PROMPT LEAD EXPOSURE OF AQUEOUS ENVIRONMENT BIOMONITORED BY PHOTOSYNTHETIC BACTERIA

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Abstract
Anthropogenic activities including industrialization, urbanization and growth of population have significantly increased concerns about detrimental effects of pollutants on health and environment. Among the heavy metal ions, lead (II) ions are especially toxic and hazardous. Here, we report the application of purple photosynthetic bacteria in biomonitoring of lead pollution in aqueous habitats. The monitoring method is based on light absorption and fluorescence responses of living microorganism to prompt appearance of lead ions in the solution. The Pb(II) ions penetrate the cell membrane immediately, attack and (in few mM external concentration) destroy the light harvesting system together with the reaction center protein. As these bacteria may act as bioaccumulators of lead, they can be also used for bioremediation of contaminated cultures. The advantages using photosynthetic bacteria for monitoring and accumulating Pb(II) pollution in aqueous environmental compartments are presented in the paper.

Introduction
The release of highly toxic and hazardous heavy metal ions (mercury, lead and cadmium) of anthropomorphic origin into the biosphere has increased today to alarming levels [1]. Out of these non-biodegradable pollutants, lead(II) ions have special significance because of their wide use in various human activities including the glass and metal industries. They are employed in batteries, paints, pigments and ammunition, cables, alloys and steels, plastics, and are still used in petrol as an anti-knocking agent. Water from industrial effluents, vehicular traffic and mixing of roadside run-offs is heavily contaminated by lead and its compounds. Due to different physiological disorders and toxicological effects caused to humans, the permissible level of lead contamination in drinking water (World Health Organization limit) is as low as 10 μg/l ≈ 50 nM.

Large efforts have been undertaken to investigate the behavior of lead in different ecosystems (particularly water, due to industrial wastewater pollution) and to work out strategies for its control, abatement and removal. Several analytical techniques have been applied to the assay of lead [2]. Voltammetry provided a reliable and cost-effective technique for its monitoring, especially in drinking water [3]. Different chemical methods including reduction and precipitation, ion-exchange, electrolysis and adsorption have been used for the removal of lead ions from water. In addition to the chemical methods, biological techniques are also available to detect and to remove the toxic heavy metal pollutants [4]. Photosynthetic bacteria were successfully used to monitor the level of mercury(II) ions in aqueous habitats [5-7].

The present study was carried out to demonstrate the possibility of detection (biomonitoring) of Pb(II) ions by purple photosynthetic bacteria in aqueous solutions and to assess the potential of these microorganisms to concentrate and to remove (bioremediate) lead contamination from the aqueous environment.

Experimental

Bacterial strains and growth conditions

The photosynthetic purple bacterium *Rubrivivax (Rvx.) gelatinosus* were grown in Siström's medium either in completely filled screw top vessels without oxygen (photoheterotrophic and anaerobic growth). The medium was inoculated from a dense batch culture (1:100) and was illuminated by tungsten lamps that assured 13 Wm^{-2} irradiance on the surface of the vessel as described earlier [8].

Chemicals

The cells were harvested at the exponential phase of the growth and bubbled by nitrogen for 15 min before measurements. Variable amounts of $Pb(CH_3COO)_2*3H_2O$ (Pb(II)-acetate) were added to the bacterial culture for heavy metal ion treatment [4]. These chemicals are highly soluble in aqueous solution under physiological conditions. 100 mM $Pb(CH_3COO)_2*3H_2O$ stock solution was prepared freshly before the experiment. The durations of the Pb(II)-acetate treatments were prompt.

Optical assays

Steady-state absorption spectrum. The absorption spectra of intact cells were measured by dual beam spectrophotometer with reference of scattering suspension of sand of similar size (~ 5 μm) and concentration (~ $1 \cdot 10^8$ particles/mL) as those of the bacteria.

Flash-induced absorption change kinetics. The kinetics of absorption changes of the whole cells induced by Xe flash were detected by a home-constructed spectrophotometer [8]. The electrochromic shift (ECS) of the carotenoids in the photosynthetic membrane were detected at 530 nm wavelength with reference to 510 nm wavelength.

