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Locust adipokinetic hormones mobilize diacylglycerols selectively

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ABSTRACT

The diacylglycerols (DG) molecular species and their fatty acid (FA) composition were investigated by electrospray mass spectrometry (ESI-MS) and by gas chromatography with flame ionisation detection (GC-FID) in haemolymph of *Locusta migratoria* after application of adipokinetic hormones Locmi-AKH-I, -II and -III. The analyses showed (1) a heterogeneous distribution of individual DGs in haemolymph after the hormone application. The results revealed that mobilization of the DGs is molecular species-specific with the highest proportion of 34:1 DG (16:0/18:1 — mw 594 Da) for all Locmi-AKHs bearing palmitic acid (C16:0) and oleic acid (C18:1) residues, and forming about 20% of the total mobilized DG content. (2) Analysis of fat body triacylglycerols revealed that all Locmi-AKHs mobilize the DGs selectively with the preference of those possessing the C18 and C16 FAs. The fat body FAs with carbon chain longer than 18 did not participate in the mobilization. (3) A distribution of FAs in the DG structures obtained by LC/ESI-MS, and FA analysis by GC-FID after transmethylation indicated a certain degree of Locmi-AKH selectivity toward the mobilized DGs and hence the FAs. The Locmi-AKH-I significantly prefers mobilization of DGs containing unsaturated FAs, while Locmi-AKH-II and -III prefer mobilization of saturated FAs.

1. Introduction

Triacylglycerols (TGs) are a major lipid class serving as a reserve of metabolic energy which can be mobilized and utilized in response to bioenergetic demands. In insects TGs occur predominantly in the fat body (FB), an organ analogous to vertebrate adipose tissue and liver, where they represent about 90% of the stored lipids (Arrese and Wells, 1994; Canavoso et al., 1998). Some TGs are also present in the haemolymph. However, diacylglycerols (DGs) predominantly bound to carrier protein lipophorins, represent the major haemolymph lipids (Chino and Downer, 1982). At least 90% of haemolymph DGs are present as the *sn*-1,2-isomers and serve as the transport lipid form in insects, in contrast to vertebrates where the lipid transport is established by free fatty acids (FAs) (Van der Horst, 1982; Arrese et al., 1996).

Insect lipid mobilization is induced by adipokinetic hormones (AKHs), a large group of neuropeptides comprising 8–10 amino acids, which are synthesized and released from the corpora cardiaca (Gäde et al., 1997). Apart from the lipid mobilization these hormones control many aspects of insect energy metabolism and as typical pleiotropic factors also a number of accompanying processes (Kodrík, 2008). Generally, AKHs behave as typical stress hormones by stimulating catabolic reactions, making energy more available, while inhibiting synthetic reactions. Their crucial role in the activation of lipids is well-known and well documented (e.g. review articles—Canavoso et al., 2001; Gäde and Goldsworthy, 2003; Van der Horst and Ryan, 2005). Currently more than 40 insect AKHs have been characterized and their physiological effects investigated, particularly in *Locusta migratoria*, a classical insect model possessing three AKHs: Locmi-AKH-I, -II and -III, from which the AKH-I is the most effective in the lipid mobilization assay (Goldsworthy, 1994).

AKHs exert their effect on lipid mobilization via a signal transduction cascade, which is initiated upon binding of AKH to its G-protein coupled receptor on a FB cell (Gäde and Auerswald, 2003). This process leads to a conformational change of the G-protein, which in turn, activates adenylatecyclase, resulting in an increase of intracellular cyclic AMP level. This second messenger together with the intra- and extracellular Ca^{2+} activate a TG-lipase and subsequent 1,2-DG production (Arrese et al., 1996, 2006). The DGs are transported to the tissues by a "shuttle" lipophorin transport system through a mechanism which is not completely understood. The transported DG molecule is moved using a lipid transport particle (LTP) (Van Heusden and Law, 1989) to high density lipophorin (HDLp) in the haemolymph. HDLp stability and capacity is increased by apolipoprotein-III (apoLp-III) and the resulting particle called low density lipophorin (LDLp), has about twice the mass of HDLp. The LDLp transports the DGs into target tissues (mostly muscles) where the DGs are hydrolyzed by a lipophorin lipase. At the end the apoLp-III dissociates from LDLp. LDLp is changed to HDLp which is cycled back to the FB to transport the next DG molecule (Canavoso et al., 2001; Van der Horst and Ryan, 2005).

