connective tissue was removed, and the secretory tissue was minced, in a meat grinder. This material was suspended in Tris-HCl buffer (0.14M, pH 8) in a 1:1 (v/v) ratio and homogenized in a Waring blendor for 30 sec. This homogenate was further rehomogenized in a small mill (Polyscience Corp., IL) for 30 sec. All manipulations were carried out at 4 C.

The final homogenate was centrifuged in a refrigerated Sorvall centrifuge using a GSA rotor (r = 5.75") at 15,000 x g for 20 min at 4 C. The supernatant was decented and strained through two layers of cheese cloth to separate the fat layer and centrifuged in a Beckman model L2-65 preparative ultracentrifuge using a type-21 fixed angle rotor at 44,000 x g for 75 min. The final supernatant was quickly frozen in a round bottom flask using a dry ice-acetone bath and lyophilized in a Virtis freeze-drier (at 0.03 mm Hg pressure at 24 C for 24 hr). The resulting powder was ground to a fine consistency and stored in plastic-capped vials at −25 C. This powder was used as the enzyme source.

Butanol:benzene wash: The lyophilized supernatant was suspended in n-butanol in a 1:10 (w/v) ratio and immediately centrifuged at 3,000 x g in a refrigerated Sorvall centrifuge for 10 min. The resulting supernatant was decanted, and the wash operation was repeated three more times. The final pellet was further washed four times with a 2:1 (v/v) butanol: benzene mixture following the same procedure used for the butanol wash. All preparations were carried at 4 C. The final pellet was dried under a stream of dry nitrogen. This powder was stored at −25 C and called the "delipidated enzyme preparation."

Preparation of phospholipid dispersions: With the exception of lyso-PC, all phospholipids (20 μmol) were first dissolved in 0.5 ml benzene followed by the addition of 5.0 ml of 0.01%(v/v) Tween 20 in water. The suspension was sonicated for 10 min in a water bath sonicator (model 8845-3, Cole-Parmer Instrument Co., Chicago, IL), and the organic solvent was then evaporated under a stream of dry nitrogen. The phospholipid suspension, free of organic solvent, was sonicated (ca. 20 min) until the opalescence disappeared.

Assay conditions: Unless otherwise stated, all assays were performed in a total volume of 375μl containing 50 mM pH 8.0 Tris-HCl and ca. 1.0 mg of protein. Stock solutions of ethanolamine were adjusted to pH 8.0 with Tris-HCl buffer. Initial velocities were obtained with 15-30 min incubations. Routine incubations were performed at 36 C in a shaking water bath, stopped with 50 μl of 1.0 M trichloroacetic acid, and subsequently centrifuged in a refrigerated Sorvall centrifuge at 5,000 x g for 5 min at 4 C. Aliquots of the 5,000 x g protein free supernatant were subsequently analyzed for radioactive substrates and products.

The rationale of the substrate competition technique used in this study to determine whether two alternate substrates are utilized by the same active site has been discussed in detail (9,10).

All kinetic constants obtained in these experiments must be considered as apparent ones, since true kinetic constants and their composition in terms of invididual rate constants can only be obtained after the kinetic mechanism has been elucidated.

Analytical: The protein was determined by the method of Lowry et al. (11) using crystalline bovine serum albumin as a standard. Choline, P-choline, and CDP-choline were separated by paper chromatography (10 x 10", Whatman 3 MM) using a modification of the solvent system described by Plageman (12). The developing solvent contained 2.7 M ammonium acetate buffer pH 5.0 and 95% ethanol in a 3:7 (v/v) ratio. The Rf's were 0.82, 0.48, and 0.27 for choline, P-choline, and CDP-choline, respectively. The same solvent system was found to resolve ethanolamine, P-ethanolamine, and CDP-ethanolamine, with Rf's of 0.75, 0.32, and 0.15, respectively. Authentic standards of choline, ethanolamine, and their respective phosphate and CDP- esters were always chromatographed along with the assay aliquots. Choline

