High Hydrostatic Pressure Tolerance of Tardigrades

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Abstract
Tardigrades are small invertebrate animals and enter a dry state called “anhydrobiosis”. Anhydrobiotic tardigrades have also been shown incredible tolerance to extreme environments such as wide range of temperatures, radiation, chemicals, vacuum and high hydrostatic pressure up to 0.6 GPa. However, there is only one report concerning high pressure tolerance in tardigrades. Thus, in order to estimate limit of their survival ability against high pressure, we here conducted additional experiments that expose a tardigrade Milnesium tardigradum to 1.2 GPa. We found that all of anhydrobiotic tardigrades survived after exposure to 1.2 GPa, whereas no hydrated ones showed any movements of body after that treatment. This result indicates that biological integrity of anhydrobiotic tardigrades is not collapsed even by 1.2 GPa of high hydrostatic pressure.

Keywords: tardigrades, Milnesium tardigradum, high pressure tolerance, anhydrobiosis

1. Introduction

Tardigrades (Phylum Tardigrada) are small animals ranging from 50 to 1,200 μm in length and are distributed in various types of environments. Most of the 400 species are terrestrial, living in the water film that surrounds moss and lichens. Terrestrial tardigrades enter an ametabolic dry state called “anhydrobiosis” when exposed to dry environments. The water content of the body can be reduced from 85% to 1 – 3% [1, 2]. The anhydrobiotic tardigrades become immobile and shrink in to a form known as tun (barrel-shape) state, showing no viable sign. When anhydrobiotic tardigrades are exposed to water, normal activities resume and viable signs can be observed. The most attractive characteristic in anhydrobiotic tardigrades will be the tolerance to extreme environments such as low (−273°C) and high (151°C) and temperatures, high dose of ionizing radiations, chemicals, and vacuum [2-7].

There is only one report on the resistance of anhydrobiotic tardigrades to high hydrostatic pressure (HHP). Survival rate (%) of active state tardigrade species Macrobiotus occidentalis in water and perfluorocarbon, inactive hydrophobic liquid, was zero after treatments at 200 MPa or more, and that of tun state in perfluorocarbon was 95% even after the treatments at 0.6 MPa [8]. From this result, there a simple question arises; how much HHP tardigrades can survive.

In the present study, therefore, we carried out HHP-treatment of anhydrobiotic tardigrades at pressures up to 1.2 GPa to estimate limit of their survival ability against HHP.
2. Materials and methods

2.1 Animals

In this study, a tardigrade species *Milnesium tardigradum* was used as an experimental animal for HHP treatment. *M. tardigradum* was gained from dry moss, *Bryum argenteum*, collected from in Ibaraki, Japan. The moss containing tardigrades was stored under desiccating condition for around one month at room temperature until use. The moss was immersed for 24 h in tap water, and animals released from the rehydrated moss were picked up via a glass pipette. Only active animals, checked under a stereomicroscope with transmitted illumination (MZ125, Leica, Solms, Germany), were used in following experiments. Large-sized animals were considered for HHP treatment.

2.2 Animal preparation for HHP treatment

In order to prepare anhydrobioic samples, a group of 41 active individuals on a piece of parafilm (Pechiney Plastic Packaging, Inc., Chicago, IL, USA) was desiccated at about 25°C under 85% relative humidity (RH) for over 24 h, and then 0% RH for 24 h. RH was controlled by 35% wt./wt. glycerol in water (Invitrogen, Carlsbad, CA, USA) for RH 85% or by silica gel for RH 0% (See [2]). Then, anhydrobiotic animals were immersed in perfluorocarbon (Fluorinate FC77, Sumitomo 3M, Tokyo, Japan) in a plastic pouch. Hydrated samples were prepared as follows: 42 active animals with a drop of around 50 µl of water were placed on a piece of a filter paper, and it was immediately put in a plastic pouch filled with perfluorocarbon. These pouches containing anhydrobiotic or hydrated samples were sealed up and were placed inside a pressure capsule (HPS-1400: Teramecs Co. Ltd., Kyoto, Japan). The samples were compressed from atmospheric pressure to 1.2 GPa at rates of about 0.1 GPa min\(^{-1}\), held at 1.2 GPa for 20 min, and then depressed to ambient pressure at around 0.1 GPa min\(^{-1}\). Temperature inside a pressure machine was 35-40°C.

2.3 Survival check

After depression, samples were immediately removed from the unit, and rehydrated with distilled water. Animals were washed with distilled water at three times to remove perfluorocarbon and preserved in water in a Petri dish (diameter 50 mm). Survival was examined at 24 h after addition of water by observation under a stereomicroscope. Animals showing any motion were judged as survivors, whereas motionless animals were considered to be dead.

2.4 Statics

Survival after HHP treatment was statistically compared between anhydrobiotic and hydrated groups by chi-square test with Yate's correction.

3. Results

We conducted the experiments that expose 1.2 GPa to anhydrobiotic or hydrated tardigrade
**Fig. 1** Survival rates of tardigrade *M. tardigradum* in anhydrobiotic (Anh.) and hydrated (Hyd.) states at 24 h after exposure to 1.2 GPa. Asterisk denotes a significant difference in survival between anhydrobiotic and hydrated groups (chi-square test with Yeats correction; \( p < 0.01 \)).

*N. tardigradum*. No hydrated animals showed any movements, while all anhydrobiotic individuals showed normal activity after at 24 h after treatment of 1.2 GPa (Fig. 1). There is a significant difference in survival between anhydrobiotic tardigrades and hydrated ones (\( p < 0.01 \)). It was indicated that high-pressure resistance of anhydrobiotic tardigrades was almost completely lost of the presence of water.

### 4. Discussion

In this study, we demonstrated that tardigrade *M. tardigradum* in anhydrobiotic state showed tolerance to HHP at 1.2 GPa on the basis of its survival ability (Fig. 1). This is the new record result that animals survived the highest pressure. No anhydrobiotic individuals died after treatment of 1.2 GPa, indicating that anhydrobiotic tardigrades can tolerate HHP over 1.2 GPa. Difference in survival ability between anhyadrobotic and hydrated tardigrades probably depends on the difference in their water contents, since original organizations of fully hydrated macromolecules or tissues are thought to be easily collapsed by exposure of such degree of HHP. Anhydrobiotic tardigrades retaining extremely little amounts of water in their body can exclude that problem and thereby show tolerance to HHP. In fact, some individuals of anhydrobiotic tardigrade *Echiniscus japonicus* died after exposure 0.6 GPa when they had been dehydrated inadequately and had been considered to retain much water than normal anhydrobiotic ones before HHP treatment [8].

The outstanding tolerance of anhydrobiotic tardigrades to HHP shown in this study suggest that multi-cellular organisms potentially have a novel system to preserve their complicated biological integrity after exposure of extreme high pressures. In other words, even nervous system or reproductive organs can be stored without any damages after experience of extreme environments if these are in dry state. Researches on anhydrbiosis in tardigrades have thought to reveal the survival strategies against extreme environmental conditions and to provide an insight into preserving techniques concerning complicated biological materials, including foods, organs, and whole bodies. There, however, have been limited numbers of investigations on physiology of anhydrosis in tardigrades [1, 9, 10], due to difficulties of
their artificial rearing [11]. In order to use tardigrades for future experimental purposes, it is needed to establish culture system for them.

5. Conclusion

In conclusion, we demonstrated that all individuals of anhydrobiotic tardigrade *M. tardigradum* survived 1.2 GPa, indicating that the ability to lose body water confer them on high-pressure tolerance.

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7. References