Apidologie 32 (2001) 25–35

Original article

Genetic, ontogenetic, and tissue-specific variation of aminopeptidases of *Apis mellifera*

Marco Antonio DEL LAMA[a]*, Renata Maria BEZERRA[a], Ademilson Espencer EGEA SOARES[b], Maria Cláudia Colla RÚVOLO-TAKASUSUKI[a]

[a] Departamento de Genética e Evolução, Universidade Federal de São Carlos, Rodovia Washington Luiz km 235, 13565.905, São Carlos, SP, Brazil
[b] Departamento de Genética, Faculdade de Medicina de Ribeirão Preto (USP), Av. Bandeirantes s/n, 14049.900, SP, Brazil

(Received 13 April 2000; revised 24 July 2000; accepted 14 August 2000)

Abstract – Four aminopeptidases were detected in *Apis mellifera* by starch gel electrophoresis. These enzymes were characterized on the basis of their substrate preference, effect of inhibitors, tissue and ontogenetic developmental distribution. Lap-A activity was present at all tissues and developmental stages. Lap-P was characterized by a more intense activity during the pupal stage. Lap-G activity was concentrated in the midgut and was detected in association with the presence of food inside the digestive tract. Lap-D was more proeminent in the reproductive tract of adult drones, where its activity appeared to be concentrated in the mucus. Four electrophoretic variants of Lap-D were observed, with an uncommonly high intralocus heterozygosity level. Segregational analyses demonstrated the absence of close linkage between *Lap-D* and *Est-1a*, *Est-2, Est-5, Est-6, Mdh-1, Hk-1* and *Pgm-1* loci of *Apis mellifera*.

aminopeptidases / *Apis mellifera* / polymorphism / tissue distribution / reproduction

1. INTRODUCTION

Aminopeptidases (α-aminoacyl-peptide hydrolase, EC 3.4.11) are exopeptidases that hydrolyze single amino acids from the N-terminus portion of the polypeptide chain. They are classified according to their dependence on metal ions (usually Zn^{++} or Mn^{++}) and their substrate preference.

Gut aminopeptidases from some insect species have been characterized biochemically

* Correspondence and reprints
E-mail: dmdl@power.ufscar.br

(Terra and Ferreira, 1994) and their genetic characterization occurred mainly after the development of zymogram technique.

Several animal species of all vertebrate classes have been submitted to aminopeptidase characterization (Del Lama, 1977; Del Lama et al., 1992). Among insects, the exopeptidases of *Drosophila* are the enzymes that have been most extensively studied (Beckman and Johnson, 1964; Hall, 1988; Sakai et al., 1969; Walker and Williamson, 1980; Walker et al., 1981).

Despite their presumptive physiological importance, little information is available about the biochemical and genetic properties of the exopeptidases present in bees. Two distinct groups of aminopeptidases were characterized in pupal extracts of 14 bee species by Del Lama and Mestriner (1984).

The objective of the present study was to characterize the aminopeptidases of *Apis mellifera* L. on the basis of their substrate preference, the effect of inhibitors on their activity, their tissue and ontogenetic developmental distribution, and the differences in aminopeptidase patterns observed in drones, queens and workers. We also present data about the genetic variation detected at a locus that exhibits its greatest expression in the reproductive tract of adult males. The genetic determination of this polymorphism, the frequency of the different variants, and data about genetic linkage with other biochemical markers of *Apis mellifera* are also presented.

2. MATERIALS AND METHODS

2.1. Material

Apis mellifera workers, drones and queens of different stages of ontogenetic development were obtained from the apiary of the Department of Genetics, Faculty of Medicine of Ribeirão Preto (FMRP), SP, Brazil.

2.2. Sample preparation

Head-thorax or abdomen extracts from workers, queens and drones were prepared separately in 0.2 ml of 0.2% 2-mercaptoethanol solution and centrifuged at 3500 *g* for 15 min at room temperature, and the supernatants obtained were used for electrophoretic analysis.

2.3. Electrophoretic analysis

Horizontal electrophoresis was carried out on 14% corn starch gel (Penetrose 30™, Refinações de Milho Brasil S/A) using tris-citrate-borate buffer (0.017M tris + 0.0023M citric acid, pH 8.0, and 0.3M boric acid, pH 8.3, for the gel and electrodes, respectively). The gels were exposed to a constant current at 2 mA/cm for 4 h at 10 °C.

