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White Pupa: a *Ceratitis capitata* Mutant Lacking Catecholamines for Tanning the Puparium

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The *white pupa* mutant of the Mediterranean fruit fly, *Ceratitis capitata*, fails to tan the puparium, but develops normal larval and adult cuticular structures. We found that the puparium of this mutant underwent minor stiffening at the beginning of pupariation, but subsequently did not increase further in stiffness. By the end of puparium formation, it was fivefold less resistant to compression than the wild type strain. Scanning electron microscopy of cross-sections of puparial exuviae revealed a dense sclerotized cuticle in the wild type, whereas the *white pupa* cuticle was quite distinct, with the inner two-thirds consisting of unsclerotized lamellae and the outer third being a dense, nonlaminar, amorphous layer. Puparial catecholamine levels were also very low in the *white pupa* when compared with the wild type strain, in which *N*-*β*-alanyldopamine (NBAD) predominated. However, in mutant hemolymph, NBAD, *N*-acetyldopamine (NADA), and dopamine were about 10 times more concentrated than in the normal phenotype. By injecting 1-^{14}C-*β*-alanine as a tracer, we confirmed that *N*-*β*-alanyldopamine incorporation into the puparium was much lower in the *white pupa* than in the wild type strain. However, insoluble cuticle phenoloxidase activity was similar in the two strains. Tanning occurred *in vitro* when *white pupa* puparial cuticle, free of epidermis, was incubated with either NBAD or NADA, and melanization occurred when the cuticle was incubated with dopamine, demonstrating that tanning enzymes, but not substrates, were present in *white pupa* puparial cuticle. Solid state ^{13}C nuclear magnetic resonance spectroscopy revealed that more chitin as well as less protein, catechols and *β*-alanine were present in the *white pupa* cuticle relative to the wild type. We conclude that the *white pupa* mutant is defective in the mechanism that provides hemolymph catecholamines to the puparial cuticle; this defect prevents normal sclerotization and pigmentation.

Catecholamines Cuticle tanning Sclerotization Puparium *Ceratitis capitata* Mediterranean fruit fly Transport *white pupa* Solids NMR

INTRODUCTION

Sclerotization is a process that hardens and stiffens insect cuticles. Pigmentation also may occur by formation of light yellow to dark brown sclerotins and black melanic pigments. When accompanied by the acquisition of a brown color, the sclerotization process is frequently called "tanning". These physical changes may occur by covalent cross-linking between *N*-acyldopamine quinones and certain amino acid residues of cuticular polypeptides and probably also chitin (Andersen, 1985; Schaefer *et al.*, 1987; Hopkins and Kramer, 1992). The quinone-sclerotizing agents are oxidation products of two catecholamines, *N*-acetyldopamine (NADA) (Karlson and Sekeris, 1962), and *N*-*β*-alanyldopamine (NBAD) (Hopkins *et al.*, 1982). A third catecholamine, dopamine (DA), appears to participate as the main precursor for melanin in cuticle (Hiruma *et al.*, 1985; Roseland *et al.*, 1987; Czapla *et al.*, 1990; Hopkins and Kramer, 1991). Catecholamines were reported to be synthesized by several insect tissues, but most of the available experimental work suggested that they are synthesized mainly in the epidermis (Maranda and Hodgetts, 1977; Hirsh, 1989; Krueger *et al.*, 1989; Hiruma and Riddiford, 1990). In hemolymph, catecholamines accumulate to relatively high concentrations

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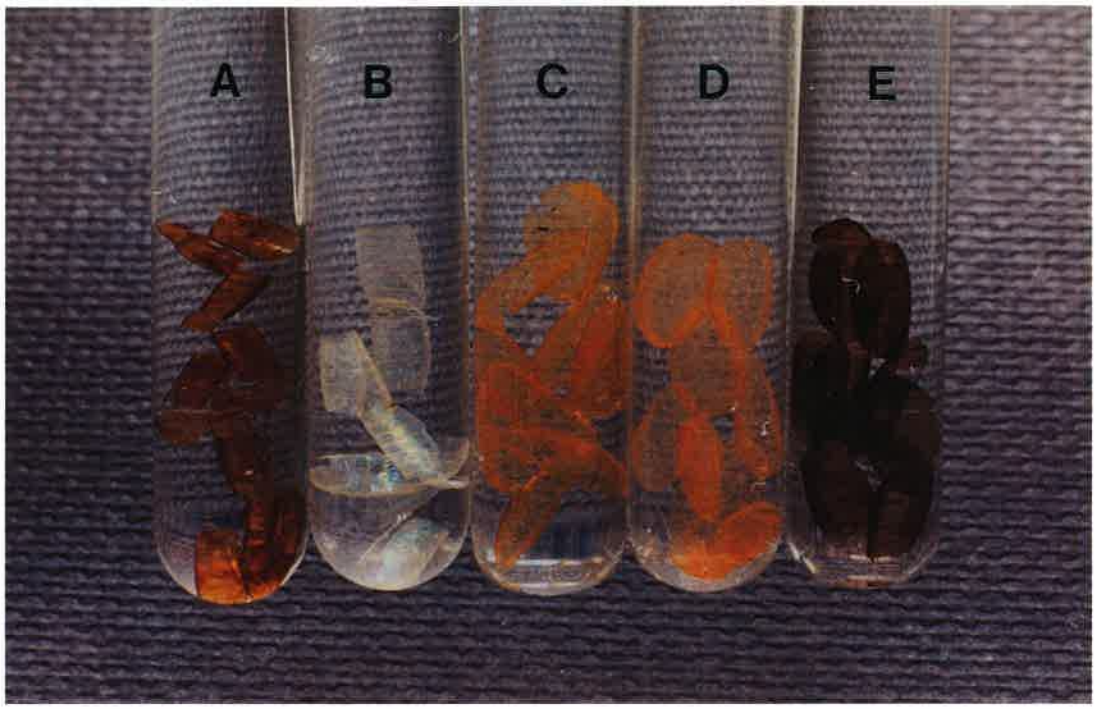

