DIACYLGlycerols: structure, composition, function and analysis

DIACYLGlycerols

STRUCTURE, COMPOSITION, FUNCTION AND ANALYSIS

1. Diacylglycerols – As Components of Oils and Fats

Diacylglycerols (or “diglycerides”) are esters of the trihydric alcohol glycerol in which two of the hydroxyl groups are esterified with long-chain fatty acids. They can exist in three stereochemical forms (see our web document on Triacylglycerols for a discussion of nomenclature) -

\[
\begin{align*}
& \text{sn-1,2-diacylglycerol} \\
& \text{sn-2,3-diacylglycerol} \\
& \text{sn-1,3-diacylglycerol}
\end{align*}
\]

A racemic mixture of sn-1,2- and 2,3-diacylglycerols are sometimes termed α,β-diacylglycerols, while sn-1,3-diacylglycerols may be designated α,α'-diacylglycerols.

α,β-Diacylglycerols are formed as intermediates in the hydrolysis of triacylglycerols by pancreatic lipase and other hydrolytic enzymes in animal tissues, and they are generated in seed oils by the action of plant lipases. They are important technologically in commercial seed oils, as small amounts can have a profound influence on the physical properties.

Edible oils consisting of 80% 1,3-diacylglycerols are marketed in Japan as nutritional supplements. It is claimed that they are metabolized in a different way from triacylglycerols with beneficial nutritional effects. The 1(3)-monoacylglycerols formed when they are digested are absorbed into tissues relatively poorly, apparently limiting the accumulation of fats in body tissues.

It should be noted that it is easy to generate diacylglycerols artefactually on storing and extracting tissues if inappropriate methods are used. Often, attempts are made to analyse 1,2-/2,3- and 1,3-diacylglycerols separately, but the data may not be meaningful as acyl migration occurs rapidly until an equilibrium mixture is formed that contains about 67% of the 1,3-isomer. All diacylglycerols will isomerize slowly on standing in inert solvents or in the dry state even at low temperatures.

Diacylglycerols can be recovered from tissues with minimal isomerization, if this is necessary, by extracting the tissues with non-alcoholic solvents such as diethyl ether or chloroform, taking care not to heat extracts at any stage. When pure positional isomers are required, it is necessary to chromatograph the partial glycerides on TLC plates coated with silica gel G impregnated with boric acid at a level of 10% of the adsorbent), using a solvent system of chloroform (alcohol-free)-acetone (96:4, v/v).

Routine determination of molecular species of diacylglycerols in oils and fats can be accomplished by various chromatographic methods of which high-temperature GC seems most appropriate, since information on the composition as well as the absolute amount is obtained in this way.
2. *sn*-1,2-Diacylglycerols in Tissues – Biological Functions

*sn*-1,2-Diacylglycerols tend to be minor components of most tissues in quantitative terms, but they are very important in animal tissues, as they function as second messengers in many cellular processes, modulating vital biochemical mechanisms. They arise by several mechanisms. For example, they are formed as intermediates both in the biosynthesis and catabolism of *triacyl*-sn-glycerols and in the biosynthesis of certain phospholipids. Thus, *sn*-1,2-diacylglycerol is a key intermediate in the formation of phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine. In this instance, phosphatidate phosphatase is an important enzyme, which converts phosphatidic acid to *sn*-1,2-diacylglycerol (and is discussed in greater detail in the web page dealing with *triacylglycerol biosynthesis*). The reverse reaction in which phosphatidic acid is produced by the action of a diacylglycerol kinase is also of great biological importance (see below).

Most of the precursor phosphatidic acid is generated via the *Kennedy pathway*, but a second mechanism involves the action of a specific phospholipase D on phosphatidylcholine. The latter can also be a precursor for diacylglycerol production via the action of phospholipase C, or by an exchange reaction with ceramide in the biosynthesis of sphingomyelin (a related pathway may be important in some pathogenic fungi). These reactions occur more slowly but are of longer duration.

More significantly in relation to their signalling function, *sn*-1,2-diacylglycerols are formed along with the important signalling molecules, the water-soluble inositol phosphates, by the action of the enzyme phospholipase C (a family of at least thirteen related enzymes in four sub-families exist) on phosphatidylinositol and the polyphosphoinositides and especially phosphatidylinositol-4,5-bisphosphate (see our web pages on these lipids for further discussion of this topic). These enzymes are activated by agonists at receptors on the cell surface, of which over one hundred types have been identified. The response is immediate if short-lived.

The fatty acid compositions of the diacylglycerols formed by these various routes then reflect the composition of the parent phospholipids. In particular, those derived from phosphatidylinositol are highly enriched in molecular species containing stearic in position *sn*-1 and arachidonic acid in position *sn*-2. There is evidence that the diacylglycerols in most cells and organelles must contain polyunsaturated fatty acids to fulfill their function as messengers optimally. However, in the cell nucleus it appears that there are two distinct pools of diacylglycerols with very different
compositions produced from phosphatidylinositides (polyunsaturated) and phosphatidylcholine (saturated and monoenoic) by specific stimuli, and these may have different functions.