For the genetic linkage studies, esterase phenotypes were determined in phosphate, pH 6.7, buffer gels, while Mdh-1, Hk-1 and Pgm-1 phenotypes were determined in tris-EDTA-maleate-$MgCl_2$, pH 7.4, buffer gels. Further details about the electrophoretic procedures for the determination of these phenotypes have been published elsewhere (Del Lama et al., 1985, 1993).

2.4. Detection of aminopeptidase activity

Aminopeptidase activity was detected by incubating the gels for 2 h at 37 °C in 100 ml tris-maleate buffer (0.1M tris + 0.1M maleic anhydride), pH 5.5, containing 30 mg substrate (aminoacyl derivatives of β-naphthylamine) and 40 mg Fast Garnet GBC salt (Beckman et al., 1964).

2.5. Enzymatic inhibition

The effect of EDTA and 1,10-phenanthroline on aminopeptidase activity was investigated by first incubating the gel for 20 min in tris-maleate buffer, pH 5.5,

containing 10, 5 or 1 mM EDTA or 1 mM 1,10-phenanthroline. The enzymatic activity was then developed in the presence of these inhibitors. The control consisted of another gel slice submitted to previous incubation in the absence of inhibitors and then stained with the developing mixture.

2.6. Tissue distribution in adult individuals

Adult workers, virgin and inseminated queens and immature or sexually mature drones of *Apis mellifera* were dissected under a stereomicroscope. The digestive tract of all insects and the reproductive tract of drones (testes, mucus glands, ejaculatory bulb, seminal vesicles, and semen) and queens (ovaries/oviduct and spermatheca) were removed and kept in an ice bath, homogenized in 0.2% 2-mercaptoethanol (10 mg/20 µl) and centrifuged, and the resulting supernatants were submitted to electrophoresis.

2.7. Developmental distribution

Samples of eggs, 2–6 day larvae, young and pigmented pupae and adults were homogenized in a 2-mercaptoethanol solution (10 mg/20 µl), centrifuged and submitted to electrophoretic analysis.

2.8. Genetic variants

The occurrence of genetic variants of Lap-D was investigated in the abdomen extracts of adult drones from 52 *Apis mellifera* colonies of an apiary of FMRP-USP located near Luiz Antonio, SP, Brazil. At least 12 drones per colony were analyzed so that the genotype of the queen could be determined. The genetic basis of variation detected was confirmed by a homogeneity test of the phenotypic segregations produced by queens of the same genotype. The frequency of variants observed was estimated from the queen's genotype, which in turn was determined by analysis of their respective drone progenies.

2.9. Genetic linkage

Drones produced by naturally inseminated doubly heterozygous queens for *Lap-D* and another biochemical marker (*Est-1a*, *Est-2*, *Est-5*, *Est-6*, *Mdh-1*, *Hk-1*, and *Pgm-1*) were analyzed electrophoretically to detect genetic linkage between these loci. Expected segregational distribution was analysed by the chi-square test.

3. RESULTS

Four aminopeptidases were detected in *Apis mellifera* which were designated Lap-A, Lap-P, Lap-D and Lap-G according to their similarities with aminopeptidases of *Drosophila*. They are presumably products of four distinct structural gene loci and their relative electrophoretic mobilities can be seen in Figure 1.

Table I shows the results of the tests for substrate preference. It can be seen that the aminopeptidases of *Apis mellifera* did not demonstrate absolute specificity for the β-naphthylamide derivatives employed, although their highest relative activity was observed with leucyl-β-Na as substrate.

The inhibition studies showed that the four *Apis* aminopeptidases were completely inhibited in the presence of 10 mM EDTA and partially inhibited by 5 mM or 1 mM EDTA. Partial Lap-A inhibition and total Lap-P, D and G inhibition occurred with 1 mM 1,10-phenanthroline.

3.1. Developmental distribution

Table II shows the developmental distribution of *A. mellifera* aminopeptidases. Lap-A activity was present at all developmental stages, from newly deposited eggs to aged drones and workers, whereas Lap-P

was detected from the larval to the adult stage of both sexes, although its activity was more intense in pupae. Lap-D was observed in young (0–2 day-old) male and female larvae and in adult males. Lap-G was detected in both sexes from 2-day larvae to adults and was always observed when food was present in the digestive tract.

