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[REVIEW]

Cryptobiosis in the African Chironomid: Physiological Mechanism to Survive Complete Dehydration

Takashi OKUDA[1)], Masahiko WATANABE[1)], Takahiro KIKAWADA[1)], Akihiko FUJITA[1)] and Ewa FORCZEK[2)]

[1)] *National Institute of Agrobiological Sciences, Owashi 1–2, Tsukuba, Ibaraki 305–8634, Japan*
[2)] *Jagiellonian University, Department of Entomology, Ingardena 6, 30–060 Kraków, Poland*
E-mail: oku@affrc.go.jp (TO)

It is well known that some microscopic organisms, such as bacteria, fungal spore, yeast cells, nematodes, tardigrades and cysts of crustacean animals are fully desiccated to go into a complete metabolic arrest. Once desiccated, such dormancy often called "cryptobiosis" or "anhydrobiosis" may last over 100 years unless given water (Keilin, 1959). The present review describes recent works done by Watanabe *et al.* (2002, 2003) on the physiological mechanism allowing an insect to survive complete dehydration to provide some hints to understand "cryptobiosis" a peculiar biological state.

Cryptobiosis of the sleeping chironomid

The sleeping chironomid, *Polypedilum vanderplanki*, is the highest and largest multicelluler animal with cryptobiotic ability (Hinton, 1951, 1960a). This species breeds in small and shallow temporal rock pools in semi-arid regions in Africa (Fig. 1). When the pools dry up, the larvae also dry up and go into a cryptobiotic state until the next rain. The cryptobiotic larvae show extremely high thermal tolerance from −270 to 102℃ and can recover soon after

Fig. 1 Habitat of *Polypedilum vanderplanki* larvae in semi-arid regions in Nigeria. Arrows indicate the shallow temporal pools where the larvae breed.

prolonged dehydration for even up to 17 years (Hinton, 1960a, b; Adams, 1985).

Surprisingly nobody has investigated physiological mechanisms of cryptobiosis in this unique chironomid for more than 40 years after Hinton's works. In 2000, as our group succeeded in continuous rearing of this chironomid in the laboratory, we started studying physiological analysis of the cryptobiosis.

Factors inducing cryptobiosis

When *P. vanderplanki* larvae are dehydrated either over 2 days with 0.44 ml of distilled water or over 7 days with 1.5 ml of distilled water, most of the dehydrated larvae recovered within an hour after rehydration (Fig. 2A). During desiccation, the larvae accumulated a large amount of trehalose, a kind of disaccharide (*ca.* 20 to 25% of the dry body weight) (Fig. 3A, B). On the other hand, larvae dehydrated quickly within 1 day did not accumulate trehalose so much (less than 3% of the dry body weight), and did not recover after rehydration. Slow desiccation may provide a lag time to accumulate enough trehalose to succeed cryptobiosis induction.

During desiccation, cryptobiotic tardigrades contract into a structure resembling a small tun (Wright, 1989). The tun formation is essential in the preparation for cryptobiosis by helping to reduce transpiration. If tardigrades are dried under anesthesia (Crowe, 1972), they do not form a tun but shrink into a flat formless skin and do not survive. Similarly, if they are dried at low relative humidity (RH), water loss is too fast and they are unable to form tun. Tardigrades start accumulating trehalose abruptly after tun formation (Westh and Ramlov, 1991). Trehalose is known as a common compatible solute among cryptobiotic organisms (Ingram and Bartels, 1996; Clegg, 2001).

Significance of trehalose on cryptobiosis

It is of interest to discuss why trehalose was selected for a compatible solute for cryptobiosis in various groups of organisms such as bacteria, plants and animals. A non-reducing sugar, trehalose, is less harmful for cells and tissues even at extremely high concentration. Trehalose appears to have two functions in the desiccating organism (Burke, 1986; Crowe *et al.*, 1987, 1998; Green and Angell, 1989). One is its high ability for water-replacement, *i. e.*, trehalose can maintain structures of cell membrane and proteins as substitute for bound and free water. The other role is vitrification. Glasses of trehalose may fill space in tissues during dehydration and serve to inhibit additional increases in tissue collapse, aggregation of biological molecules and solute concentration. High and stable viscosity of trehalose

