Biochemistry of Development in Insects

Stereospecific Incorporation of Fatty Acids into Triacylglycerols

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(Received July 15/September 1, 1975)

1. Previous experiments showed that fatty acids were incorporated into triacylglycerols by homogenates of Ceratitis capitata larvae far more efficiently than by pharate adult homogenates. This metabolic behaviour of both stages of development of the insect has been interpreted throughout the existence of a different acyltransferase activity. To obtain new data on the acyltransferase mechanism, a time-course of the stereospecific incorporation of labelled myristic, palmitic, oleic and linoleic acids into the sn-positions of triacylglycerols has been followed.

2. Studies on the stereospecific incorporation of labelled fatty acids confirmed previous results. Palmitic acid was mainly incorporated into sn-1 and sn-3 positions whereas position 2 exhibited a low incorporation. Myristic acid acylated sn-3 position at a higher rate than it acylated the other sn-positions. Oleic acid was more specifically distributed than palmitic acid and linoleic acid was more efficiently incorporated than the monounsaturated acid. All these data reflect substrate differences in the acyltransferase activity of larval homogenates.

Pharate adult homogenates incorporated fatty acids very scarcely and mainly into positions (1 + 3).

3. Kinetics of incorporation of labelled fatty acids into the sn-positions points to a non-random distribution with respect to the major saturated and unsaturated fatty acids in triacylglycerols of larvae of Ceratitis capitata.

Among the differences [1–8] in the metabolic behaviour of the different stages of development of the insect Ceratitis capitata, the incorporation of fatty acids into triacylglycerols is one of the most outstanding facts regarding the lipid metabolism [9]. All fatty acids were more efficiently incorporated by larval homogenates than by pharate adult homogenates. Using a series of saturated and unsaturated labelled fatty acids, most of the radioactivity remained as free fatty acids in the presence of pharate adult homogenates whereas fatty acids were efficiently incorporated by larval homogenates into triacylglycerols [9]. This difference could not be interpreted through variations in the acyl-CoA synthetase activity of the insect as this enzyme activity was notably higher in pharate adult homogenates than in the larval ones whatever could be the nature of the fatty acid assayed. However, double-label experiments using [14C]glycerol 3-phosphate and 3H-labelled fatty acids suggested the existence of different acyltransferase activity levels in the various stages of development of the insect [9].

The purpose of the study reported herein was to obtain further information on the participation of the different positions of the glycerol moiety in the acyltransferase mechanism. With this aim, the stereospecific distribution of fatty acids in the triacylglycerols obtained after incubating larval and pharate adult homogenates with myristic, palmitic, oleic and linoleic acids was investigated. The stereospecific incorporation of these labelled fatty acids was followed at different incubation intervals.

MATERIALS AND METHODS

Materials

ATP, CoA, NADPH, liquid scintillation reagents and Crotalus atrox venom were purchased from Sigma Chemical Co. (St. Louis, Mo.). Pancreatic lipase was obtained from Calbiochem (Los Angeles, Cal.). (1-14C)-labelled myristic, palmitic, oleic and linoleic acids were obtained from The Radiochemical Centre (Amersham, Bucks).
**Rearing of Insects**

*Ceratitis capitata* (Wiedemann) was used during the larval and pharate adult stages of development. Conditions of culturing were previously reported [1].

**Incubation Mixture**

Insects were directly homogenized according to the method previously reported [2,8]. The composition of the incubation mixture was previously reported [9] and contained 22.6 μCi of the labelled fatty acid (sp. act. 40–60 Ci/mol) per 2.5 ml of total volume. Each ml of mixture contained 10 mg protein. Incubations were made in a shaker at 37 °C for the intervals described in the experiments. At the end of the incubation intervals the reaction was stopped by adding 1 vol. of chloroform and 2.1 vol. of methanol. The crude lipid extract was obtained and purified according to the reported methods [1,8]. Lipid extracts were fractionated on thin-layer plates of Kieselgel G and hexane/diethyl ether/acetic acid (70/30/1, v/v/v) as solvent system. Triacylglycerols were recovered from the corresponding bands with chloroform.

**Stereospecific Analysis of Triacylglycerols**

An adaptation of the procedures devised by Brockerhoff [10] and Christie and Moore [11] was used for the stereospecific analysis of triacylglycerols. Triacylglycerols (40 mg) were decyated by using ethyl magnesium bromide according to Christie and Moore [11]; 1,2(3)-diacylglycerols were purified on thin-layer plates of Kieselgel H using hexane/ethyl ether/acetate (60/40, v/v) as solvent system. Triacylglycerols were recovered from the corresponding bands with chloroform.

**Measurement of Radioactivity**

Lipid bands scraped from the plates were directly transferred to liquid scintillation vials containing 10 ml of the mixture of 10.5 g PPO, 0.5 g POPOP, 150 g naphthalene diluted to 1.5 l with dioxane and 300 ml of water.

**Calculation of Fatty Acid Incorporation**

Calculation of labelled fatty acid incorporation into the stereospecific positions of triacylglycerols (mol fatty acid/mol triacylglycerol) was carried out by the formula mol fatty acid/mol triacylglycerol = X'(mol triacylglycerol × specific activity of fatty acid) in which X is the radioactivity calculated for a stereospecific position according to X = X'(a × b), being X' the radioactivity measured for the corresponding lipid fraction; a, the radiochemical yield of the reactions of phosphorylation and hydrolysis; b, the radiochemical yield of the deacylation reaction.