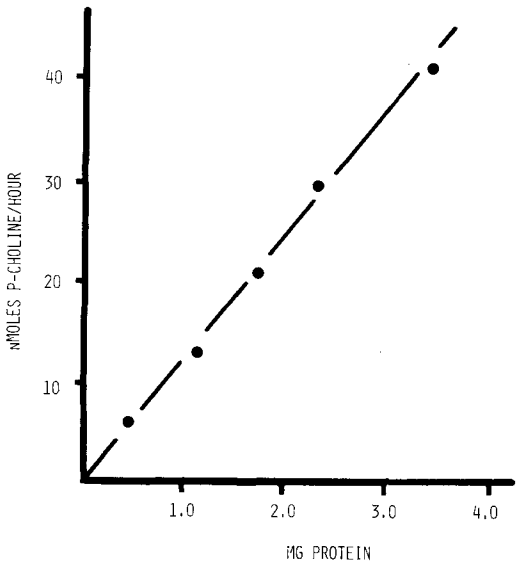

FIG. 1. Bovine mammary choline kinase activity as a function of protein concentration. The specified amounts of high speed supernatant from mammary tissue were incubated in medium containing Mg-ATP^{-2}, 3.3 mM, free Mg^{2+}, 0.4 mM, and (Me-^{14}C) choline, 0.15 mM. Other experimental procedures as in "Methods."

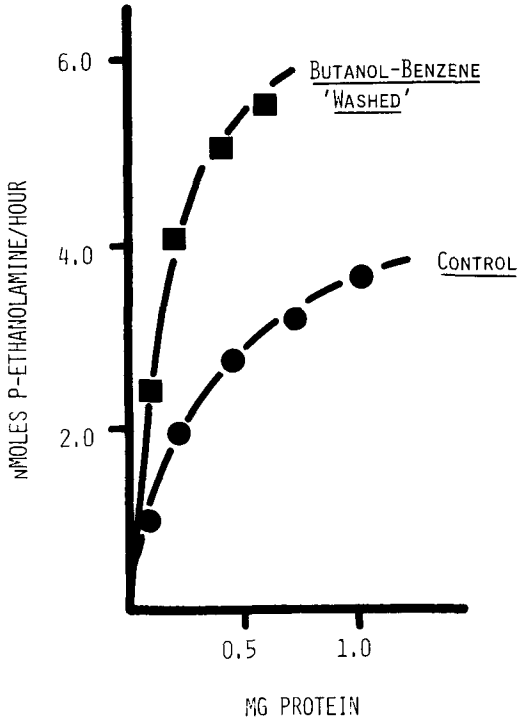

FIG. 2 Activity of bovine mammary ethanolamine kinase as a function of protein concentration. The specified amounts of protein from high speed supernatant (normal and butanol:benzene "washed") were incubated in a medium containing Mg-ATP^{-2}, 3.6 mM, free Mg^{2+}, 0.43 mM, and (1,2-^{14}C)-ethanolamine, 0.37 mM. Other experimental procedures as in "Methods."

spots were detected by iodine vapors. The phosphate ester was detected by the phosphorus spray of Hanes and Isherwood (13) as modified by Dawson (14). Ethanolamine and P-ethanolamine were detected by a ninhydrin spray. CDP-choline and CDP-ethanolamine were visualized under ultraviolet light. Following identification of the standards, the appropriate spots were cut from the paper chromatograms and counted in a Packard scintiallation spectrometer (15).

Radiopurity of ^{14}C-labeled compounds and recovery of radioactivity in the assays were routinely checked, and both were 98%. CDP-(^{14}C)-choline or CDP-(^{14}C) ethanolamine were not detected in the assays.

Calculations of Mg-ATP^{-2} and free Mg^{2+} concentrations were made using the Mg-ATP^{-2} stability constant of 20 mM^{-1} (16).

RESULTS

Choline kinase activity assayed with a limiting concentration of choline (0.15 mM) was directly proportional to protein concentration over a tenfold range, i.e., 0.36-3.6 mg of protein (Fig. 1). This indicated that no detectable concentrations of endogenous substrates, inhibitors, or activators were present in the enzyme preparation at the protein levels used.

