

# Physiological changes leading to anhydrobiosis improve radiation tolerance in *Polypedilum vanderplanki* larvae

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## Abstract

High tolerance against various extreme environments exhibited by some anhydrobionts might be due to being almost completely desiccated, a state where little or no chemical reactions occur. We have shown that anhydrobiotic larvae of *Polypedilum vanderplanki* have higher tolerance against both high- and low-linear energy transfer (LET) radiation than hydrated larvae. It is of great interest to know how the desiccating larvae gain radiation tolerance. We therefore examined effects of high-LET radiation on four kinds of larvae: (1) normal hydrated (intact) larva, (2) intermediates between the anhydrobiotic and normal hydrated state, (3) almost completely dehydrated (anhydrobiotic) larvae, and (4) immediately rehydrated larvae that are assumed to have a similar molecular profile to anhydrobiotic larvae. The intermediates and immediately rehydrated larvae survived longer after high-LET radiation than intact larvae, indicating that radiation tolerance could be enhanced even in hydrated larvae. Physiological changes toward anhydrobiosis, e.g. accumulation of protectants or increasing damage repair capacity, correlate with improved radiation tolerance in hydrated larvae. In addition, almost complete desiccation further enhanced radiation tolerance, possibly in a different way from the hydrated larvae.

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## 1. Introduction

Anhydrobiosis is defined as the state of an organism when it shows no visible signs of life and when its metabolic activity is hardly measurable or comes reversibly to a standstill under almost completely dehydrated conditions (Keilin, 1959; Clegg, 2001). These dehydrated organisms frequently tolerate various extreme environments (Wharton, 2002a). Anhydrobiosis is achieved by controlled dehydration and complex physiological changes (Wharton, 2002b; Watanabe, 2006). Upon desiccation, a number of stress-responsive genes are known to be upregulated (Browne et al., 2004; Kikawada et al., 2006) and, as a

consequence, inter- and intracellular environments are drastically changed. Concurrently, many types of anhydrobiotic organisms are shown to accumulate non-reducing disaccharides, e.g. sucrose and trehalose (Crowe et al., 1992; Ingram and Bartels, 1996; Womersley et al., 1998). These factors are assumed to provide protection against desiccation stress, and probably also against other types of stress including low temperature, freezing, oxidation, and ionizing radiation (Storey and Storey, 1991; Crowe, 2002).

An African chironomid, *Polypedilum vanderplanki*, is found in temporal rock pools in semi-arid Africa, and the larvae enter anhydrobiosis when the pools dry out (Hinton, 1951). During entry into anhydrobiosis, the larvae accumulate trehalose up to approximately 20% of dry body weight (Watanabe et al., 2002, 2003), and various genes are induced to express (Kikawada et al., 2006).

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The anhydrobiotic larva shows cross-tolerance to various extreme conditions (Hinton, 1960a, b). We recently demonstrated that *P. vanderplanki* larvae increased tolerance against both high- and low-linear energy transfer (LET) radiation by entry into anhydrobiosis (Watanabe et al., 2006a, b). Generally, low-LET radiation like X-rays and gamma-rays hits water and generates free radicals and excited molecules, which attack biomolecules such as DNA, proteins, and phospholipids (indirect effect), whereas high-LET radiation like ionizing beams directly attacks biomolecules (Hall, 1994). Since it is believed that DNA is a primary target of radiation damage (Hall, 1994), we assumed that the anhydrobiotic state would effectively protect DNA (Watanabe et al., 2006a), or that entry into anhydrobiosis would increase DNA repair capacity (Watanabe et al., 2006b). However, no biochemical basis for this radiation tolerance is known.

The anhydrobiotic state is markedly different from the hydrated one with regard to physiological, biochemical, and physicochemical properties. Therefore, understanding when and how the desiccating larvae gain radiation tolerance is of great interest. Because trehalose content increases with increasing time after starting dehydration (Watanabe et al., 2002, 2003), we assumed that it provides an indication of the ever-changing physiological state of desiccating larvae. In the present study, we therefore set up four kinds of larvae with different trehalose contents, and examined the biological effects of high-LET radiation in desiccating larvae of *P. vanderplanki*.

## 2. Materials and methods

### 2.1. Insect rearing

*P. vanderplanki* were reared on a 1% agar diet containing 2% commercial milk under controlled light (13-h light:11-h dark) and temperature (27–28 °C) according to Watanabe et al. (2002). Final instar larvae of approximately 1 mg wet body weight were used for all experiments.

