

The main sclerotization precursor of insect brown cuticles is also an essential component of the innate immunity

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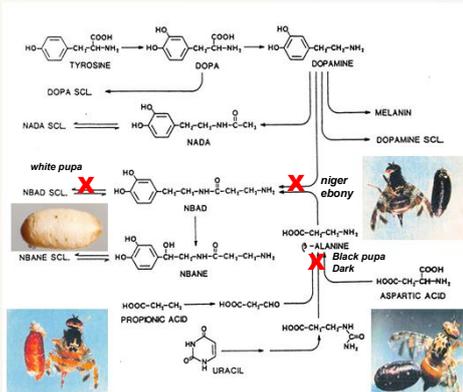
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Introduction

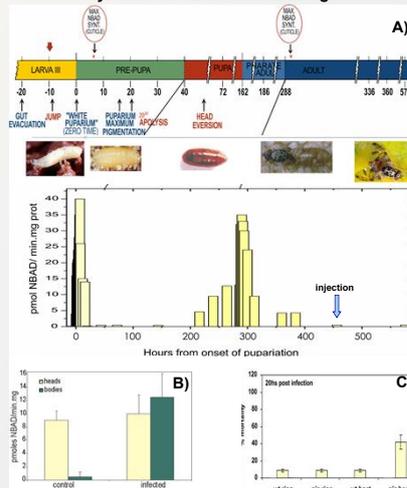
N-β-alanyldopamine (NBAD) is the main sclerotization precursor of insect brown cuticles. The enzyme conjugating dopamine and β-alanine (NBAD-synthase) is induced in epidermis only when the insect needs to sclerotize and tan the cuticle i.e. larval to pupal transition and adult ecdysis. We demonstrated that this enzyme is present in nervous tissue in a constitutive manner. We show here, in two holometabolous insects *Tenebrio molitor* and *Ceratitis capitata*, that this enzymatic activity is activated in the epidermis when the cuticle is disrupted and pathogens enter into the haemocoel. We demonstrated in cell free extracts that NBAD-synthase exhibits the same properties of the ecdysone-induced enzyme expressed only at the time of cuticle sclerotization. In *C. capitata* the mutant *niger* is unable to synthesize NBAD. This mutant shows higher mortality than wild types flies when they are infected with bacteria. Finally we demonstrated the antimicrobial properties of NBAD *in vitro*.

Metabolic pathway for the synthesis of sclerotization precursors



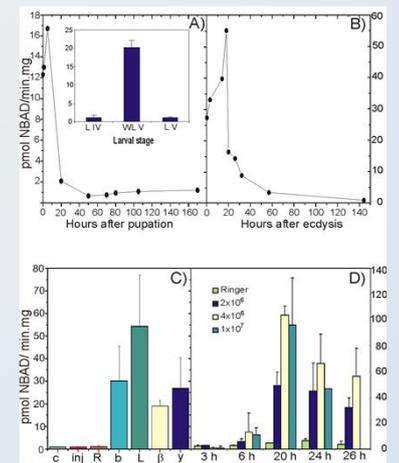
Metabolic way for the synthesis of cuticle sclerotization precursors. The red crosses show the steps that cannot be solved by *C. capitata* mutants, *niger*, *Dark/Black pupa* and *white pupa* and the *Drosophila melanogaster ebony* and *Black* mutants.

Main events of *C. capitata* life cycle and induction of NBAD-synthase after bacterial challenge



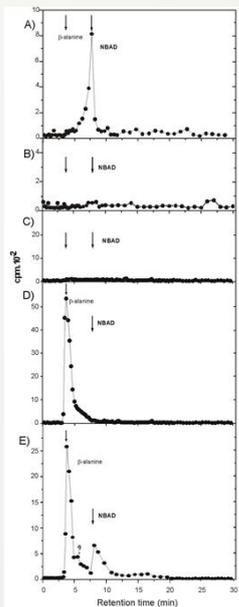
A) Main biochemical events during *C. capitata* metamorphosis (upper panel) and the activity profile of NBAD-synthase in the medfly life cycle. B) Induction of NBAD-synthase in *C. capitata* adults, activity in heads and body carcasses 20 h after *Escherichia coli* injection. C) Mortality rates of wt and *niger* mutant (*nig*) 20h after bacteria injection.

Induction of NBAD-synthase after microbial challenge in *Tenebrio molitor*.



