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Cloning and expression of rat liver CTP:phosphocholine cytidylyltransferase: An amphipathic protein that controls phosphatidylcholine synthesis

(polymerase chain reaction/amphipathic α-helix/COS cell transfection)

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ABSTRACT CTP:phosphocholine cytidylyltransferase (EC 2.7.7.15) is a key regulatory enzyme in the synthesis of phosphatidylcholine in higher eukaryotes. This enzyme can interconvert between an inactive cytosolic form and an active membrane-bound form. To unravel the structure of the transferase and the mechanism of its interaction with membranes, we have cloned a cytidylyltransferase cDNA from rat liver by the oligonucleotide-directed polymerase chain reaction. Transfection of the rat clone into COS cells resulted in a 10-fold increase in cytidylyltransferase activity and content. The activity of the transfected transferase was lipid-dependent. The central portion of the derived protein sequence of the rat clone is highly homologous to the previously determined yeast cytidylyltransferase sequence [Tsukagoshi, Y., Nikawa, J. & Yamashita, S. (1987) *Eur. J. Biochem.* 169, 477–486]. The rat protein sequence lacks any signals for covalent lipid attachment and lacks a hydrophobic domain long enough to span a bilayer. However, it does contain a potential 58-residue amphipathic α-helix, encompassing three homologous 11-residue repeats. We propose that the interaction of cytidylyltransferase with membranes is mediated by this amphipathic helix lying on the surface with its axis parallel to the plane of the membrane such that its hydrophobic residues intercalate the phospholipids.

In higher eukaryotes CTP:phosphocholine cytidylyltransferase (CT; EC 2.7.7.15) catalyzes a rate-limiting reaction in the synthesis of phosphatidylcholine, the major membrane phospholipid. CT activity is a key determinant of cell membrane synthesis and of the supply of lipid second messengers such as fatty acids and diacylglycerols (1). In most cells CT is distributed between the cytosol and membrane fractions. Interconversion between the inactive cytosolic form and the active membrane-bound form can be promoted by a variety of effectors of phosphatidylcholine synthesis (reviewed in ref. 2). This amphitropism (3) links CT with a class of proteins that includes protein kinase C, arachidonate 5-lipoxygenase, and mammalian diacylglycerol kinase (4–6). Despite extensive study, the precise mechanism of interaction of any of these proteins with membranes has yet to be defined.

The association of CT with membranes has four intriguing features: (*i*) it is promoted by specific lipids such as fatty acids, diacylglycerols, and anionic phospholipids (7–10) and is antagonized by aminolipids such as sphingosine (11); (*ii*) it is reversible (10, 12, 13); (*iii*) it involves hydrophobic interactions (10); and (*iv*) it appears to be influenced by the phosphorylation state of the enzyme (14).

These features of the membrane–protein interaction have enticed us to begin to decipher the structure of the transferase, and in particular its lipid binding domain. A cDNA encoding the yeast CT had been isolated previously (15). Little is known with regard to the regulation of the yeast enzyme. Most of the activity is membrane-associated (15), but its mode of interaction has not been investigated. There is no evidence for a regulatory role for yeast CT in the control of the CDP-choline pathway for phosphatidylcholine synthesis. We expected substantial differences between the rat and yeast protein sequences.

In this paper we report the isolation of a CT cDNA from rat liver and its expression in COS cells. Based on structures predicted from the protein sequence we propose a model for membrane interaction that accounts for some of the known features of the CT–membrane association.¶

MATERIALS AND METHODS

Protein Purification and Peptide Isolation and Sequencing. CT was purified from rat liver by the method of Weinhold *et al.* (7) with modifications (16). Protein digestion and sequencing was performed on 7–10 μg of purified enzyme. The samples were concentrated using a Centricon (Amicon) followed by acetone precipitation. After electrophoresis in an SDS/12% polyacrylamide minigel the protein was electroblotted onto nitrocellulose with 20% methanol/0.195 M glycine/25 mM Tris Cl, pH 8.2/0.005% SDS as the transfer buffer (17). The nitrocellulose filter was stained with amido black, the region where CT was bound was sliced out, and the bound enzyme was digested on the matrix with trypsin (17). The resulting peptides were separated by HPLC (17) using a Vydac C_4 column (250 × 2.1 mm) in a Waters peptide analyzer. Four prominent peptides were sequenced on an Applied Biosystems model 477A sequenator. Their sequences are underlined in Fig. 1*D*.

