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Original article

A member of the short-chain dehydrogenase/reductase (SDR) superfamily is a target of the ecdysone response in honey bee (*Apis mellifera*) caste development

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Abstract – Many aspects in caste polyphenism result from hormonally controlled differential gene expression. A DDRT-PCR screen for ecdysteroid-regulated genes in ovaries revealed a set of ESTs coding for metabolic enzymes. For a cDNA encoding a short-chain dehydrogenase/reductase (SDR) we obtained the complete coding sequence (246 amino acids), revealing the protein motifs typical of insect SDRs. Its initially high expression in early fifth-instar larvae vanished in prepupae. Expression levels in worker larvae were higher than in queen larvae, suggesting negative regulation by the caste-specific ecdysteroid titer. This finding was confirmed by in vitro exposure of competent worker ovaries to makisterone A. In contrast to whole body RNA extracts, two SDR transcripts were detected in the ovaries. Both had their expression downregulated by makisterone A. Hormonal regulation and tissue-specific expression pattern makes this SDR an interesting enzyme for comparative molecular studies on social insect caste polyphenisms.

***Apis mellifera* / differential display PCR / ecdysteroid / alcohol dehydrogenase / caste polyphenism**

1. INTRODUCTION

Division of labor in highly eusocial insects is associated with a pronounced polyphenism, especially between the queen and worker caste. In the honey bee, *Apis mellifera* L., female larvae receive a caste-specific diet and consequently enter different developmental pathways, leading to an adult queen or a worker (for review, see Hartfelder and Engels, 1998). The most distinctive caste character in honey bees is the ovary phenotype. An adult queen ovary consists of approximately 180 ovarioles, whereas that of an adult worker contains only 2–12 ovarioles. Up to the fourth larval instar, the ovaries of both castes still exhibit equally high numbers of undeveloped ovarioles. However, during the last (fifth) larval instar, most of the ovarioles in the worker ovary exhibit signs of programmed cell death and completely degenerate before the pupal molt (Hartfelder and Steinbrück, 1997; Schmidt Capella and Hartfelder, 1998). Thus, the differentiation process leading to the two adult ovary phenotypes is part of the general metamorphosis program in honey bee development and, consequently, is ruled by juvenile hormone (JH) and ecdysteroids. Caste-specific titer profiles have been demonstrated for JH and also for ecdysteroids (Rachinsky et al., 1990), with makisterone A as the major ecdysteroid compound in honey bee hemolymph (Feldlaufer et al., 1985).

Little is known of the direct mode of action of hormones at the molecular level in honey bee caste development, with the exception of a

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report of caste-specific differences in the synthesis of two ecdysteroid-regulated proteins in the larval honey bee ovary (Hartfelder et al., 1995). These two ecdysteroid-regulated proteins were detected by two-dimensional fluorography analysis of ovarian proteins synthesized in the presence or absence of makisterone A. Indirect evidence for JH effects on caste- and stage-specific gene activity patterns was provided initially by Severson et al. (1989), based on analyses of in vitro translation products of whole body mRNA extracted from honey bee larvae. More powerful methods, such as suppression subtractive hybridization (Evans and Wheeler, 1999, 2000) and semi-quantitative analysis by reverse transcription-PCR (Corona et al., 1999) identified a large set of caste-specifically expressed genes in whole body extracts of queen and worker larvae of different ages. Many of these exhibited sequence similarity with genes encoding metabolic enzymes and storage proteins, and for genes related to RNA processing and translation. It is interesting to note that no genes with regulatory functions were isolated in these screens, but were detected in a differential expression study on the response of larval ovaries to a caste-specific makisterone A pulse (Hepperle and Hartfelder, 2001). While providing insight into overall patterns of caste-specific gene expression in vivo, such tissue-specific responses to metamorphic hormones were apparently diluted out in screens based on whole-body mRNA. This indicates that the elucidation of the precise mode of action of metamorphic hormones in caste development of social insects should be refined by single tissue analysis and corroborated by in vitro studies.

The present study was initiated as a differential-display reverse-transcription PCR (DDRT-PCR) approach (Liang and Pardee, 1992) to screen for ecdysteroid-regulated genes during a critical period in the development of the honey bee ovary. We focused on ecdysteroid-regulated genes and on a specific tissue because the molecular cascade of ecdysteroid action in insect metamorphosis is fairly well established, particularly for *Drosophila melanogaster* and the tobacco hornworm, *Manduca sexta* (Thummel, 2002; Henrich et al., 1999; Riddiford et al., 1999), and because stage- and tissue-specific responses to an ecdysone pulse are commonplace (Andres and Cherbas, 1992; Woodard et al., 1994; Baehrecke and Thummel, 1995). In the ovary of late fifth-instar worker larvae, we detected a repressive effect of makisterone A on the expression of transcripts putatively coding for enzymes involved in different aspects of energy metabolism. An expressed sequence tag (EST) coding for a short-chain dehydrogenase/reductase (SDR) was chosen for a more detailed study because of the general importance of this large enzyme family and the profound knowledge on SDR structure/function relationships (for recent reviews see, Kallberg et al., 2002; Tanaka, 2001). This family includes the short-chain alcohol dehydrogenase (ADH) of *Drosophila melanogaster* (Benach et al., 2001) which is one of the best-studied enzymes in insects.

