D-[2-14C]glucose and D-[6-14C]glucose also appeared in the cell lipid fatty acyl chains.

We conclude that L-threonine is a preferred source of lipid carbon for culture form T. brucei under the conditions used. This appears to be unusual since the organism is neither energy- nor carbon- and nitrogen-limited in our growth medium (compare McGilvray & Morris, 1969). We also believe that this is the first report of the 'aminoacetone' pathway being utilized by a eukaryotic cell for the generation of acetate units to be used in lipid biosynthesis.


Biosynthesis of Phosphatidylcholine and Phosphatidylethanolamine by Germinating Soya Bean

JOHN L. HARWOOD

Department of Biochemistry, University College, P.O. Box 78, Cardiff CF1 1XL, Wales, U.K.

Phosphatidylcholine is the major phospholipid in almost every plant tissue that has been examined (Galliard, 1973). Not only is it an important component of cellular membranes but it may also play a role as acceptor (Vijay & Stumpf, 1971) or as substrate during fatty acid desaturation (Gurr et al., 1969; Roughan, 1970; Willemot & Verret, 1973).

In spite of these important functions, study of the synthesis of phosphatidylcholine has been rather neglected in higher plants. The molecule can be synthesized, via methylation, from phosphatidylethanolamine or by a CDP-base pathway. The latter can also form phosphatidylethanolamine, though it is not known whether the same enzymes are involved throughout (Galliard, 1973). We have studied these reactions by using the germinating soya bean, a plant which has a high content of the nitrogen-containing phospholipids (Harwood, 1975; Wagner & Wolff, 1964).

Phosphatidylcholine and phosphatidylethanolamine were both rapidly labelled, after an initial lag phase, from [32P]orthophosphate or [1-14C]acetate. Their specific radioactivities and the inhibition, by protein-synthesis inhibitors, of [1-14C]acetate incorporation into them were similar (Harwood, 1975). However, there were small differences in both radioactive and total fatty acid patterns between the two lipids.

The time-courses of labelling of phosphatidylcholine from [Me-14C]choline and of phosphatidylethanolamine from [2-14C]ethanolamine are shown in Fig. 1. In both cases there was a rapid increase in the labelling at about 16h germination, at a time when esterification of free fatty acids into phospholipids also increases (Harwood, 1975). Labelling then increased at a more steady rate, corresponding to emergence of the radicle. The rapid rates of labelling are surprising in view of the fact that the soya bean contains a large amount of stored phospholipid. The limiting factor for incorporation of the isotopes does not appear to be the entry of the radioactive precursors into the seed, since these are present in large amounts after very short times. Initially, the rate of entry of isotope corresponds to the amount of water imbibed. There was no labelling of phosphatidylcholine from [2-14C]ethanolamine, indicating the absence of the methylation pathway at this stage of germination.

The lag phase in labelling of phosphatidylethanolamine and phosphatidylcholine from radioactive precursors could be due to a need for enzyme synthesis to take place. The effect of the protein-synthesis inhibitors chloramphenicol and cycloheximide, neither of which caused much lowering of [14C]acetate incorporation (Harwood, 1975), was therefore tested. Cycloheximide (10 μg/ml) and chloramphenicol (50 μg/ml) inhibited labelling of the phospholipids to different extents, but these
Fig. 1. *Time-course of the incorporation of [Me-14C]choline into phosphatidylcholine and [2-14C]ethanolamine into phosphatidylethanolamine*

Results are expressed as a percentage of the values at 48 h germination and are the means of results for two experiments. (a) [14C]Choline uptake into phosphatidylcholine (○) and water-soluble compounds (●). (b) [14C]Ethanolamine uptake into phosphatidylethanolamine (△) and water-soluble compounds (▲).

Effects were not due to an inhibition of transport, since the uptake of [2-14C]ethanolamine and [Me-14C]choline was unaffected.