Induction and relaxation of bacteriochlorophyll (BChl) fluorescence. The induction and subsequent decay of the BChl a fluorescence of intact cells were measured by a home built fluorometer [5]. The light source was a laser diode (808-nm wavelength and 2W light power) that produced rectangular shape of illumination and matched the 800 nm absorption band of the LH2 peripheral antenna of the cells. The BChl *a* fluorescence (centered at 900 nm in mature cells) was detected in the direction perpendicular to the actinic light beam, with a near infrared sensitive, large area (diameter 10 mm) and high gain Si-avalanche photodiode (APD; model 394-70-72-581; Advanced Photonix, Inc., USA) protected with an 850-nm high-pass filter (RG-850) from the scattered light of the laser. The usually very small deviation of the kinetics of the excitation from the rectangular shape was corrected by detection of the kinetics of extracted BChl *a* in organic solvent. The induction of fluorescence rise was measured during the actinic laser light and the subsequent dark-relaxation was tested by attenuated short (3 μs) laser pulses distributed according to geometrical series in time.

Results and discussion

The physiological properties of purple photosynthetic bacterium *Rubrivivax gelatinosus* show abrupt changes upon addition of Pb(II) acetate pollutant to the culture. While the I_{50} values (half lethal dose) of bacteria exposed to prolonged heavy metal ion contamination are low (e.g. I_{50} = 2 μM for Hg^{2+}), prompt addition of lead to the culture evokes much (about 10^3 times) less changes due to slow kinetics of lead uptake through the cell wall (Fig. 1). Both red absorption bands characteristic of the peripheral (800 nm) and core (860 nm) antenna complexes demonstrate prompt decomposition after treatment with lead acetate in the few mM concentration range. The lead(II) ions can pass the cell wall of the bacteria in the short time range of exposure, and attack immediately the essential protein-pigment complexes including the peripheral and core light harvesting complexes. There is no preferential damage of the macromolecules as the observed rates of BChl degradation due to Pb(II) contamination are the same for the two antenna complexes.

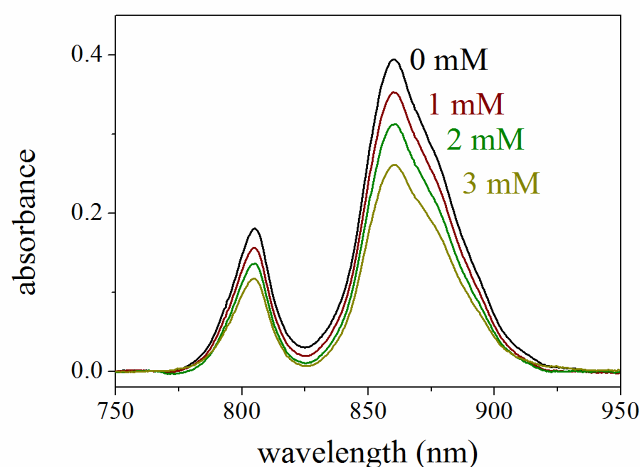

Fig. 1. Steady state red absorption spectra of intact cells of photosynthetic bacteria *Rubrivivax gelatinosus* after prompt addition of Pb(II) acetate to the culture.

The electrochromic signals due to absorption change of carotenoid pigments evoked by the membrane potential describe similar changes upon exposure to lead(II) ions (Fig. 2). The amplitude and not the kinetics of the flash-induced absorption change is sensitive to the Pb(II) treatment indicating that the magnitude of the membrane potential and not the pathways of disappearance of the initial charge pairs is primarily influenced by lead(II) ions.

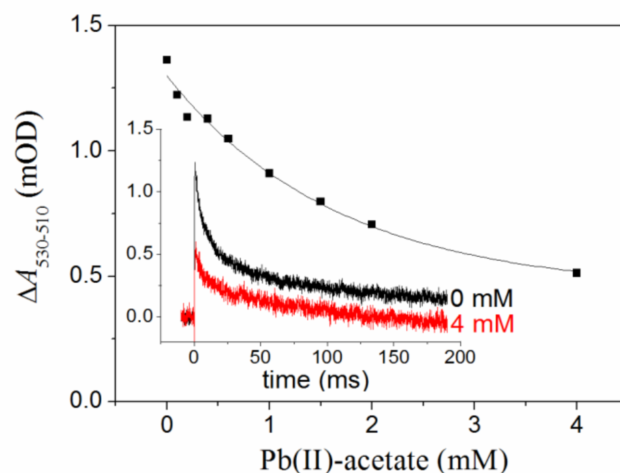

Fig. 2. Kinetics (inset) and lead(II)-dependent amplitude of flash-induced electrochromic response of the carotenoids in whole cells of *Rubrivivax gelatinosus* upon treatment with 0-4 mM Pb(II) measured by absorption change at 530 nm (vs. 510 nm).