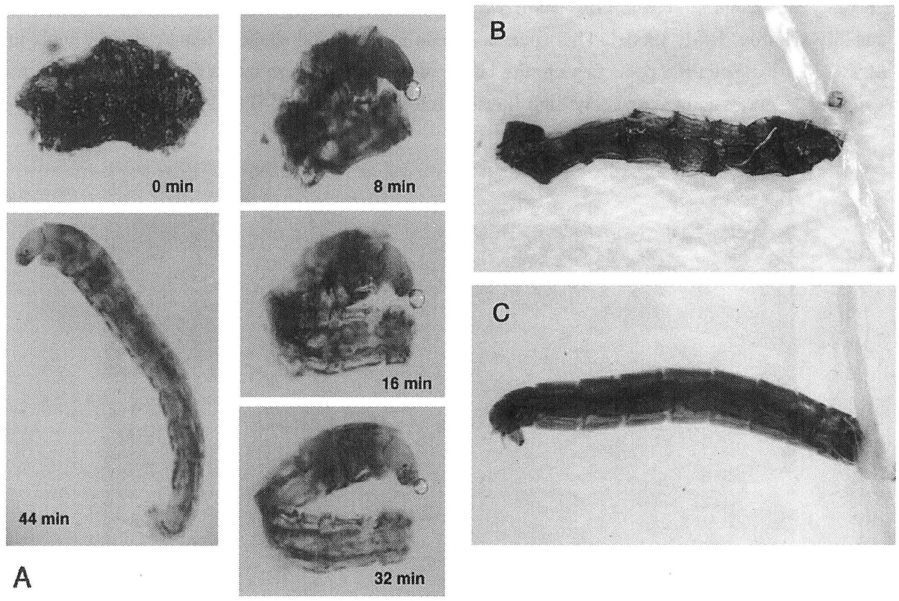

Fig. 2 *Polypedilum vanderplanki* during recovery from cryptobiosis. A. larva recovering from cryptobiosis at 0, 8, 16, 32 and 44 min after rehydration. B. Decapitated cryptobiotic larva. C. Actively moving decapitated larva on the second day after rehydration. From Watanabe *et al.* (2002).

glasses may also stop all chemical reactions that require molecular diffusion. Furthermore, recent study shows that this compound protects cellular fatty acids and proteins most efficiently from damage by oxidation (Benaroudj *et al.*, 2001; Oku *et al.*, 2003). Thus, the cooperative actions of these superior traits in trehalose appear to enable organisms to enter cryptobiosis.

Internal factors triggering explosive trehalose synthesis

It is also interesting to know what is the cue triggering the rapid synthesis of trehalose during cryptobiosis in *P. vanderplanki*. Rapid trehalose synthesis occurred when the body water content decreased below 75%, indicating that changes of osmolarity occurring in the body are the likely cue for the explosive trehalose synthesis. Indeed, exposure to high salinity effectively triggered an explosive accumulation of trehalose even without desiccation treatment (Figs. 4, 5). In the yeast, *Saccharomyces cerevisiae*, exposure to high extracellular osmolarity induced an osmosensor to activate the HOG and MAP kinase cascades, and finally caused accumulation of glycerol (Maeda *et al.*, 1994; Posas and Saito, 1997). This activation response is induced by high osmolarity regardless of the kinds of solute (Maeda, 1999). Similar osmosensor has already been found in a higher plant, *Arabidopsis thaliana* (Urano *et al.*, 1999), and signal transduction cascades of the osmotic response are activated under desiccation and high salinity stress (Shinozaki and Yamaguchi-Shinozaki, 1997; Urano *et al.*, 1998). In the case of *P. vanderplanki* larvae, exposures to both desiccation and solutions containing salt or other substances at high concentrations were able to cause an internal increase of salt and other solute concentrations. The change is caused by gradual evaporation of water from the body in the former and invasion of solutes into the body in the latter. These treatments allowed larval tissues and cells become exposed to high osmolarity and high concentrations of various solutes. Unlike the yeast and *A. thaliana*, rapid accumulation of