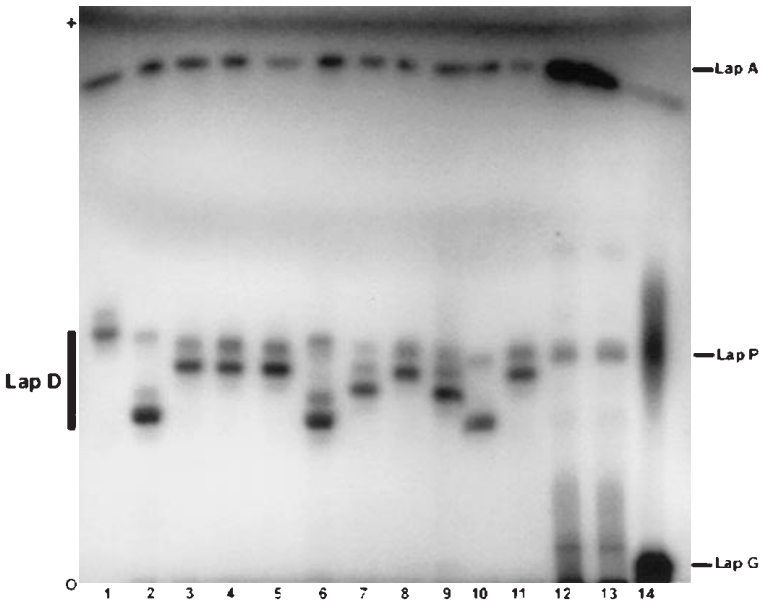

Figure 1. Starch gel electrophoretic profile of aminopeptidases of *Apis mellifera* detected in extracts from reproductive tracts of adult drones (samples 1 to 11), from worker pupae (12 and 13) and from the intestinal lumen content of adult workers (14). The four phenotypes of Lap-D are also shown: Lap D 107 (sample 1), Lap-D 100 (3, 4, 5, 8, 11), Lap D 90 (7, 9) and Lap D 82 (samples 2, 6, 10).

Table I. Relative activity of *Apis mellifera* aminopeptidases on six aminoacyl-derivatives of β-naphthylamide. (+) .... (++++) indicate subjective estimates of increasing activity and (–) indicates no activity.

	AMINOPEPTIDASES			
SUBSTRATES	LAP-A	LAP-P	LAP-D	LAP-G
Ala-β-Na	+++	+	++	++
Arg-β-Na	++	+	+	+
Phe-β-Na	+	–	+	+
Leu-β-Na	++++	++	+++	+++
Lys-β-Na	++	+	–	–
Tyr-β-Na	+	–	–	+

Table II. Aminopeptidase activity in the different ontogenetic developmental stages of drones (D), workers (W) and queens (Q) of *Apis mellifera*. (+) indicates activity and (–) means no activity.

		AMINOPEPTIDASES			
STAGE	SEX	LAP-A	LAP-P	LAP-D	LAP-G
Eggs	D	+	–	–	–
	W	+	–	–	–
Larvae	D	+	+	+	+
	W	+	+	+	+
Pupae	D	+	+	–	–
	W/Q	+	+	–	–
Adults	D	+	+	+	+
	Q	+	+	–	+

3.2. Tissue distribution in adults

The distribution of *A. mellifera* aminopeptidases in the tissues of workers, queens and adult drones is found in Table III. Lap-A was detected in all drone tissues and body parts analyzed, including the antennae, cerebral ganglia and Malpighian tubules, but not in semen. This was also the only aminopeptidase present in the hemolymph of adults and in the ovary/oviduct and spermatheca extracts of queens.

Lap-P was observed in the digestive tract and a residual activity was also observed in the thorax extracts of males and females.

Lap-D was visualized only in the reproductive tract of drones. Electrophoretic analysis of some tissue extracts (testes, seminal vesicle, mucus glands and ejaculatory bulb) demonstrated that Lap-D is present in all parts of the reproductive tract of drones. However, the highest activity was detected in the mucus gland and/or ejaculatory bulb,

Table III. Tissue distribution of aminopeptidase activity in adult drones (D), workers (W) and queens (Q) of *Apis mellifera*. (+) indicates activity and (–) means no activity.