2. MATERIALS AND METHODS

2.1. DDRT-PCR of ecdysone-responsive genes in honey bee ovaries

A detailed protocol for differential-display reverse-transcription PCR employed in the detection of ecdysone-responsive genes in the developing ovaries of last instar worker larvae of the honey bee (*Apis mellifera* L.) has previously been described (Hepperle and Hartfelder, 2001). In brief, ovaries were dissected from fifth-instar worker larvae and incubated for 3 h in honey bee-specific medium in the presence or absence of makisterone A. RNA extracted from batches of 120 ovaries per experiment was used in permutational combinations of DDRT-PCR reactions (GeneEx-Screen primer kit, Biometra, Göttingen). The radiolabeled PCR products obtained in identical primer combinations from makisterone A-treated and control ovaries were separated in parallel on 6% native acrylamide sequencing gels.

Bands containing differentially expressed transcripts were excised from the gels, reamplified, cloned and sequenced. The sequences of identified honey bee cDNA fragments were named according to the respective downstream (D) and upstream (U) primers, as well as fragment size, and were submitted to the GenBank EST database (accession numbers BG149167, BG149168 to BG149173; http://www.ncbi.nlm.nih.gov/Genbank/index.html).

2.2. Cloning and sequencing of honey bee short-chain reductase/ dehydrogenase (SDR)

Gene-specific primers designed against the 3'- (SDR1a: 5' TTA CTG GAG CTA ACT CGG 3') and 5'-ends (SDR1b*:* 5' CGA GTT GTG GAG CAA TTC 3') of the EST coding for a putative honey bee SDR (accession number BG149171) were employed in RACE-PCR protocols to obtain a full length coding sequence. For this purpose, total RNA was extracted from individual late-feeding stage worker larvae (L5F3) utilizing TRIzol reagent (Invitrogen). 3' RACE reactions were performed with an oligo-dT primer for first-strand cDNA synthesis (Invitrogen), followed by PCR (94 °C for 2 min, 30 cycles of 94 °C, 30 s, 50 °C, 1 min, 72 °C, 2 min, and an elongation step at 72 °C for 10 min) with the sense primer (SDR1a) and the oligo-dT primer. For the 5'-RACE reactions, we made use of the 5'-RACE-System (Rapid Amplification of cDNA Ends, Version 2.0, Invitrogen). PCR reactions (94 °C for 2 min, 30 cycles of 94 °C, 30 s, 54 °C, 1 min, 72 °C, 2 min, and an elongation step at 72 °C for 10 min) were performed with the polyC-adaptor primer and SDR1b.

PCR products were purified by agarose gel electrophoresis (1%), extracted from the gels (QIAquick-Gel Extraction kit, Qiagen), ligated into pGEM-T-Easy vector (Promega) at 16 °C for 12-16 hours, and cloned in competent *E. coli* DH5α-cells. Sequencing was performed by the dideoxynucleotide chain-termination method. Clones were sequenced on both strands using the M13F and M13R sequencing primers and internal, specific primers, in an automated fluorescence sequencing system ABI Prism 310 (Applied Biosystems).

2.3. Sequence comparison and analysis

The overlapping sequences obtained from the different 5' and 3' clones were joined into a single contig (Phred/Phrap/Consed) which was then submitted to a *blastx* search. ClustalW analyses with members of the SDR and MDR families retrieved from the *blastx* search aand protein motif analysis were performed online (http://clustalw.genome.ad.jp; http://meme.sdsc.edu/meme/website/). Database searches against characteristic motifs of protein families were submitted to PFAM and PRINTS (http://www.sanger.ac.uk/Software/Pfam/; http://bioinf.man.ac.uk/dbbrowser/PRINTS/). The cDNA sequence of the putative *Apis mellifera SDR* was submitted to GenBank (accession number AY217747). In addition to the deduced open-reading-frame sequence, it inludes information on the complete 3'- and partial 5'-UTR.

2.4. Northern Blot analysis of honey bee SDR expression

SDR expression was analyzed in two separate experiments. In the first one we analyzed the developmental patterns of SDR expression in whole body RNA extracts of fifth-instar queen and worker larvae, to gain information on caste-specific expression profiles during this critical period of caste differentiation. In the second experiment, RNA was extracted from fifth-instar worker ovaries exposed in vitro to makisterone A, i.e. the same developmental stage and set-up as used in the DDRT-PCR screen, to validate the DDRT-PCR results and to obtain information on tissue-specific expression and ecdysone response of honey bee SDR.

