ABSTRACTS (MASTER THESIS)

Measuring stress by biogenic amine analysis using LC/MS with a subterranean termite *Coptotermes formosanus* Shiraki

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Introduction

Stress response has been widely studied and become one of the more important research topics in entomology. There have been few reports, however, on the stress response of wood-attacking insects such as termites. The influence of the homeostasis disturbance caused by stress affects the behavior and mortality of insects. Thus, the impact of stress should not be disregarded in bioassays of termites.

The purpose of this study was to establish the protocol for measuring stress in termites. Biogenic amines, dopamine and octopamine were target chemicals, measured by liquid chromatography-mass spectrometry (LC/MS). The two amines had the same molecular weight of 153.18, and the special protocol was indispensable for LC/MS analysis.

Materials and methods

Mature workers of a subterranean termite (*Coptotermes formosanus* Shiraki) were collected from a laboratory colony maintained in the Deterioration Organisms Laboratory (DOL), RISH, at Kyoto University. The insects were exposed to 4 kinds of stresses: physical (vibration), nutritional (starvation), social (isolation) and physical/social (isolation by a stainless-mesh). They were then killed quickly by dipping into liquid nitrogen, and their brains were carefully collected with a pair of fine forceps. The brains were deproteinized by the mixing ethanol precipitation-centrifugation method, and the obtained samples served for LC/MS analysis.

Results and discussion

By investigating many combinations of mobile phases and columns, a protocol for analyzing dopamine and octopamine in the termite brain was established. Octopamine and dopamine were successfully separated from the samples by this protocol, and were quantitatively analyzed. The brains of the freshly collected *C. formosanus* workers contained high levels of octopamine and low levels of dopamine.

When the termites experienced stress, octopamine levels were significantly decreased, regardless of stressor. In contrast, dopamine level change depended on the stressor. Dopamine level was increased by vibration (physical stress), decreased by starvation (nutritional stress), and did not change by isolation (social stress). Interestingly, when facing combined stress (physical/social stresses by isolation), the insects showed a combined reaction against the two stresses. Octopamine levels were more stable than those of dopamine. The results were consistent with those of other insects such as German cockroaches.

Breeding with more than 5 individuals can reduce stress in termites. This clearly shows stress relaxation by chemical communication with nest mates in termite societies, suggesting that results of laboratory bioassays, such as evaluation of the termiticidal effectiveness of chemicals and anti-termite performance of building materials, might depend on the population of test insects. Collecting data on the relationships between other biogenic amines and other types of stressors will help us understand the physiology of termites, an extremely economically important insect, in more detail.

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