		AMINOPEPTIDASES			
TISSUES	SEX	LAP-A	LAP-P	LAP-D	LAP-G
Digestive tract	D	+++	+	–	++
	W	+++	+	–	++
Reproductive tract	D	++	–	+++	–
	Q	++	–	–	–
Cerebral ganglia	D/W	++	–	–	–
Thorax	D/W	++	++	–	–
Antenae	D/W	+	–	–	–
Malpighian tubules	D	+	–	–	–

depending on where mucus was present in largest amounts. This association between the presence of mucus in the ejaculatory bulb/mucus gland and Lap-D activity was confirmed by analysis of mucus extracts isolated from these tissues.

Lap-G was detected in the digestive tract of adults whenever food was present. Digestive tracts of males and females were isolated, washed, homogenized and these extracts were used for electrophoresis analysis. The results indicated that Lap-G activity seems to be concentrated in the intestinal lumen.

Electrophoretic analysis of seminal vesicle extracts demonstrated the presence of an additional region of aminopeptidase activity. The low relative activity of this enzyme, however, impaired its detection and appropriate characterization.

3.3. Genetic variants and linkage analysis

Among the four aminopeptidases detected, electrophoretic variants were observed only for Lap-D. Because of its particular tissue distribution, this variation was characterized by electrophoretic analysis of abdomen extracts of adult males from 54 colonies. Four electrophoretic variants were detected and named according to their anodal mobility in relation to the most frequent variant taken as reference (Fig. 1).

Analysis of drone progenies from presumably heterozygous queens demonstrated that these queens segregated two phenotypes at the expected 1:1 ratio. Table IV shows the homogeneity chi-square values estimated to demonstrate that queens of the same genotype produced similar phenotypic segregation in males, supporting the genetic determination of the variation observed.

The genotypes of the queens from each colony were inferred from the phenotypes observed in their males, resulting in the determination of the following allele frequencies: $Lap\text{-}D^{82}$ = 0.157; $Lap\text{-}D^{90}$ = 0.278; $Lap\text{-}D^{100}$ = 0.463 and $Lap\text{-}D^{107}$ = 0.103. The chi-square values (χ^2 = 4.834, d.f. = 6; $p = 0.50 < P < 0.70$) revealed that this queen sample is in Hardy-Weinberg equilibrium for this locus.

A. mellifera young larvae also presented a region of mobility similar to that of Lap-D observed in the reproductive tract of adult males. The correspondence between the two regions was confirmed by electrophoretic analysis of worker and drone larvae and adult drone progenies of these queens.

Table IV. Segregational analysis to demonstrate the inheritance of the Lap-D enzyme variants in adult drone progenies. Data from different queens (n) of *Apis mellifera* with the same phenotype were pooled to test homogeneity.

Queen phenotype	n	82	90	100	107	χ^2_{hom}	d.f.	*P*
82	02	37						- - -
82/90	04	28	30			2.35		$0.50 < P < 0.70$
82/100	06	114		115		3.30	05	$0.50 < P < 0.70$
82/107	03	11			26	0.13	02	$0.70 < P < 0.80$
90	04		66					- - -
90/107	04		35		36	2.58	03	$0.30 < P < 0.50$
90/100	14		147	150		4.17	13	$0.98 < P < 0.99$
100	14			222				- - -
100/107	02			24	20	0.05	01	$0.80 < P < 0.90$
107	01				11			- - -

Electrophoretic analysis of worker larvae aged approximately 2 days, collected from colonies of *Apis mellifera ligustica* (four colonies) and *Apis mellifera carnica* (three colonies) revealed the presence of the variants 82, 90 and 100. These findings suggest that Lap-D polymorphism is well established in different *Apis mellifera* subspecies. The heterozygote pattern observed in these larvae consisted of two bands, typical of a monomeric protein.

Table V shows the linkage studies involving *Lap-D* and *Est1-a*, *Est-2*, *Est-5*, *Est-6*, *Hk-1*, *Mdh-1* and *Pgm-1* loci. The estimated chi-square values indicate that these genes are not closely linked.

