Türk. entomol. derg., 1998, **22** (2) : 93-99
ISSN 1010-6960

The distribution in lipid classes of fatty acids biosynthesized by the black cricket ***Melanogryllus desertus*** Pall. (Orthoptera: Gryllidae)

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Summary

The fatty acid compositions of various lipid classes such as phospholipid, monoacylglycerol, diacylglycerol and triacylglycerol in one-day old adults of the black cricket ***Melanogryllus desertus*** reared on fat-free artificial diet based on casein were analyzed by gas chromatographic methods.

There were significant differences among various lipid classes with respect to concentrations of some fatty acids biosynthesized by the insect, with linoleic acid being much more abundant in phospholipid fraction (50 %) than other fractions. In the monoacylglycerol and diacylglycerol fractions, palmitic acid was the most actively biosynthesized fatty acid. In the triacylglycerol, oleic acid predominated.

Key words: Black cricket, fatty acids, lipid classes, distribution

Anahtar sözcükler: ***Melanogryllus desertus***, yağ asitleri, lipid sınıfları, dağılım

Introduction

Fatty acids are compounds of basic significance of biology. Their roles in metabolic energy storage, cell and biomembrane structure, and regulatory physiology appear to apply, in a general way to most organisms. These general patterns apply to insects as well.

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Alınış (Received): 18. 8. 1997

Lipids play key roles in insects biochemistry as sources of energy, structural components and as hormones (Stanley-Samuelson et al., 1988). Generally triacylglycerols are major sources of energy storage while the polar lipids, especially phospholipids, function as structural components of membranes. In most insects, the largest part of the total fatty acids is associated with triacylglycerol. Triacylglycerols are used to meet the energy requirements of developing eggs, of insects that hibernate at one or another stage of development, and of locomotor activities. Fatty acids are also associated with the phospholipids. Phospholipids are component of cellular and subcellular biomembranes, where they make up part of the structure of cells and tissues. In that biomembranes are physical perimeters of organelles and cells, as well as physiological surfaces, fatty acids, particularly in phospholipids, are structurally and functionally involved in cell biology.

Triacylglycerols formed the major lipid class in ovaries, fat-body and newly-laid eggs, whereas diacylglycerols and phospholipid predominate in the haemolymph (Grapes et al., 1989). In insect using lipid as an energy source during flight or extended periods of rapid movement, diacylglycerols are the usual form in which fatty acids are transported in the haemolymph (Beenakers et al., 1985).

Linoleic acid, a polyunsaturate fatty acid, is known to be required for normal development of most insects (Dadd, 1985) and this fatty acid is a precursor to a number of biologically active compounds including prostaglandins and other eicosanoids, special attention was given to the patterns of its biosynthesis and its distribution in lipid classes (Cripps and Reñobales, 1988). There is good evidence that 15 species of insects from the orders Orthoptera, Isoptera, Homoptera and Neuroptera can synthesize linoleic acid de novo (Stanley-Samuelson et al., 1988).

Recently, we analysed fatty acids of unfractionated total lipids of the black cricket ***Melanogryllus desertus*** Pall. (Orthoptera; Gryllidae). Black cricet grown on fat-free synthetic diet by gas chromatographic methods and demonstrated the de novo biosynthesize of linoleic acid in the black cricket (Başhan and Çelik, 1995; Başhan, 1996).

Very few studies of the fatty acid composition of the lipid classes of the lipid classes have been performed for insects in general (Fast, 1970; Stanley-Samuelson et al., 1988) although fatty acid profiles are known to vary with lipid classes (Thompson and Barlow, 1983; Stanley-Samuelson and Dadd, 1983; Stanley-Samuelson and Pipa, 1984).

In this study, the total lipids of ***M. desertus*** grown on fat-free artificial diet based on casein were fractionated as phospholipid, monoacylglycerol, diacylglycerol, and triacylglycerol and these fractions were then compared in terms of their quantitative fatty acid compositions. So it was tried to determine the distribution of linoleic acid and other fatty acid in different lipid classes, which are biosynthesized by ***M. desertus.***

Materials and Methods

Rearing of insects

The eggs obtained from a stock culture (Başhan and Emre, 1988) were sterilized for 30 min in 0.025 % sodium hypochlorite solution containing Triton X, washed with sterile distilled water and 70 % ethanol (Getzin, 1962), and then transferred to moist, streile sand in a dish and incubated at 30°C for 11 days to obtain first instar nymphs. Nymphs were transferred to sterile plastic cups containing fat-free artificial diet based on casein (Başhan and Emre, 1988) and held at 30±1°C in the darkness. Relative humidity was 55±5%. One-day old adult crickets were used for fatty acid analyses of lipid classes.

