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Cation-exchange capacity of algae and cyanobacteria: a parameter of their metal sorption abilities

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SUMMARY

The potential metal sorption abilities of algae and cyanobacteria were estimated as cation-exchange capacities, using a potentiometric titration method. Unicellular cyanobacteria *Anacystis nidulans*, *Synechocystis aquatilis*, and the green microalga *Stichococcus bacillaris* revealed a higher maximal capacity (205–825 μeq g^{-1} dry wt) than filamentous macroalga *Vaucheria* sp. (Xanthophyceae, 41 μeq g^{-1} dry wt). The cation-exchange capacity decreased when external pH decreased. Different ion-exchange properties of cell surfaces of cyanobacteria and algae were observed.

INTRODUCTION

Unicellular prokaryotic cyanobacteria and eukaryotic algae are essential components of aquatic ecosystems and compose a large proportion of seston biomass in marine and freshwater environments. These microorganisms can accumulate heavy metals from solution [21], and they play an important role as biosorbents influencing metal concentration and metal speciation [9]. Metal uptake by algal cells is a complex process, involving passive and/or active steps [1,12,16,17]. However, from the quantitative point of view, surface sorption (of an ion-exchange nature) seems to be particularly important [2]. Surface sorption may be the largest proportion of the total metal uptake [10,15]. The large surface area of microalgae can highly affect the metal equilibrium concentration in the surrounding water solution and in consequence metal availability to other living organisms. This phenomenon is important for self-cleaning processes in contaminated waters [6] and is also of current biotechnological interest, because different microorganisms (including algae) are considered as efficient biosorbents for treatment of toxic and radioactive waters and for recovery of valuable metals [5]. Little is known of the metal sorption capacity of unicellular algae and cyanobacteria. Since sorption is connected with ion-exchange properties of the cell surface, we have attempted to characterize these properties in four different algal species.

MATERIALS AND METHODS

Organisms and cultivation

The unicellular green alga *Stichococcus bacillaris* Näg., obtained from the Institute of Microbiology, Warsaw University, Poland, was grown in sterilized, modified Prat's culture medium (urea 0.3 g, KH_2PO_4 0.135 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $FeSO_4 \cdot 7H_2O$ 0.003 g, sodium citrate 0.0057 g, H_3BO_3 2.14 mg, $MnCl_2 \cdot 4H_2O$ 1.81 mg, $ZnSO_4 \cdot 7H_2O$ 0.22 mg, $(NH_4)_2MoO_4$ 0.002 mg, $CuSO_4 \cdot 5H_2O$ 0.07 mg, $Co(NO_3)_2 \cdot 6H_2O$ 0.08 mg, NH_4VO_3 0.01 mg and 1 L double-distilled water) as described previously [18]. The unicellular cyanobacterium *Synechocystis aquatilis* VAARA 1978/CB-3 (from the Culture Collection of the Institute of Botany, Treboń, Czech Republic) and *Anacystis nidulans* (*Synechococcus leopoliensis*) from Göttingen University, Germany were cultivated in nutrient medium Z ($NaNO_3$ 0.467 g, $Ca(NO_3)_2 \cdot 4H_2O$ 0.059 g, $MgSO_4 \cdot 7H_2O$ 0.025 g, KH_2PO_4 0.025 g, Na_2CO_3 0.021 g, $FeCl_3 \cdot 6H_2O$ 0.0027 g, H_3BO_3 0.25 mg, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 0.007 mg, $ZnSO_4 \cdot 7H_2O$ 0.022 mg, $Co(NO_3)_2 \cdot 6H_2O$ 0.0117 mg, NH_4VO_3 0.00076 mg, $Na_2WO_4 \cdot 2H_2O$ 0.024 mg, $MnSO_4 \cdot 4H_2O$ 0.178 mg, KBr 0.0095 mg, $CuSO_4 \cdot 5H_2O$ 0.01 mg, $Cr(NO_3)_3 \cdot 7H_2O$ 0.003 mg, $Al_2(SO_4)_3 \cdot K_2SO_4 \cdot 24H_2O$ 0.038 mg, KI 0.0066 mg and 1 L double-distilled water) [20] pH 7.3, at 25 °C, in cycles of 16-h light (25 $\mu Em^{-2}\ s^{-1}$), 8-h darkness. Sterile air was bubbled through the media. The filamentous macroalga *Vaucheria* sp. was obtained from the Department of Ecology and Ecotoxicology, Free University of Amsterdam, Netherlands and cultivated as described previously [19] in modified liquid Woods Hole MBL medium ($NaNO_3$ 0.16 g, HEPES 0.2 g, $MgSO_4 \cdot 7H_2O$ 0.154 g, $CaCl_2 \cdot 6H_2O$ 0.069 g, $Na_2SiO_3 \cdot 4H_2O$ 0.005 g, K_2HPO_4 0.0175 g, Na_2CO_3 0.02 g, Fe (III) citrate 3 mg, citric acid 0.003 g, Na_2EDTA 0.006 g, $CoCl_2$ 0.01 mg, $CuSO_4 \cdot 5H_2O$ 0.016 mg, $Na_2MoO_4 \cdot 2H_2O$ 0.008 mg, $MnCl_2 \cdot 4H_2O$ 0.266 mg, $ZnSO_4 \cdot 7H_2O$ 0.035 mg, soil extract 0.2 ml, thiamine 1 mg, cyanocobalamine 0.5 μg and 1 L double-distilled water).