For the developmental time course of SDR expression, fifth-instar worker larvae in the appropriate stages were picked from brood frames of *Apis mellifera* colonies (Africanized stocks kept in the Experimental Apiary of the Dept. Genetics, Univ. São Paulo at Ribeirão Preto). Queen larvae were produced according to standard apicultural procedures, by transfer of first-instar larvae into queen cups and rearing to the appropriate stages in two-story hives separated by a queen excluder. The TRIzol extraction protocol for lipid-rich samples (Invitrogen) was adopted to extract whole body RNA from individual late-feeding-stage fifth-instar larvae, late-spinning-stage larvae and a mid-prepupal stage, corresponding to L5F3, L5S3 and PP2 of the stage classification of Rachinsky et al. (1990).

For probing the ecdysone response in ovaries in vitro, batches of 50 L5F3 worker larvae per sample were dissected and their ovaries were incubated for 6 h at 34.5 °C in 250 µL larval honey bee medium (Rachinsky and Hartfelder, 1998) supplemented with makisterone A (final conc. 5×10^{-7}M) (Hartfelder et al., 1995). Control ovaries were incubated for the same time without hormone. After removal of the medium, the ovaries of each incubation were lysed in 1 mL TRIzol for RNA extraction.

The RNA samples were run on a denaturing gel (1.2% agarose, 0.74% formaldehyde in MOPS buffer) and stained with ethidium bromide to visualize ribosomal RNAs. The gel was rinsed in 10 X SSC before transfer to a nylon membrane (Biodyne, Pall) by means of a high-salt buffer system (20 X SSC, overnight). After transfer, the RNAs were fixed by UV-exposure of the membrane for 4 min.

After pre-hybridization treatment of the membranes for 2 h at 65 °C in 5 X SSC supplemented with 0.1% dextrane sulfate and blocking solution (Amersham Biosciences), the

respective denatured probes were added and hybridization continued at 65 °C for 16 h. The probe used for detection was derived from the 800 bp clone corresponding to the 3'-end of *Apis mellifera SDR*, which was fluorescein-labeled following the kit protocol of Gene Images Random Prime (Amersham Biosciences). Stringency washes were performed for 15 min at 65 °C in 1 X SSC and 0.1 X SSC, both containing 0.1% SDS.

The detection procedure employed the Gene Images CDP-Star module (Amersham Biosciences) and exposure to Kodak XR-Omat film. Normalization was performed by reprobing the membranes with a fluorescein-labeled probe against 28S rRNA and by comparison to the corresponding ethidium-bromide stained rRNA bands in the agarose gels.

3. RESULTS

3.1. DDRT-PCR screen for ecdysone-responsive gene expression

When ovaries of late feeding-stage worker larvae were exposed to a queenlike makisterone A pulse in vitro and subsequently had their RNAs subjected to a DDRT-PCR protocol, we detected several ecdysteroid-responsive cDNA fragments. From this set of ecdysteroid-responsive ESTs, the ones exhibiting the most prominent differences were reamplified, cloned and sequenced. By *blastx* analyses, we could assign 6 cDNA fragments to 3 major functional groups (Tab. I). The other 13 amplification products could not be matched to known transcripts and thus may represent novel genes, or at least novel ESTs. Only two of these had their expression upregulated by makisterone A, while the rest were downregulated, as were all the 6 transcripts that could be assigned to functional groups.

Even though only a limited number of cDNAs could be retrieved in this tissue-specific screen for differentially expressed genes in honey bee caste development, we detected members of two functional groups that were not covered by the much larger cDNA screen on whole-body extracts of honey bee larvae (Evans and Wheeler, 1999, 2000; Corona et al., 1999). Sequences and putative regulatory properties of the transcription factors FTZ-F1-like and CUT-like have already been published (Hepperle and Hartfelder, 2001). A third honey bee EST, D5/U10-217, could be putatively assigned to the immunoglobulin superfamily of transmembrane proteins and growth factors (Tab. I). This 217 bp fragment aligned with *klingon* and *kekkon1*, encoding two *Drosophila* transmembrane proteins (Mussacchio and Perrimon, 1996; Butler et al., 1997; Adams et al., 2000; Duffy JB, unpublished data).

The other ecdysone-responsive honey bee ESTs that could be assigned to known protein families showed sequence similarites with enzymes catalyzing redox reactions. All of them were repressed by makisterone A in the ovaries of late feeding-phase worker larvae. The first one, the honey bee cDNA fragment D5/U14-530, exhibited a domain of 40 amino

Table I. Functional group assignment of ESTs corresponding to ecdysteroid-regulated genes in the ovary of larval honey bee workers. 19 cDNA fragments identified as differentially expressed in a DDRT-PCR screen on makisterone-A effects in ovary development were cloned and sequenced. GenBank accession numbers BG149167, and BG149169 to BG149173.