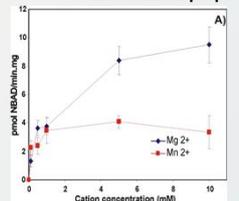
Enzymatic activity of NBAD-synthase in *T. molitor*. A) activity in pupae, time zero indicates the onset of pupation. Inset: activity in non-sclerotizing larva IV and V (LIV, LVLV) and sclerotizing white larva V (WLIV). B) activity after adult ecdysis. C) activity after the infection with different antigens or microbes. c: control (without injection); inj: injured; R: injected with Ringer; b: *E. coli*; L: bacterial lipopolysaccharide; β: algal β-1-3 glycan; y: *S. cerevisiae*. D) Time dependent induction of NBAD-synthase.

In vivo synthesis of NBAD in *Tenebrio molitor* larvae infected with bacteria

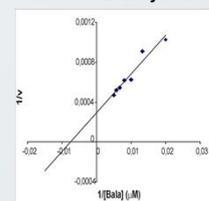


In vivo induction of NBAD synthesis in *T. molitor* larvae injected with [¹⁴C] β-alanine with or without *E. coli*. A) injection with bacteria; B) control, same as A) but without bacteria; C-D) injection as in B) but with dopamine; E) injection as in A) plus tyramine.

Biochemical properties of induced NBAD-synthase

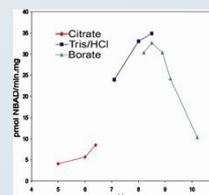


Divalent cation dependence of NBAD-synthase.



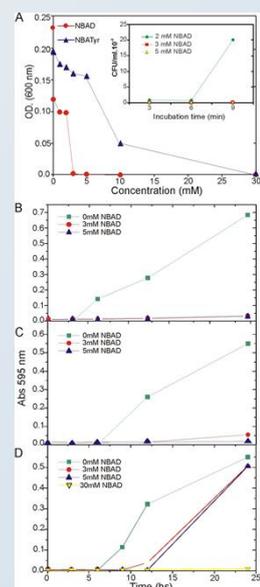
Reciprocal plot for NBAD-synthase kinetic parameters with β-alanine as substrate. $K_{M, \beta}$ 130 μM, V_{max} 330 pmole/min.mg.

Time dependent decay of NBAD-synthase activity, preincubated at 22°C, using dopamine (NBAD) or norepinephrine (NBANE) as substrate.

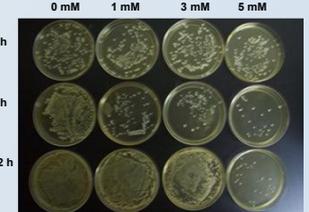


Activity of NBAD-synthase at different pH

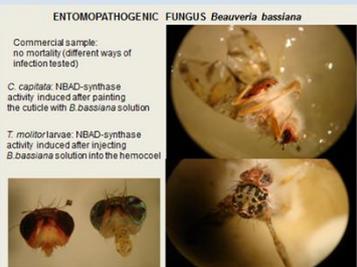
Antimicrobial activity of NBAD



Antimicrobial activity of NBAD. A) concentration-dependent inhibition of *E. coli* cultures in presence of NBAD or sarcophagine (NBATyrosine). Inset: time dependent colony formation capacity after culture with NBAD. B-D) time-dependent effect of NBAD in *E. coli* (B), *Bacillus megaterium* (C) and *S. cerevisiae* (D) cultures.



S. cerevisiae colonies cultivated with different concentration of NBAD. An aliquot of the cultures were plated out after different times of culture.



ENTOMOPATHOGENIC FUNGUS *Beauveria bassiana*

Commercial sample no mortality (different ways of infection tested)

C. capitata: NBAD-synthase activity induced after painting the cuticle with *B. bassiana* solution

T. molitor larvae: NBAD-synthase activity induced after injecting *B. bassiana* solution into the haemocoel

Conclusions

- The synthesis of NBAD was activated in epidermis after infection with microorganisms.
- The activated enzyme showed the same properties of the ecdysone-induced epidermis enzyme.
- The neural tissue enzymatic activity was not enhanced.
- The *niger* mutant was unable to express the activity after microbial challenge. This mutant showed higher mortality than wt flies.
- NBAD showed antimicrobial properties *in vitro*.