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pH titration

The cells of microalgal species from early exponential phase cultures (48 h) were harvested by centrifugation, rinsed five times with 1 mM $NaNO_3$ and resuspended in the same solution. Cell densities were 2.6 mg dry wt ml^{-1}, 7.2 mg dry wt ml^{-1} and 2.75 mg dry wt ml^{-1} for *S. bacillaris*, *S. aquatilis* and *A. nidulans*, respectively. The biomass of *Vaucheria* sp. (50–70 mg dry wt) from laboratory cultures was separated by filtration and prepared for experiments as described above. Dry weight was determined by filtering the algae on Whatman GF/C filters after which cells were washed with double-distilled water and dried at 80 °C. The pH of the cell suspensions (V = 16 ml) was adjusted to 11 using 0.1 N NaOH. Argon was constantly bubbled through the suspension to remove dissolved CO_2. Next, the suspensions were titrated with 0.02 M HCl in the range of pH 11–3. Acid was constantly added by peristaltic pump (100 μl min^{-1}) and pH changes were recorded using a pH-meter (N 517, Elwro, Poland) with a glass electrode. A blank titration curve of 0.00125 N NaOH was also prepared. Three to four trials were carried out for each alga.

RESULTS

The studies were carried out on unicellular algal and cyanobacterial species which are ubiquitous in aquatic ecosystems, and on a filamentous macroalga *Vaucheria* sp. In order to assess the potential cation-exchange capacity of the organisms, the amount of protons bound to the cell surface was determined by pH titration. The titration curves of suspensions of intact cells are presented in Fig. 1. All curves for cell suspensions have different patterns than the NaOH titration curve. Only the upper parts of these curves were similar when an excess of NaOH was neutralized at the beginning of the titration. At about pH 10, the titration curves dropped rapidly; however, titration curves of the unicellular alga and cyanobacteria were less steep than the blank NaOH curve. This indicates a reaction between added protons and functional groups on the algal surface. On the basis of the amount of acid used for the titration, the cation-exchange capacity of each alga was calculated. As shown in Table 1, the maximal capacities of the unicellular alga and cyanobacteria were significantly higher than that of the filamentous macroalga *Vaucheria* sp. The highest capacity was observed in the smallest cyanobacterium *A. nidulans*. The ion-exchange capacity was strongly influenced by external pH. Its value (measured for Na^+/H^+ exchange) decreased with pH decrease. In all four species at pH 7, 70–80% of the sodium cations were bound to the algal cell surface. At pH 4, only 10–20% of Na^+ was sorbed on the cyanobacteria and no sorption to other algae was observed. Fig. 2 presents ratios of the amount of H^+ bound to the cell surface to the measured pH change, vs pH. These plots are first derivatives of the titration curves, and correspond to cation-exchange properties of algal surfaces. The existence of functional groups able to disssociate at different external pH values is responsible for various cation-exchange properties of the algal surface. As shown in Fig. 2, the cyanobacteria *A. nidulans* and *S. aquatilis* have very similar cation-exchange properties (almost identical patterns) and were completely different from *S. bacillaris* (Chlorophyceae) or *Vaucheria* sp. (Xanthophyceae). The considerably higher values of $\Delta H^+/\Delta pH$ at pH <6 than at pH >7 in cyanobacteria suggest that in the cation-exchange process, more groups with a low dissociation constant, pK_a, are involved than those with pK_a >7.

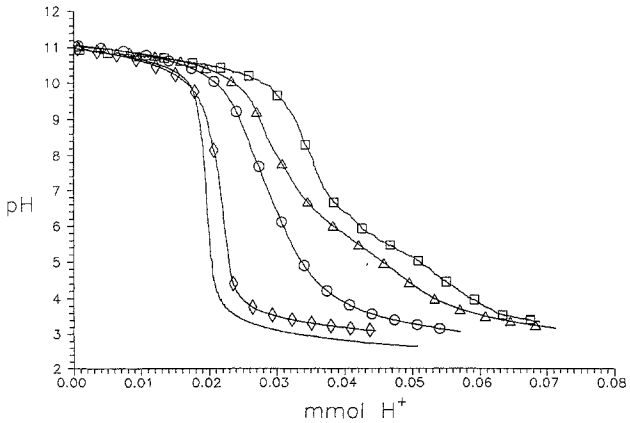

Fig. 1. pH-titration curves of intact cells of *A. nidulans* (Δ), *S. aquatilis* (□), *S. bacillaris* (○), *Vaucheria* sp. (◇) and NaOH (—).