4. DISCUSSION

The four aminopeptidases detected showed greater activity towards leucyl-β-naphthylamide and therefore may considered to be leucylaminopeptidases.

The difference in sensitivity exhibited by Lap-A and the other aminopeptidases toward the two chelators, EDTA and 1,10-phenanthroline, was significant. EDTA had a more pronounced effect on Lap-A activity, whereas 1,10-phenanthroline had a greater inhibitory effect on Lap-P, Lap-D and Lap-G. Studies of the effects of these reagents on the aminopeptidase activity of *Drosophila* demonstrated that EDTA had

Table V. Segregational analysis of adult drone progenies of doubly heterozygous queens to test genetic linkage between *Lap-D* and other biochemical markers of *Apis mellifera*.

MARKERS	COLONY	PHENOTYPES				χ^2	*P*
Lap D × *Est-1a*	137	90/S	90/F	100/S	100/F		
		32	30	23	40	4.70	$0.20 < P < 0.10$
	147	90/S	90/F	100/S	100/F		
		05	14	13	10	4.67	$0.20 < P < 0.10$
Lap D ×*Est-2*	145	82/S	82/F	100/S	100/F		
		43	42	31	32	3.30	$0.50 < P < 0.30$
	147	90/S	90/F	100/S	100/F		
		07	12	13	10	2.00	$0.70 < P < 0.50$
Lap D × *Est-5*	162	90/+	90/–	100/+	100/–		
		13	14	14	16	0.33	$0.98 < P < 0.95$
Lap D × *Est-6*	145	82/S	82/M	100/S	100/M		
		29	21	16	18	4.67	$0.20 < P < 0.10$
Lap D × *Hk-1*	68	82/S	82/F	100/S	100/F		
		09	13	09	11	1.05	$0.80 < P < 0.70$
	162	90/S	90/F	100/S	100/F		
		12	15	14	16	0.61	$0.80 < P < 0.90$
Lap D × *Mdh-1*	137	90/M	90/F	100/M	100/F		
		26	19	21	25	1.44	$0.70 < P < 0.50$
	150	100/S	100/F	107/S	107/F		
		06	10	08	06	1.47	$0.50 < P < 0.70$
Lap D × *Pgm-1*	150	100/M	100/F	107/M	107/F		
		05	11	08	06	2.80	$0.50 < P < 0.30$

a marked inhibitory effect on Lap-A but did not affect Lap-D activity, whereas 1,10-phenanthroline did not affect Lap-A activity but strongly inhibited Lap-D activity (Walker and Williamson, 1980; Walker et al., 1981).

Aminopeptidases are usually more active than carboxypeptidases in insects (Terra and Ferreira, 1994) and must play an important role in the intermediary digestion of proteins. The tissue distribution of aminopeptidase activities would be expected to be intimately tied to their physiological roles. The nearly ubiquitous occurrence of Lap-A activity through most tissues, body parts and developmental stages points to a rather general metabolic role, such as regulating the concentrations of amino acids and small peptides in the hemolymph and other tissues. This enzyme is probably an aminopeptidase of general use in tissues and in the hemolymph and should be important in protein anabolism and maintenance of osmotic stability. Its presence in the embryo suggests that this enzyme not only acts on larval tissue histolysis (Muhs, 1975), but is probably active early in development for the hydrolysis of yolk protein. This enzyme is probably synthesized in the ovary and not formed "de novo" from a stable maternally-derived message since unfertilized eggs (drones) produce zymogram patterns identical to those of early embryos, contributing to the maintenance of a continuous supply of amino acids for protein anabolism (Collet, 1976a, b; Laurie-Ahlberg, 1982). This assumption is supported by the fact that Lap-A was the only aminopeptidase detected in queen ovary extract.