Oligonucleotides. Degenerate oligonucleotides were used as primers for polymerase chain reactions (PCRs). CT1 (5′-GA$^{C}_{T}$TT$^{C}_{T}$GT$^{I}_{C}$GC$^{I}_{C}$CA$^{C}_{T}$GA$^{C}_{T}$GA$^{C}_{T}$AT-3′), corresponding to a portion of tryptic peptide 1, was complementary to the antisense strand, and CT4 (5′-CTT$^{C}_{T}$TC$^{G}_{A}$TTIAT$^{G}_{A}$AAI-$^{CAC}_{GT\,I}$AC$^{G}_{A}$TT-3′), corresponding to a portion of tryptic peptide 4, was complementary to the sense strand. Primers complementary to vector sequences (18) were the forward

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Abbreviations: CT, cytidylyltransferase; PCR, polymerase chain reaction.
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¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. M36071).

sequencing primer (5'-GTTTTCCCAGTCACGAC-3') and reverse sequencing primer (5'-CAGGAAACATCTATGAC-3').

RNA Isolation and Preparation of cDNA Libraries. Total RNA was extracted from the liver of a 200-g male Sprague–Dawley rat (19). The poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose chromatography and used as a template with random hexamer primers (20) to generate double-stranded cDNA. After size fractionation by agarose gel electrophoresis, cDNAs of 900–3500 nucleotides were ligated into *Sma* I-digested pUC19 (18). The cDNA library was transformed into *Escherichia coli* DH5α (BRL).

PCR and Cloning. Plasmid DNA from amplified libraries was digested with *Pvu* I and used as a template for PCR (21). The PCR reaction mixture (25 μl) contained 20 ng of plasmid DNA, 70 mM Tris Cl (pH 8.8 at 20°C), 7 mM $MgCl_2$, 18 mM ammonium sulfate, 10 mM 2-mercaptoethanol, 0.1% Triton X-100, 160 μg of bovine serum albumin per ml, 10% dimethyl sulfoxide, 0.5 mM each deoxynucleoside triphosphate, 25 μg of CT-specific primers (CT1, CT4) and/or 5 μg of sequencing primers (18) per ml, and 2 units of *Thermus aquaticus* (*Taq*) polymerase (Promega). Conditions for reaction mixtures containing primers CT1 and CT4 were 30 cycles of 92°C for 30 sec, 52°C for 2 min, and 72°C for 2 min. Conditions for mixtures containing CT4 and the forward sequencing primer or CT1 and the reverse sequencing primer were 30 cycles of 92°C for 30 sec, 48°C for 2 min, and 72°C for 4 min. The PCR products (P14, P4f, and P1r) were purified by agarose gel electrophoresis and electroeluted. P14 DNA was blunt-ended with T4 DNA polymerase and inserted into *Sma* I-cut pUC19. P4f DNA was cut with *Sac* I and inserted into *Sac* I-cut pUC19. P1r DNA was cut with *Eco*RV and *Hin*dIII and inserted into *Sma* I- and *Hin*dIII-cut pUC19.

DNA Sequence Determination and Protein Sequence Analysis. The complete DNA sequence was determined on both strands by using Sequenase 2 according to the manufacturer (United States Biochemical). Comparisons between the amino acid sequence of CT and other polypeptides were done with GAP and BESTFIT (22) or FASTA (23) for data base searches. Secondary structure, hydrophobicity, and helical hydrophobic moment analyses were provided in the program PEPPLOT (22).