The BChl fluorescence is especially sensitive to lead(II) contamination. Both the kinetics and the magnitude of fluorescence induction (F_{max}) show major changes upon Pb(II) treatment (Fig. 3). The severe drops of F_{max} and F_0 (the initial fluorescence) indicate substantial loss of BChl pigments together with decoupling of the light harvesting antenna systems from the reaction center.

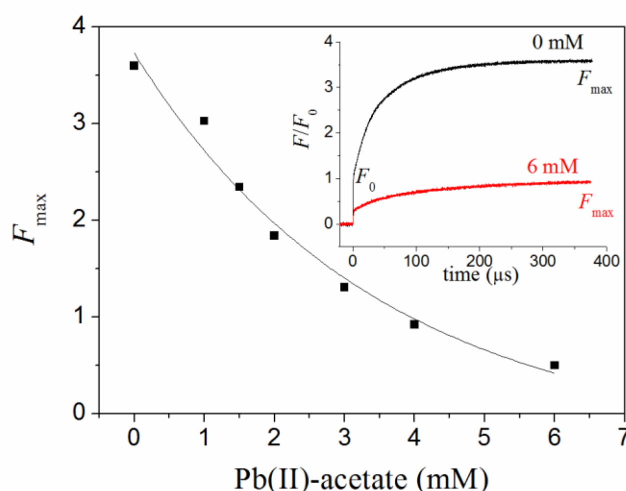

Fig. 3. BChl fluorescence (*F*) induction kinetics (inset) and lead-dependent changes of the maximum fluorescence (F_{max}) in intact cells of photosynthetic bacteria *Rubrivivax gelatinosus* grown in the light upon addition of Pb(II) acetate to the culture. The kinetic traces are normalized to the initial F_0 fluorescence level of the untreated cells.

While the BChl fluorescence induction probes the intactness of the light harvesting system and primary photochemistry taking place in the reaction center protein, the relaxation of the BChl fluorescence gives information about the pathways of re-opening of the closed reaction centers in the dark (Fig. 4).

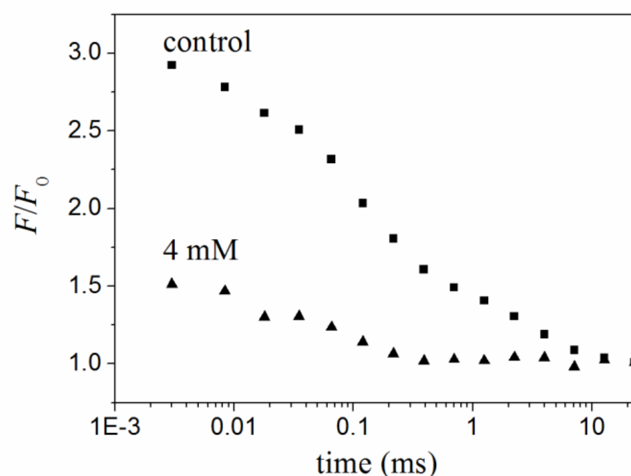

Fig. 4. Relaxation of the BChl fluorescence after flash excitation in intact cells of photosynthetic bacteria *Rubrivivax gelatinosus* grown in the light upon addition of Pb(II) acetate to the culture.

The prompt effect of lead(II) ions is expressed as significant decrease of the maximum fluorescence and minor acceleration of re-reduction of the oxidized BChl dimer in the

reaction center. This technique is also suited to track the diverse structural and functional changes in the photosynthetic apparatus of the intact cell caused by Pb(II) contamination.

Conclusion

Steady state absorption spectra and kinetics of flash-induced absorption changes and fluorescence of intact photosynthetic bacteria are sensitive bioindicators of lead(II) contamination of aqueous cultures. After fast penetration through the cell wall, the Pb(II) ions cause prompt and detectable physiological changes by disconnection and damage of the antenna pigments followed by graduate destruction of the photosynthetic machinery of the bacteria.

Acknowledgements

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