The Biosynthesis of Cytidine Diphosphate Diacylglycerol in the Mammalian Cerebral Cortex

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The liponucleotide CDP-diacylglycerol can be regarded as an activated form of phosphatidic acid, which in eukaryotic systems is believed to be a precursor in phosphatidylinositol, phosphatidylglycerol and cardiolipin synthesis (G. A. Thompson, 1973). The formation of CDP-diacylglycerol and its participation in phosphatidylinositol synthesis apparently proceeds thus:

\[
\text{CTP} + \text{phosphatidic acid} \rightarrow \text{CDP-diacylglycerol} + \text{pyrophosphate} \quad (1)
\]

\[
\text{CDP-diacylglycerol} + \text{inositol} \rightarrow \text{phosphatidylinositol} + \text{CMP} \quad (2)
\]

Although the above reactions were originally proposed many years ago (Agranoff et al., 1958; Paulus & Kennedy, 1960), the liponucleotide has only very recently been isolated from biological systems. In bacteria, where CDP-diacylglycerol serves as a precursor to all common phospholipids (Ambron & Pieringer, 1973), the cytidine liponucleotide pool has been shown to be an approximately equimolar mixture of CDP-diacylglycerol and dCDP-diacylglycerol (Raetz & Kennedy, 1973). Radioactively labelled CDP-diacylglycerol has been detected in the pineal gland (Hauser & Eichberg, 1975), and large-scale extraction procedures have led to the isolation of the liponucleotide from bovine liver and brain (Thompson & MacDonald, 1975, 1976). The above reports are the first clear demonstration that CDP-diacylglycerol actually exists in vivo. In both bacteria and mammalian tissues the intracellular concentration of liponucleotide appears to be very low, representing about 1-5% of the concentration of phosphatidic acid itself, and thus making it likely that the supply of CDP-diacylglycerol is the rate-limiting step in the pathways in which it participates (Raetz & Kennedy, 1973; Thompson & MacDonald, 1976).

It has been known for some years that cell nuclei isolated from the mammalian cerebral cortex actively incorporate CTP into an acid-insoluble product (Mandel et al., 1967). Following the development of subcellular fractionation methods for the isolation of cell-specific populations of nuclei from the brain (Løvtrup-Rein & McEwen, 1966; Kato & Kurokawa, 1967; Austoker et al., 1972; R. J. Thompson, 1973), it was noted that CTP incorporation was especially active in nuclei believed to be derived from neurons rather than from glial cells (Kato & Kurokawa, 1970; Austoker et al., 1972). The product of CTP incorporation by a population of neuronal nuclei derived from the rabbit cerebral cortex, the N1 nuclear population (R. J. Thompson, 1973), has been identified as CDP-diacylglycerol (Thompson, 1974, 1975), and preliminary evidence has been presented localizing this activity to the nuclear envelope (Thompson, 1976). Further experiments (R. J. Thompson, unpublished work) have shown that the CTP-phosphatidate cytidylyltransferase (EC 2.7.7.41) associated with the nuclear envelope will also form dCDP-diacylglycerol in an exactly analogous manner and appears to show no specificity towards CTP or dCTP. Unlike the situation in bacteria (Raetz & Kennedy, 1973), no dCDP-diacylglycerol could be detected in rat pineal gland (Hauser & Eichberg, 1975) or in bovine brain or liver (Thompson & MacDonald, 1975, 1976); possibly this reflects low concentrations of dCTP in vivo.

Since active incorporation of CTP seemed to be a property of neuronal nuclei (Kato & Kurokawa, 1970; Austoker et al., 1972), the rates of CDP-diacylglycerol synthesis by nuclei from other sources were examined to determine whether neuronal nuclei were unique in this activity. The nuclear separation method introduced for the rabbit cerebral cortex (R. J. Thompson, 1973) yields a second nuclear population, the N2 nuclear population, which consists of nuclei with the morphological characteristics of glial-cell nuclei. These nuclei were only approximately one-fifth as active.
Table 1. *Differential sensitivity of microsomal and mitochondrial CDP-diacylglycerol synthesis to Triton X-100*

The assay medium contained (final concentrations): Tris/HCl buffer, pH7.0; MgCl₂, 20mM; phosphatidic acid, 1mM; [H³]CTP (10μCi/μmol), 1mM. CDP-diacylglycerol synthesis was measured by the filter-disc method (Goldfine, 1966). Mitochondrial and microsomal fractions were isolated as described by Whittaker (1965).