Initial velocity experiments with choline as the variable reactant produced nonlinear double reciprocal plots at choline concentrations below 0.07 mM. Therefore, the substrate competition experiments in this study were performed above this level of choline. Detailed studies on the kinetic mechanism of mammary choline kinase will be published separately.

The activity of ethanolamine kinase was not linear with protein concentration, suggesting that an inhibitor(s) was present in the preparation (Fig. 2). When the lyophilized preparation was washed with a butanol:benzene mixture, ethanolamine kinase activity increased threefold and deviation from linearity decreased (Fig. 2), indicating that part of the inhibition was being removed by the solvent treatment. In contrast, choline kinase showed only a slight increase (10%) in activity after the same solvent wash, suggesting that no inhibitors nor activators of this enzyme were present in the preparation at the concentration of protein used (Table I). This agrees with the observed linearity of choline kinase activity as a function of protein

TABLE I

Effects of Solvent Extraction of Crude Enzyme on Choline and Ethanolamine Kinase Activities[a]

Enzyme treatment	Specific activity	
	Choline kinase	Ethanolamine kinase
Normal preparation	22	4.2
Benzene extracted	17	4.1
Butanol extracted	21	12
Benzene:butanol extracted	25	13

[a]Incubation medium contained (Me-^{14}C) choline, 0.3 mM for the choline kinase assay; (1,2-^{14}C) ethanolamine, 2.5 mM for the ethanolamine kinase assay. Mg-ATP^{-2} and free Mg^{2+} concentrations were 3.6 and 0.43 mM, respectively, in both enzyme assays. Other experimental procedures as in "Methods." Specific activity is indicated in nmol phosphorylcholine or phosphorylethanolamine formed per mg protein per hour.

concentration.

Choline kinase activity was remarkably stable in freeze-dried preparations, showing only 5% loss in activity after 15 months storage at −25 C. Its stability in buffer solution at 4.0 C was limited to ca. 5 hr, after which the activity decreased according to a first order reaction ($k = 5.1$ min^{-1}) over a 32 hr period, with the half life ($t_{½}$) of 8.2 hr (Fig. 3). Perhaps the high protein concentration of the holding stock solution contributed to the stability of the enzyme. The initial 5 hr stability period was of sufficient duration for performance of experiments. The stability of choline kinase at 24 C was more limited. Activity diminished over a 32 hr period following first order rate kinetics ($k = 10$ min^{-1}), with $t_{½}$ equal to 4.0 hr. The stability of

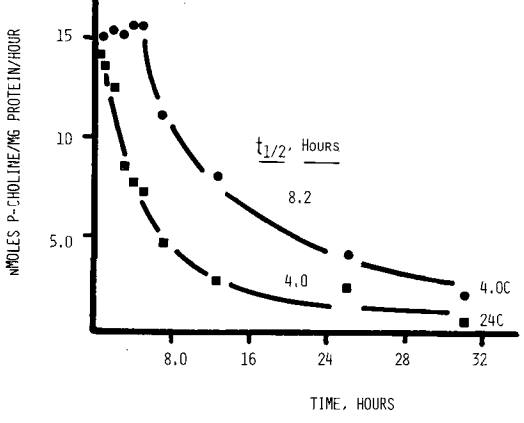

FIG. 3. Stability of choline kinase activity from bovine mammary tissue at 4 and 24 C. Enzyme solutions containing 75 mg protein per ml of Tris-HCl buffer (0.3 M pH 8.0) were held at the indicated temperatures for the specified periods. The activity of aliquots containing 1 mg protein was assayed in a medium containing Mg-ATP^{-2}, 3.3 mM, free Mg^{2+}, 0.41 mM, and (Me-^{14}C)-choline, 0.15 mM. Other procedures as in "Methods."

ethanolamine kinase in solution followed the same pattern as choline kinase, and freeze-dried preparations retained ethanolamine kinase activity over a period of 15 months.

