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Zinc Biosorption from Aqueous Solution by a Halotolerant Cyanobacterium *Aphanothece halophytica*

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Abstract. We have investigated the characteristics of zinc biosorption by *Aphanothece halophytica*. Zinc could be rapidly taken up from aqueous solution by the cells with an equilibrium being reached within 15 min of incubation with 100 mg L^{-1} $ZnCl_2$. The adsorbed zinc was desorbed by treatment with 10 mM EDTA. The presence of glucose, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), and N,N′-dicyclohexylcarbodiimide (DCCD) did not affect the uptake of zinc. The specific uptake of zinc increased at low cell concentration and decreased when cell concentration exceeded 0.2 g L^{-1}. The binding of zinc followed Langmuir isotherm kinetics with a maximum zinc binding capacity of 133 mg g^{-1} and an apparent zinc binding constant of 28 mg L^{-1}. The presence of an equimolar concentration of Mn^{2+}, Mg^{2+}, Co^{2+}, K^+, or Na^+ had no effect on zinc biosorption, whereas Ca^{2+}, Hg^{2+}, and Pb^{2+} showed an inhibitory effect. The biosorption of zinc was low at a pH range from 4 to 6, but increased progressively at pH 6.5 and 7.

Pollution of water, air, and soil by heavy metals has been one of the most important environmental problems worldwide. Heavy metals usually form compounds that can be toxic even at very low concentrations. Traditional technologies for the removal of heavy metals, such as ion exchange or precipitation, are often inefficient and very expensive when used for the reduction of heavy metal ions to very low concentrations [18]. Alternative methods of heavy metal removal and recovery based on biological materials have received increased attention in recent years because of their potential application in environmental protection [8, 17, 18].

Metallic zinc has been used extensively for a variety of industrial applications. This extensive use of zinc without its recovery has caused contamination of soil and fresh water habitats [16]. Biosorption of zinc has been studied in various organisms, but most of the work documented so far used algae, fungi, and bacteria [13, 18, 19]. Only rarely has a report appeared in which cyanobacteria were used as a zinc biosorbent [1]. Recently we have shown that cyanobacteria and green algae were efficient biosorbents to remove cadmium, lead, and mercury from aqueous solution [10]. In the present work we used a halotolerant unicellular cyanobacterium *Aphanothece halophytica* to study its properties for biosorption of zinc from aqueous solution. We showed that *A. halophytica* could adsorb almost all the zinc to its cell surface with a moderate capacity of 133 mg g^{-1}.

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Materials and Methods

Organism and culture. *Aphanothece halophytica*, originally isolated from Solar Lake in Israel, was obtained in axenic culture from T. Takabe (Nagoya University). The cyanobacterium was grown photoautotrophically in BG 11 medium supplemented with 18 mM $NaNO_3$ as described previously [7]. Cells were grown in cotton-plugged 500 ml conical flasks containing 200 ml medium on a rotary shaker at 30° C without CO_2 supplementation. Continuous illumination was provided by cool-white fluorescent lamps at an irradiance of 60 μE m^{-2} s^{-1}.

Zinc biosorption studies. Log-phase cells of *A. halophytica* were harvested by centrifugation (7700 *g*, 10 min). The collected cells were washed once with 10 mM piperazine-N,N′-bis [2-ethanesulfonic acid] (Pipes) buffer, pH 6.5, containing 0.6 M sorbitol to prevent cell breakage. Washed cells were then suspended in the same buffer with a cell density of 0.2 g L^{-1} in the presence of 100 mg L^{-1} $ZnCl_2$. The reaction mixture was incubated with gentle mixing for 1 h at 30°C and was stopped by rapid centrifugation at 10,500 *g*. The cells were washed once with Pipes–sorbitol buffer, and the pellets were rinsed with distilled deionized water before being dried in an oven at 110°C. The dried cells were treated with concentrated HNO_3 and were analyzed for zinc with a Shimadzu atomic absorption spectrophotometer. The su-