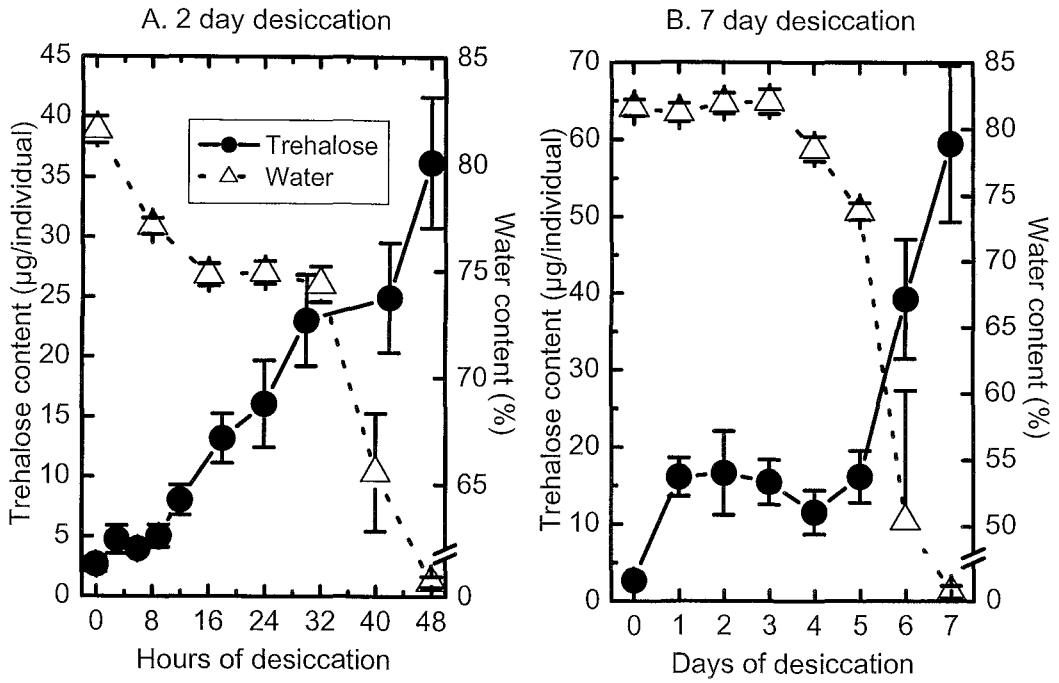

Fig. 3 Changes of trehalose and water contents in *Polypedilum vanderplanki* larvae during desiccation over 2 (A) and 7 days (B). Solid lines with closed symbols, trehalose content; dotted lines with open symbols, water content. n = 6–10 for trehalose and 12 for water. Last instar larvae with around 1 mg of wet body weight were used for all experiments. Each of five to seven larvae was placed on pieces of filter paper with 0.44 or 1.5 ml of distilled water in a glass Petri dish (diameter, 65 mm; height, 20 mm). These dishes were immediately transferred to a desiccator (<5% RH) at room temperature (24–26℃). In the desiccator, the dishes were gradually dehydrated over 2 days with 0.44 ml and 7 days with 1.5 ml of distilled water. Trehalose and water content were measured as described by Watanabe *et al.* (2002, 2003).

trehalose was not a simple osmotic response in *P. vanderplanki* larvae, *i. e.*, the explosive production occurred mainly in high concentration of salt solutions, and depended on the kinds of cation in solution. Thus, increase of internal ion concentration triggers the explosive trehalose synthesis associated with cryptobiosis in this species.

Figure 5 shows trehalose content in larvae incubated in various salt and carbohydrate solutions for 1 day at the same osmotic pressures (342 mOs). There was no significant difference in trehalose content between untreated larvae (control) and those treated in solutions of glycerol, and dimethyl sulfoxide (DMSO). All of the salt solutions except for KCl and K_2SO_4 were more effective in eliciting trehalose accumulation than those of carbohydrates such as mannitol, glycerol and DMSO. Salt molecules dissociate into cations and anions in solution. Effects of cations on trehalose content between molecules with the same kinds of anions were compared. Na^+ tended to be more effective in stimulating trehalose accumulation than K^+, especially with Cl^- and NO_3^-, suggesting that an increase of extracellular