Optimum pH of choline and ethanolamine kinase activities: The pH profiles of both choline and ethanolamine kinase activities had characteristic bell shapes, but each showed a distinctly different pH optimum. Choline kinase showed its maximum activity at pH 9.0, with a steep decrease in activity on the acid side of the curve; a 17-fold difference was observed between pH 6 and 9 (Fig. 4A). Ethanolamine kinase reached its maximum activity at pH 8.0, showing only a 60% lower rate at pH 6.0 (Fig. 4B). The buffer N-(2-acetamido) iminodiacetic acid (ADA) was strongly inhibitory to both enzymes. This inhibition is consistent with its Mg^{2+} binding properties, i.e., log $K_{assoc} = 2.5$ (17). The other buffers used have negligible metal binding constants (18), thus their competition for Mg^{2+} can be ruled out.

Effect of temperature on activity of choline and ethanolamine kinases: The effect of temperature on the activity of both enzymes was studied between 3.5 C and 40.7 C. From rate data obtained between incubation temperatures of 3.4 C and 31.3 C, Arrhenius activation energies (Ea) of 29 joules ($Q_{10} = 1.5$) and 31 joules ($Q_{10} = 1.5$) were calculated for choline kinase and ethanolamine kinase, respectively (Fig. 5). Above 31.3 C, the Arrhenius plot deviated from linearity for both enzymes. Suggesting that denaturation was occurring.

Initial velocity patterns with ethanolamine and choline was variable reactants: An apparent Km of 17 mM for ethanolamine, as the variable reactant, was obtained from a double reciprocal plot of initial velocities (Fig. 6A). Because this Km was abnormally high, the presence of an endogenous competitive inhibitor in the enzyme preparation was suspected. Consistent