### 2.2. Desiccation and rehydration of larvae

Four groups of larvae having different water and trehalose contents (Fig. 1) were prepared as follows. Intact larvae were designated as “W-low” meaning wet larvae with a low trehalose level. Hydrated larvae with a medium trehalose level (W-mid) were prepared by placing W-low larvae in a plastic Petri dish (diameter 50 mm, height 10 mm) and incubating them at 100% relative humidity (r.h.) for 24 h. Following further treatment with 76% r.h. and 5% r.h., each for a day, the larvae were almost completely dehydrated (approximately 3% of body water content) and accumulated a large amount of trehalose (D-high). Hydrated larvae with a high trehalose level (W-high) were prepared by supplying D-high larvae with a sufficient amount of distilled water and then rehydrated them for 1 h. Using this procedure, most non-irradiated

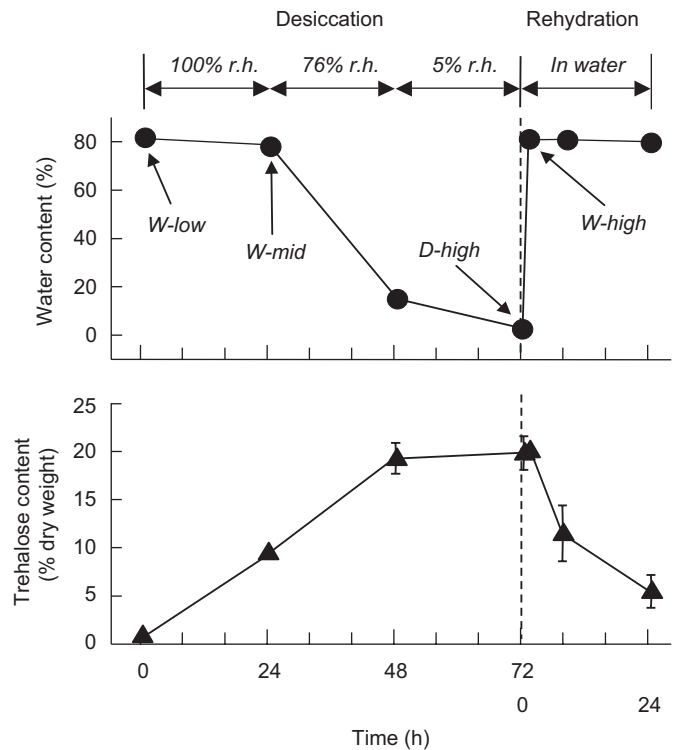


Fig. 1. Temporal changes in larval water and trehalose contents during 72-h desiccation and the following rehydration in larvae of *P. vanderplanki*. Larvae were exposed to a series of desiccation treatments over 3 days: 100% r.h., 76% r.h., 5% r.h., for each day. W-low, no-desiccation; W-mid, desiccation at 24 h; D-high, desiccation at 72 h; W-high, rehydration at 1 h.  $N = 7-10$  in trehalose content and 5–20 larvae  $\times$  5 replicates for water content.

larvae successfully recovered from anhydrobiosis. Larval body water was gravimetrically determined and trehalose was quantified by high-performance liquid chromatography according to Watanabe et al. (2003).

### 2.3. Heavy-ion irradiation

Dehydrated larvae (D-high) were sandwiched between two pieces of polyimide film (7  $\mu$ m in thickness, Kapton<sup>®</sup>, Dupont-Toray, Tokyo), and the sandwiches were put on the bottom of a plastic Petri dish (diameter 50 mm, height 10 mm). Hydrated larvae (W-low, W-mid, and W-high) were placed on moistened filter paper (diameter 47 mm) in a Petri dish, covered by polyimide film to avoid drying. These dishes were sealed with Parafilm<sup>®</sup> (Pechiney Plastic Packaging Inc., Chicago, IL).

These samples were exposed to 1–6000 Gy of 50 MeV <sup>4</sup>He (LET <sub>$\infty$</sub>  = 16.2 keV/ $\mu$ m) ion beam delivered from the azimuthally varying-field (AVF) cyclotron at the Takasaki Ion accelerators for Advanced Radiation Application (TIARA) facility of the Japan Atomic Energy Agency (JAEA), Japan (Watanabe et al., 2006b). LET <sub>$\infty$</sub>  values shown above in parentheses are calculated according to the kinetic energy loss ( $E_{\text{loss}}$ ) at the target assuming water equivalence. To convert particle fluence to dose in Gy, the

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