TRIACYLGLYCEROL BIOSYNTHESIS IN OILSEED RAPE

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Introduction

Recently Roughan and Stack (1982) proposed a mechanism for the synthesis of triacylglycerols (TAG) in seeds having oils rich in polyunsaturated fatty acids (FA) with phosphatidyl choline (PC) as a key intermediate. PC served as both a substrate in the desaturation of oleic acid (18:1(9)) to linoleic (18:2(9,12)) and 18:2(9,12) to linolenic acid (18:3(9,12,15) and a donor of polyunsaturated FA for the acylation of diacylglycerols (DAG). Further PC was the precursor of DAG which were subsequently acylated to form TAG. Seeds with oils containing mainly long chain monoenoic acids (eicosenoic acid (20:1(11)) and erucic acid (22:1(13)) were thought to synthesise TAG by a classical Kennedy-type pathway (Gurr, Blades, Appleby, Smith, Robinson and Nichols, 1974; Roughan and Slack, 1982).

Some preliminary work has been carried out in an attempt to elucidate the mechanism of TAG biosynthesis in immature seed of a high erucic acid rapeseed cultivar.

Experimental and Discussion

Time-course and pulse-chase experiments were carried out using [2-^{14}C]-acetate as tracer supplied to immature seeds at a developmental stage when oil was accumulating rapidly. Lipds were extracted from the tissues and separated into the following lipid classes, TAG, phospholipid (PL), DAG and

free fatty acids (FFA). Individual components of the PL fraction were isolated. TAG were separated into their component molecular species. FA mass and radiolabel (when sufficient activity was available) composition was determined on fractions and individual components as appropriate.

^{14}C-acetate was rapidly incorporated into TAG and even after the shortest incubation period of a few minutes duration, these glycerides were the predominantly labelled lipids. As expected, the major TAG molecular species were the heaviest labelled components of this fraction. 22:1(13) and to a much lesser extent 20:1(11) contained the highest proportion of radiolabel. Presumably these FA were labelled in the terminal carbons (adjacent to the carboxyl group) in a manner described by Downey and Craig, (1964). Incorporation of radioactivity in PL and DAG was slow and very much lower than the TAG.

Phosphatidyl choline (PC) was the highest labelled PL. After short incubation times 18:1(9) was the most radioactive FA in PC but with time the activity appeared almost quantitatively in 18:2(9,12) and 18:3(9,12,15) although the former was always more strongly labelled than the latter. 22:1(13) and 20:1(11) were only weakly labelled in PC. This somewhat incomplete data is being taken as indicative that PC may be involved in the desaturation of 18:1(9) and 18:2(9,12).

Less label was incorporated into DAG than PL. Further the FA mass and radiolabel composition of this fraction was in all cases different from that of PC. There was a close similarity in the FA mass and radiolabel composition of this fraction. DAG or individual species thereof may be important in TAG biosynthesis. The FA mass and radioactive composition of the FFA were different from each other and did not resemble that of any other major lipid fraction.

The data obtained although incomplete are taken to indicate that the major TAG in rapeseed are synthesised by a classical Kennedy-type pathway and not via DAG derived from PC. Supporting evidence for this is provided by the large differences in FA mass and radiolabel composition of PC as a lipid class and DAG derivative thereof that could possibly act as a precursor of any major TAG molecular species. In addition, no individual molecular species of PC was identified with a FA mass composition resembling a possible DAG precursor of any of the three major TAG molecular species. From the FA mass and radiolabel data, the possibility exists that the DAG or more likely individual molecular species of this fraction may serve as precursors for TAG biosynthesis. Finally analyses of the major TAG molecular species revealed that the radiolabel was restricted almost entirely to 22:1(13) and 20:1(11) in positions 1 and 3 of the molecules even after prolonged incubation times. FA in position 2 of these TAG were always weakly labelled and from FA mass analysis were 18:1(9), 18:2(9,12) and 18:3(9,12,15).

This work will be reported in detail elsewhere.

References

Roughan, P.G. and Slack, C.R. (1982). Ann. Rev. Plant Physiol., 33, 97-132.

Gurr, M.I., Blades, J., Appleby, R.S., Smith, C.G., Robinson, M.P. and Nichols, B.W. (1974). Eur. J. Biochem. 43, 281-290.

Downey, R.K. and Craig, B.M. (1964). J. Am. Oil. Chem. Soc. 41, 475-478.