DISCUSSION

Interactions of metals and protons with filamentous macroalgae have been intensively studied [2–4]. The metal sorption and ion exchange capacities of those macroalgal species varied widely. In this paper the ion-exchange capacities of some unicellular algae and cyanobacteria and filamentous macroalga *Vaucheria* sp. are presented (Table 1). Structural and chemical variation of cell surface components as well as various cell morphologies can be responsible for the different sorption capacities observed. Unicellular algae and cyanobacteria have higher surface/volume ratios than macroalgae. This may be one of the reasons for the higher ion-exchange capacity of these two cyanobacteria and the green alga *S. bacillaris* than of *Vaucheria* sp.

Using pH-titration it was possible to obtain information on potential metal sorption abilities of the algae. The ion-exchange capacity of *S. bacillaris* (at pH 7) determined on the basis of Na^+/H^+ exchange was 175 μeq g^{-1} dry wt, and is in good agreement with that calculated from a ^{115m}Cd sorption isotherm (195 μeq g^{-1} dry wt) [17]. At low pH, the ion-exchange capacity decreased significantly (Table 1), although it was stronger in the eukaryotic algae than in the cyanobacteria. Similar differences among algae were observed when heavy metal sorption was measured directly. In the green algae *Chlorella vulgaris*, *Ankistrodesmus braunii* [7] and *S. bacillaris* [17], at pH 4, Cd sorption was close to zero, whereas in the cyanobacterium *Chroococcus paris* [10] considerable Cd-, Cu- and Zn-binding was observed. The differences in algal and cyanobacterial cell walls [13] can be responsible for differences in binding capacity.

Functional groups such as carboxyl, phosphate, hydroxyl, and amino can be involved in metal–cell interactions [8]. Metal cations of various valency and other metal species may bind to the cell surface. In the case of *Sphagnum russowii* cell

TABLE 1

Na^+/H^+ exchange capacity of algal cells at different pH values

Alga	Ion-exchange capacity (μeq g^{-1} dry weight)			
	maximal	at pH 7	at pH 5	at pH 4
A. nidulans (Cyanophyceae)	825 ± 89	635 ± 120	335 ± 28	165 ± 12
S. aquatilis (Cyanophyceae)	205 ± 61	140 ± 26	55 ± 8	20 ± 4
S. bacillaris (Chlorophyceae)	260 ± 32	175 ± 60	80 ± 17	0
Vaucheria sp. (Xanthophyceae)	41 ± 11	31 ± 19	15 ± 4.5	0

Data are expressed as means ±SD of three separate experiments.

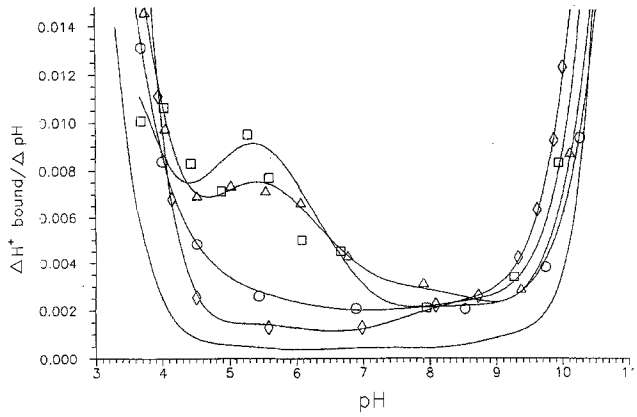

Fig. 2. Differences in cation-exchange properties of algal cell surfaces *A. nidulans* (Δ), *S. aquatilis* (□), *S. bacillaris* (○), *Vaucheria* sp. (◇) and NaOH (—).

wall material, uni-, di- and trivalent-cation binding was more or less dependent on pH [14]. The maximal sorption capacity (μeq g^{-1} dry wt) determined by titration of *S. russowii* in the presence of Na^+, Ca^{2+} or La^{3+} was similar [14].

The pH-titration of cells and calculation of the first derivatives of titration curves (Fig. 2) enabled characterization of the ion-exchange properties of the algal and cyanobacterial cell surface. The exchange capacity depends on the amount and dissociation degree of functional groups. As shown in Fig. 2, in cyanobacteria functional groups of dissociation constants pK_a at pH values lower than 6 are mostly involved in ion exchange processes. Many carboxyl groups have such pK_a values [8]. In *Vaucheria* sp. and *S. bacillaris* functional groups with pK_as from 5 to 9 can contribute equally to cation exchange. Majidi et al. [11], using ^{113}Cd NMR spectroscopy, stated that in dead cells of *S. bacillaris*, at pH 3.3–5.8 carboxylic groups are most likely responsible for Cd-binding. However, alga–metal interactions seem to be also possible through various other functional groups. At present, the total structure of the cell surface and its sorption properties in most algae and cyanobacteria has not been completely elucidated.

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