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Arsenate uptake by Pi transport system of an arsenate-resistant mutant, AR3, of *Chlamydomonas*

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Introduction

Arsenic is much contained in the environment and is toxic for organisms. Some organisms are harboring the arsenate resistance. The resistance to arsenicals is mediated by the expression of arsenite efflux [Silver et al., 1993] or methylation of arsenic [Kaise et al., 1988]. Arsenate is taken up through Pi transport system in many organisms. It was reported that arsenate tolerant strain of higher plant *Holcus lanatus* showed low activities of arsenate uptake and Pi uptake [Meharg et al., 1992]. We generated arsenate-resistant and sensitive mutants by the tagging method of *Chlamydomonas* [Fujiwara et al., 2000]. Here we show characteristics of arsenate uptake and report that one of the arsenate-resistant mutants, AR3, enhances the activity of Pi uptake.

Materials and Methods

Chlamydomonas reinhardtii CC125 (wild type *mt+*) and the arsenate-resistant mutant, AR3, which was obtained by random insertional mutagenesis [Fujiwara et al., 2000], were grown mixotrophically in TAP medium (containing 1 mM Pi). Cultures in the flasks were agitated on a gyratory shaker (120 rpm) under continuous illumination at 80 µmol photons m^{-2} s^{-1} with fluorescent tubes. Before physiological analyses, AR3 was backcrossed three times with CC125 or CC124 (wild type *mt-*). For Pi-starvation experiments, cells were transferred to Pi-free (TAP-P) medium and then incubated for 24 h. Total arsenic in cells was measured using an atomic absorption spectrophotometer (Spectra AA220, Varian, Australia) as described previously [Fujiwara et al., 2000]. For determination of Pi uptake, ^{32}Pi (specific radioactivity = 7 kBq

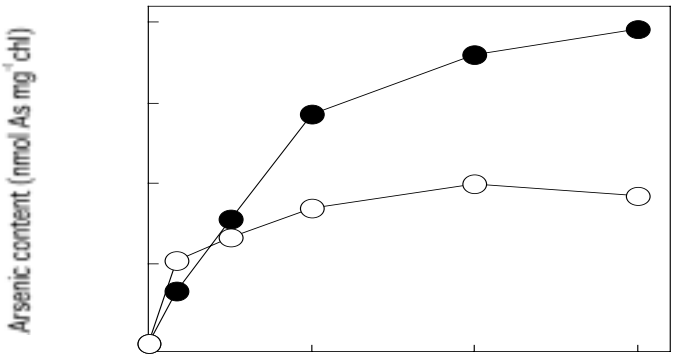

Fig. 1 Changes in intracelllar arsenic content. 1mM arsenate was added to the culture at the logarithmic phase. Closed circle; CC125. open circle; AR3.

Table I. The rates of arsenate uptake in arsenate-preincubated cells. Cells were incubated in the medium containing 1 mM arsenate for 80 min. The rate of arsenate uptake was measured after arsenate in the medium was removed.

Strain	Arsenate uptake (nmol mg^{-1}chl min^{-1})	
	control	As-preincubated cells
CC125	4.77 ± 0.35 (n=4)	3.98 ± 0.43 (n=9)
AR3	9.79 ± 0.58 (n=4)	3.70 ± 0.19 (n=9)

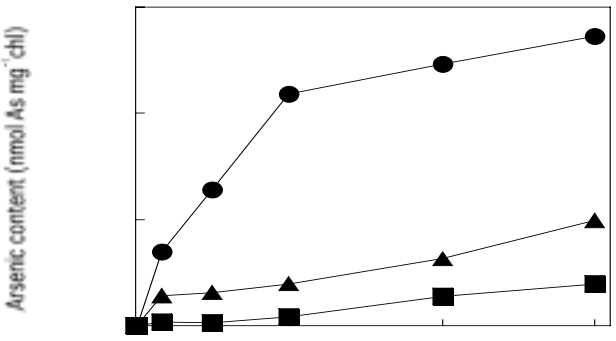

Fig. 2 Effects of Pi concentrations on arsenate uptake. CC125 cells were incubated in Pi-depleted medium for 20 min, and 1mM arsenate was added to it with various concentrations of Pi.

