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ORIGINAL PAPER

On the mechanism of uranium binding to cell wall of *Chara fragilis*

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Abstract Biosorption of uranium from nuclear waste liquids and contaminated surface waters and soils has recently attracted special interest. However, the detailed mechanism of uranium uptake by plants is not well understood. The aim of this work is to investigate the role of cell wall components of the freshwater alga Chara fragilis in uranium sequestration from its solution. Three types of algae preparations: extract of cell wall polysaccharides, dried and live algae were subjected to uranium solutions of different concentration and pH. FTIR and X-ray diffraction were used to assess both potential binding sites and the form of the uranyl sequestered by algae. Sorption of uranium by live and dry algae shows remarkable differences both in terms of overall uptake and mechanisms involved. All experiments are consistent with the conclusion that coprecipitation of uranyl species with CaCO3 is the major binding mechanism in uranium sequestration by Chara fragilis, while the direct exchange of Ca2+ with UO22+ has a minor role. Live algae are twice as efficient in sequestering uranium from solution than dried ones due to the formation of different crystalline forms such as aragonite and rutherfordine forming in live algae in the presence of the uranyl species in solution. It therefore appears that metabolic processes such as photosynthesis, most likely through the regulation of pH, play a key role in the uranium uptake by plants. Further understanding of the complex mechanism of metabolic control of the uranium uptake by plants is needed before the planning of bioremediation of this element.

Keywords Uranium sequestration · Algae · Phytoremediation

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Introduction

Uranium represents one of the major long-term environmental contaminants due to its long half-life (4.5×10^9 years), the long decay chain and high chemical toxicity. Despite its high atomic number, uranium is not a rare element—average concentration of uranium in the earth's crust is 3 mg/kg of dry soil. Unlike in soil, the concentration of uranium in surface water is rather low (average 1 ppb), but in some pond waters can be over 1 ppm (Aleissa et al. 2004). Significantly increased concentrations of uranium are usually found in waters near uranium mines and phosphate fertilizer plants. Methods for uranium removal from contaminated water can be divided into two major groups. Physicochemical methods of uranium separation include direct chemical methods, electrochemical treatments and ion exchange separation (Le Cloirec et al. 1995; Rivas et al. 2006). Those methods are time-consuming and very expensive. The second group of methods, which are a relatively cheap alternative to physicochemical methods, involves biosorption and bioaccumulation of uranium by various bacteria, fungi and plant species (mosses and algae). The principal difference between these methods is that in the latter case, removal can be metabolically mediated. Biosorption of heavy elements, especially uranium, from nuclear waste liquids

and contaminated surface waters and soils has recently attracted special interest (Bhainsa and D'Souza 1999; Dushenkov et al. 1997; Kalin et al. 2005; Goldberg et al. 1998; Malekzadeh et al. 2002). It has been found that biosorption includes several mechanisms such as ion exchange, chelation, adsorption, diffusion through the cell wall and membranes, depending on the species of biosorbent and environmental factors such as pH, temperature, metal concentration, and total dissolved salts.

The most common and diverse group in the aquatic environment are the algae, which come in various shapes and sizes and grow in some of the most extreme habitats. Uranium sequestration by algae is mainly mediated by the structure and composition of the plant cell wall (charge of cell wall, number and type of binding functional groups) and uranium speciation in solution (Yang and Volesky 1999a). Material obtained by drying of algae contains almost intact cell wall components (Vieira and Volesky 2000), so the mechanism of uranium binding should be the same for live and dry specimens. pH has the major impact on the binding of this metal and its influence is bidirectional: alteration of uranium speciation in solution (Ebbs et al. 1998) and the type and availability of binding sites (Davis et al. 2003a, b). On the other hand, live algae possess the ability of adaptation imposed by environmental conditions, such as pH variations and variations of media composition (Dubinsky and Rotem 1974). One such process is the compensations of pH changes up to three units (Schiewer and Volesky 1995), because of H^+ uptake by the cell wall and/or, in case of live algae, bicarbonate utilization that accompanies photosynthesis. The latter process in some algae such as *Chara* is responsible for $CaCO_3$ deposition in the cell wall (McConnaughey and Falk 1991). All the above-mentioned implies the assumption that live algae have a somewhat different and complex mechanism of uranium binding than dry algal material. However, so far, little attention has been paid to components and metabolic control of that mechanism.