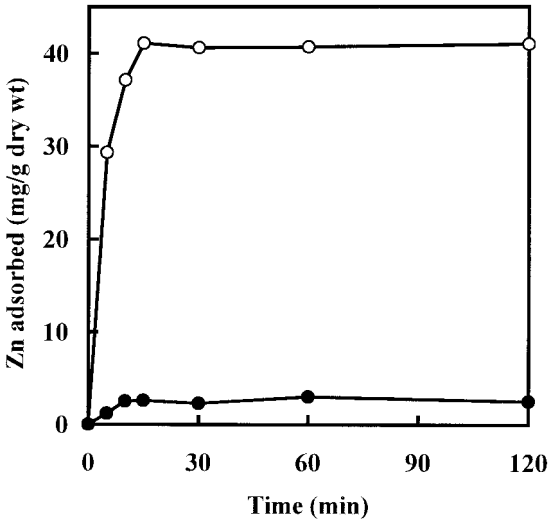

Fig. 1. Time course of zinc biosorption by *A. halophytica*. At indicated times after incubation of cells with the metal as described in Materials and Methods, cells were treated for 5 min with 10 mM Pipes–sorbitol buffer pH 6.5 with (●) or without (○) 10 mM EDTA.

pernatants after incubation with the metal were also analyzed for equilibrium zinc concentrations. The values shown in the figures represent the mean of two independent experiments.

Results

Biosorption of zinc to the cell surface. The time course of zinc biosorption by *A. halophytica* and the effect of EDTA are shown in Fig. 1. Zinc was rapidly taken up by the cells; within 15 min an equilibrium was attained. When the cells with the adsorbed zinc were treated with 10 mM EDTA, it was found that less than 10% of zinc remained associated with the cells. This suggests that the rapid uptake of zinc is ascribed to surface binding of metal to the cell wall and other material external to the cells. This process was independent of the energy requirement and was further substantiated by studies using glucose and metabolic inhibitors. Figure 2A shows that the supply of glucose to the reaction mixture did not appear to increase the amount of zinc taken up by the cells. Moreover, the presence of either CCCP or DCCD hardly affected zinc uptake by the cells (Fig. 2B).

The effects of cell concentration on both zinc-adsorbing capacity and extent of zinc removal from the mixture containing 100 mg L^{-1} $ZnCl_2$ during 60 min of incubation were also analyzed (Fig. 3). The amount of zinc adsorbed by the cells reached a maximum at a cell concentration of 0.2 g L^{-1} and then decreased with increasing cell concentrations, simply owing to dilution of the metal with the added cells. In contrast, the extent of zinc removal from the mixture increased with increasing cell concentrations, and more than 90% of zinc could be removed at a cell concentration of 2.5 g L^{-1}.

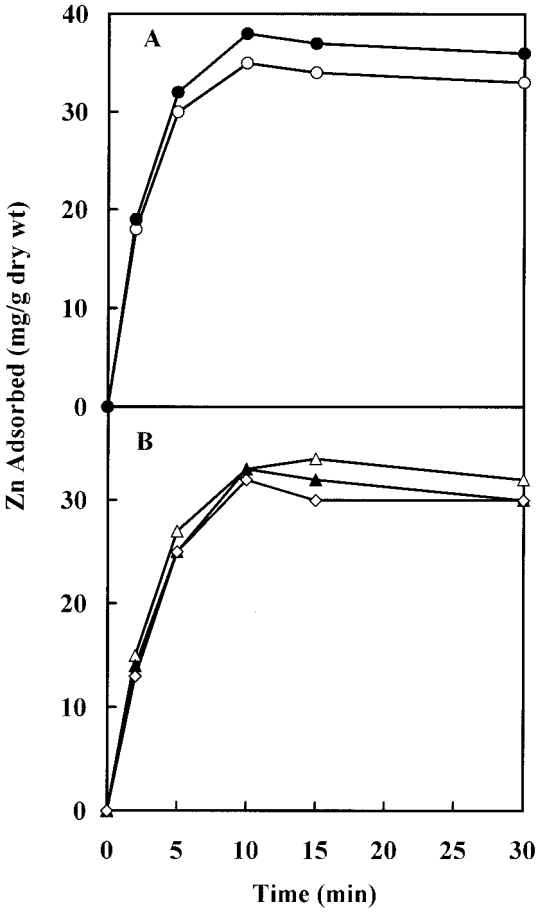

Fig. 2. Effects of glucose (**A**) and metabolic inhibitors (**B**) on zinc biosorption by *A. halophytica*. Cells were pretreated with (●) or without (○) 50 mM glucose and with 40 μM CCCP (▲) or with 40 μM DCCD (◇) or without inhibitor (△) for 30 min before the addition of $ZnCl_2$ and incubated at indicated time intervals.