Construction of a Full-Length CT cDNA for Expression. A cDNA encoding the entire sequence of rat CT was constructed by joining the 5' *Dde* I–*Sst* I fragment from pP4f and the 3' *Sst* I–*Hin*dIII fragment from pP1r. The constructed cDNA coding region of rat CT was inserted at the *Sma* I cloning site of the expression vector pAX111 to generate pAX-RCT. The pAX111 vector includes a human cytomegalovirus immediate-early promoter for transcription of inserted cDNAs and a simian virus 40 origin that drives rapid replication of the vector in COS cells (R.J.K. and K. Humphries, unpublished work).

DNA Transfection and Assay of CT in Cellular Subfractions. COS cells were grown and transfected with pAX111 or pAX-RCT by using DEAE-dextran as described (24), with a 20% (vol/vol) glycerol shock for 2 min. Three days after transfection the cells were harvested in phosphate-buffered saline with 2.5 mM EDTA and were homogenized in 10 mM Tris Cl, pH 7.4/1 mM EDTA/2 mM dithiothreitol/0.1 mM phenylmethylsulfonyl fluoride (25). Solid NaCl was added to a concentration of 0.1 M after homogenization. Cytosol and microsomes were isolated as described (25). Extracts were assayed (16) at three different protein concentrations.

Western Blot. Three hundred micrograms of homogenate protein from transfected COS cells and 2 μg of purified CT (7) were resolved in a 12% polyacrylamide/SDS gel. Protein was electroblotted to nitrocellulose as described above. CT was detected on the blot by reaction with chicken polyclonal anti-CT antibody and subsequent reaction with anti-chicken IgG-horseradish peroxidase conjugate (Sigma). 4-Chloro-1-naphthol was used as substrate (26).

RESULTS

Peptide Sequences. Our strategy for cloning a cDNA encoding CT depended on obtaining the amino acid sequences of several peptides derived from the purified rat liver enzyme (7). The N terminus of CT is blocked (7); thus N-terminal sequences of four internal tryptic peptides were obtained (see Fig. 1*D*). Peptides 1 and 4 were significantly homologous to peptides in the yeast CT protein. These peptides in the yeast CT are separated by just 64 amino acids (see Fig. 2).

cDNA Cloning by PCR. To generate a DNA probe that would exactly match the CT cDNA sequence, we first amplified the DNA in the region between the sequences encoding peptides 1 and 4. Plasmid DNA prepared from a pUC19 library of 2 million rat liver cDNAs was used as a template. PCR amplification (21) using degenerate oligonucleotides encoding segments of peptides 1 and 4 resulted in an obvious ≈230-base-pair (bp) product, which was of the expected size assuming equivalent separation of peptides 1 and 4 in the rat and yeast proteins. The amplified DNA was purified and cloned in pUC19. Sequencing identified one 230-bp clone (P14) encoding a peptide of 74 amino acids that was 70% identical to the sequence in the yeast CT between amino acids 201 and 265 (Figs. 1*A* and 2). The amino acids encoded at the 5' and 3' ends of this clone were identical to the sequences of tryptic peptides 1 and 4, respectively (Fig. 1*A*). This confirmed that P14 was derived from a cDNA encoding rat CT.

Southern blots of plasmid DNA probed with the P14 insert indicated that there was only a single CT clone in the 2-million-member library (data not shown). Rather than attempting to isolate this clone by colony screening on a very large scale, we amplified the entire CT cDNA in two separate PCRs. The 5' portion of the CT cDNA in the pooled plasmid library was amplified by PCR with oligonucleotide CT4 and the forward sequencing primer, which the Southern blots had indicated to be adjacent to the 5' end of the cDNA insert. The 3' portion of the cDNA was amplified by PCR with CT1 and the reverse sequencing primer (Fig. 1*B*). The products of these two reactions, which were very heterogeneous in size, were then inserted into pUC19 and CT clones were identified by colony screening using the P14 fragment as a specific probe. By this approach we obtained 5' (P4f) and 3' (P1r) clones that contained sequences encoding tryptic peptides 2 and 3, respectively (Fig. 1*D*). The complete 1342-bp sequence of the rat liver CT cDNA assembled from the overlapping 5' and 3' clones is shown in Fig. 1*D*.