Lap-P is more active in the pupal stage. Studies on other insects have suggested that this type of enzyme is synthesized during early development and is stored in its inactive form to be later used in a more advanced stage of development (Walker and Williamson, 1980). Hall (1986, 1988) reported the occurrence of three distinct aminopeptidases in *Drosophila*. One of them, denoted Lap-P, was predominantly present during the pupal stage and its probable function was the histolysis and mobilization of the food stock. Furthermore, appreciable amounts of the enzyme were present in the exuvial fluid and pupal capsule during hatching. Although *Apis* Lap-P has been so designated because it is more proeminent in pupae, as is the case for Lap-P of *Drosophila*, it is also present in larvae and adults. In *Drosophila* females, most Lap-P activity is found in the ovaries, suggesting that this enzyme may be involved in the oviposition process, and in embryos, where it is probably involved in the digestion of yolk protein. However, the Lap-P of *Apis* is not present in ovary or egg extracts, indicating that these enzymes perform different functions in these two species.

Lap-D exhibited tissue- and sex/caste-specific distribution; it was observed in young larvae (less than three days) of drones and workers. Its most important characteristic, however, was its presence in extracts of the reproductive apparatus of drones, especially in mucus glands or ejaculatory bulb extracts.

Lap-G was detected only in larvae and in digestive tract extracts of adults. The activity of this enzyme is related to the presence of food inside the digestive tract. The presence of a high Lap-G activity in the midgut, which is the major site of digestion and absorption, is consistent with the assumption that the primary function of this aminopeptidase is digestion of dietary polypeptides to diffusible peptides in the digestive tract. A similar enzyme was observed in *Drosophila* (Walker and Williamson, 1980), where it is present in the gut of larvae and adults and predominantly acts in the midgut, even though some activity has also been observed in the Malpighian tubules of larvae.

Among the four aminopeptidases detected, only Lap-D showed electrophoretic variants. The allelic nature of these variants was confirmed by analysis of colonies in which queens, presumably heterozygous, produced

two types of drones at the expected 1:1 Mendelian proportion. Furthermore, drone analysis did not reveal any heterozygous patterns and the homogeneity tests showed that queens of the same genotype produced similar drone progenies.

Four different Lap-D phenotypes were observed, indicating the occurence of four alleles. The allele frequencies obtained by inference of the queen genotypes were used to demonstrate that the genotypic proportions expected according to the model of genetic equilibrium were indeed observed in this sample. Young worker larvae showed a heterozygous pattern suggesting a monomeric structure for this enzyme.

Two facts should be emphasized with respect to Lap-D. First, this polymorphism also occurs in European bees; secondly, Lap-D represents the biochemical marker with the highest intra-locus heterozygosity yet reported for *Apis mellifera*. Furthermore, this gene presents a highly peculiar expression in tissue and during development – although present in young worker and drone larvae, this gene has its greatest expression in the reproductive tract of adult drones. In animal spermatozoa and eggs, endopeptidases have been isolated and suggested to play important roles in the process of fertilization. Thus, acrosin isolated from mammalian sperm, two trypsin-like enzymes (acrosin and spermosin) from ascidian sperm and a chymotrypsin-like enzyme from sea urchin and frog sperm, are involved in sperm penetration through the vitelline coat of the eggs, functioning in elevation of the fertilization envelope and in the establishment of the block to polyspermy. However, the role of aminopeptidases in fertilization has not been determined in animals, although some of its properties and its relationship with fertilization have been demonstrated in bull and sea urchin sperm (Yasuhara et al., 1990). Evidence obtained from the effect of inhibitors on the respiration and motility of sea urchin sperm suggests that these may act on the aminopeptidase activity present in sperm (Yasuhara et al., 1991).

The elucidation of the relationship between Lap-D activity in the mucus and its possible functional role in the process of fertilization in *Apis mellifera* needs further intensive examination.

ACKNOWLEDGMENTS

We would like to thank Isabel C. Godoy, Adelino Penatti and Jairo de Souza for technical assistance. This research was supported by the Brazilian Research Council (CNPq).