For this work we selected *Chara fragilis*, a freshwater green macroscopic alga, which grows in various conditions and is abundant in all continents. The choice of *C. fragilis* as a model for uranium binding was also guided by the fact that the structure and composition of the cell wall of the Characea family is similar to mosses and lichens (Popper and Fry 2003) hence elucidation of the uranium sequestration mechanism could be valid for those organisms as well.

In this work we investigated the binding of uranium by *C. fragilis*, using methods of FTIR spectroscopy and X-ray diffraction, under conditions of variable uranium concentration and pH. FTIR spectroscopy was used to study binding sites on the *Chara* cell wall and to identify their role in uranium binding. X-ray diffraction was used to characterize composition of calcite deposits prior and after uranium sequestration.

Materials and methods

Cell wall of Characeae

The cell wall of Characeae consists of the primary (PCW) and secondary cell wall (SCW). Principal building blocks of PCW are cellulose and hemicellulose fibrils which are embedded in a matrix of the complex polysaccharide pectin. In the case of *Chara* it is a polymer made of oligomers of glucouronic and galactouronic acid, with absence of mixed-linkage glucan (Popper and Fry 2003). Polymer chains are interconnected by Ca^{2+} bridges (Fig. 1) (Carpita 2007). Carboxyl groups of glucouronic and galactouronic residues are at some positions esterified, which makes them unavailable for binding ions from solutions. Cellulose and chemicelluloses contain only hydroxyl groups which are in general very poor binders of metal ions. The cell wall also contains $CaCO_3$ deposits, which are the product of metabolism of *Chara* cells.

Sample preparation

Chara fragilis was collected at Banja Vrujci, Serbia, and transferred to water tanks (volume 25l) for further growth. Tanks were kept under fluorescent light at a 12-h day/night regime. Thalli were harvested 3 weeks after seeding and three types of preparations were used for experiments on uranium uptake: (a) thalli of live algae which were washed with degassed tap water and placed for 2 h, for adaptation

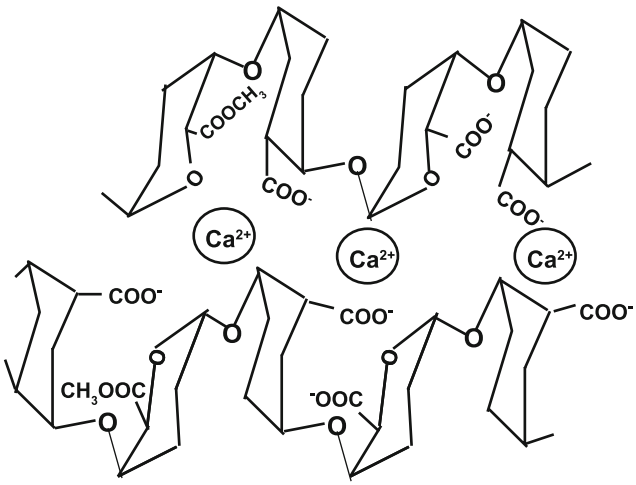

Fig. 1 The segment of pectine polymer chains in the cell wall of Charae with interconnecting Ca^{2+} bridges between polymer chains (Carpita 2007)

reasons, in nutritional growth solution for *Chara*, prepared according to (Andersen 2005), prior to exchange experiments; (b) thalli for dry algae specimens were washed four times with deionized water (MiliQ®) and placed in an oven for 24 h at 80°C. Dry algae were ground in mortar and kept in desiccators; (c) *Chara*'s matrix polysaccharide extraction was performed with a 2% solution of Na_2CO_3 at 80°C. The solution was then filtered and treated with 0.1 M HCl to precipitate the extract. The extract was separated from the solution by filtration, washed four times with deionized water and dried in an oven at 80°C for 24 h.

Uranium exchange experiments

All exchange experiments were performed at room temperature, in polyethylene bottles containing a total of around 50 mg of dry weight of algae samples and 50 ml of solution containing *Chara* growth solution and the appropriate amount of uranium (1–12 ppm). Uranium solutions were prepared by dissolving analytical grade $UO_2(CH_3COO)_2 \cdot 2H_2O$ (Merck, Darmdstat, Germany) into nutrient solutions for *C. fragilis* growth. The initial pH value of solutions with different uranium concentrations was adjusted to 5.60 (also pH of the nutritive solution) using 0.1 M NaOH and 0.1 M HCl solutions. pH values before and after the experiments were controlled with a Fisher Accumete pH-meter. Acidity dependence of uranium binding was performed by setting the initial pH values of the solution from 4 to 9, which is the range in which *Chara* grows in nature (Dubinsky and Rotem 1974; Andersen 2005). Duration of exchange experiments was 24 h under fluorescent light at a 12-h day/night regime.