Features of the Rat CT Sequence and Homology to Yeast CT. The rat CT cDNA contains an open reading frame encompassing 367 amino acids. The first ATG codon is in an ideal context for translation initiation (27) and is immediately preceded by a termination codon, suggesting that it encodes the true N terminus. The predicted protein molecular weight of 41,720 compares well to a molecular weight of 42,000 estimated from SDS gels of purified rat liver CT (14, 16). The encoded protein contains one potential N-linked glycosylation site at Asn-231; however, this residue was within the sequenced peptide fragment 4, and the sequence analysis gave no evidence for an oligosaccharide linkage at this residue. The rat CT cDNA does not contain an N-terminal leader peptide or any sequences denoting the covalent attachment of lipid, such as a hydrophobic C terminus for glycosyl-phosphatidylinositol (28), a C-terminal Cys-Ala-Ala-Xaa for an isoprenyl group (29), or a glycine in position 2 for N-myristoylation (30). The longest stretch of hydrophobic amino acids is 5 (residues 108–112). The rat cDNA contains three homologous 11-mer peptides, repeated in

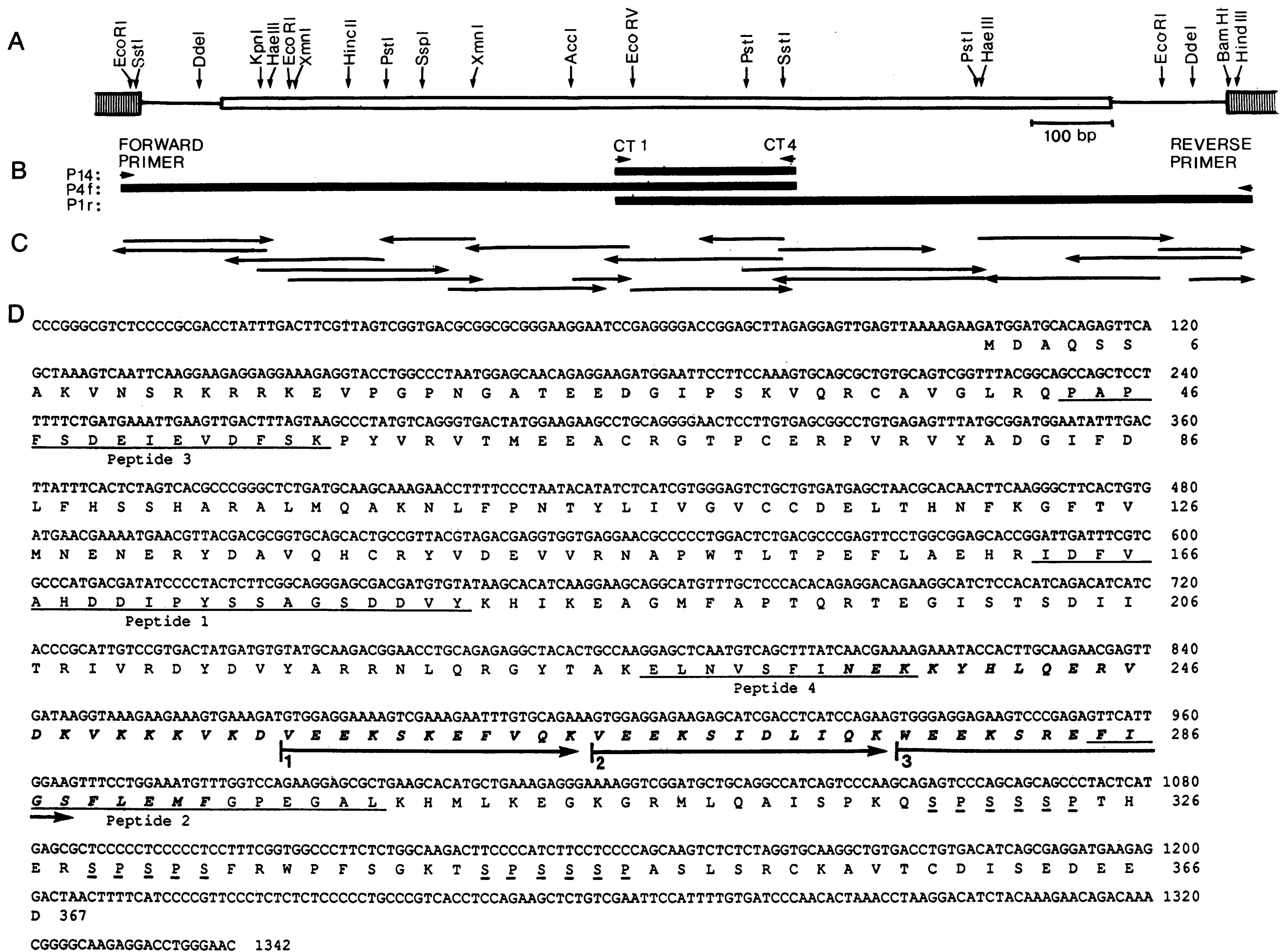

FIG. 1. (*A*) Restriction map of the CT clone in pUC19. Hatched box, pUC19 sequence; open box, open reading frame of 1101 nucleotides; solid line, untranslated region of the CT clone. (*B*) CT clones generated by PCR. Solid bars represent PCR products. The arrows above the solid bars indicate the positions and orientations of the oligonucleotides used in cDNA amplification. (*C*) Sequencing strategy. Restriction enzyme sites used in obtaining fragments for sequencing are indicated in *A*. (*D*) Nucleotide sequence and translated amino acid sequence of the CT cDNA. Amino acids are represented by the single-letter code. Positions of the four tryptic peptides are underlined. The amphipathic α-helix is in boldface and italics. The 11-mer repeats within the amphipathic helix are indicated by arrows. The Ser/Pro motifs are indicated by dashed underlines.