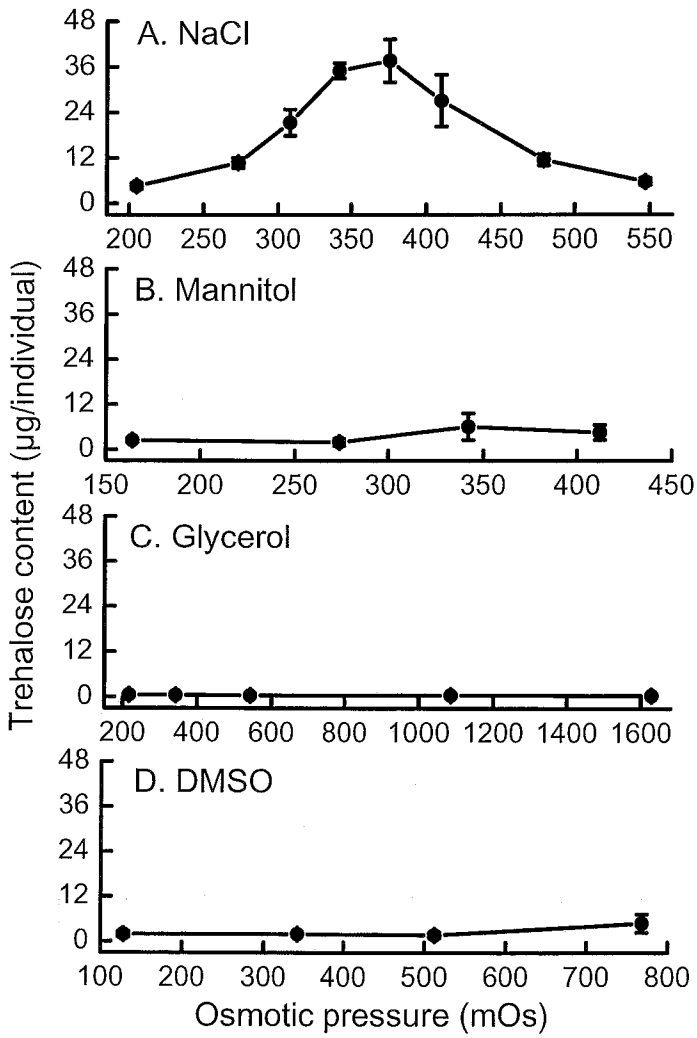

Fig. 4 Trehalose content of *Polypedilum vanderplanki* larvae incubated for 1 day in NaCl, mannitol, glycerol and DMSO solutions at various osmotic pressures. One percent of NaCl solution is 342 mOs. n = 4–10 each. Groups of five to 20 larvae were submerged in *ca.* 20 ml of various kinds of solution in the glass Petri dish, and incubated for 24 h at room temperature. Solutions of NaCl, mannitol, glycerol and DMSO were applied at various osmotic pressures (205 to 547 mOs in NaCl; 164 to 412 mOs in mannitol; 217 to 1628 mOs in glycerol; 128 to 768 mOs in DMSO). From Watanabe *et al.* (2003).

Na^+ due to dehydration appeared to trigger trehalose synthesis.

Mechanisms regulating induction and termination of cryptobiosis

Many insects not only in temperate but also tropical regions enter diapause at various developmental stages to survive adverse seasons such as winter and drought. The brain is the common prime regulator for induction, maintenance and/or termination of diapause (Denlinger, 1985). The corpora allata and the prothoracic gland are main endocrine organs for regulating diapause. Therefore, the core of regulation and endocrine system are distributed in head and thorax parts. To elucidate the possible role of the brain, suboesophageal ganglion (SG) and thoracic ganglia (TG) in induction of cryptobiosis of *P. vanderplanki*, we used larvae deprived of these endocrine organs after ligation and followed by desiccation over 3 days (Fig. 2B). When completely dehydrated larvae were later submerged in water, most of the decapitated larvae (n = 20, 95%) and a half of those from which the head and thorax were removed (n = 8, 50%) were able to recover (Fig. 2C). Some of the decapitated larvae moved actively and survived for more than 2 weeks after rehydration. During desiccation, those treated larvae also accumulated a large amount of trehalose, although only about half amount of the intact larvae (–H, dry and –HT, dry in Fig. 6). When the decapitated larvae were continuously incubated in water, they did not accumulate trehalose (–H, wet). The brain, SG and TG did not affect the induction and termination of cryptobiosis, *i. e.*, cryptobiosis occurs without the regulation of the brain, SG and TG. We did not exclude a possibility that the abdominal ganglia (AG), a part of central nervous system might regulate the cryptobiosis. At least for the termination of cryptobiosis it is obvious that the central nervous system is not involved at all due to an evidence that even a small piece of desiccated larval body or tissues could revive after rehydration (Hinton, 1960a). We recently found that the excised fat body could synthesize a great amount of trehalose during desiccation *in vitro* (unpublished data), which could be an evidence that the AG have little effect on the induction of trehalose synthesis. It is therefore most probable that the central nervous system is not involved in both induction and termination of cryptobiosis in *P. vanderplanki*.