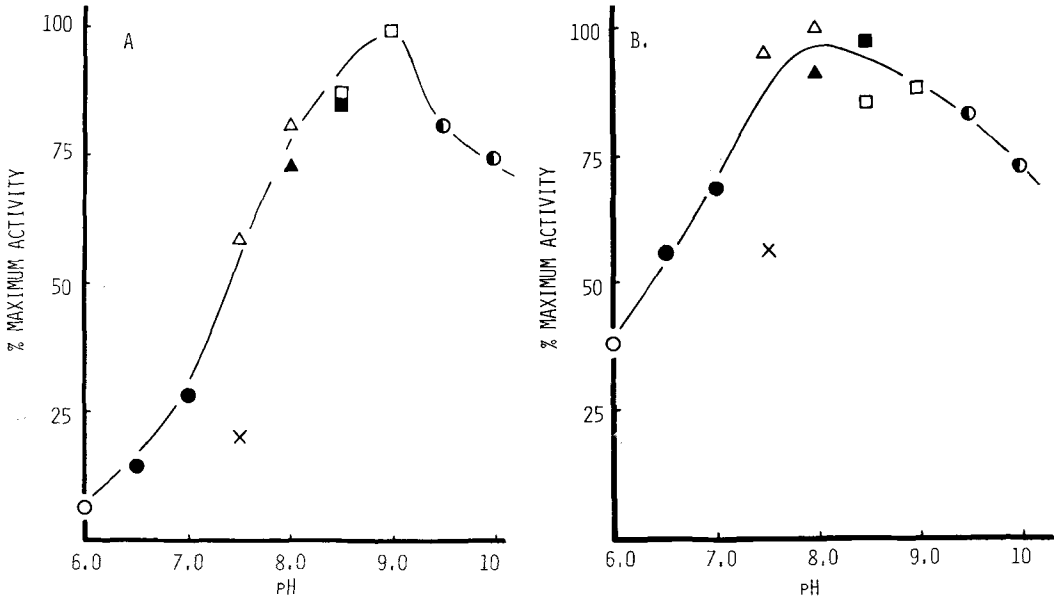

FIG. 4. Phosphoryl transfer activity as a function of pH. Assay medium for choline kinase (A) contained Mg-ATP^{-2}, 3.6 mM, free Mg^{2+}, 0.43 mM, and (Me-^{14}C) choline, 2.37 mM. Assay medium for ethanolamine kinase (B) contained Mg-ATP^{-2}, 3.6 mM; free Mg^{2+}, 0.43 mM, and (1,2-^{14}C) ethanolamine, 1.9 mM. Buffer concentration was 80 mM; —○—, 2-(N-morpholino) ethane sulfonate (MES); —●— N,N-bis (2-hydroxymethyl)-2-aminoethane sulfonate (BES); —△— N-2-hydroxyethyl piperazine-N-2-ethane sulfonate (HEPES); —X— N-(2-acetamido) iminodiacetate (ADA); —▲—, 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris); —■—, N,N-bis (–2-hydroxyethyl) glycine (BICINE); —□—, glycyl glycine (GLY-GLY); —◐— glycine. Other experimental procedures as in "Methods."

with this was the fact that activity was not linear with increasing protein concentration (Fig. 2). Ethanolamine kinase activity was tripled when the lyophilized enzyme preparation was washed with butanol or butanol: benzene while the choline kinase activity remained relatively constant. Kinetic studies revealed that the butanol:benzene extraction of the enzyme preparation caused a large decrease in the apparent Km for ethanolamine of ethanolamine kinase, i.e., from 17 mM to 2.5 mM (Fig. 6B), while the Vmax remained unaffected. These data indicated that a kinetically competitive inhibitor was being removed from the enzyme preparation by the solvent wash.

The phospholipids 3-sn-phosphatidylcholine, monoacyl-sn-glycero-3-phosphoryl-choline, and 3-sn-phosphatidylethanolamine, in Tween 20 (final concentrations, 1.3 mM and 0.03%, respectively), were not inhibitors of ethanolamine kinase because, when added to the delipidated enzyme preparation, they caused no inhibition with respect to the control containing Tween 20 (Table II). The surfactant Tween 20 (0.03%) produced 37% inhibition of ethanolamine kinase. On the other hand, choline kinase was unaffected by the butanol:benzene wash, phospholipids, or Tween 20.

Substrate competition experiments showed that choline was a strong competitive inhibitor (apparent Ki = 0.50 mM) of ethanolamine kinase activity (Fig. 6B), while choline kinase activity (apparent Km = 0.25 mM) was only slightly inhibited by ethanolamine (apparent Ki = 21 mM). This inhibition was also competitive (Fig. 7A).

To minimize the effect of possible activators and/or inhibitors, the same substrate competition studies were done using the delipidated enzyme preparation. The activity of choline kinase was not changed, its apparent Km for choline being 0.28 mM, and it was slightly inhibited by ethanolamine, i.e., apparent Ki = 19 mM (Fig. 7B). In the case of ethanolamine kinase, its ethanolamine apparent Km decreased to 2.5 mM (Fig. 6B). The inhibition by choline was slightly increased compared to the original preparation, i.e., apparent Ki = 0.33 mM compared to an apparent Ki of 0.50 mM, respectively (Fig. 6A).

DISCUSSION

Previous studies indicated that the lactating mammary gland synthesized phospholipids de novo and that the classical Kennedy pathway

