Triacylglycerol Biosynthesis in High Erucate Rapeseed

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Introduction

Two pathways for the biosynthesis of TAG in oilseeds have been described (Roughan and Slack, 1982). In both routes, the TAG are produced by the acylation of DAG acceptors by acyl-CoA. These DAG acceptors are obtained by two distinct pathways. In the Kennedy or Glycerol-3-phosphate pathway, the DAG acceptors are derived from phosphatidate which is formed by the sequential acylation of G3-P by acyl-CoA species (Kennedy, 1961). This route has been implicated in TAG biosynthesis in a number of oilseeds including those containing oils rich in long chain monoenoic acids [eicosenoic 20:1 (11c); docosenoic (erucic) 22:1 (13c)] such as Crambé (Gurr, Blades, Appleby, Smith, Robinson and Nichols, 1974) white mustard (Mukherjee, 1983) and rapeseed (Harris and Norton, 1983). Alternatively the DAG acceptor may be derived directly from phosphatidylcholine. This pathway has been shown to occur in oilseeds with TAG rich in polyunsaturated FA such as safflower, soyabean and linseed (Roughan and Slack, 1982). In these seeds it was shown that PC served both as a substrate for the desaturation of oleic acid [18:1 (9c)] to linoleic [18:2 (9c, 12c)] and linoleic [18:3 (9c, 12c, 15c)] acids in processes occurring in the ER and also as a donor of PUFA for the acylation of DAG to the TAG. At this time it is not known whether PUFA in the TAG of other oilseeds are produced in a similar way.

Of the other FA present in the TAG in oilseeds, palmitic acid (16:0) is synthesised de novo as the ACP-derivative and this is elongated to stearoyl-ACP (18:0) which in turn is desaturated to produce oleoyl-ACP. These processes are thought to occur in the soluble phase of the plastids of a number of oilseeds (Stumpf, 1977). Oleic acid is exported to the cytosol where it can be elongated as the CoA derivative to 20:1 (11c) and 22:1 (13c) in the soluble phase (Downey & Craig, 1964) or desaturated as indicated above.

Information on the synthesis of TAG, FA and other acyl lipids in rapeseed

is scanty despi the worldwide importance of the crop. The work reported here represents an extension of the preliminary findings on TAG biosynthesis in a high erucate variety (Harris and Norton, 1983).

Experimental

Excised embryos from immature seeds of oilseed rape (B.napus. spp. oleifera cv, Victor) grown in the field were used in time-course and pulse-chase experiments involving [1-^{14}C]-acetate and/or [^{3}H]-glycerol. Generally, these seeds were at the stage of rapid oil accumulation. After incubation the seeds were killed in a large excess of boiling propan-2-ol and the lipids extracted following homogenisation in $CHCl_3$/MeOH (2:1 v/v) at room temperature. Several changes of solvent were used in a protracted extraction. Lipid extracts (Christie 1982). The lipids were separated by tlc into the following classes: PL, 1,2-DAG(1,3-DAG), FFA and TAG. Individual PL components were isolated and the molecular species of PC, 1,2-DAG and TAG obtained by argentation tlc. Individual molecular species of 1,2-DAG and TAG were subjected to stereospecific analysis when quantities permitted. Throughout, FA and radiolabel (radioactivity permitting) composition of fractions and individual lipids was determined.

Results and discussion

[1-^{14}C]-acetate was rapidly incorporated into the acyl groups only of acyl lipids of immature rapeseed. Even after short incubation periods (5 min.) the TAG accounted for over 60% of the total activity incorporated into the lipids. The radioactivity assimilated into the individual TAG molecular species was proportional to the mass of these species in the mature seed. Thus species M_3, M_2D, M_2T accounted for almost 75% of the radioactivity found in the TAG fraction. Between 70-80% of the radiolabel was located in 20:1 (11c) and 22:1 (13c) in the proportions 1:4-5. 18:1 (9c) was the only other FA labelled appreciably. Presumably 20:1 (11c) and 22:1 (13c) were labelled in carbon 1 or carbons 1 and 3 respectively in the manner described by Downey and Craig (1964).

The kinetics and extent of labelling of the 1,2-DAG and PL were almost identical but completely different from those of the TAG. Both the DAG and PL pools were completely saturated within 30 min. whereas the incorporation of radiolabel into the TAG was linear over 180 min. PC was the major and highest labelled PL. After short incubation times (5 min) 18:1 (9c) was the highest labelled FA with relatively much smaller amounts in 18:2 (9c, 12c)

20:1 (11c) and 22:1 (13c). 18:3 (9c, 12c, 15c) was very weakly labelled. In time (180 min.) 18:2 (9c, 12c) and 18:1 (9c) were almost equally labelled with 20:1 (11c), 22:1 (13c) and 18:3 (9c, 12c, 15c) still weakly radioactive.