FIGURE 1. Effect of catecholamines on puparial cuticle tanning *in vitro* in *C. capitata white pupa* mutant. Cuticles dissected 2 h after the onset of pupariation and cleaned of epidermis. (A) Fully tanned wild type puparial exuviae showing a reddish brown color; (B) *white pupa* puparial exuviae, which are colorless; (C) *white pupa* puparial cuticles incubated with 0.3 mM *N*-β-alanyldopamine; (D) *white pupa* puparial cuticles incubated with 0.3 mM *N*-acetyldopamine. Puparia in C and D exhibit a color similar to that of 8 h-tanned wild type puparia. (E) *white pupa* puparial cuticles incubated with 0.3 mM dopamine showing a dark brown-black color that indicates melanization. Scale bar is 2 mm.

prior to cuticle tanning and usually are conjugated as glucosides or sulfates depending upon the species (Kramer and Hopkins, 1987; Hopkins and Kramer, 1991). At the time of cuticle tanning, hemolymph catecholamines are released from their conjugates and are transported by unknown mechanisms into the cuticle (Hopkins and Kramer, 1992).

The puparium of the Mediterranean fruit fly, *Ceratitis capitata*, is reddish brown after tanning has been completed (Fig. 1A). Starting with a genetic approach, we have studied several puparial color mutations in this fly. One of these mutations, the *white pupa*, exhibits a relatively uncolored, pale white puparium (Fig. 1B), whereas cuticular structures in the larval and adult stages of the mutant exhibit normal coloration (Rossler, 1979). In this study, we report about the distribution and function of catecholamines in wild type and *white pupa* strains and also on differences in mechanical properties between their puparial cuticles. The data support the hypothesis that a defect exists in the mechanism of supply or transport of hemolymph catecholamines into the puparial cuticle of the *white pupa*, which results in an untanned and less sclerotized exoskeleton.

MATERIALS AND METHODS

Insects

Medfly larvae were reared in the Argentinian laboratory as described by Terán (1977) or were provided kindly by Fanny Manso from the Instituto de Genética INTA, Castelar. Adults were fed a mixture of yeast and sugar. The growth chamber was maintained at 23°C and 55–80% relative humidity with a light:dark regime of L16:D8 h. The reference strain in all the experiments was the wild type strain, ARG-17.

Chemicals

N-β-Alanyldopamine was synthesized as described in Yamasaki *et al.* (1990). Dopamine, α-methyldopa and *N*-acetyldopamine were obtained from Sigma. 1-^{14}C-β-Alanine (54.5 mCi/mmol) was from New England Nuclear.

Mechanical resistance assay

Puparial cuticle was dissected immediately before testing to prevent drying. The 8th and 9th segments at various times up to 72 h after the onset of pupariation were sliced into ring-shaped structures with a razor blade, being careful not to fracture the puparium or tear the cuticle at the edge of the cut pieces. Soft tissues were removed by scraping the rings immersed in Ringer's solution (Chen, 1968) with a bent needle and cleaning with filter paper. Each dissected puparial ring was attached immediately on the ventral side to the roof of the compression device (Fig. 2). To measure compression resistance, a load was applied to the puparial ring until 50% of the original diameter was attained.

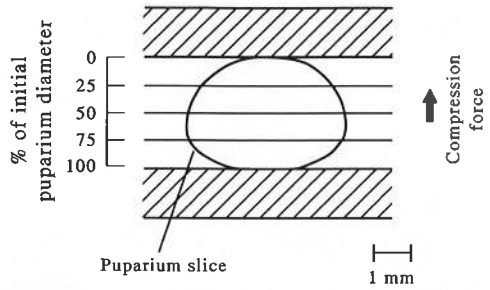

FIGURE 2. Device for measurement of puparial cuticle resistance to compression. The apparatus was built from a one-plate balance. The load applied on the plate is proportional to the vertical force made by the balance arm, which compresses the puparial ring.

Scanning electron microscopy (SEM)

Puparial exuviae were cleaned ultrasonically in a detergent solution, rinsed in distilled water, dried, and mounted on aluminum studs with silver paste. Cross-sections of the cuticle were exposed by breaking off pieces of cuticle, sputter coating with gold–palladium and viewing in an ETEC Autoscan electron microscope.