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The DG molecular species are characterized by individual FAs. Several dozen FAs have been described in the insect haemolymph, however, the major proportion is represented just by 8–9 FAs possessing 12–18 carbon atoms and including saturated FAs: lauric (C12:0), myristic (C14:0), palmitic (C16:0) and stearic (C18:0), the monounsaturated FAs: myristoleic (C14:1), palmitoleic (C16:1) and oleic (C18:1) and polyunsaturated FAs: linoleic (C18:2) and linolenic (C18:3). The monounsaturated FAs are composed primarily of the cis-Δ-9-isomer and the C18 polyunsaturated FAs are the cis-Δ-9-12- and cis-Δ-9-12-15 isomers (Downer, 1985; Schneider and Dorn, 1994).

Although the physiological, biochemical and metabolic roles of AKHs in an insect body have been intensively studied, as far as we know, the effect of AKHs on mobilization of the particular DG molecular species is undisclosed or have not been published yet. Therefore this study deals with the characterization of DGs mobilized from FB of the model species – *L. migratoria* – into the haemolymph after the injection of the Locmi-AKH-I, -II and -III. The results could contribute to solving of an interesting phenomenon that is not understood properly — why certain insect species possess more than one AKH.

2. Materials and methods

2.1. Chemicals

The *L. migratoria* adipokinetic hormone-I (Locmi-AKH-I: pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH_2) was purchased from Bachem (Switzerland). The adipokinetic hormones-II and -III (Locmi-AKH-II: pGlu-Leu-Asn-Phe-Ser-Ala-Gly-Trp-NH_2 and Locmi-AKH-III: pGlu-Leu-Asn-Phe-Thr-Pro-Trp-Trp-NH_2) were commercially synthesised by Dr. L. Lepša from the Vidia Company (Praha, Czech Republic). TG and DG standards, and other chemicals used for lipid extraction and HPLC, ESI-MS and GC-FID analyses, were purchased from Sigma-Aldrich (Praha, Czech Republic).

2.2. Experimental insects

Adult males and females of *L. migratoria*, 18 days after adult eclosion, were taken from the stock colony. The insects were reared on a standard diet of grass and carrot, supplemented with bran. They were kept under crowded conditions at a temperature of 30 °C under 12 h photoperiod.

2.3. Hormonal treatment and preparation of samples

A dose of 10 pmol Locmi-AKH-I, -II or -III dissolved in 5 μL 20% methanol in Ringer saline (Kodrík et al., 2000) was injected into the abdomen of experimental locusts. Control locusts were injected with 5 μL of solvent only. Haemolymph samples for evaluation of lipid mobilization were taken just before and 90 min after the injection from 10 locusts (5 males and 5 females). Haemocytes from the samples were removed by centrifuging at 13,000 *g* for 2 min at 4 °C. Fat bodies (FB) were dissected from the same aged intact locusts in a common way. Ten μL of the pooled haemolymph samples ($n = 3$; each n from 10 locusts) and the weighted FB samples (usually 40–50 mg from each locust/analysis, $n = 10$) were taken immediately after the dissection for lipid extraction using chloroform:methanol (ratio — 2:1) solution according to the method of Folch et al. (1957) as modified by Košťál and Šimek (1998).