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<th></th>
<th>Mitochondrial</th>
<th>Microsomal</th>
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<tbody>
<tr>
<td>Control</td>
<td>4.7</td>
<td>3.8</td>
</tr>
<tr>
<td>+Triton X-100 (0.5%, v/v)</td>
<td>4.9</td>
<td>0.24</td>
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in CDP-diacylglycerol synthesis as the N¹ nuclear population. Rabbit liver nuclei resembled glial nuclei rather than neuronal nuclei, but nuclei from the cerebellum, which largely originated from small granule-cell neurons, were significantly more active than glial-cell nuclei or liver nuclei, but less active than cortical neuronal nuclei (R. J. Thompson, unpublished work). CDP-diacylglycerol synthesis is not therefore confined to neuronal nuclei, but is possibly more active in nuclei derived from neurons than from other cell types. Significant CDP-diacylglycerol synthesis has been detected in rat liver nuclear preparations (Van Golde *et al.*, 1974).

The outer nuclear membrane is continuous with the endoplasmic reticulum (Kay & Johnston, 1973), and at least in guinea-pig liver (Carter & Kennedy, 1966; Davidson & Stanacev, 1974) and rat liver (Davidson & Stanacev, 1974; Van Golde *et al.*, 1974) the endoplasmic reticulum appears to be the main site of CDP-diacylglycerol synthesis. The CDP-diacylglycerol synthesis occurring in cortical nuclei could therefore reflect a general similarity between the nuclear envelope and the endoplasmic reticulum. A report on embryonic chick brain (Petzold & Agranoff, 1967) has proposed that CDP-diacylglycerol synthesis is largely mitochondrial, and a further report on rat brain preparations has claimed that CDP-diacylglycerol synthesis is entirely mitochondrial, and the microsomal fractions are devoid of activity (Cotman *et al.*, 1971). Table 1 shows that, at least with fractions prepared from rabbit cerebral cortex, both mitochondria and microsomal fractions show CDP-diacylglycerol synthesis. However, in the presence of 0.5% Triton-X-100 microsomal CDP-diacylglycerol synthesis is abolished, whereas mitochondrial activity is unaffected (Table 1). The use of a non-ionic detergent in the assay medium (Petzold & Agranoff, 1967; Cotman *et al.*, 1971) would therefore obscure microsomal synthesis and give the impression of an exclusively mitochondrial activity.

As nuclear CDP-diacylglycerol synthesis is also abolished by low concentrations of detergent (Thompson, 1975), it appears that the CDP-diacylglycerol synthesis associated with the nuclear envelope reflects generalized endoplasmic-reticulum activity. Bishop & Strickland (1976) have reported a similar detergent-sensitivity of rat microsomal preparations. At least some bacterial forms of CTP-phosphatidate cytidylyltransferase have been reported to require a non-ionic detergent for activity (McCaman & Finnerty, 1968); possibly mitochondrial CDP-diacylglycerol synthesis may be performed by a prokaryotic-type enzyme distinct from the enzyme in the endoplasmic reticulum.

The pioneering studies of Thompson & McDonald (1975, 1976) have shown that the fatty acid composition of CDP-diacylglycerol isolated from bovine liver and brain is unexpectedly similar to that of phosphatidylinositol in showing a marked preponderance of stearate and arachidonate, and is distinct from the fatty acid composition of tissue phosphatidate and cardiolipin. This suggests that the mitochondrial pool of CDP-diacylglycerol, which presumably serves as a precursor for phosphatidylglycerol and cardiolipin, either forms only a minor proportion of total

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cellular CDP-diacylglycerol or is not being isolated by the procedures used. The distinct fatty acid composition of CDP-diacylglycerol suggests several possibilities for its metabolic origin (Thompson & McDonald, 1975). First, CTP-phosphatidate cytidylyltransferase, catalysing reaction (1) above, could be selective for arachidonate-containing species of phosphatidic acid. The studies by Bishop & Strickland (1976) on CDP-diacylglycerol synthesis by rat brain preparations show very little selectivity, and this possibility is therefore unlikely. Secondly, CDP-diacylglycerol could arise by a reversal of reaction (2) above. This possibility has been suggested (Petzold & Agranoff, 1965), but reversibility of reaction (2) has never been convincingly demonstrated and attempts to show CDP-diacylglycerol synthesis from CMP and phosphatidylinositol have been unsuccessful (R. J. Thompson, unpublished work).

Thirdly, CDP-diacylglycerol could arise from a discrete pool of phosphatidic acid rich in stearate- and arachidonate-containing species. A final possibility is that the liponucleotide itself could undergo a deacylation and reacylation cycle to produce its distinctive composition.

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Thompson, R. J. (1973) J. Neurochem. 21, 19–40
Thompson, R. J. (1975) J. Neurochem. 25, 811–823
Thompson, R. J. (1976) Exp. Brain Res. 24, 21