Analysis of insects

In the analyses,mixed one-day old adults of ***M. desertus*** were used because the fatty acid compositions of males and females of black cricket were virtually identical (Başhan, 1996). Total lipids of insects were extracted by the method of Bligh and Dyer (1959). Autoxidation of unsaturated fatty acid was minimized by addition of 50 µl butylated hydroxytoluene (2 % in chloroform, w/v). For analysis of fatty acids in lipid classes, total lipids were applied to thin layer chromatography (TLC) plates (Silika Gel G, 20x20 cm, 0.25 mm gel thickness). The plates were developed in petroleum ether: diethyl ether: acetic acid (80:20:1, v/v) (Stanley-Samuelson and Dadd, 1983).

Fractions were visualized by spraying with 2'7'-dichlorofluorescein and viewing under UV light. Then bands corresponding in R_f to various lipid classes standards by using authentic standards (Sigma Chemical Co., St Louis, Mo.) were scraped into reaction tubes. The fatty acids in each fraction then transmethylated to fatty acid methyl esters by refluxing in acidified method for 9 min (Stanley-Samuelson and Dadd, 1983). Fatty acid methyl esters were extracted from the reaction mixture with hexane, concentrated and analysed by gas chromatography as described below.

Gas chromatography

The fatty acid methyl esters were concentrated, then analysed by gas chromatography. The methyl esters were chromatographed on a Ati Unicam 610 series capillary column (0.35mmx15m, 0.15µm film thickness, J&W Scientific, CA), a flame ionization detector and a Unicam 4815 recording integrator. All GC runs used temperature programming from 180 to 200 °C at 3 °C/min, with an initial 2 min hold period. Injections were made in split mode (25:1) and separations were carried out with N_2 carrier. Flow rates of gases: N_2+make up, 30 ml/min; hydrogen, 33 ml/min; dry air, 330 ml/min. Components were identified by comparisons of retention times with authentic standards.

Gas chromatographic analyses of fatty acid compositions in different lipid classes were evaluated statistically using variance analysis (Snedecor and Cochran, 1967). Duncan's (1955) Multiple Range Test was used to determine significance of the difference between means.

Results and Discussion

The fatty acid compositions of various lipid classes such as phospholipids, monoacylglycerols, diacylglycerols, and triacylglycerols derived from one-day old adults of black cricket ***M. desertus*** reared on fat-free artifical diets based on casein are set forth in Table 1. Qualitatively major fatty acids in the lipid classes of insects include palmitic (16:0), palmitoleic (16:1), stearic (18:0) and linoleic (18:2n-6) as expected from the analyses of other Gryllidae (Fast, 1967; Grapes et al., 1989) and most other insect groups (Thompson, 1973; Stanley-Samuelson et al., 1988).

Table 1. Proportions of fatty acids as percentages of total fatty acids associated with various lipid classes prepared from one-day old adult ***M. desertus*** reared on fat-free synthetic diet.

Fatty Acids	Phospholipid	Monoacylglycerol	Diacylglycerol	Triacylglycerol
12:0*	-	-	-	2.76 ± 0.73
14:0	-	-	-	6.64 ± 1.12
16:0	20.10 ± 1.18a	34.76 ± 1.31b	42.59 ± 1.33c	24.64 ± 1.16d
16:1	1.58 ± 0.13a	4.65 ± 0.68b	3.70 ± 0.23cc	4.28 ± 0.13b
18:0	11.11 ± 1.16a	5.01 ± 0.71b	9.25 ± 0.92c	6.78 ± 0.72d
18:1	16.93 ± 1.57a	33.69 ± 1.13b	29.62 ± 1.18c	35.24 ± 1.15b
18:2	50.26 ± 1.24a	21.50 ± 1.03b	15.74 ± 1.03c	20.35 ± 1.86b

Values are mean proportions (SD); n:3 analyses of individual each.