Catecholamine analysis

Puparia were dissected under deionized water saturated with phenylthiourea (PTU) and cleaned as described above. After blotting onto filter paper, the puparial cuticles were weighed, homogenized in 1.2 M HCl containing 5 mM ascorbic acid and centrifuged. The supernatant was used for catecholamine analysis. To collect hemolymph, six prepupae were punctured through the cuticle with an insect pin and immediately placed on a nylon tissue attached to the top of a centrifuge tube containing a few crystals of PTU at the bottom. After centrifugation at 4000 rpm in an Eppendorf 5415C microcentrifuge for 4 min, hemolymph free of fat body (4–6 μl) was collected, heated in 10% HCl for 10 min at 100°C, and extracted with one volume of chloroform, which was discarded. Catecholamines from either cuticle or hemolymph were extracted and analyzed by reversed phase HPLC with electrochemical detection using a C-18 column (Hopkins *et al.*, 1984). The mobile phase was 0.21% sodium octyl sulfate, 2.5 mM KCl, 0.04% Na_2EDTA, 0.6% phosphoric acid (v/v) and 6% acetonitrile. α-Methyl DOPA was used as an internal standard (Morgan *et al.*, 1987).

Phenoloxidase assay and in vitro tanning

Insoluble cuticular phenoloxidase activity was measured according to Andersen (1979) with minor modifications. Cuticles were dissected 2 h after the onset of pupariation in cold 1% potassium tetraborate and cleaned as described above. The cuticles were weighed immediately after blotting and stored in 1% potassium tetraborate at 4°C for several hours until phenoloxidase determinations were made. 2 mg of cuticle were incubated at 23°C in 6 ml of a stirred solution containing 0.0015% ascorbic acid; 0.03% Na_2EDTA; and 0.3 mM of either NBAD, NADA, or dopamine in 0.2 M sodium acetate buffer (pH 5.5). Absorbance at 265 nm was recorded every 5 min for a 25-min period in a Gilford spectrophotometer. UV spectra of the reaction mixture taken before and after incubation were compared. Controls with no cuticle also were incubated to assess the rate of spontaneous oxidation. To check that insoluble phenoloxidases were indeed measured, an aliquot of the supernatant was removed from the reaction mixture after 10 min, and absorbance was recorded 15 min later. No change in absorption was detected, demonstrating that no soluble enzymes were catalyzing the reaction.

For observation of tanning *in vitro*, 0.3 mM substrates were incubated with the puparial cuticles for 30 min at pH 5.5, and color development of the cuticle phenotypes was recorded 30 min later.

1-^{14}C-β-alanine injection and radioactivity distribution

Zero time prepupae were injected ventrally through the last intersegmental groove as described by Rabossi *et al.* (1991) with 0.5 μl (10^5 cpm) of 1-^{14}C-β-alanine dissolved in Ringer's solution. 24 h after injection, the puparia were dissected on filter paper, and the body contents spread out on the paper with a small spatula. Puparial cuticles were washed with distilled water and blotted on paper. Then, individual body contents and puparia were dried under an infrared lamp and introduced into liquid scintillation vials containing scintillation fluid for radioactivity determination. The percentage of radioactivity in the puparium was calculated based on the amount of radioactivity incorporated into the whole insect.

Nuclear magnetic resonance

Cross polarization, magic angle spinning ^{13}C NMR spectra were obtained on dried and powdered puparial samples ($<850\ \mu$m size) at room temperature at 50.3 MHz (Schaefer *et al.*, 1987). A single, 9 mm diameter, radio-frequency coil was connected by a low-loss transmission line to a quadruple resonance tuning circuit. The 1 kW ^{1}H and ^{13}C tuned transmitters produced maximum radio-frequency-field amplitudes of 120 and 75 kHz, respectively. Cross polarization transfers were performed at 50 kHz and proton dipolar decoupling at 100 kHz. Rotors with 0.35 g capacities were made from ceramic (zirconia) barrels fitted with plastic (Kel-F) end caps and supported at both ends by air-pumped journal bearings. Magic angle spinning was at 5 kH. In these experiments, 0.1 g samples were positioned in the center of the rotor with Kel-F spacers. Chemical shift assignments for chitin, protein, and catechols were the same as reported in Kramer *et al.* (1991). Relative percent normalized compositions of chitin, protein, catechol and lipid were estimated from integrated intensities of chemical shifts that were weighted by using an average number of carbons per subunit (Kramer *et al.*, 1989).