The uranyl ion shows complex behavior in water solution depending on pH and composition of the solution (Ebbs et al 1998) and it is essential to determine uranyl ion speciation in any particular solution. We applied Phreeqc software (v. 3.17 USGS) (Vrabel and Glynn 1998) to asses uranyl ion speciation in solutions used here for the exchange experiment. As an input for calculation, we took into account all species present in the solution, including ingredients of nutrient solution, pH and the temperature of the solution. The software uses equilibrium constants of forming of all possible chemical structures that may appear in the solution or may precipitate under given conditions. The program gives as an output concentrations of all species of uranium taking into account variables such as different pH, temperature or any other user defined parameter (see Fig. 2).

After finalization of exchange experiments, the remaining solution was removed by filtration and algae were washed four times with deionized water and dried in an oven at 80°C for 24 h. Dry specimens were ground in a mortar and used for FTIR and X-ray diffractometry. Tablets for FTIR spectroscopy were prepared by pressing 2 mg of the algae specimen and 150 mg of KBr. Spectra were recorded on a Thermo Nicolete 6700 FTIR with resolution of 4 cm^{-1} and transparency measurement absolute error of 0.01%. Absolute values of transparencies of particular vibration bands used for ratio calculations were determined in the Microcal Origin™ v. 7.5 software with an error of 0.5 %. Powder diffraction patterns of ground dry algae were obtained on a Phillips PW1050 X-ray diffractometer, with the step of 0.01.

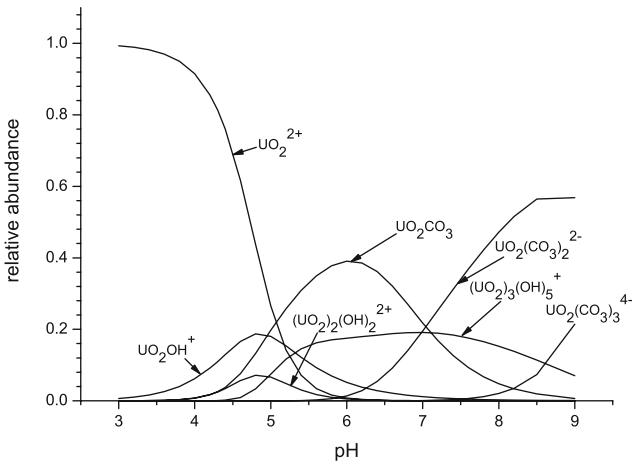

Fig 2 The relative abundance of uranyl species in nutrient growth solution for Charae algae containing 12 ppm of uranium and dissolved CO_2 of 15 ppm (from air, corresponding to normal atmospheric pressure)

Results and discussion

Distribution of uranyl species in water solution used for exchange experiments is given in Fig. 2. At low concentration of CO_3^{2-}, the free uranyl ion is a major specie at lower pH values (pH < 5), while at higher pH values the dominant species are complexes with hydroxyl ions and carbonate complexes, which under natural conditions become the most abundant ones. Since the cell wall of *Chara* (and plants in general) is negatively charged, good candidates for binding should be positively charged species, such as free UO_2^{2+} and hydroxyl complexes. However, considering the ratio of the hydroxyl complex and pore radius within the cell wall (Berestovsky et al. 2001) uranyl