*: Means followed by the same latter are not significantly different ($p>0.05$).

As indicated by Thompson (1973), the qualitative fatty acids of all the insect orders are similar. Quantitatively, fatty acid compositions vary between the orders, families and species. The most striking characteristics of the fatty acid compositions of the insects are the high levels of palmitoleic acid (16:1) in dipterous insect and the high levels of myristic acid (14:0) in hemipterous insects (Thompson 1973; Stanley-Samuelson et al., 1988).

The same fatty acids generally predominate in all lipid classes from crickets analysed, but the percentage contributions differ between lipid classes in the ***M. desertus*** (Table 1).

The major fatty acids comprising the phospholipid fraction were 16:0, 16:1, 18:0, 18:1 and 18:2n-6 the most abundant component, accounting for 50 % phospholipid fatty acid. The triacylglycerols are composed primarily of 16:0, 18:1, and 18:2n-6, with 18:1 the predominant fatty acid (35 %). Also this fraction included at low proportions of lauric acid (12:0), myristic acid (14:0) were not detected in the other fractions.

The high proportions of palmitic acid (16:0) was found in the monoacylglycerol and diacylglycerol. This fatty acid, comprised 42 % of diacylglycerol and 34 % of monoacylglycerol.

No polyunsaturated fatty acids beyond linoleic acid (18:2n-6) and odd-chain components were detected in all lipid classes (Table 1).

It is substantially documented that the common C18 polyunsaturated fatty acids, especially linoleic acid, tend to occur preferentially in insect phospholipids (Pagani et al., 1980; Lambremont and Dial, 1980; Dikeman et al., 1981), and my data accord with this general situation, since linoleic acid was a considerably higher proportion for the phospholipid fatty acids than for those of the other lipid classes. High levels of this polyunsaturate fatty acid in phospholipid could be based on a number of physiological factors. One of the major functions of linoleic acid and polyunsaturated fatty acids in general, is a structural component of membranes to maintain proper fluidity and permeability. Linoleic acid also a precursor to prostaglandins in insects (Stanley-Samuelson and Loher, 1986).

In recent studies, we showed that the black cricket was able to biosynthesize linoleic acid, by introduction of a second double bond into the most common monounsaturated fatty acid, oleic acid (Başhan and Çelik, 1995; Başhan, 1996), as only 15 insect species have been found to synthesize this fatty acid (Stanley-Samuelson et al., 1988). In this single step, previously believed not to occur in any animal cell, a monounsaturated fatty acid is converted into a polyunsaturate.

The most abundant fatty acid in monoacylglycerol and diacylglycerol is palmitic acid that is the intermediate product of fatty acid synthetase. This is an expected result because saturated fatty acids such as palmitic acid are mainly accumulated in these fractions as shown in the other cricket, ***Acheta domesticus*** (Grapes et al., 1989) and other Dictyopteran ***Periplaneta americana*** (Borgeson et al., 1991).

Özet

Melanogryllus desertus **Pall. (Orthoptera: Gryllidae)'un sentezlediği yağ asitlerinin lipid sınıflarındaki dağılımı**

Kazein esaslı yağsız sentetik besin üzerinde yetiştirilen ***Melanogryllus desertus'*** (karaçekirge) un bir günlük ergin bireylerinin, fosfolipit, monoaçilgliserol, diaçilgliserol ve triaçilgliserol gibi çeşitli lipit sınıflarının yağ asiti içerikleri gaz kromatoğrafisi metodu ile analiz edildi.

Çeşitli lipit sınıfları arasında, böcek tarafından sentezlenen bazı yağ asitlerinin konsantrasyonları bakımından önemli farklar bulundu. Linoleik asit (18:2n-6), diğer fraksiyonlara oranla fosfolipit fraksiyonunda daha fazladır (% 50). Monoaçilgliserol ve diaçilgliserol fraksiyonlarında en aktif sentezlenen yağ asiti palmitik (16:0) asittir. Triaçilgliserolde ise en fazla bulunan yağ asidi ise oleik (18:1) asittir.

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