tandem at residues 256–288. A duplicated motif, Ser-Pro-Ser_3-Pro, is found at residues 319–324 and 343–348, and a similar sequence, Ser-Pro-Ser-Pro-Ser, at residues 329–333 (Fig. 1*D*). These repeats are not found in the yeast CT.

CT is phosphorylated *in vivo* in HeLa cells (31). Purified CT is a substrate for cAMP-dependent protein kinase, and phosphorylation correlates with a reduction in enzyme activity (14). A search for the consensus phosphorylation sequence Arg-Arg-Xaa-Ser(or Thr)-Xaa (32) identified Arg-Val-Thr-Met (residues 61–64), which resembles the cAMP kinase phosphorylation site 1 on acetyl-CoA carboxylase (Arg-Met-Ser-Phe; ref 33). There are six potential sites for phosphorylation by protein kinase C [Ser (or Thr)-Xaa-Lys (or Arg)] (34). The repeated Ser/Pro-rich domains located C-terminal to the amphipathic helix resemble the site of RNA polymerase II phosphorylation by a cell cycle control protein kinase (35), and the sites for phosphorylation by other cell cycle-dependent protein kinases such as the MBP kinase from sea star (J. Sanghera and S. L. Pelech, personal communication).

Residues 72–234 of the rat protein and 101–260 of the yeast protein share 75% identity (Fig. 2). By contrast, the N-terminal domains share only 23% identity, with 2 gaps in the alignment. The C-terminal domains (after residue 234 of the rat sequence) are also only weakly homologous (37% identity with 5 gaps in the alignment). An exception is the final 6 amino acids of the rat sequence, Ser-Glu-Asp-Glu-Glu-Asp which resembles the yeast sequence at residues 401–406, Ser-Glu-Asp_4. The serine in this sequence is a potential site for phosphorylation by casein kinase II (36).