Graphic data represent an average of three determinations (P < 0.05).

**RESULTS AND DISCUSSION**

Experiments were designed for incorporation in vitro of the labelled fatty acids, myristic, palmitic, oleic and linoleic acids into triacylglycerols by larval and pharate adult homogenates of the insect *Ceratitis capitata*. Labelled triacylglycerols were isolated after different incubation intervals by preparative thin-layer chromatography and then submitted to a stereospecific analysis procedure. Thus, radioactivity data are expressed as μmol of the corresponding fatty acid incorporated into each sn-position of the glycerol moiety of triacylglycerols synthesised over the time range 2 – 120 min.

Fig.1 shows the stereospecific distribution of oleic and palmitic acids into triacylglycerols by larval homogenates. After a rapid incorporation rate, maximal contents were attained in each position at about 10 – 20 min of incubation interval. Once the maximal incorporation levels were reached, the incorporation profile varied afterwards according to the position and the nature of the fatty acid. Palmitic acid was mainly incorporated into sn-1 and sn-3 positions in approximately equal overall abundance; after the maximum was reached, a slow decrease of the incorporation levels occurred. Position 2 exhibited a low incorporation of palmitic acid that remained practically unchanged during the whole incubation time.
The incorporation of oleic acid was more specifically distributed than palmitic acid, the sn-3 position having the highest content followed by the sn-2 and sn-1 positions. The rate of decrease of the oleic acid incorporation into each glycerol position was higher than that of the incorporation of palmitic acid.

These results confirmed previous experimentation [9] on the fatty acid specificity of the acyltransferase activity exhibited by larval homogenates.

Fig. 2 shows the stereospecific distribution of myristic and linoleic acids incorporated into triacylglycerols by larval homogenates; their incorporation patterns exhibited some similar characteristics to those of oleic and palmitic acids. The relative acylation rates by linoleic acid of the sn-positions of the glycerol moiety and the maximal abundance of the fatty acid at 10–20 min of incubation were very similar to those found for oleic acid. There was, however, some marked differences in the content change of linoleic acid in its stereospecific distribution in triacylglycerols during the incubation assays when compared to that of oleic acid. Levels of linoleic acid incorporated into triacylglycerols were higher than those of oleic acid and remained practically constant during incubation with larval homogenates. This observation may reflect substrate differences in the acyltransferase activity of larval homogenates.

Myristic acid acylated sn-3 position at a notably higher rate than it acylated the sn-1; however, its incorporation into position sn-1 continued at a uniform rate during the whole experiment. Myristic acid acylated position sn-2 at marked lower levels than the other two positions, as it was shown for the incorporation of palmitic acid.

Fig. 3 shows the distribution of palmitic and oleic acids incorporated into triacylglycerols by pharate adult homogenates of the insect. The low fatty acid incorporation into different positions of triacylglycerols catalyzed by pharate adult preparations confirmed previous results on the different metabolic activities
[1–8] among the several stages of development of the insect. On the other hand, this low radioactivity incorporation in the fatty acids of the triacylglycerols from pharate adults represented only a very small fractions of the precursor and it made difficult the accurate analysis of the stereospecific distribution; thus, the labelling was determined in positions 2 and (1 + 3). The presence of oleic acid in positions (1 + 3) exceeded that of palmitic acid by a factor of about two. The incorporation of both fatty acids, oleic acid and palmitic acid, was very scarce in position 2.

Fig. 4 summarizes the kinetics of incorporation of labelled myristic (14:0), palmitic (16:0), oleic (18:1) and linoleic (18:2) acids into the sn-positions of the triacylglycerols isolated from larval homogenates. In Fig. 4 data are also given of the stereospecific analysis of mass triacylglycerols of the insect larvae expressed as distribution percentages of a given fatty acid among the three positions. The evidence points to a non-random distribution with respect to the major saturated and unsaturated fatty acids in triacylglycerols. The stereospecific distribution changed during incubation intervals; at the earliest time point, the presence of the labelled fatty acids at the position sn-3 was the highest one to exhibit, afterwards, a rapid decrease towards the mass values of triacylglycerols. This decrease was counterbalanced by an increase of both the saturated fatty acids at the position sn-1 and the unsaturated fatty acids at the position sn-2. This increase exhibited also a clear tendency towards the higher mass analytical values. Percentages of saturated fatty acids did not change practically at the position sn-2 during the incubation intervals; changes in the percentages of unsaturated fatty acids at the position sn-1 were not also significant.

These data, as well as the previous results from double-label experiments [9], could be consistent with an initial rapid labelling of the position sn-3
that most likely accounted for by acylation of endo-
genous diacylglycerols. The lower initial labelling at
positions sn-1 and sn-2 could suggest the fatty acid
incorporation by a synthesis of diacylglycerols de novo
followed by an acyl-exchange mechanism that points
to a non-random distribution with respect to the
major saturated and polyunsaturated fatty acids.

These results suggest a non-random distribu-
tion in the triacylglycerols of the insect in agreement
with the positional specificity in diacylglycerol 3-phos-
phate biosynthesis pointed out by several authors [14–
19].

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