RESULTS

Mechanical resistance to deformation of puparial cuticle

Sclerotized cuticle may range in coloration from clear to dark brown or black depending upon the types of catecholamine metabolites available in the cuticle. Colorless or lightly colored stiff cuticles are dependent mainly upon NADA metabolism, whereas dark brown cuticles usually are due to NBAD (Hopkins *et al.*, 1982; Hopkins and Kramer, 1992). To determine whether the uncolored puparium of the *white pupa* mutant undergoes an increase in stiffness indicating the occurrence of sclerotization, we measured the resistance of the puparial cuticle to dorso-ventral compression until 50% of the original diameter was obtained. We compared the wild type and *white pupa* cuticles at different times during the prepupal and pupal stages. At the onset of pupariation (zero time, Rabossi *et al.*, 1991), shrinkage of the larval cuticle was almost completed (Rabossi *et al.*, 1992), and the ring of cuticle to be used for the compression assay, composed of the 8th and 9th segments, collapsed from its own weight. However, 15 min after zero time, the cuticle became stiff enough to maintain its oval shape. Thereafter, it was possible to measure the compression force necessary to attain 50% of the diameter. Resistance to compression of the wild type puparium increased rapidly while tanning proceeded and reached a maximum level approximately 24 h later, whereas the *white pupa* puparium did not substantially increase its resistance to compression after the initial puparial stabilization (Fig. 3). At 24 h, the wild type puparium was approximately 5 times more resistant to compression than the mutant puparium. We thus conclude that, after an initial period of stabilization of the puparial cuticle of the *white pupa* mutant, no sclerotization occurs thereafter, in contrast to the high degree of sclerotization which takes place in the wild type puparium.

SEM of puparial exuviae

Cross-sections of puparial exuviae of wild type and *white pupa* showed striking differences in the amount of sclerotization. Wild type cuticle appeared to be highly sclerotized, with the lamellae fused into a continuous dense, closely packed structure (Fig. 4A). *White pupa* cuticle, however, was made up of two distinct regions. The inner two-thirds was composed of well defined thin sheets or lamellae that appeared to be unsclerotized, because they separated when the cuticle was fractured (Fig. 4B). The sheets appeared to be interconnected by a fibrous network. The outer region of the cuticle was a dense amorphous secretion lacking any of the lamellar structure visible in the wild type cuticle.

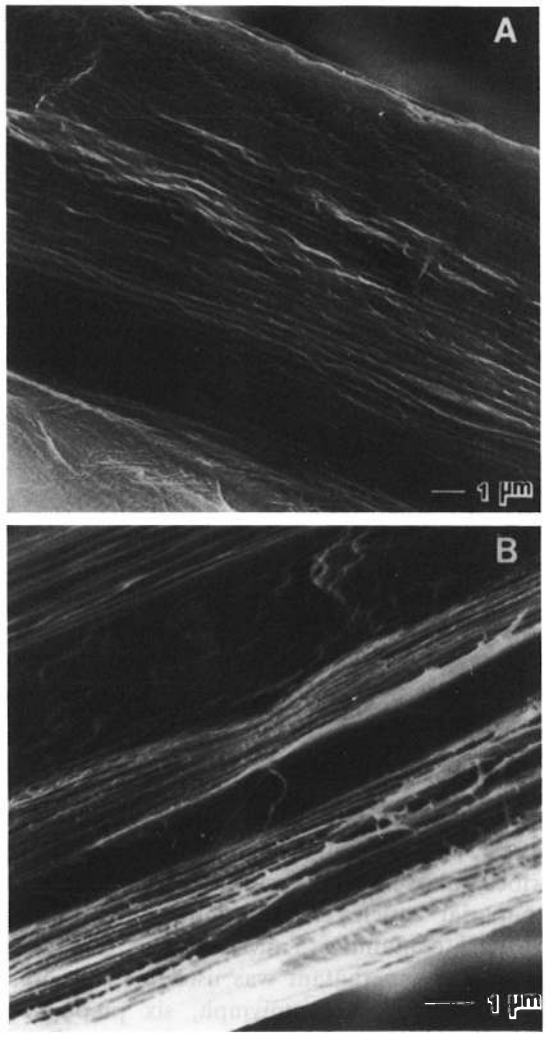

FIGURE 4. Scanning electron micrographs of cross-section of the puparial exuviae of wild type (A) and *white pupa* (B). The outer region of the cuticle is in the upper area.

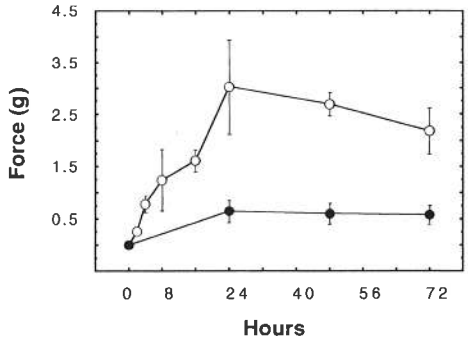

FIGURE 3. Force needed to cause 50% dorso-ventral compression of a ring of puparial cuticle. Zero time is the onset of pupariation. ○, wild type; ●, *white pupa*. Each point is the mean value from at least five determinations.