Résumé – Variation génétique, ontogénétique et spécifique des tissus des aminopeptidases chez *Apis mellifera*. Les aminopeptidases sont des enzymes qui hydrolysent les acides aminés situés en bout de la chaine polypeptidique. Malgré leur importance physiologique présumée, il existe peu d'informations concernant leurs propriétés biochimiques et génétiques chez les abeilles. Les aminopeptidases d'*Apis mellifera* ont été caractérisées après électrophorèse sur gel d'amidon selon leur préférence vis-à-vis des substrats, l'action d'inhibiteurs, leur répartition dans les tissus et au cours de l'ontogenèse et leur variation génétique. Quatre aminopeptidases – Lap-A, Lap-P, Lap-D et Lap-G – , produits des quatre locus de gène de structure, ont été détectées. Aucune d'elles n'a montré de spécificité absolue vis-à-vis des substrats testés, bien que l'activité relative la plus élevée ait été observée avec la leu-β comme substrat. Les études d'inhibition enzymatique ont montré que la Lap-A était plus affectée par l'EDTA, alors que la 1,10-phenanthroline réduisait plus particulièrement l'activité des autres aminopeptidases. L'activité de la Lap-A était présente dans tous les tissus et chez tous les stades de développement, depuis l'œuf jusqu'à l'adulte âgé. L'activité de la Lap-G était concentrée dans la lumière de l'intestin moyen et a été détectée associée à la présence de nourriture dans le tube digestif. La Lap-D, bien que présente dans les extraits de jeunes larves

de mâles et femelles, a été principalement détectée dans le tube digestif des mâles adultes, où son activité semblait se concentrer dans le mucus présent dans la glande à mucus ou dans le bulbe de l'organe copulateur. Quatre variants électrophorétiques de Lap-D déterminés génétiquement ont été observés et leur fréquence relative nous a permis d'estimer un niveau d'hétérozygotie singulièrement élevé à l'intérieur d'un locus chez *Apis*. La détection d'au moins trois variants chez les abeilles d'origine européenne laisse penser que ce polymorphisme doit être largement répandu chez les sous-espèces d'*A. mellifera*. Le profil hétérozygote observé chez les jeunes larves d'ouvrières suggère que la Lap-D a une structure de monomère. Les analyses de ségrégation par électrophorèse ont montré l'absence de liaison génétique entre ce locus et les marqueurs biochimiques *Est-1a, Est-2, Est-5, Est-6, Mdh-1, Hk-1,* et *Pgm-1* d'*Apis mellifera.*

***Apis mellifera* / aminopeptidase / polymorphisme / répartition dans les tissus / reproduction**

Zusammenfassung – Genetik, Ontogenese und gewebespezifische Unterschiede von Aminopeptidasen bei *Apis mellifera* L. Aminopeptidasen von *Apis mellifera* wurden nach der Auftrennung durch Stärkegel – Elektrophoresen auf Grund ihrer Substratpräferenz und der Wirkung von Inhibitoren charakterisiert. Weiter wurde sowohl ihre Verteilung im Gewebe und während der Ontogenese als auch ihre genetische Variation untersucht. Vier Aminopeptidasen – Lap-A, Lap-P, Lap-D und Lap-G –, Produkte von 4 unterschiedlichen strukturellen Genloci wurden nachgewiesen. Keines von ihnen zeigte eine absolute Substratspezifität, ihre größte relative Aktivität ergab sich beim Substrat leu-β-Na. Die Untersuchungen über Enzymhemmungen zeigte, dass Lap-A am stärksten auf EDTA reagierte, während die Aktivität der anderen Aminopeptidasen stärker durch 1.10-Phenanthrolin eingeschränkt wurde. Die Lap-A Aktivität fand sich in allen Geweben und allen Entwicklungsstadien, von frisch gelegten Eiern bis zu alten adulten Bienen. Für Lap-P war eine intensivere Aktivität während der Puppenstadien typisch. Die Aktivität von Lap-G konzentrierte sich auf das Lumen des Mitteldarms und wurde in Verbindung mit Nahrung im Verdauungstrakt nachgewiesen. Lap-D kam zwar auch in Extrakten von jungen männlichen und weiblichen Larven vor, sie wurde aber vor allem in den Reproduktionsorganen der adulten Drohnen nachgewiesen, wo sich ihre Aktivität auf den Schleim der Mucusdrüsen und im Bulbus des Begattungsorgans zu konzentrieren schien.