We have studied the distribution of molecular species of phosphatidylcholine and phosphatidylethanolamine by a combination of phospholipase A or phospholipase C digestion and AgNO₃-t.l.c. of the intact phospholipids. These lipids were also labelled from 14C-labelled precursors and separated into molecular species which were then compared for radioactivity. The most saturated and the most highly unsaturated molecular species of phosphatidylcholine did not contain incorporated [14C]choline. On the other hand, all molecular species of phosphatidylethanolamine were significantly labelled. However, one, which was characterized by large amounts of linoleic acid and oleic acid, contained 58% of the total radioactivity incorporated from [2-14C]ethanolamine. Thus, although both phospholipids are made by a CDP-base pathway, the molecular species and protein-synthesis inhibitor results indicate that there is at least one enzyme in the pathway which is specific for each lipid.

The final enzyme of the CDP-base pathways to both phosphatidylcholine and phosphatidylethanolamine (CDP-choline–diglyceride phosphorylcholine transferase, EC 2.7.8.2, and CDP-ethanolamine–diglyceride phosphorylethanolamine transferase, EC 2.7.8.1) have been demonstrated in subcellular fractions prepared from germinating soya bean. Both enzymes are most active (on a protein basis) in the

Vol. 4
microsomal fraction. The properties of the microsomal phosphorylcholinetransferase enzyme are similar to those reported for that from spinach leaves (Devor & Mudd, 1971), except that exogenous diglyceride was necessary for maximal rates, presumably because the activity of endogenous lipases was not high enough to generate a sufficient amount.

These results indicate that, whereas the CDP-base pathway operates to phosphatidylcholine and phosphatidylethanolamine in soya bean, methylation of the latter does not take place during the first 48 h of germination. Since there were a number of differences in synthesis of the two phospholipids, there is a need to elucidate which enzymes are different and which common in the two CDP-base pathways.

Devor, K. A. & Mudd, J. B. (1971) *J. Lipid Res.* 12, 403-411

A Partial Reaction of δ-Aminolaevulinate Synthetase from *Rhodopseudomonas spheroides*

A. LAGHAI and P. M. JORDAN

*Department of Physiology and Biochemistry, Medical and Biological Sciences Building, University of Southampton, Southampton SO9 3TU, U.K.*

δ-Aminolaevulinate synthetase (EC 2.3.1.37) (Jordan & Shemin, 1972) catalyses the reaction shown in eqn. (1). The enzyme requires pyridoxal phosphate for full catalytic activity.

\[
\begin{align*}
\text{CO}_2\text{H} & \quad \text{COCH}_2\text{CH}_3\text{CO}_2\text{H} \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{CH}_2\text{CO}_2\text{H} + \text{CH}_2 & \rightarrow \text{NH}_2 + \text{CO}_2 + \text{CoASH} \\
\text{NH}_2 & \quad \text{COSCoA} \\
\text{Glycine} + \text{succinyl-CoA} & \quad \delta\text{-aminolaevulinate}
\end{align*}
\]

Recent work on the mechanism of δ-aminolaevulinate synthetase (Akhtar & Jordan, 1968; Zaman et al., 1973; Abboud et al., 1974) has revealed many interesting features about the mechanism and stereochemistry of the reaction. From these studies it was predicted that, after the initial formation of the enzyme–pyridoxal phosphate–glycine Schiff-base complex, one of the α-hydrogen atoms of the glycine would be labilized to form a stabilized carbanion. To detect such a reaction it was necessary to have available a homogeneous preparation of the enzyme. With the use of *Rhodopseudomonas spheroides* (70g), grown semi-anaerobically in the light, the method of Warnick & Burnham (1971) was followed up to the (NH₄)₂SO₄ stage. Thereafter the procedure was modified by the utilization of a heat-treatment stage in the presence of pyridoxal phosphate and glycine and subsequent chromatography on DEAE-cellulose, calcium phosphate and DEAE-Sephadex (A-25). The enzyme obtained from the DEAE-Sephadex was in two fractions, fractions I and II (Tuboi et al., 1970). The specific activity of fraction II (1.05mg) was 132μmol/h per mg. Both fractions were shown to be homogeneous by polyacrylamide-gel electrophoresis. Fraction II was used for all experiments as it was very stable compared with fraction I during prolonged incubation.

1976