Diacylglycerols accumulate transiently in membranes, where they bind via strong hydrophobic interactions to particular proteins, and then cause changes in the physical properties of the bilayer. As their polar head group is small, they tend to form inverted micellar structures. In practice, this means that they introduce small areas of unstable negative curvature in membranes that facilitate membrane fission or fusion. By exposing small areas of the apolar regions of neighbouring lipids, they improve the hydrophobic interactions with proteins within these membrane regions thereby affecting their activities. The fusion of biological membranes is of great importance for the proper functioning of cells, and diacylglycerols in membranes are able to facilitate this process partly via their specific physical properties and partly through activation of certain proteins.

It appears that the formation of diacylglycerols may be initiated at the plasma membrane, but subsequently the reaction at internal membranes becomes more important. In particular, there is a significant production of diacylglycerols at the nucleus in response to stimuli. It is thought that diacylglycerols produced for purposes other than signalling are segregated spatially within the cell.

An especially important function of sn-1,2-diacetylglycerols, and in particular those derived from phosphatidylinositol, is that they affect vital processes in cell physiology by activating members of the protein kinase C family of enzymes, often acting in concert with the soluble phosphoinositides. The sn-1,2-configuration is essential for this activity. Diacylglycerols appear to function then by increasing the concentration of calcium ions in the cell, which stimulates the translocation of the various iso-enzymes of protein kinase C to the inner face of the plasma membrane. They are bound in a 1:1 ratio to the enzymes at a highly conserved cysteine-rich ‘C1’ domain, which consists of a sequence of 50 amino acids with a characteristic motif. Other conserved regions such as the ‘C2’ domain assist in membrane recruitment of the kinase by interaction with specific phospholipids, especially phosphatidyserine, often by a mechanism triggered by Ca²⁺. Thus, kinases regulated by lipid second-messengers contain one or more membrane-targeting modules, which result in protein kinase activation, typically by relieving autoinhibitory constraints.

The protein kinase C enzymes are involved in both short- and long-term modifications of normal cellular physiology with more than a hundred substrates identified to date. Of particular importance is the finding that the tumour-promoting phorbol esters mimic the activity of diacylglycerols and activate the same enzymes. Diacylglycerols therefore have a key role in the pathophysiology of cancer and other disease states. In addition, the identification of non-kinase receptors of sn-1,2-diacetylglycerols, many but not all of which have the conserved C1 domain, has revealed new and strategic functions in regulating cellular responses and in cytoskeletal remodelling.

Diacylglycerols also bind to protein kinase D, a cytosolic serine-threonine kinase that in turn binds to the trans-Golgi membrane network and regulates transport of proteins to the cell surface. Protein transport is blocked in the absence of diacylglycerols.

There appears to be little evidence for the existence of a diacylglycerol signalling pathway in higher plants. These do not have enzymes of the protein kinase C family, although there are some proteins with similar functions. Rather diacylglycerols generated by the action of phospholipase C are rapidly phosphorylated by diacylglycerol kinases to phosphatidic acid, which appears to be the key second messenger in plants.

In insects, although lipids are stored in the form of triacylglycerols in fat bodies, they are transported in hemolymph (the insect equivalent of plasma) in the form of sn-1,2-diacetylglycerols bound to the lipoprotein lipophorin to those tissues where they are required as a source of energy (see our webpage on lipoproteins).
3. Deactivation of sn-1,2-Diacylglycerols in Tissues

sn-1,2-Diacylglycerols serve as precursors for biosynthesis of phosphatidic acid via the action of diacylglycerol kinases (as illustrated above) and of 2-arachidonylglycerol (see our webpage on these lipids), both of which also have second messenger functions. In particular in mammals, there is a family of at least ten diacylglycerol kinase isoenzymes (in five subfamilies), which are structurally related to the sphingosine kinase, and each of which may have slightly different properties and functions. They each may be segregated in distinct cellular organelles and activated by different means. Some are cytosolic, some are associated with membranes and some are located within the nucleus. In the brain different isoenzymes are expressed in different types of neuron, some of which have several isoenzymes. These enzymes have a negative effect on signalling by diacylglycerols by reducing their concentrations in cells, but they are believed to generate phosphatidic acid with specific signalling functions, rather than simply to serve as a precursor of other lipids.

Diacylglycerols are also formed in bacteria as a by-product of the biosynthesis of lipoteichoic acids and membrane-derived oligosaccharides from phosphatidylglycerol. These have the potential to be disruptive to membranes and are rapidly converted to phosphatidic acid and thence to other phospholipids by diacylglycerol kinases.

Recommended Reading


**William W. Christie**

*James Hutton Institute (and Mylnefield Lipid Analysis), Invergowrie, Dundee (DD2 5DA), Scotland*

Last updated: April 26th, 2013