Catecholamines in hemolymph and cuticle

Because the protein composition of the *white pupa* puparium is similar to that of the wild type puparium (Rabossi *et al.*, 1991; P. Wappner *et al.*, unpublished data), the lack of tanning and sclerotization in the mutant puparium could be due to a diminished concentration of catecholamines, in either the whole body or the cuticle, or to a defective enzyme that utilizes the catecholamines as substrates for sclerotization. To determine whether diminished levels of catecholamines could account for the observed phenotype, we measured the amounts of NBAD, NADA, and dopamine extractable

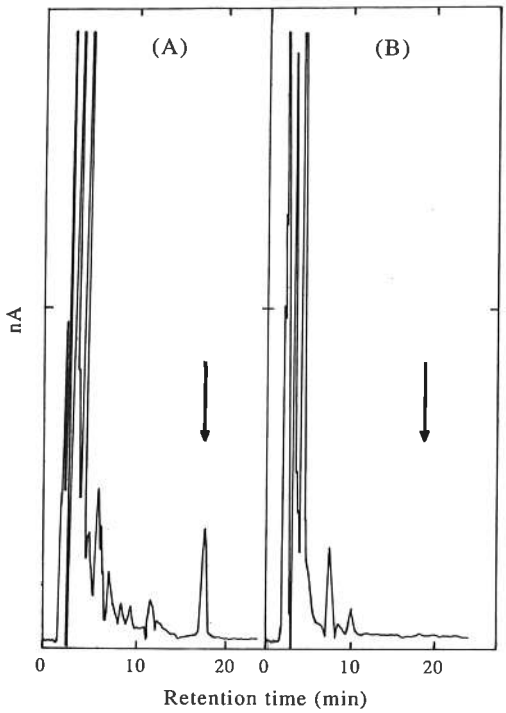

FIGURE 5. Reversed phase HPLC-EC profile of catecholamines extracted from 48 h puparia of (A) wild type strain and (B) *white pupa* mutant. The arrow indicates the retention time of *N*-β-alanyldopamine. Mobile phase composition is 0.21% sodium octyl sulphate, 2.5 mM KCI, 0.04% Na_2EDTA, 0.6% phosphoric acid (v/v), and 6% acetonitrile. α-Methyl DOPA was used as the internal standard.

from the puparium 48 h after the onset of pupariation when sclerotization had been completed, as well as the concentrations of catecholamines in hemolymph at different times during the pupariation process (Fig. 5). Whereas NBAD was the major catecholamine present in the wild type puparium, only a trace level was detected in the *white pupa* puparium (Table 1). In contrast, catecholamines in mutant hemolymph or whole body extracts were about 10 times more concentrated than the levels in the wild type strain throughout the 24 h developmental period (Fig. 6). Apparently, catecholamine synthesis occurs in the *white pupa*, but these potential precursors for sclerotizing agents do not reach the puparium.

In another experiment, 1-^{14}C-β-alanine was injected into wild type and mutant prepupae at the onset of pupariation, and, radioactivity in the puparium and remaining tissues was measured 24 h later. The data showed that, in the wild type strain, $91 \pm 4\%$ ($n = 10$) of

TABLE 1. Catecholamine levels in sclerotized puparial cuticles of *Ceratitis capitata* 48 hours after the onset of pupariation*

	NBAD	DA	NADA
Wild type	42.8 ± 3.2	4.4 ± 0.8	tr
white pupa	tr	3.5 ± 0.4	tr

*Mean value $\pm$ SEM ($n = 3$). Units = pmol/mg puparium. NBAD: *N*-β-alanyldopamine; DA: Dopamine; NADA: *N*-acetyldopamine; tr: trace level (<1 pmol) detected.

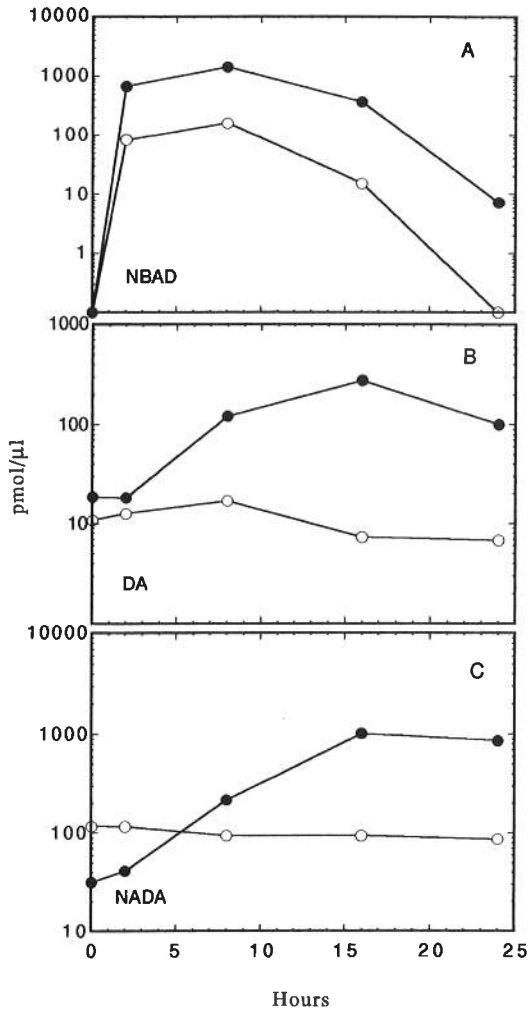

FIGURE 6. Temporal profile of catecholamine concentrations in *C. capitata* hemolymph during development. Zero time is the onset of pupariation. ○: wild type; ●: *white pupa*. Panel (A) *N*-β-alanyldopamine; (B) dopamine; (C) *N*-acetyldopamine. Concentrations were calculated by comparing hemolymph and standard catecholamines resolved by HPLC. α-methyl-DOPA was used as the internal standard to assess the recovery of catecholamines from alumina (see Materials and Methods). Each point is the mean value of two determinations.

the radioactivity incorporated into the body was found in the puparium, whereas only $11 \pm 1\%$ ($n = 10$) was recovered in the *white pupa* puparium. Presumably, the radiolabel in these puparia was derived from incorporation of ^{14}C-NBAD.