Expression of CT cDNA in COS Cells. A cDNA corresponding to the entire sequence of CT was constructed from P4f and P1r and inserted into the expression vector pAX111. Transfection of COS cells with the pAX-RCT construct resulted in a 10-fold increase in CT activity in cell homogenates (Table 1). The transfected cytosolic activity was stimulated 25-fold by exogenous lipid (Table 1), suggesting that our isolated clone encodes an enzyme with the regulatory properties that have been established for rat liver CT. The CT content of the cells also increased dramatically as detected by Western analysis (Fig. 3).

CT Messages. Two major CT mRNAs were detected in rat liver, estimated from Northern blots to be 1500 and 4000 bases (data not shown). When we probed poly(A)$^+$ extracts from other mammalian sources, such as the murine erythroleukemic cell line GM979, the larger band predominated or was the only band detectable. The CT mRNAs in rat liver are very rare, estimated to constitute from 0.0005% to 0.005% of

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                 1 MDAQSSAKVNSRKRRKEVPGPNGATEEDGIPSKVQ R
                    :   |  :   | :::         ||   || |
  1 MANPTTGKSSIRAKLSNSSLSNLFKKNKNKRQREETEEQDNEDKDESKNQ Y

 36 ....RCAVGLRQPAPFSDEIEVDFSKPYVRVTMEE........ACRGTPC R
           :  |    :: |:|    :    :  |              |
 51 DENKDTQLTPRKRRRLTKEFEEKEARYTNELPKELRKYRPKGFRFNLPPT Y

 74 ERPVRVYADGIFDLFHSSHARALMQAKNLFPNTYLIVGVCCDELTHNFKG R
    :||:|:||||:|||||  | : | | |  |||  |||||  | :|| :||
101 DRPIRIYADGVFDLFHLGHMKQLEQCKKAFPNVTLIVGVPSDKITHKLKG Y

124 FTVMNENERYDAVQHCRYVDEVVRNAPWTLTPEFLAEHRIDFVAHDDIPY R
    :||: : :| : : ||| ||||| |||| :||||| ||:|| ||||||||
151 LTVLTDKQRCETLTHCRWVDEVVPNAPWCVTPEFLLEHKIDHVAHDDIPY Y

174 SSAGSDDVYKHIKEAGMFAPTQRTEGISTSDIITRIVRDYDVYARRNLQR R
     || |||:|| ||| | |  |||| |:|||||||:|:|||| |  ||: |
201 VSADSDDIYKPIKEMGKFLTTQRTNGVSTSDIITKIIRDYDKYLMRNFAR Y

224 GYTAKELNVSFI....NEKKYHLQERVDKVKKKVKDVEEKSKEFVQKVEE R
    | |  |||||::     | | |::|    :||   ::   |:::   | |
251 GATRQELNVSWLKKNELEFKKHINEFRSYFKKNQTNLNNASRDLYFEVRE Y

270 KSI..DLIQKWEEKSREFIGSFLEMFGPEGALKHMLKEGKGRMLQAISPK R
      :   |  |:  |   :||  |           :| :   | |
301 ILLKKTLGKKLYSK...LIGNELKKQNQRQRKQNFLDDPFTRKL...... Y

318 QSPSSSPTHERSPSPSFRWPFSGKTSPSSSPA.................. R
            :| ||   |   |:|  |   ||
342 .......IREASPATEFANEFTGENSTAKSPDDNGNLFSQEDDEDTNSNN Y

350 ....SLSRCKAVTCDISEDEED 367                         R
        | |     :   |||::|
385 TNTNSDSDSNTNSTPPSEDDDDNDRLTLENLTQKKKQSAN 424       Y
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FIG. 2. Comparison of rat liver (R) and yeast (Y) CT protein sequences. Vertical lines indicate identical matches, and colons indicate conservative changes. Gaps in alignment are indicated by dots.

the total liver mRNA. CT mRNAs were quantified by comparison to autoradiographic signals from known amounts of denatured restriction enzyme fragments of the CT cDNA, which were coelectrophoresed and blotted with the RNA.