Es konnten 4 genetisch unterschiedliche Varianten von Lap-D nachgewiesen werden, und ihre relative Häufigkeit erlaubte uns, ein ungewöhnlich hohes Niveau von Heterozygotie innerhalb eines Locus in *Apis* zu berechnen. Der Nachweis von mindestens 3 der 4 Varianten in Honigbienen europäischen Ursprungs lässt vermuten, dass dieser Polymorphismus in *Apis mellifera* Rassen weit verbreitet sein muss. Auf Grund der heterozygoten Muster bei jungen Larven der Arbeiterinnen wird vermutet, dass Lap-D eine monomere Struktur aufweist. Analysen mit elektrophoretischer Auftrennung ergaben, dass es keine enge Verbindung zwischen diesem Locus und den biochemischen Markern *Est-1a, Est-2, Est-5, Est-6, Mdh-1, Hk1* und *Pgm-1* von *Apis mellifera* gibt.

Aminopeptidasen / *Apis mellifera* / Polymorphismus / Verteilung im Gewebe / Reproduktion

REFERENCES

Beckman L., Johnson F.M. (1964) Genetic control of aminopeptidases in *Drosophila melanogaster*, Hereditas 51, 221–230.

Beckman L., Bjorling G., Christodoulou C. (1964) Genetics of leucine aminopeptidase isozymes in maize, Genetics 50, 899–904.

Collet J.I. (1976a) Small peptides, a life-long store of amino acid in adult *Drosophila* and *Calliphora*, J. Insect Physiol. 22, 1433–1440.

Collet J.I. (1976b) Peptidase mediated storage of amino acids in small peptides, Insect Biochem. 6, 179–185.

Del Lama M.A. (1977) Padrões Eletroforéticos de Exopeptidases em Abelhas, Ms. thesis, Faculdade de Medicina de Ribeirão Preto, USP, São Paulo, Brazil.

Del Lama M.A., Mestriner M.A. (1984) Starch gel electrophoretic patterns of exopeptidase phenotypes in 14 different species of bees, Brazil. J. Genet. 7, 9–20.

Del Lama M.A., Soares A.E.E., Mestriner M.A. (1985) Linkage studies in *Apis mellifera* using biochemical and morphological markers, J. Hered. 76, 427–430.

Del Lama M.A., Mascarenhas R., Soares A.E.E. (1993) Further linkage studies with biochemical markers in *Apis mellifera*, J. Hered. 84, 129–131.

Del Lama S.N., Del Lama M.A., Mestriner M.A., Mortari N. (1992) Peptidase B polymorphism in cattle erythrocytes, Biochem. Genet. 30, 247–255.

Hall N.A. (1986) Peptidases in *Drosophila melanogaster*. I. Characterization of dipeptidase and leucine aminopeptidase activities, Biochem. Genet. 24, 775–793.

Hall N.A. (1988) Peptidases in *Drosophila melanogaster*. II. The variation of peptidase activities during development, Insect Biochem. 18, 145–155.

Laurie-Ahlberg C.C. (1982) Genetic, ontogenetic, and tissue-specific variation of dipeptidases in *Drosophila melanogaster*, Biochem. Genet. 20, 407–424.

Muhs J.-J. (1975) Isoenzyme frequencies in long term selection lines of *Drosophila melanogaster*. II. Isoenzyme frequencies of leucine aminopeptidases (LAP) in selection lines under different temperature conditions, Theor. Appl. Genet. 46, 123–128.

Sakai R.K., Tung D.A., Scandalios J.G. (1969) Developmental genetic studies of aminopeptidases in *Drosophila melanogaster*, Mol. Gen. Genet. 105, 24–29.

Terra W.R., Ferreira C. (1994) Insect digestive enzymes: properties, compartmentalization and function, Comp. Biochem. Physiol. B 109, 1–62.

Walker V.K., Williamson J.H. (1980) Ontogeny and tissue distribution of leucine aminopeptidase in *Drosophila melanogaster*, Insect Biochem. 10, 535–541.

Walker V.K., Williamson J.H., Church R.B. (1981) Differential characterization of two leucine aminopeptidases in *Drosophila melanogaster*, Biochem. Genet. 19, 47–60.

Yasuhara T., Yokosawa H., Ishii S. (1990) Purification and characterization of an aminopeptidase from sperm of the sea urchin, *Strongylocentrotus intermedius*, Ca^{2+}-dependent substrate specificity as a novel feature of the enzyme, J. Biochem. 107, 273–279.

Yasuhara T., Yokosawa H., Hoshi M., Ishii S. (1991) Involvement of a sperm aminopeptidase in fertilization of the sea urchin, Experientia 47, 100–103.