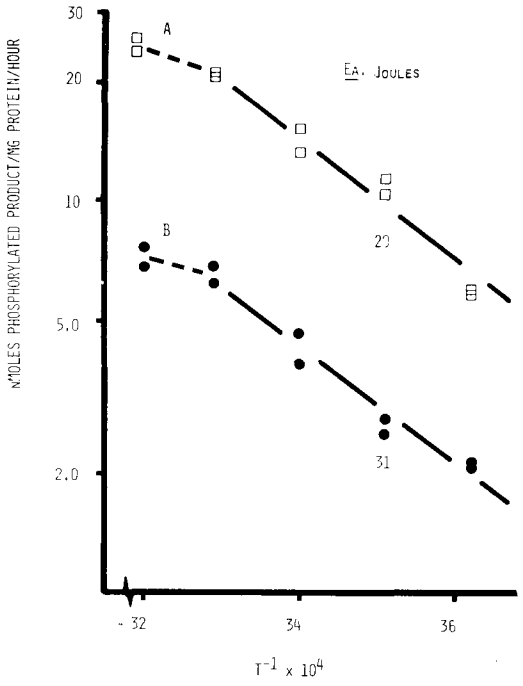

FIG. 5. Arrhenius plot of bovine mammary choline kinase and ethanolamine kinase activities. Assay medium for choline kinase (A) contained Mg-ATP^{-2}, 3.6 mM, free Mg^{2+}, 0.43 mM, and (Me-^{14}C) choline, 0.30 mM. Assay medium for ethanolamine kinase (B) contained Mg-ATP^{-2}, 3.6 mM; free Mg^{2+}, 0.43 mM, and (1.2-^{14}C) ethanolamine, 2.3 mM. Other experimental procedures as in "Methods."

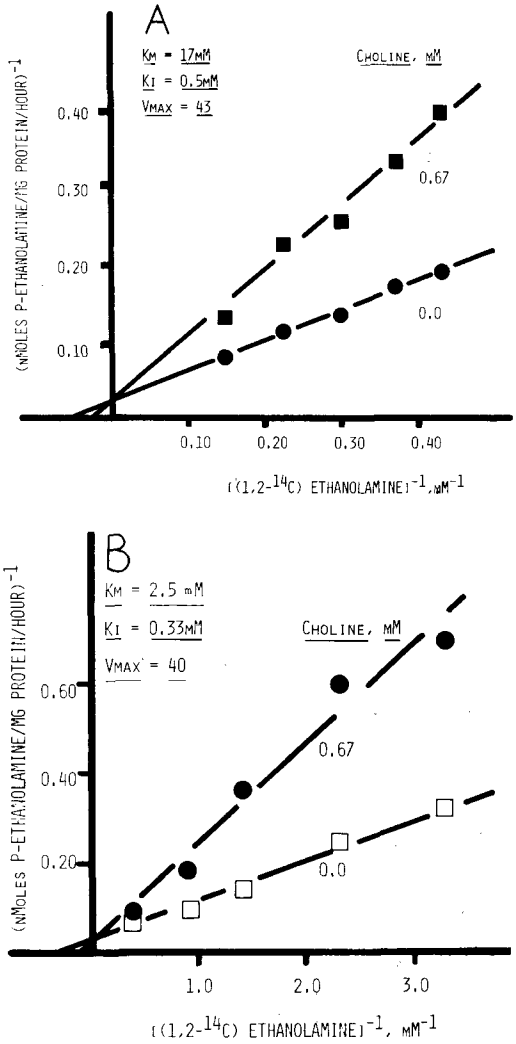

FIG. 6. Double reciprocal plots of initial velocities of bovine mammary ethanolamine kinase activity in the presence of choline. Assay medium contained Mg-ATP^{-2}, 3.6 mM, free Mg^{2+}, 0.43 mM, and varying levels of (1,2-^{14}C) ethanolamine. Other experimental procedures as in "Methods." A. Unwashed enzyme. B. Butanol:benzene washed enzyme.

was the major route (1,19,20). The present data corroborate this.

The broad alkaline pH optima for choline and ethanolamine kinases from bovine mammary tissue are in agreement with reports for these enzymes from other tissues (2,3,21-26). However, there are no comparative studies on the pH optima for these two enzymes under the same conditions. The fact that, when identical assay conditions were used in this study, ethanolamine kinase activity showed a lower pH curve than choline kinase activity suggests that these bases are not phosphorylated by the same active site. These data are also consistent with the possibility that a cationic nitrogen is needed for the binding of ethanolamine and choline to the enzyme active site. In the case of choline, the quaternary ammonium cation ($-N^+-(CH_3)_3$) holds a permanent charge independent of pH. Ethanolamine, however, has a dissociable proton on the primary amine nitrogen (pK = 9.2) at 36 C (27). At the optimum pH (pH 8.0), 94% of the ethanolamine would be in the protonated form ($-H_3N^+$), conceivably providing maximum binding to the enzyme active site. At pH 9.0, however, only 62% of the ethanolamine species would be in the cationic form. The possibility that a single active site could have different pH optima for these two substrates cannot be excluded if a cationic nitrogen on the base is needed for binding reaction to occur. These data suggest that an anionic group on both enzymes is involved in the binding of both choline and ethanolamine.