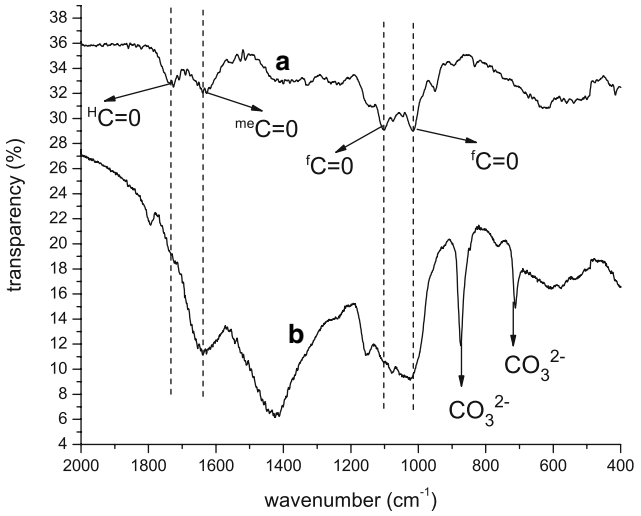

Fig. 3 The comparison of IR spectra of the extract of cell wall matrix polysaccharides (**a**) and dry algae (**b**). Assignations of stretching vibrations of C = 0 from COO^- and CO_3^{2-} groups are according to Nakamoto (1997) Superscripts in the assignation: *H* protonated group, *F* free ionized group, and *Me* occupied by Ca^{2+}

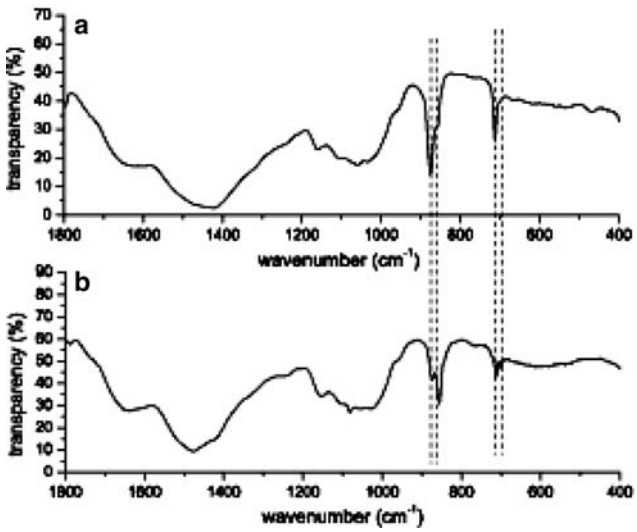

Fig. 4 The region of carbonate bands of IR spectra of dry (**a**) and live (**b**) algae specimens following the exchange in 12 ppm uranium solution (pH 5.60, dissolved CO_2 concentration of 15 ppm). New bands are clearly visible on the IR spectrum of live algae

hydroxyl ions must eliminate OH^- from its coordination sphere in order to bind to the cell wall, which is an energetically unfavourable process. In addition, UO_2CO_3, present in the solution as neutral specie, may co-precipitate in the process of $CaCO_3$ formation in the cell wall.

Infra red spectra of the matrix polysaccharides extracted from the untreated cell wall were recorded to identify potential binding groups for uranium within the polysaccharide matrix (Fig. 3a). Spectrum of the extract shows three types of bands arising from stretching vibrations of C = 0 in carboxyl groups: protonated COO^- group (1724 cm^{-1}), free COO^- group (1102 and 1017 cm^{-1}), and Ca^{2+}-loaded COO^- group (1633 cm^{-1}). Comparison with the spectrum of dry algae (Fig. 3b) shows that bands arising from free and protonated carboxyl groups can be observed only as shoulders features on wider bands, leading to the conclusion that under natural conditions, nearly all COO^- group sites are occupied with Ca^{2+} ions in agreement with previous findings for other algae (Yang and Volesky 1999b). The other functional group that could potentially bind uranyl ions is the hydroxyl group which is highly unlikely since the hydrogen from OH can not be easily exchanged within the studied pH range. Consequently, the extract of matrix polysaccharides is not a good model for studying live algae, at least not in the case of *Chara*. Data for dry algae show that the principal mechanism should be the Ca^{2+}–UO_2 ion exchange on the Ca^{2+} occupied carboxyl groups. This is supported by the fact that the complex between uranyl and acetate groups has a higher stability constant than the corresponding complex of Ca (pK = 2.38 vs. pK = 0.6) (Dean 1999). The spectrum of untreated dry algae also shows the presence of carbonate bands at 872 and 713 cm^{-1}, which correspond to vibrations in carbonate groups of $CaCO_3$ in the cell wall and are not present in the spectra of the extract.