Functional group	Number of bee ESTs	blastx results*	Effect of makisterone A
transcription factor **	2	E = 1.3 (P33244)	inhibitory
		E = 4.9 (P53565)	inhibitory
cell signaling and growth factor	1	E = 17 (AAF56071)	inhibitory
metabolic enzyme	3	$E = 4e^{-07}$ (AAF48012.1)	inhibitory
		E = 1.3 (Q33568)	inhibitory
		E = 2.0 (AAD11826.1)	inhibitory
no match (unknown)	13		inhibitory and inducing

* In parentheses, accession number to blast match corresponding to the respective score; ** details in Hepperle and Hartfelder (2001).

Figure 1. Multiple alignment (ClustalW) of the deduced amino acid sequences of *Apis mellifera SDR* cDNA (GenBank AY217747) with *Drosophila melanogaster alcohol dehydrogenase* (*GenBank* AE003485) and *Anopheles gambiae Adh* (*GenBank* AAAB01008981). The detected sequence similarity of 30–32% (amino acid positions on grey background) is typical for insect SDRs. Bold-printed residues mark protein signature motifs characteristic of SDRs (bars above aligned sequences). Amino acids marked by # constitute the putative catalytic triad, while those marked by asterisks correspond to elements of the NAD(H)-coenzyme binding site.

acids with sequence similarity to NADH-oxidoreductases (GenBank accession number BG149170). The second one, cDNA fragment D3/U4-360, showed similarity to the family of cytochrome proteins. For the third one, the honey bee EST D9/U22-250, a *blastx* search revealed sequence similarity with short-chain dehydrogenases/reductases; the best fit ($E = 4e^{-7}$) being with an antennal-specific short-chain dehydrogenase/reductase of *Drosophila melanogaster* (Wang et al., 1999).

3.2. The cDNA of *Apis mellifera* short chain dehydrogenase/reductase

The *blastx* results permitting assignment of the EST D9/U22-250 to the SDR family of proteins and the fact that insect SDR alcohol dehydrogenases are among the molecularly best-characterized enzymes (Savakis and Ashburner, 1985) prompted us to fully sequence the cDNA of this enzyme by cloning 3'- and 5' cDNA fragments obtained by RACE-PCR. The assembled sequence of *Apis mellifera SDR* spanned 856 nucleotides. Conceptual translation of the nucleotide sequence gave a complete open reading frame encoding for 247 amino acids. In addition, the submitted honey bee *SDR* sequence (GenBank, accession number AY217747) also includes the complete 3'-UTR and 101 bp of the 5'-UTR.

At the 3'-end of the assembled SDR nucleotide sequence we found embedded (with 98% identity) the previously published EST sequence of clone 52A9 (*oxidoreductase-like protein* mRNA; dbEST-*GenBank accession number* AF134819) from the suppression-subtractive hybridization screen for caste- and stage-specific gene expression in honey bee larvae (Evans and Wheeler, 1999). ClustalW multiple alignment of the deduced protein sequence for *A. mellifera SDR* with *Drosophila melanogaster* a*ntennal-specific short-chain dehydrogenase/reductase* (*GenBank accession number* AE003485) and *Anopheles gambiae Adh* (*GenBank accession number* AAAB01008981) revealed identities of 30–32% (Fig. 1). Such relatively low degrees of similarity in protein sequence are typical for

members of the SDR family (Jörnvall et al., 1995; Kallberg et al., 2002).

To reveal conserved domains and signature motifs in *Apis mellifera SDR* we made use of online search tools. Submitting the deduced protein sequence to a PFAM search resulted in a fit to an alcohol dehydrogenase consensus sequence from residues 6 to 240. A conserved motif search by PRINTS detected the three conserved signatures characteristic of short-chain dehydrogenase/reductases. These covered similar domains as those revealed by the MEME/MAST search tools. The best conserved domain, which spans from residues 162 to 178, contained the motif YXXXK. This is the most characteristic signature of enzymes belonging to the SDR family (EC 1.1.1.). The second most relevant domain detected by PRINTS was a divided one, from residues 9 to 21 and 83 to 93, the first part containing the signature GXXXGXG, and the second one the NNA sequence of the SDR family.

3.3. Ecdysone response in the caste- and tissue-specific expression of honey bee SDR

Northern blot analysis of SDR expression in whole body extracts detected a single transcript of approximately 1.5 kb whose expression decreased markedly during the last larval instar of honey bee queens and workers (Fig. 2a). In both castes, expression levels were drastically reduced as the ecdysteroid titer increased during the late spinning and especially prepupal stages (Rachinsky et al., 1990). In addition, we noted clear caste-specific differences in SDR expression levels at the beginning of the fifth instar. These were considerably higher in worker larvae than in queens. Similarly, the Northern blot signal detected in spinning-stage worker larvae appeared much stronger than that in the corresponding developmental stage of queens. It was only in the mid-prepupal stage when SDR expression was reduced to undetectable levels in both castes.