DISCUSSION

The identity of our clone as a bona fide CT cDNA is based on four criteria: (*i*) Extensive homology to the yeast protein sequence in the central domain, (*ii*) inclusion of all four of the sequenced tryptic peptides in the translated protein sequence, (*iii*) a 10-fold increase in the lipid-dependent specific activity of the transferase after transfection of COS cells with an expression vector containing the cloned cDNA, and (*iv*) increased levels of a protein recognized by anti-CT antibody in the COS cells after transfection with the cloned cDNA.

The central region of the rat CT may house the active site, based on the close sequence homology with the yeast CT. Homologies to other nucleotidyltransferases in this region are not strong and do not identify any highly conserved sequences. Protein kinase C shares some regulatory properties with CT in that anionic phospholipids, diacylglycerols, and fatty acids activate it and sphingosine is inhibitory (37). However, homologies between CT and protein kinase C were restricted to the Ser/Pro-rich motifs that occur in the C-terminal region of rat CT (Fig. 1*D*) and in the variable region V_3 of several protein kinase subtypes (38). Two zinc finger-like domains are present in all protein kinase subtypes (38) and appear to be required for phorbol ester binding (39). There is also a potential zinc finger of the form Cys-Xaa_4-Cys-Xaa_{15}-His-Xaa_2-His at residues 68–92 of rat CT.

Table 1. CT activity in COS cells transfected with pAX111 or pAX-RCT

	Lipid*	CT specific activity	
		pAX111	pAX-RCT
Homogenate	−	0.76 ± 0.19	2.6 ± 0.8
	+	1.46 ± 0.05	15.1 ± 3.0
Cytosol	−	0.2 ± 0.1	1.2 ± 0.22
	+	2.2 ± 0.6	22.8 ± 3.4
Microsomes	−	0.71 ± 0.01	2.15 ± 0.05
	+	0.8 ± 0.26	7.2 ± 2.2

Enzyme specific activity (mean ± SD of three separate transfections) is expressed as nmol/min per mg of protein.
*Sonicated vesicles (0.2 mM) consisting of phosphatidylcholine and oleic acid, 1:1 molar ratio (ref. 16).

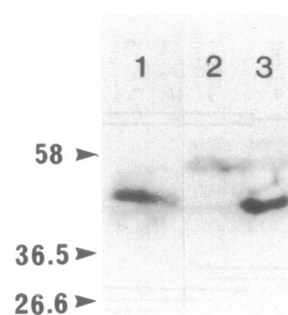

FIG. 3. Western blot of transfected COS cell homogenates. Lane 1, purified rat liver CT (2 μg); lane 2, homogenate protein (300 μg) from COS cells transfected with pAX111 vector; lane 3, homogenate protein (300 μg) from COS cells transfected with pAX-RCT. Molecular weights ($M_r \times 10^{-3}$) of prestained protein standards (Sigma) are indicated.

The most striking feature of the rat sequence was found at the point of divergence from the yeast sequence. This region from Lys-228 to Gly-287 was predicted to be an unbroken α-helix by the algorithm of Garnier *et al.* (40). Similarly, the Chou–Fasman algorithm (41) predicted an α-helix between Asn-236 and Gly-294 interrupted briefly by β structure at residues 272–276. Within this domain an 11-mer motif is repeated three times in tandem: VEEKSKEFVQK VEEKSIDLIQK WEEKSREFIGS (residues 256–288). A strongly asymmetric distribution of polar and hydrophobic residues is evident when this region is displayed as a helical wheel (42), as shown in Fig. 4. The hydrophobic face is interrupted by 3 serines, aligned 11 residues apart at positions 260, 271, and 282, which are included in the 11-mer repeat. The polar face has an extraordinary concentration of charged residues. From position 239 to 286 there are 26 charged residues,