The 1,2-DAG fraction contained considerably more label than PC. In terms of FA mass and radiolabel composition the DAG fraction was appreciably different from PC. After prolonged incubation times (360 min.) the FA mass and radiolabel of the DAG were similar apart from 18:3 (9c, 12c, 15c) which was always low in radiolabel. Molecular species analysis of this fraction enabled specific precursors of the TAG to be identified. In neither short nor long-term pulse [^{14}C-acetate] chase experiments could radiolabel transfer from either PC or DAG to the TAG be detected. In view of the different kinetics and pattern of labelling of TAG compared with PC (PL generally) and 1,2-DAG it must be concluded that in short term labelling experiment, TAG are formed by the acylation of preformed and non-radioactive acceptor DAG.

[^{3}H]-glycerol incorporation into the acyl glycerols bore little qualitative or quantitative relationship to ^{14}C-labelling. The 1,2-DAG were rapidly labelled with the PL fraction slightly slower but similar in pattern. Both incorporation patterns were exponential. TAG labelling was slow and almost linear with time although calculated rates of TAG synthesis using incorporation data agreed closely with the measured rates of seeds in pods. Labelling of the TAG was again in proportion to the mass composition of the individual molecular species with M_3, M_2D and M_2T accounting for almost 70% of the activity incorporated. The PA pool in the tissue was found to be extremely small (mass and radiolabel) and clearly very active. Even after short incubation times PC was the predominantly labelled PL.

The labelling pattern obtained with [^{3}H]-glycerol is consistent with the biosynthesis of the TAG via the classical Kennedy pathway. 1,2-DAG became labelled extremely rapidly (1 min.). It is presumed that these were formed from the very small but equally active PA pool. Incorporation of glycerol (DAG) into TAG was slow and this may be indicative that acylation of the 1,2-DAG precursor occurs at a location remote from the 1,2-DAG synthesis site. This suggestion is consistent with the [^{14}C]-acetate labelling data since the radiolabel is almost entirely restricted to 22:1 (13c) and 20:1 (11c). It is known that these FA are located in equal proportions in positions 1 and 3 of the TAG (Norton and Harris, 1983) and it must be concluded that an acceptor 1,2-DAG is acylated by the heavily labelled 22:1 (13c) and 20:1 (11c) [formed

by elongation of 18:1 (9c)]. This incorporation would lead to asymmetrically labelled TAG (position 3) in short term ^{14}C incorporation studies. Stereo-specific analysis of TAG showed this to be the case. Acetate labelling data also revealed that the elongation site of 18:1 (9c) is remote from the de novo FA synthesis site which is closer to the PL and 1,2-DAG synthesis locations. In rapeseed, the incorporation of long chain monoenoic acids with 1,2-DAG (and PL) is very slow and always weak and must be taken to indicate that these FA are slow to enter the FA pool involved in PL and 1,2-DAG formation. 18:1 (9c), on the other hand, enters this pool relatively quickly but is slow to enter the FA pool involved in the final acylation of the DAG.

None of the experimental evidence supported the involvement of PC as a direct precursor of the 1,2-DAG acceptor for TAG biosynthesis. The FA mass and radiolabel composition of PC as a lipid class and the individual PC molecular species precluded such a role. Further, the labelling kinetics of PC and 1,2-DAG with either [^{14}C]-acetate or [^{3}H]-glycerol were inconsistent with PC playing a major role in the formation of DAG acceptors. PC, however, may be involved in the desaturation of 18:1 (9c) to 18:2 (9c, 12c) and 18:3 (9c, 12c, 15c) which could then be included in the TAG. These three FA occupy position 2 of the three major TAG and are only weakly labelled by [^{14}C]-acetate in order of decreasing intensity: 18:1 (9c) 18:2 (9c, 12c) 18:3 (9c, 12c, 15c). Even after prolonged incubation periods 18:3 (9c, 12c, 15c) was only weakly radioactive. Thus in TAG synthesis in rapeseed the 18 carbon FA are specifically directed to position 2 of the molecule whereas positions 1 and 3 are occupied by monoenoic acids, principally 22:1 (13c) and 20:1 (11c) but also 18:1 (9c) and saturated FA to a much lesser extent (Norton and Harris, 1984).

The labelling data also indicates that individual FA are synthesised at different sites within the cell. 18:1 (9c) and presumably 18:0 and 16:0 are synthesised de novo and slowly enter two distinct pools of FA. One pool is involved in the formation of PL and DAG. 18:1 (9c) is desaturated to 18:2 (9c, 12c) and 18:3 (9c, 12c, 15c) in association with PC. The other pool of FA is involved in the final acylation of 1,2-DAG to yield TAG. This location is close to and probably identical with the elongation site for 18:1 (9c) to 20:1 (11c) and 22:1 (13c) [and possibly 18:0 to 20:0 and 22:0] since these FA are both labelled and incorporated rapidly into the TAG. 20:1 (11c) and 22:1

(18:1 enter the first pool extremely slowly and labelling of 1,2-DAG and PL with these two acids is always weak. The incorporation of 18:1 (9c) into these lipids is more significant indicating that this FA has more ready access to this pool compared with the second pool. These labelling patterns are similar to those obtained for other cruciferous seeds (Pollard and Stumpf, 1980; Mukherjee, 1984).

The following scheme is proposed for the biosynthesis of FA and TAG in oilseed rape.

References

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Biosynthesis of Fatty Acids and Triacyglycerols in Oilseed Rape.