Phenoloxidase determinations

Since some embryos of phenoloxidase-defective lethal mutants of *Drosophila melanogaster*, which lack any kind of cuticle sclerotization, show elevated levels of whole-body catecholamines (Ritzki *et al.*, 1985), we tested whether a stage-specific defect in phenoloxidase activity could account for the *white pupa* puparial phenotype. For this purpose, we measured insoluble cuticular phenoloxidase activity at the beginning of pupariation. To determine cuticular activity, equal weights of 2-h-old wild type or mutant puparia free of epidermis were incubated with ascorbic acid and either NBAD, NADA,

or dopamine, and the relative rate of oxidation was determined by the decrease in absorbance at 265 nm as a function of time (Fig. 7). The results demonstrated that little or no differences existed in the insoluble activity when either of the three substrates was tested. For cuticular enzymes of both the wild type and *white pupa*, the relative rate of oxidation was in the order of NBAD > DA > NADA (Fig. 7). Interestingly, after 1 h of incubation with NBAD or NADA, *white pupa* cuticles acquired a reddish brown color (Fig. 1C and D), which was similar to the color of partially sclerotized (8-h-old) wild type puparia. When dopamine was used as the substrate, dark brown or black puparia were obtained (Fig. 1E). *In vitro* results confirmed that normal tanning occurred in the *white pupa* mutant when *N*-acyl-catecholamines were made available to the puparium, thus indicating that tanning enzymes were present in the mutant cuticle. All of these results indicate that the *white pupa* fails to incorporate catecholamines into the puparium, which consequently accumulate in the hemolymph.

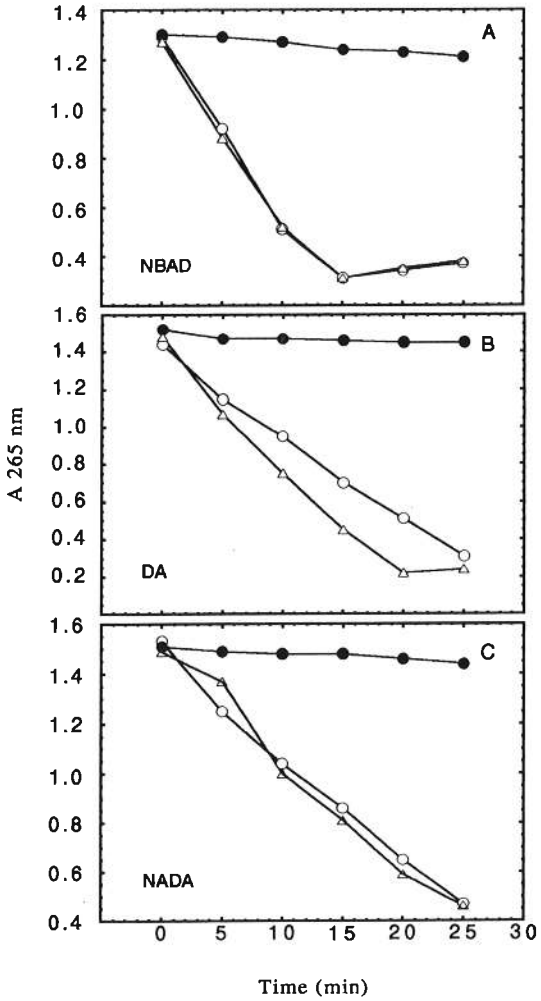

FIGURE 7. Cuticular phenoloxidase activity of 2-h-old puparia of *C. capitata*. Activity was assessed as the decrease in absorbance at 265 nm. ○: wild type strain; △: *white pupa* mutant. ● are controls without any cuticle added. Substrates were (A) *N*-β-alanyldopamine; (B) dopamine or (C) *N*-acetyldopamine. The reaction mixture contained 0.0015% ascorbic acid, 0.03% Na_2EDTA, and 0.3 mM of the substrate in 0.2 M sodium acetate buffer pH 5.5. Two mg of cuticle were used in each assay. Each point is the mean value of two determinations.