Kinetic characterization. The adsorption equilibria of zinc on *A. halophytica* were analyzed by measuring the zinc adsorption at various zinc concentrations. A constant amount of cells was incubated with $ZnCl_2$ (0–100 mg L^{-1}), and after 60 min of incubation the amount of zinc bound to the cells at equilibrium was determined, as well as that in the supernatant. The binding data at equilibrium, shown in Fig. 4, seemed to fit the Langmuir isotherm equation $[Zn]_b = Q_{max} \times [Zn]_f/(K_b+[Zn]_f)$, where $[Zn]_b$ is the amount of zinc adsorbed (mg g^{-1}), Q_{max} is the maximum zinc-binding capacity of the cells (mg g^{-1}), K_b is the apparent binding constant (mg L^{-1}), and $[Zn]_f$ is the equilibrium zinc concentration (mg L^{-1}). A linear transformation of the Langmuir equation enabled us to determine the Q_{max} and K_b values to be 133 mg g^{-1} and 28 mg L^{-1}, respectively (Fig. 4, inset).

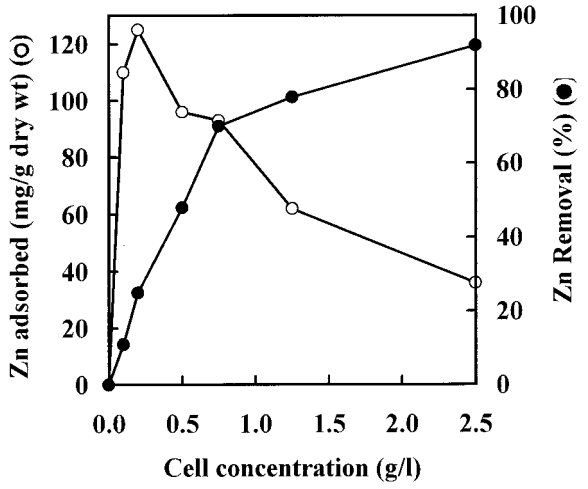

Fig. 3. Effect of cell concentration on zinc biosorption to the cells (○) and the extent of zinc removal from the aqueous solution (●). The binding of zinc to varying concentrations of cells and the contents of zinc in the supernatant were analyzed as described in Materials and Methods.

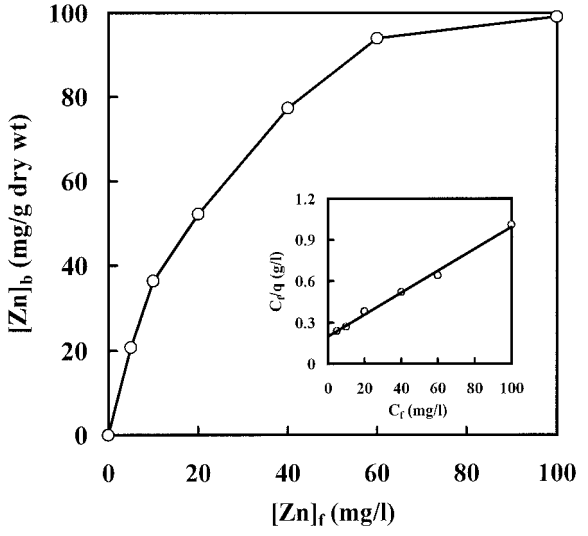

Fig. 4. Biosorption of zinc by *A. halophytica* at various zinc concentrations. Cells were incubated with varying concentrations of $ZnCl_2$, and the analysis of zinc bound to the cells was as described in Materials and Methods. $[Zn]_f$ is the equilibrium zinc concentration, and $[Zn]_b$ is zinc bound to the cells. The inset shows the linear plot of the Langmuir isotherm equation as described in the text.

Effects of cations and pH on zinc biosorption. Six divalent and two monovalent cations at the same molarity as $ZnCl_2$ were tested for their possible inhibitory effects on zinc biosorption. Ca^{2+}, Hg^{2+}, and Pb^{2+} could reduce the amount of zinc adsorbed to the cells (data not shown). Pb^{2+} seemed to be the most potent inhibitor, resulting in about one-third of the zinc remaining adsorbed to the cells as compared with the control, whereas Ca^{2+} and Hg^{2+} reduced zinc biosorption to about one-half. Mn^{2+}, Mg^{2+}, Co^{2+}, K^+, and Na^+ did not inhibit zinc biosorption.