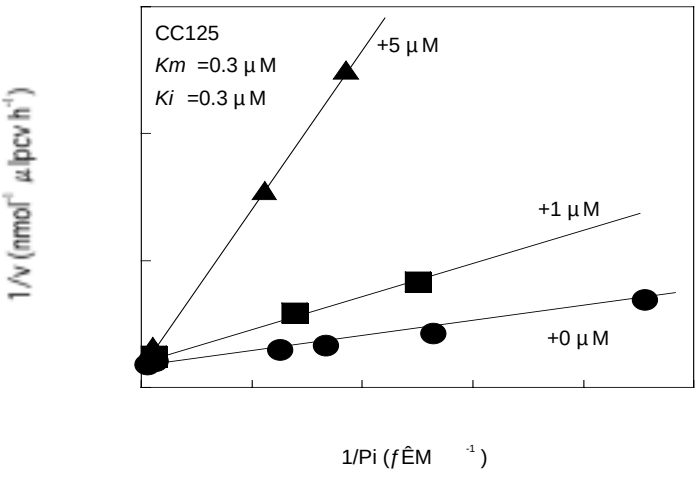

Fig. 3 Lineweaver-Burk plots of high-affinity Pi transport activity in CC125. Various concentrations of Pi were added to Pi-starved cells with 0 M arsenate, 1 µM , or 5 µM *Vmax* (57.1 µ nmol^{-1} pcv h^{-1}), *Km* (0.3µM), *Ki (0.3* µ*M*

nmol^{-1}) was added to the cell suspension to a proper concentration. Pi taken up by the cell and intracellular P content were determined by quantification of radioactivity of ^{32}Pi.

Results and Discussion

The suppression of arsenate uptake in AR3

In AR3, though intra-cellular arsenic content at 10 min after the addition of arsenate was higher almost twice as high as that of CC125, arsenic content at 60 min was about a half that of CC125 (Fig. 1). At 80 min after the addition of arsenate, the rates of arsenic excretion from cell to the medium in CC125 and AR3 were 1.17 and 0.34 nmol mg^{-1}chl min^{-1}, respectively. This suggests that low content of arsenic in AR3 is not due to the enhanced efflux. Though the initial rate of arsenate uptake in AR3 was 9.79 nmol mg^{-1}chl min^{-1}, which was 2-fold higher than that of CC125, that at 80 min decreased to 3.70 nmol mg^{-1}chl min^{-1} (Table I). This result indicates that AR3 worked out the suppression of arsenate influx.

Effects of Pi concentrations on arsenate uptake in CC125

Intracellular arsenic contents of CC125 after the addition of 1 mM arsenate with various concentrations of Pi (0, 1 or 10 mM) were measured (Fig. 2). One hour later, arsenic content at 1 mM Pi was 18% of that in the absence of Pi. At 10

mM Pi, arsenate was hardly taken up by the cell. It suggests that Pi in the medium suppressed arsenate uptake in *Chlamydomonas*.

Kinetics of Pi uptake

In Pi-starved *Chlamydomonas*, the high-affinity Pi transport component is induced, and its activity dominates Pi uptake [Shimogawara et al., 1999]. However, that was inhibited by external arsenate (Fig. 3). Inhibition constant (*Ki*) of arsenate to Pi uptake was almost the same value as *Km* of Pi uptake. Pi uptake in AR3 was also competitively inhibited by arsenate (data not shown). Our data support that arsenate was a competitive inhibitor of Pi uptake.

P content and high-affinity Pi transporter

In Pi-replete condition, there are the low-affinity and the high-affinity Pi transport components in *Chlamydomonas*, and the low-affinity one mainly works out to acquire the external Pi. The rates of Pi uptake were 12.8 and 42.5 nmol mg^{-1}chl min^{-1} in CC125 and AR3, respectively, and intracellular P content of AR3 was twice as high as that of CC125. These phenomena were considered to be due to the high activity of the high-affinity Pi transport system in AR3, since the rates of the high-affinity one in CC125 and AR3 were 3.8±1.3 (n=4) and 18.3±1.3 (n=4) nmol mg^{-1} chl min^{-1}, respectively. When intracellular Pi level was much higher in AR3, arsenate would not be so harmful. Thus, we conclude that the arsenate resistance in AR3 is attributed to the high P content maintained by the activated Pi transport system, in addition to the specific suppression of arsenate uptake via the Pi transport system.

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