Solid state NMR

The natural abundance ^{13}C-NMR spectra of puparial cuticles from the *white pupa* and the wild type strain are shown in Fig. 8, together with their difference spectrum. Chitin was by far the major organic component in both types of puparia. The wild type exuviae was composed of approximately 86% chitin, whereas the pale mutant had 93% as evidenced by the sharp peaks between 20 and 105 ppm in the difference spectrum. There were only 10 and 5% protein, and 3 and 1% catechols in the respective puparia. The broad negative signal from 30 to 50 ppm and the negative signal at 175 ppm indicated that the wild type puparial exuviae had more β-alanine, presumably as NBAD, than the *white pupa* (Kramer *et al.*, 1989). The broad aromatic signal from 110 to 150 ppm showed that wild type cuticle had more aromatic and catecholic carbons as well. The lipid content in both types of puparia was low, only about 1%.

DISCUSSION

We have described a *C. capitata* mutant, *white pupa*, lacking both stiffness and pigmentation of the puparium. Analysis of cuticular catecholamines indicated that NBAD was the main precursor for sclerotizing agents in the *C. capitata* puparium, although relatively high levels of both NADA and NBAD were present in hemolymph. This finding also was supported by the fact that cuticular phenoloxidase activity was higher for NBAD than for NADA or dopamine. Results from preliminary experiments indicate that approximately 60% of the phenoloxidase activity is of the laccase type. NBAD is also the preferred substrate for phenoloxidases in the dark brown cuticle of the tobacco hornworm, *Manduca sexta* (Hopkins *et al.*, 1982; Kramer *et al.*, 1983). However, *white pupa* puparia incubated *in vitro* with NADA showed a reddish brown color similar to that of puparia incubated with NBAD.

Side chain desaturation of NADA to 1,2-dehydro NADA is one pathway for synthesis of sclerotizing agents and has been observed in both locusts with light colored cuticles and puparia of other Diptera (Andersen, 1989a, b; Saul and Sugumaran, 1989). NADA and NBAD also can be involved in ring and β-carbon cross-linking reactions with proteins in brown sclerotized cuticles (Morgan *et al.*, 1987; Andersen, 1989c, 1990). It seems likely that, in the *C. capitata* puparium, enzymes from the ring and β-carbon sclerotization pathway are active, even with NADA as a substrate.

Although *white pupa* puparial cuticle did undergo some initial stiffening at the beginning of pupariation, the resistance to compression did not increase substantially and remained approximately one-fifth that of the wild type puparium. The nature of this initial stabiliz-

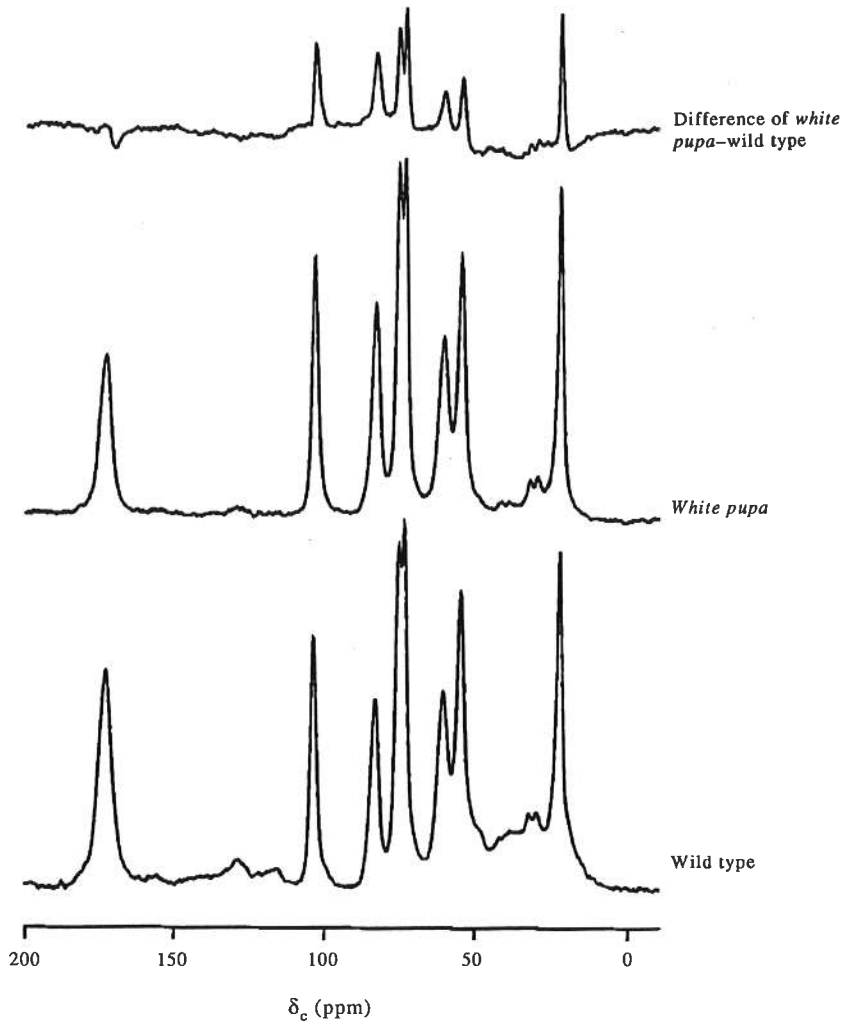

FIGURE 8. Natural abundance 50.3 MHz ^{13}C-NMR spectra of *Ceratitis capitata* puparial exuviae. Bottom spectrum, wild type; middle spectrum, *white pupa*; and top, difference spectrum of wild type subtracted from *white pupa*. Magic angle spinning was at 5 kHz.