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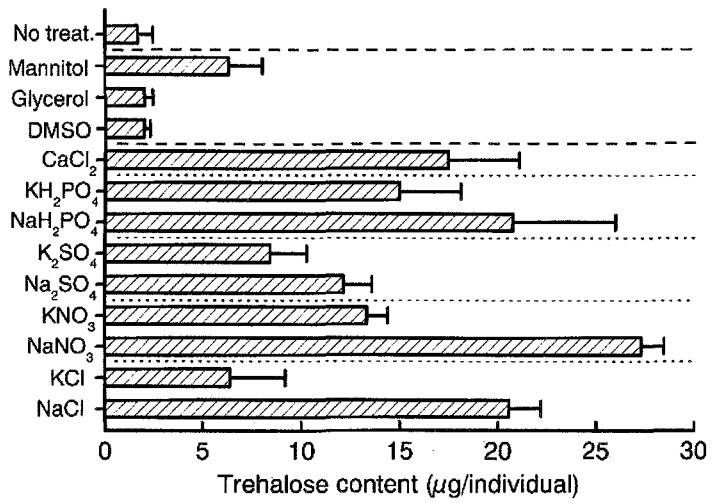

Fig. 5 Effect of various salt solutions on trehalose content of *Polypedilum vanderplanki* larvae. Larvae were incubated for 1 day in the same osmotic pressure (342 mOs) of each solution. Control shows the content of untreated larvae ("no treatment"). n = 5–8 each. From Watanabe *et al.* (2003).

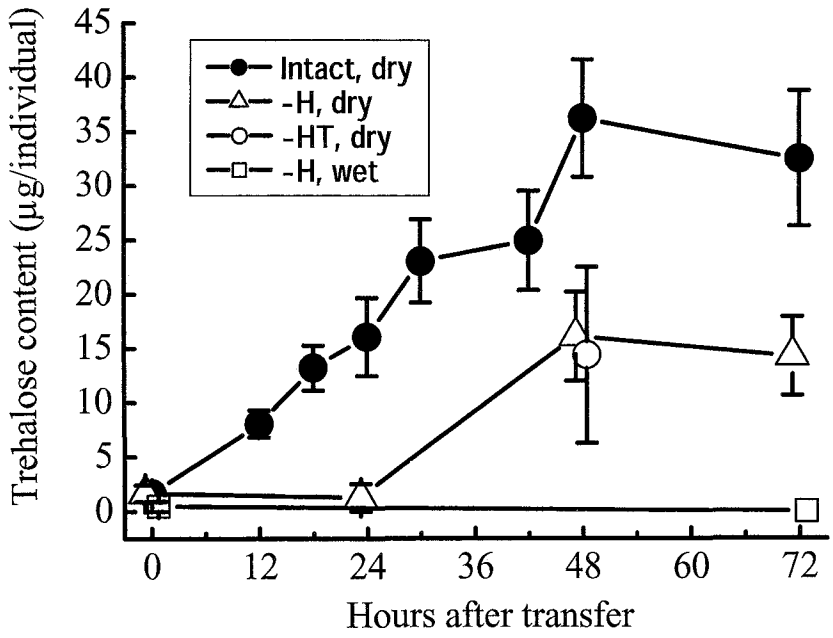

Fig. 6 Change of trehalose content in *Polypedilum vanderplanki* larvae during desiccation over 2 days. Intact, dry: intact larvae (closed circles), –H, dry: decapitated larvae (open triangles), –HT, dry: larvae from which the head and thorax were removed (open circles), –H, wet: decapitated larvae in water (open squares). Each point shows mean ± SD. n = 3–9. After ligation (applied behind the head or thorax), the head or head- and thorax-segments were severed from larvae in iced water. The remaining body parts were incubated in distilled water for 1 day at room temperature, and then completely dried for 2 days in a desiccator or subsequently treated for 2 days in distilled water. From Watanabe *et al.* (2002).

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