2.4. HPLC/ESI-MS and GC-FID analyses

High performance liquid chromatography (HPLC) combined with electrospray ionization mass spectrometry (ESI-MS) was performed on a quadrupole ion trap LCQ mass spectrometer (Thermo, San Jose, CA, USA) coupled to a Rheos 2000 ternary HPLC system (Flux, Basel, Switzerland), equipped with a FAMOS autosampler and Thermos thermostat (both LC Packings-Dionex, Amsterdam, The Netherlands) as described in our earlier paper (Tomčala et al., 2006). The stored dry samples were dissolved in 1 mL methanol and 5 μL aliquots were injected into a 150 × 2.0 mm i.d. 3 μm Gemini HPLC column (Phenomenex, Torrance, CA, USA). The mobile phase was composed of (A) 5 mmol·L^{-1} ammonium acetate in methanol, (B) water, and (C) isopropanol. A linear gradient of A:B:C changing from 92:8:0 to 50:0:50 within 37·min was used with a flow rate of 150 μL min^{-1}. The column temperature was maintained at 30 °C. The mass spectrometer was operated in the positive ion detection mode at +4 kV with capillary temperature at 220 °C. Nitrogen was used as both the shielding and the auxiliary gas. Mass range of 440–1100 Da were scanned every 0.5 s to obtain the ESI mass spectra of the respective DGs and TGs. For the investigation of the DG and TG structures the CID (collision induced dissociation) multi-stage ion trap tandem mass spectra MS^2 were recorded with respective 5 and 3 Da isolation windows. Maximum ion injection time was 100 ms and the normalized collision energy was set to 30% for the CID MS^2 scan type. Neither DGs nor TGs posses any ionisable functional group and only sodium or ammonium adducts are observed in their ESI spectra. In mobile phases containing ammonium ion species only the ammonium adducts of molecules, $[M + NH_4]^+$ are observed in positive ESI, which enables the molecular weight of each DG or TG molecular species to be determined. No abundant ions corresponding to $[M + H]^+$ or $[M + Na]^+$ are observed. Number of carbons and double bonds can be calculates from molecular weight of particular DG or TG. The CID of $[M + NH_4]^+$ ions results in the neutral losses of NH_3 (i.e. molecular ion $[M + H]^+$ is observed) and acyl side-chain (as a carboxylic acid $[M\text{-}RCOO]^+$) to generate monoacyl product ion. This fragmentation is characteristic for all molecular species of DGs and TGs. For example two DGs with *m/z* 638 ($[M + NH_4]^+$ — DG of mw 620 Da) were present in all samples. Inspection of the CID MS^2 spectra revealed two isomeric DGs, namely DG 18:1/18:1 and DG 18:0/18:2 at 15.7 min and 16.2 min, respectively. The diagnostic fragment ions in the CID ESI-MS^2 spectra represented in former case mass *m/z* 339 (loss of C18:1), and in latter case masses *m/z* 337 (loss of C18:0) and *m/z* 341 (loss of C18:2) (see Mu and Hoy, 2000).

Samples of haemolymph for the gas chromatography (GC) analysis were extracted as mentioned above (chap. 2.3.) and the lipid classes were then separated by SPE (solid phase extraction). The fraction of non-polar lipids was treated with sodium methoxide and the FAs, released as methyl esters, were extracted into hexane by using a method described earlier (Hodková et al., 1999). Separation of the FAs

Table 1
Characterization of the Locmi-AKH-I, -II and -III stimulated haemolymph DGs. Their composition is characterized by a pair of FAs bound to the glycerol bone. FAs are described by the follow ratio — number of carbon atoms: number of double bonds in the molecule; the percentual proportion of two different FA variants within the DG molecules was deduced from the ESI-MS^2 fragmentation.

DG mw (Da)	Theoretical FA composition	Relative proportion of FA variants for individual AKHs (%)		
		AKH-I	AKH-II	AKH-III
566	14:0/18:1 or 16:0/16:1	50:50	0:100	50:50
568	16:0/16:0 or 14:0/18:0	60:40	60:40	60:40
590	16:0/18:3	100	100	100
592	16:0/18:2 or 16:1/18:1	80:20	100:0	33:66
594	16:0/18:1	100	100	100
596	16:0/18:0	100	100	100
612	18:3/18:3 or 18:2/18:4	50:50	50:50	66:33
614	18:2/18:3	100	100	100
616	18:2/18:2 or 18:1/18:3	50:50	33:66	33:66
618	18:1/18:2 or 18:0/18:3	66:33	50:50	50:50
620	18:1/18:1 or 18:0/18:2	33:66	66:33	66:33
622	18:0/18:1	100	100	100

Summary of identified FAs: 14:0 myristic, 16:0 palmitic, 18:0 stearic, 16:1 palmitoleic, 18:1 oleic, 18:2 linoleic, 18:3 linolenic, 18:4 stearidonic.