To validate the results of the DDRT-PCR screen and to establish a functional link between decreasing SDR expression and the increasing late-larval ecdysteroid titer, we carried out in vitro experiments with larval worker ovaries. The stage chosen in this experiment (L5F3) corresponded to that used in the differential display analysis. The in vitro response to ecdysteroids observed in ovaries

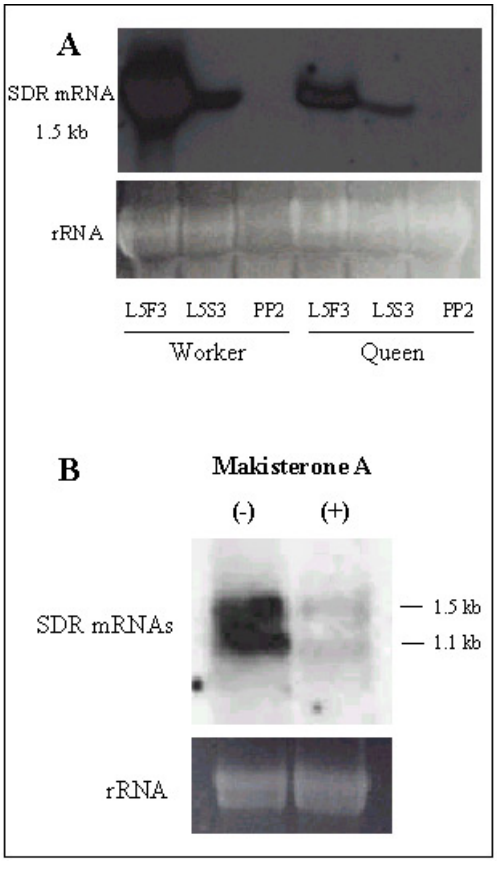

Figure 2. Developmental profile and ecdysone response of honey bee SDR expression detected by Northern-blot hybridization. (A) Whole-body extracts of total RNA prepared from different developmental stages of last-larval instar *Apis mellifera* workers and queens. SDR expression decreases concommittantly with the caste-specific increase in the hemolymph ecdysteroid titer (Rachinsky et al., 1990). Each lane contained 60 μg total RNA. (B) In vitro effect of makisterone A on the expression of honey bee SDR in ovaries of last-instar worker larvae (L5F3). Dissected ovaries were incubated for 6 h in medium containing 5×10^{-7}M makisterone A (+), or in control medium (-). The in vitro response to makisterone A corresponds to that observed in vivo. In the ovaries, two transcripts of different size were detected, the larger one corresponding to that of whole-body RNA extracts. Each lane contained 10 μg total RNA. Gel-loading controls are represented by ethidium bromide-stained 28S rRNA.

of this stage corresponded to the in vivo characteristics in ecdysteroid-regulated protein synthesis (Hartfelder et al., 1995). In the Northern blots of ovary RNA extracts this ecdysone response was clearly visible in the near complete reduction of SDR expression as a result of a 6 h exposure to 5×10^{-7}M makisterone A (Fig. 2b). This finding validates the results of the DDRT-PCR screen and also corroborates the idea that SDR expression is directly regulated by ecdsteroids in honey bee development. Interestingly, hybridization of ovary mRNA with the SDR probe detected the presence of two SDR transcripts. In the ovary preparations both were expressed at practically equal levels and showed the same response pattern to makisterone A. The larger transcript (1.5 kb) corresponded to that described for RNA extracts of whole larvae, whereas the smaller one (1.1 kb) may be germline-specific.

4. DISCUSSION

In our screen for ecdysteroid-regulated genes in larval honey bee ovaries, 19 differentially expressed cDNA-fragments were cloned and sequenced. The fact that three transcripts – that is 50% of those that could be assigned to functional groups – exhibited sequence similarity to redox-reaction enzymes indicates a marked metabolic effect of makisterone A.

For a closer look at this link between ecdysteroids and metabolism, we cloned and obtained the full length coding sequence corresponding to the honey bee EST D9/U22-250. The deduced amino acid sequence of this cDNA fragment showed significant sequence similarity to the short-chain dehydrogenase/reductase family, especially to an antennal-specific alcohol dehydrogenase of *Drosophila melanogaster*.

Drosophila short-chain ADH is one of the molecularly best characterized enzymes in insects. It is encoded by a single gene whose expression is controlled by two promotors, generating transcripts of different size during development (Benyajati et al., 1983; Savakis and Ashburner, 1985). The smaller, larval-specific transcript is controlled by the proximal promotor. It gradually accumulates from the first up to the middle of the third instar before its expression becomes drastically reduced. At the same time, the switch to the distal promotor leads to a peak of expression of the larger, adult transcript. Expression levels of this transcript decrease as the prepupal and pupal ecdysteroid peaks build up (Murtha and Cavener, 1989; Andres et al., 1993).