The Arrhenius activation energies (EA's) obtained for choline and ethanolamine, i.e., 29 and 31 joules, respectively, correspond to a

TABLE II

Effects of Tween-20 and Phospholipids on Choline and Ethanolamine Kinase Activities from Bovine Mammary Tissue[a]

Addition		Enzyme activity (nmol product formed mg^{-1} hr^{-1})	
Tween-20 (% v/v)	Phospholipid (mM)	Choline kinase	Ethanolamine kinase
0.0	0.0	25	13
0.03	0.0	26	8.2
0.03	3-sn-PC, 1.3	25	10
0.03	Lyso-PC, 2.7	26	10
0.03	3-sn-PE, 1.3	22	9.1

[a]Incubation medium contained (Me-^{14}C) choline, 0.3 mM for choline kinase assay; (1,2-^{14}C) ethanolamine, 2.5 mM for the ethanolamine kinase assay. Mg-ATP^{-2} and free Mg^{2+} concentration were 3.6 and 0.43 mM, respectively, in both assays. Solvent extracted enzyme preparation was used. Other experimental procedures as in "Methods." PC and PE denote phosphatidylcholine and phosphatidylethanolamine, respectively.

Q_{10} of 1.5. Choline kinase from brain tissue showed an EA of 13 joules (3.0 Cal) (25). The almost identical activation energies for the two mammary kinases reported here are consistent with the hypothesis that these two activities are associated with the same protein. However, these data can also be rationalized on the grounds that, since EA is a function of the bond energies involved in the reaction from the same phosphoryl donor (Mg-ATP^{-2}) to similar acceptors (choline or ethanolamine) they should have similar EA's. This assumes that the effect of temperature on the active conformation of the enzyme is negligible or of equal magnitude in both instances, if the enzymes are different proteins. The parallel loss of linearity of the Arrhenius plot observed above 31 C for the activity of the two enzymes is also consistent with the above suggestions.

The weak inhibition of choline kinase by ethanolamine (apparent Ki = 21 mM) is in line with the results obtained by Sung and Johnstone (3) for the enzyme from Ehrlich ascites cells and rat brain tissue. These authors also reported that ethanolamine did not inhibit choline phosphorylation in mouse liver, spleen, and kidney tissue.

The apparent Km (2.5 mM) obtained for ethanolamine kinase is probably higher than the real Km since the washed preparation may have still contained residual amounts of a competitive inhibitor. This strengthens the argument for the presence of two active sites, as the actual differences between the ethanolamine Km and Ki may be even larger than the experimentally observed ones. Ethanolamine kinase preparations may contain inhibitors; e.g., Weinhold and Rethy (23) suggested that choline was the endogenous inhibitor in their crude supernatant from rat. This does not seem to be the case for the mammary enzyme since ethanolamine kinase decreased its apparent Km for ethanolamine sevenfold (from 17 to 2.5 mM)