Finally, we examined the pH effect on zinc biosorption and found that zinc biosorption was low and unchanged at a pH range from 4 to 6 (data not shown). A significant increase of zinc biosorption was observed when the pH was raised to 6.5 and 7.

Discussion

The present study demonstrates that *A. halophytica* can efficiently adsorb zinc from aqueous solution. The EDTA treatment and energy-dependent studies reveal that the process of zinc biosorption by *A. halophytica* is ascribed to the binding of zinc to the cell surface (Figs. 1, 2). Cyanobacteria cell walls are polyanionic in nature and effectively act as cation exchangers [9]. Such surface binding is characterized by its independence on energy requirement and insensitivity to metabolic inhibitors. Our results in Figs. 2A and 2B strongly supported this notion. The provision of energy in the form of glucose, the addition of either CCCP, an uncoupler, or DCCD, an inhibitor of ATPase, did not affect the uptake of zinc. In many cases cell surface binding exhibits a low degree of specificity. The results that Ca^{2+}, Hg^{2+}, and Pb^{2+} inhibited zinc adsorption also suggest that the anionic groups on the cell surface of *A. halophytica* did not specifically bind to zinc. The inhibitory effect of Ca^{2+} on zinc biosorption has been reported for a fresh water cyanobacterium *Oscillatoria anguistissima* [1]. Also in *O. anguistissima*, Mg^{2+} was found to inhibit zinc biosorption, in contrast to our results in *A. halophytica* showing no effect of Mg^{2+}. Taken together, these observations suggest that different cyanobacteria may contain different functional groups in cell surface proteins and sugars. Moreover, careful comparison between the uptake of zinc by *A. halophytica* and by *O. anguistissima* also revealed that almost all the zinc bound to the cell surface of the former, whereas certain amounts of zinc were taken up inside the cells of the latter.

It is clearly evident that the removal of zinc from aqueous solution by *A. halophytica* was increased as the cell concentration increased (Fig. 3). Nevertheless, the capacity of cells to adsorb the metal was reduced upon raising the cell concentration, except at low cell concentrations up to 0.2 g L^{-1}. This reduced zinc adsorption would be a result of less cell surface available for the binding of zinc at high cell concentration. It was also likely that higher cell concentration might lead to the formation of cell aggregates, thereby reducing the effective biosorption area [2]. In fact, *Aphanothece* cells are usually surrounded by a mucilage sheath, which can

facilitate cell aggregation [14]. Similar results on the effect of cell concentration on the adsorption of metals to the cell surface have been reported for various microorganisms [4, 9, 12].

Aphanothece halophytica could efficiently take up zinc from aqueous solution with a maximum capacity of 133 mg g^{-1} (Fig. 4). This biosorption capacity was very close to that reported for a brown alga, *Sargassum* sp. (Q_{max} = 118 mg g^{-1}), but was much higher than those in other microorganisms, including fungi [3, 15, 19]. The recent study in a fresh water cyanobacterium, *Oscillatoria anguistissima*, showed a very high capacity for the biosorption of zinc at 641 mg g^{-1} [1]. Again, the large difference in zinc biosorption between *O. anguistissima* and *A. halophytica*, a halotolerant cyanobacterium, reflects the difference in cell wall components of these two cyanobacteria. It is worth noting that in the present study 0.6 M sorbitol was present in the biosorption assay medium to maintain the turgor pressure of *A. halophytica*. The possibility that sorbitol might interfere with metal binding by blocking the exposed binding sites cannot be ruled out. A recent genetic engineering study in *E. coli* could identify the peptide sequence of *E. coli* surface organelles responsible for zinc adherence [11]. Similar approaches can be undertaken by using *A. halophytica* with the aim to enhance the metal biosorptive capacity of the cells.

The biosorption of zinc by *A. halophytica* was rather low and not affected by an acidic pH ranging from 4 to 6. This was in contrast to zinc biosorption by green algae, *Chlorella vulgaris* and *Scenedesmus quadricauda*, which showed pH-dependent biosorption in the pH range from 3 to 7 [6]. The observed low zinc biosorption by *A. halophytica* at acidic pH was likely owing to the strong competition from hydrogen ions for binding sites on the cell surface. Increasing the pH further to 6.5 and 7 resulted in a progressive linear increase in metal adsorption. This suggests that an ionic charge bonding is responsible for zinc biosorption by *A. halophytica*. The effect of pH higher than 7 could not be studied owing to the existence of insoluble Zn $(OH)_2$, which might precipitate at the cell surface [5].

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