In its ecdysone response characteristics, the transcription profile of the putative honey bee SDR is very similar to that of the adult-specific *Adh* transcript of *Drosophila*. We did not detect a second transcript, corresponding to a larval-specific ADH, in whole body RNA extracts of honey bee larvae, possibly because the RNAs used in our Northern blots were from relatively late stages in the fifth larval instar. Such a second transcript, however, made its appearance in the larval ovaries, indicating a tissue-specific expression pattern. The presence of two *Adh* trancripts in gonads was previously described in *Drosophila* ovaries, where both transcripts were detected in ovaries at approximately equal expression levels (Savakis and Ashburner, 1985). Further tissue-specificity in *Adh* transcription has also been reported for larval fat body and midgut of *Drosophila melanogaster* (Benyajati et al., 1983).

Based on our sequence data for the putative honey bee SDR, we ran a series of protein motif analyses which revealed the three characteristic SDR motifs in our sequence. Further, more detailed comparison with protein structure data for SDR family members confirmed the protein database motif searches and the inclusion of the honey bee SDR as a member of this large family of proteins. In terms of functional signatures, we focused on the catalytic and coenzyme binding sites.

Bacterial 3β/17β-hydroxysteroid dehydrogenase and the short-chain ADH of *Drosophila melanogaster* have been established as model SDRs by mutation analyses on functional sites and by detailed X-ray crystallography which revealed their 3D structure. In both SDRs, the catalytic center is positioned in a single domain structure, represented by a triad of amino acid residues at Ser_{138}, Tyr_{151} and Lys_{155} (Jörnvall et al., 1995, 1999; Benach et al., 2001; Filling et al., 2002). In the deduced amino acid sequence of honey bee SDR we find such a constellation at Ser_{148},

Tyr_{162} and Lys_{166}, within motif 1 detected by MEME/MAST algorithms (Grundy et al., 1997). Besides the catalytic triad, we also detected the putative NAD(H) coenzyme binding site of *Apis mellifera* SDR at Thr_{13}, Asp_{65}, Asn_{91}, Asn_{92} and Ala_{93}, corresponding to that of the bacterian SDR (Thr_{12}, Asp_{60}, Asn_{86}, Asn_{87} and Ala_{88}). In honey bee SDR, Thr_{13} is located within motif 2 detected by MEME/MAST analysis, just one position upstream from the GXXXGXG sequence which constitutes another conserved element in SDR sequences (Jörnvall et al., 1995, 1999; Filling et al., 2002). In *D. melanogaster* ADH, the corresponding motif is GXGGXG, which is more glycin-rich and thus more similar to medium-chain (MDR) alcohol dehydrogenases (Benach et al., 2001). Yet its preference for NAD(H) instead of NADP(H) is determined by Asp_{37}, for which we found a corresponding residue in honey bee SDR at position 42.

It is important to remember that SDR enzymes are involved in a variety of cell functions besides detoxifying formaldehyde-like molecules and converting ethanol. Amongst others, such functions include the regulation of steroid hormones synthesis and metabolism and the alternation between active and inactive forms of these ligands in the binding to their respective hormone receptors (Filling et al., 2001). To our knowledge, there has so far been only one study on honey bee ADH (Martins et al., 1977) which investigated genetic polymorphism by isozyme electrophoresis. Yet, based on their activity profiles, these enzymes appear to differ from the SDR analyzed in our study.

Effects on energy metabolism by steroid hormones and by ligands to other members of the nuclear receptor superfamily are well established in vertebrates (Demonacos et al., 1996; Desvergne and Wahli, 1999). In insects other than Drosophilids, such an interaction is little explored, except for the silkworm, *Bombyx mori,* where a modulating effect of 20-hydroxyecdysone on genes coding for enzymes of a wide variety of metabolic reactions has recently been described (Kamimura et al., 2000). Energy metabolism has been addressed in previous studies on honey bee caste development, particularly cytochrome C titers (Eder et al., 1983). Our results, as well as those obtained by Evans and Wheeler (1999, 2000) and Corona et al. (1999) indicate that the regulation of genes coding for redox reaction enzymes may be a decisive initial step in the differentiation of the caste phenotypes. The puzzle that future studies will have to sort out, is to distinguish the tissue- and stage-specific hormone responses from their role in caste differentiation. This distinction is crucial to comprehend how polyphenic systems, such as the castes of social insects, are generated ontogenetically, and how such developmental mechanisms may have evolved on the background of a modulated energy metabolism.