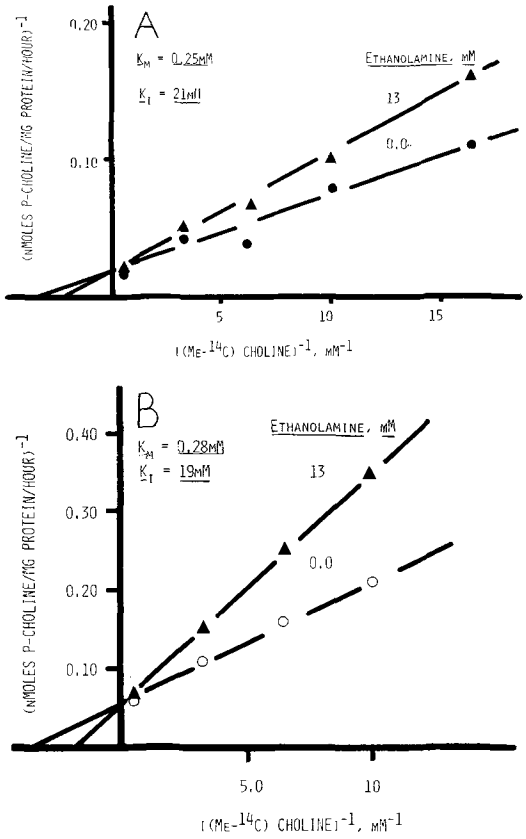

FIG. 7. Double reciprocal plots of initial velocities of bovine mammary choline kinase in the presence of ethanolamine. Assay medium contained Mg-ATP^{-2}, 3.6 mM, free Mg^{2+}, 0.43 mM, and varying levels of (Me-^{14}C) choline. Other experimental procedures as in "Methods." A. Unwashed enzyme. B. Butanol: benzene washed enzyme.

after the solvent wash, while its choline apparent Ki showed only a slight decrease (from 0.50 50 0.33 mM); thus, the small amount of choline that might have been extracted by the solvent cannot account for the large decrease in the apparent Km for ethanolamine. Furthermore, the observed linearity of choline kinase activity with increasing levels (10-fold increase) of protein, when assayed with limiting concentrations of choline (0.15 mM), suggests that little or no endogenous choline was present in the high speed supernatant in the protein range used. The possibility that endogenous ethanolamine was the inhibitor extracted by the solvent wash is very unlikely because of its insolubility in the benzene:butanol mixture.

The strong inhibition of ethanolamine kinase by choline is consistent with the results of Sung and Johnstone (3) and Weinhold and Rethy (23). The latter authors, however, reported a noncompetitive inhibition with respect to ethanolamine but competitive with respect to ATP.

From the substrate competition studies using the delipidated preparation, it is clear that choline kinase and ethanolamine kinase activity do not have a common active site. If this were not the case, the Ki of choline kinase inhibition by ethanolamine would be identical to the ethanolamine Km of ethanolamine kinase activity. This was not observed; therefore, on kinetic grounds these two activities were mediated by at least two distinct active sites. The fact that the apparent Ki of ethanolamine kinase inhibition by choline was similar to the choline Km of choline kinase suggests that the binding site of ethanolamine kinase may recognize choline with the same or higher affinity than ethanolamine. The converse, however, is not true; the binding site of choline kinase can discriminate between the two nitrogenous bases. An obvious difference between these bases is the trimethyl quaternary ammonium moiety of choline. Since the dealkylated substrate (ethanolamine) is such a weak competitive inhibitor (apparent Ki = 19 mM) of choline kinase, it is clear that this enzyme recognizes these three methyl groups which apparently are essential for the substrate binding to this enzyme. Wittenberg and Kornberg (2) showed that the Km's were directly influenced by the number of N-methyl groups of the substrate; i.e., the Km's for the N-methyl-, N,N-dimethyl-, and N,N,N-trimethyl-ethanolamine (choline) were 0.6, 0.1, and 0.02 mM, respectively.

An alternative explanation for the similarities between the choline apparent Km of choline kinase and its apparent Ki for ethanolamine phosphorylation is that the site of inhibition of the latter activity by choline is the choline kinase site. This would be possible if these two activities are associated with the same protein and a "one way negative interaction" operates between these two sites. By this mechanism, occupation of the choline kinase site would prevent the activity of the ethanolamine kinase site; however, the converse would not occur.

As the cytidine pathway is the predominant mechanism whereby 3-sn-phosphatidylcholine and 2-sn-phosphatidylethanolamine are synthesized de novo in most animal tissues (28-36), and since choline is a potent inhibitor of ethanolamine kinase, it is conceivable that the intracellular concentration of choline and of the endogenous inhibitor may control the activity of existing ethanolamine kinase. If this phosphorylation step is rate limiting, the choline:ethanolamine ratio would have the potential to regulate the synthesis of PE in vivo. This control mechanism may play a role in vivo in determining that PC is the major phospholipid in mammalian tissues, and it may explain the increased level of PE found in livers from choline deficient rats (37,38) by release of the inhibition of ethanolamine kinase activity.

ACKNOWLEDGMENTS

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