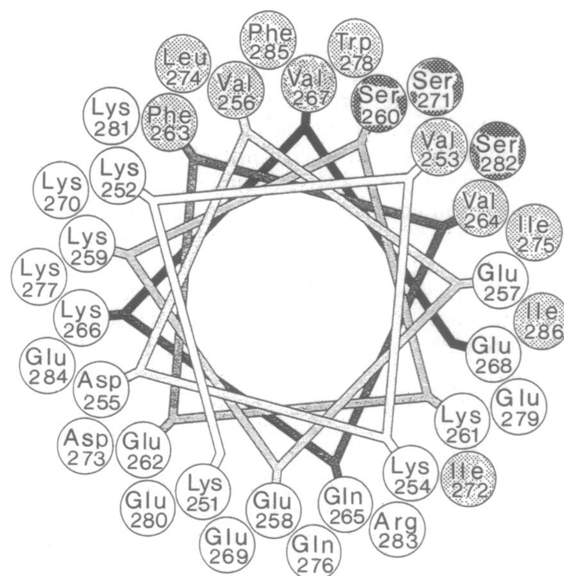

FIG. 4. Helical-wheel representation (42) of residues Lys-251 to Ile-286. Hydrophobic residues are lightly shaded, and the three interrupting serines are darkly shaded.

grouped in alternating clusters of negative and positive with the potential for numerous salt bridges between acidic and basic residues in the i and $i + 3$ or $i + 4$ positions, which would stabilize the helix (43). The average hydrophobicity on the nonpolar face is 2.8 based on a scale in which values range from −4.5 for arginine to +4.5 for isoleucine (44). The helical hydrophobic moment, $\langle\mu\rangle$ (45), a measure of the amphipathy, is high thoughout this region with a mean value per residue of 0.52. For comparison, $\langle\mu\rangle = 0.4$ for the amphipathic peptide mellitin (45).

The α-helix described above is predicted to be terminated at residues 294–297 by 2 glycines and 1 proline (41). Following this interruption another α-helical domain is predicted from Ala-298 to Ser-315 (40, 41). This potential helix is also amphipathic ($\langle\mu\rangle = 0.399$), and the ratio of basic to acidic residues is 5:1.

In the absence of a long stretch of nonpolar residues anywhere in the entire sequence, and in the absence of any signals indicative of covalent lipid attachment, we hypothesize that the amphipathic helix constitutes the membrane-binding domain, in whole or in part. Our model for the interaction of CT with membrane lipids is based on the interaction of the apolipoproteins with lipids (46, 47). Like CT, apolipoprotein A-I contains an 11-mer repeat within an extensive amphipathic helix (46). In addition, the lipid-binding domain of a lipid-requiring soluble enzyme from *E. coli*, pyruvate oxidase, lies near or within an amphipathic peptide (48, 49). We postulate that in its active, membrane-bound conformation, the helix lies on the surface of the membrane with its axis parallel to the plane of the membrane. The nonpolar face would make intimate contact with the lipids such that the hydrophobic side chains would penetrate into the lipid hydrocarbon core. This interaction, limited mainly to the membrane surface, would account for the observed hydrophobic nature of the CT–membrane interaction (10, 25) and the observation that CT can dissociate from the membrane without disintegration of bilayer structure (10, 12, 13, 25). Intercalation is supported by recent findings that activation of CT by membranes is enhanced at the lipid phase transition (R.B.C., unpublished data). This phenomenon is associated with the generation of lipid packing defects, which can facilitate peptide intercalation (50).

The amphipathic α-helix, with its many internal salt bridges, appears to be a stable structure that might be present in the conformation assumed in an aqueous environment. Both membrane-bound and cytosolic CT (L form) appear to be dimeric (16, 51). The amphipathic helix, in addition to or in place of its role in membrane binding, could mediate the protein–protein interactions of the dimer in the manner of a coiled coil (52).

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