In retrospect, it is quite plausible that energy metabolism should be a target in the hormonal control of caste polyphenism, because in most cases the initial trigger for caste development in social insects is a nutritional signal (Hunt and Nalepa, 1994). The subsequent hormonal regulation of metabolic enzyme expression could thus be interpreted as a feedback loop within the environmentally triggered process of caste development. In this context, members of the SDR family of enzymes may play a decisive role in converting dietary metabolites, especially those of the pollen-rich and often fermented diet of bee larvae. Such a role may, in fact, explain the caste-specific pattern of honey bee SDR-expression in fifth-instar worker larvae (L5F3). These are fed a pollen- and nectar-enriched larval food, in distinction to the queens which receive royal jelly exclusively during the entire larval feeding period. The higher levels of SDR expression may reflect this dietary switch in larval development, possibly as an adaptation to a diet containing fermented pollen. This hypothesis has already been proposed by Evans and Wheeler (1999, 2000), as an explanation for a set of putatively enzyme-encoding ESTs that co-segregated as worker-specific transcripts in their cluster analysis of honey bee ESTs.

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Résumé – Un membre de la super-famille des déshydrogénase/réductases à courte chaîne (SDR) est la cible de la réponse à l'ecdysone dans le développement des castes chez l'Abeille domestique (*Apis mellifera*). Les cribles d'expression différentielle des gènes sont des outils puissants pour comparer les différents états de la différenciation cellulaire et étudier les voies du développement au plan moléculaire. L'application à l'Abeille domestique de ces méthodes a déjà eu un fort impact sur la façon actuelle de considérer la différenciation des castes et le développement comportemental chez cet insecte social. Afin d'analyser le mode d'action des hormones morphogénétiques au niveau moléculaire nous avons utilisé un protocole de PCR-DDRT pour montrer les effets de la makistérone A, principal ecdystéroïde de l'hémolymphe d'abeille, sur l'expression dans l'ovaire de la larve des gènes propres à la caste. Il n'a pas été possible d'assigner à des familles de gènes connus la majeure partie des 19 fragments d'ADNc séquencés correspondants à des gènes exprimés différentiellement. Ces fragments peuvent constituer de nouveaux gènes ou au moins de nouvelles « étiquettes de séquences exprimées » (EST). Les séquences protéiques de 3 de ces EST ont montré des similitudes avec les enzymes impliqués dans la catalyse des réactions redox. L'une de ces EST correspond vraisemblablement à une déshydrogénase/réductase à chaîne courte (SDR). Elle fait ici l'objet d'une étude plus détaillée, car cette famille de gènes est particulièrement étudiée chez les insectes (alcools déshydrogénases de la drosophile).
A l'aide d'amorces spécifiques de cette EST de l'Abeille on a fait des réactions 3'- et 5' RACE, qui ont fourni une séquence codante complète du gène supposé de l'Abeille. La transcription protéique de ce gène a fourni les domaines caractéristiques des enzymes de la famille des SDR, dont les motifs YXXXK et GXXXGXG fortement conservés, ainsi que les acides aminés correctement positionnés constituant la triade catalytique et le site de liaison du coenzyme. Le fragment d'ADNc obtenu par l'étude par DDRT-PCR a pu ainsi être classé comme une SDR d'abeille de facto. Ces enzymes sont impliqués dans d'innombrables réactions métaboliques chez les insectes et les vertébrés, y compris dans le traitement des hormones stéroïdes et de leur métabolites.
L'analyse par northern blot de l'expression du gène SDR aux stades critiques du 5^{e} stade larvaire a montré que l'expression est forte au stade de la larve qui s'alimente et diminue progressivement pendant la phase de filage du cocon jusqu'à atteindre des niveaux inférieurs au seuil de détection aux stades pré-nymphaux. L'évolution temporelle de l'expression s'est révélée spécifique de la caste avec des niveaux nettement plus bas chez les reines, ce qui suggère une régulation négative de l'expression de la SDR par le taux d'ecdystéroïde, qui est plus élevé chez les reines que chez les ouvrières. Cette hypothèse a été confirmée par une expérimentation in vitro en faisant incuber des ovaires de larves d'ouvrières dans un milieu contenant 5×10^{-7}M de makistrone A. L'exposition à des niveaux physiologiques de makistérone A a fortement diminué l'expression du gène SDR. Outre le fait que cette expérimentation in vitro a pleinement confirmé le résultat initial obtenu avec l'étude par PCR-DDRT, l'analyse par northern blot a détecté dans les ovaires l'expression de deux isoformes du gène SDR, provenant peut-être d'un épissage alternatif. Elles s'exprimaient dans les ovaires à des niveaux semblables et présentaient le même profil de réponse à la makistérone A.
Les profils d'expression tissu-spécifiques observés du gène SDR d'abeille et la preuve nette de sa régulation liée à la caste par les ecdystéroïdes en circulation font de cette enzyme un marqueur potentiel pour le développement. En outre, l'expression différentielle des divers gènes codant pour les enzymes impliqués dans les réaction redox suggère que la régulation métabolique joue un rôle important dans la différenciation des castes chez l'Abeille domestique.