ation is unclear (Fraenkel and Rudall, 1940; Brunet, 1980), but normal sclerotization and pigmentation are lacking in the *white pupa*. SEM observations on the structure of the normal phenotype and *white pupa* puparial exuviae showed differences in the extent of sclerotization in the two strains that were consistent with the mechanical strength measurements. The layers or lamellae in wild type cuticle were fused uniformly into a rigid sclerotized structure, whereas those of the *white pupa* cuticle were distinct and appeared to be bound loosely to unsclerotized sheets of material. The outer dense amorphous layer was nonlaminar and it may be responsible for the stiffening of the cuticle that occurred at the beginning of pupariation in the *white pupa*. These observations were in agreement with the low levels of catecholamines found in the mutant puparium compared to the wild type and the high levels that accumulated in hemolymph. NBAD, NADA, and dopamine showed 10 times higher concentrations in mutant hemolymph relative to the wild type. The solid NMR data provided direct information about the relative abundance of organic components and showed that not only were there fewer catecholamines, but also relatively more chitin and less protein in the *white pupa* puparial cuticle. Both medfly puparial exuviae were remarkably rich in chitin. All of these results were consistent with the hypothesis that, although *N*-acylated catecholamines were available in mutant hemolymph, they did not enter into the puparial cuticle, which, therefore, neither tanned nor stiffened. The slight initial stiffening of the *white pupa* puparial cuticle might be due to chitin deposition, whereas in the wild type puparial cuticle the high degree of sclerotization and pigmentation apparently was caused by catecholamine metabolism.

Little is known about the mechanisms responsible for transporting catecholamines from the hemolymph into the cuticle (Koeppe and Gilbert, 1974). Nor is it known where synthesis of hemolymph catecholamines occurs in *C. capitata*. The fact that catecholamines were largely absent from *white pupa* puparial cuticle, but they were found in abundance in hemolymph, suggested that epidermal transport or metabolism of the catecholamines

was somehow defective. If, as was found in *M. sexta* (Krueger *et al.*, 1989), catecholamines are synthesized mainly by the epidermis, the *white pupa* epidermis may be defective in apical transport into the cuticle and, instead, may release most of the catecholamines through the basolateral surface into the hemolymph. Another lesion that would disrupt catecholamine incorporation into the puparial cuticle is a mutation in a gene that encodes or regulates a hydrolytic enzyme for catecholamine conjugates. Prior to tanning, catecholamines, which may be sequestered in the hemolymph as glucosides, phosphates, or sulfates, are liberated by glucosidases, phosphatases, or sulfatases and incorporated into the cuticle (Kramer and Hopkins, 1987; Hopkins and Kramer, 1991). If the hydrolytic enzyme did not catalyze the hydrolysis, presumably little or no free catecholamine would become available for transport into the procuticle. Whether *C. capitata* sequesters catecholamines as conjugates in the hemolymph is unknown, but tyrosine is coupled with glucose as tyrosine glucoside, which serves as a tyrosine reservoir for sclerotization of the puparium (Psarianos *et al.*, 1985).

A defect in or misregulation by hormonal or neurohormonal factors that normally modulate the steps of pupariation (Zdárek, 1985) could be responsible for catecholamine transport failure or the absence of the enzyme required to hydrolyze catecholamine conjugates. Zdárek and Denlinger (1992) used ligature and denervation methods to demonstrate that in the tsetse fly, *Glossina morsitans*, puparial tanning is inhibited transiently by the central nervous system (CNS) until morphological events leading to formation of the puparium have been completed. Apparently, the targets for the inhibition are the epidermal cells underlying the cuticle to be tanned. Considering that, during this developmental period, tanning precursors probably are already synthesized, the putative epidermal catecholamine export system might be the target for the transient inhibition. In wild type *C. capitata*, we found that anterior inhibition of tanning occurs within a narrow time frame between 1 and 0.25 h prior to the onset of puparial tanning, when morphogenetic contractions of the body occur, because larvae ligated within this period sclerotized the puparium only in the posterior half of the body (P. Wappner, unpublished data). When the *white pupa* mutant is ligated within this period, no tanning is observed in either half of the body. This observation rules out the possibility that the *white pupa* phenotype is caused by a failure to cease CNS inhibition when it is expected to occur.

According to our knowledge, mutants deficient in catecholamine epidermal transport or glucoside conjugate hydrolysis have not been described in insects previously. *D. melanogaster* mutants described to date, which do not tan, are either defective in catecholamine synthesis (Wright, 1987) or in phenoloxidase activity (Ritzki *et al.*, 1985), and in general those defects are lethal. The *C. capitata white pupa* mutant described here affords us with an opportunity to characterize more fully the biochemical basis for this lesion and perhaps to understand better how epidermal catecholamine transport and conjugate hydrolysis are regulated during insect development.

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