***Apis mellifera* / différenciation des castes / ecdystéroïde / alcool déshydrogénase / expression différentielle PCR**

Zusammenfassung – Ein Mitglied der Enzym-Superfamilie der kurzkettigen Dehydrogenasen/ Reduktasen (SDR) ist ein Ziel der Ecdyson-Antwort in der Kastenentwicklung der Honigbiene (*Apis mellifera*). Der Vergleich unterschiedlicher Zustände der Zelldifferenzierung und die Erforschung von Entwicklungswegen auf molekularer Ebene nutzten heute aussagekräftige Methoden zur Analyse differentieller Genexpression. Die Anwendung entsprechender Methoden bei Honigbienen zeitigt bereits eine starke Wirkung auf die aktuelle Betrachtungsweise der Kastendifferenzierung und der Verhaltensentwicklung in diesem sozialen Insekt. Zur Analyse der Wirkungsweise morphogenetischer Hormone auf molekularer Ebene etablierten wir ein Differential-Display-PCR (DDRT-PCR) Protokoll, mittels dessen wir die Effekte von Makisteron A, dem dominanten Ecdysteroid in der Hämolymphe von Honigbienen, untersuchen konnten. Die meisten der neunzehn von differentiell exprimierten Genen stammenden und sequenzierten cDNA-Fragmente konnten keiner der bekannten Genfamilien zugeordnet werden und stellen damit möglicherweise neue Gene oder zumindest neue Expressed Sequence Tags (ESTs) dar. Drei ESTs zeigten Sequenzähnlichkeiten mit Enzymen, die Redox-Reaktionen katalysieren. Eines dieser ESTs wurde als kurzkettige Dehydrogenase-Reduktase (SDR) notiert und wurde für eine Detailstudie ausgewählt, insbesondere auch deshalb, weil diese Genfamilie einige der beststudierten

Genprodukte enthält, nämlich die *Drosophila*-Alkoholdehydrogenasen.
Mittels genspezifischer Primer für das Honigbienen cDNA-Fragment wurden 3'- und 5'-RACE-Reaktionen durchgeführt, die eine komplette kodierende Sequenz für die putative Honigbienen SDR lieferten. In Proteinsignatur-Analysen ermittelten wir die charakteristischen Domänen für Enzyme der SDR-Familie, wie die hochkonservierten YXXXK- und GXXXGXG-Motive, sowie die Aminosäuren, die die katalytische Triade und die Coenzym-Bindungsstelle bilden. Das aufgrund der DDRT-PCR-Studie erhaltene cDNA-Fragment konnte damit als eine de facto Honigbienen SDR klassifiziert werden. Diese Enzyme katalysieren eine Vielzahl metabolischer Reaktionen, einschliesslich der Prozessierung von Steroidhormonen und ihrer Metabolite bei Insekten und Vertebraten.
Northern-Blot-Analysen zur SDR-Expression in kritischen Stadien des fünften Larvenstadiums zeigten ein hohes Expressionslevel in Fressmaden, gefolgt von einem graduellen Absinken der Expression im Spinnmadenstadium, bis hin zur Reduktion der Expression unterhalb des Detektionsniveaus in präpupalen Stadien. Im Zeitverlauf der Expression erwies sich die SDR-Expression als kastenspezifisch, mit deutlich niedrigeren Expressionslevels bei Königinnen. Dieses Muster wies auf eine negative Regulation der SDR-Expression durch den Hämolymph-Ecdysteroid-Titer hin, der bei Königinnen ab der Spinnmadenphase erhöht ist. Diese Hypothese wurde in einem in vitro-Experiment mit isolierten Ovarien von Arbeiterinnenlarven im Fressmadenstadium überprüft und bestätigt. In Ovarien, die in Medium mit 5×10^{-7}M Makisteron inkubiert wurden, war die SDR-Expression durch diese physiologische Hormonkonzentration stark reduziert. Ausser der Tatsache, dass dieses in vitro-Experiment mit larvalen Ovarien den ursprünglichen Befund der DDRT-PCR-Studie vollauf bestätigte, konnten wir in den Northern Blots mit Ovarien-RNA die Expression zweier SDR-Isoformen detektieren, die vermutlich das Resultat eines alternativen Splicing darstellen. In Ovarien waren beide Isoformen in gleichem Masse exprimiert und zeigten auch das gleiche Antwortmuster auf Ecdysteroide.
Die aufgezeigten gewebespezifischen Expressionsmuster der Honigbienen-SDR und die klaren Evidenzen für ihre Kasten spezifische Regulation durch Ecdysteroide machen dieses Enzym zu einem interessanten potentiellen Entwicklungsmarker. Ausserdem weist die differentielle Expression verschiedener für Redox-Enzyme kodierender Gene auf eine wichtige Rolle der Metabolismusregulierung in der Kastenentwicklung der Honigbiene hin.

differential display PCR / Ecdysteroide / Alkoholdehydrogenase / Honigbiene / Kastenpolyphenismus

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