Figure 4 shows the comparison of IR spectra obtained for dry and live algae after exchange in 12 ppm uranium solution. The amount of uranium sorbed by dry and live algae was 60 and 107 mg/g dry weight indicating an additional mechanism of uptake in live algae. Carbonate bands (872 and 713 cm^{-1}) are present in both IR spectra arising from calcite (Nakamoto 1997) which is the normal constituent of the *Chara* cell wall (McConnaughey and Falk 1991). Comparison of IR spectra of dry algae after exchange and that of untreated ones shows no significant differences in the used concentration range. However, when the exchange was performed with live algae, two new bands at 856 and 700 cm^{-1} appeared near carbonate bands indicating their dependence on certain metabolic processes present in *Chara*.

It is difficult to unequivocally assign these bands to different crystalline forms of calcium and/or uranium carbonates; hence X-ray diffraction was performed on these samples. Figure 5 shows the powder diffraction patterns for dry and live algae after exchange in 12 ppm uranium solution. There was no difference between patterns for untreated and treated dry algae (not shown here) and their spectra only showed the presence of the pure calcite form of $CaCO_3$. X-ray diffraction spectrum for live algae shows the presence of calcite and aragonite (crystal forms of $CaCO_3$) but also rutherfordine (UO_2CO_3) in treated live algae. This agrees with the assignation of the band at 856 cm^{-1} corresponding to the forbidden stretching

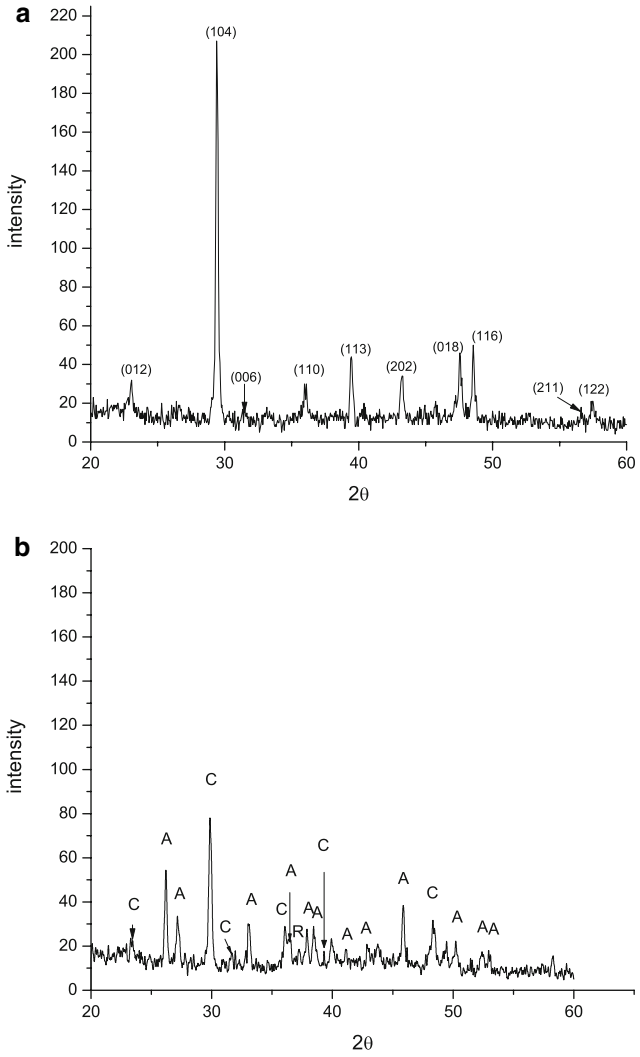

Fig. 5 The X-ray diffraction patterns for **a** dry and **b** live algae after exchange in 12 ppm uranium solution. *A* aragonite; *C* calcite; *R* rutherfordine

vibration of U–O, probably from UO_2CO_3 (Chernorukov et al. 2002), while the band at 700 cm^{-1} corresponds to the appearance of aragonite, a crystal modification of $CaCO_3$ (Vagenas et al. 2003). Consequently, both IR spectra and X-ray diffraction suggest that the uranyl in the cell wall is incorporated in some form of crystal lattice. The fact that the processes of crystal formation are different for dry and live algae is in agreement with the suggestion that the formation of calcite deposits in the cell wall of algae is in direct connection with the process of photosynthesis (McConnaughey and Falk 1991).

It is difficult to quantify changes of uranium sequestration by the cell wall as a function of uranium concentration or pH using either X-ray diffraction or absolute values of transparency in IR spectra. Hence we used the transparency ratio between bands at 872 and 856 cm^{-1} and 713–700 cm^{-1} in

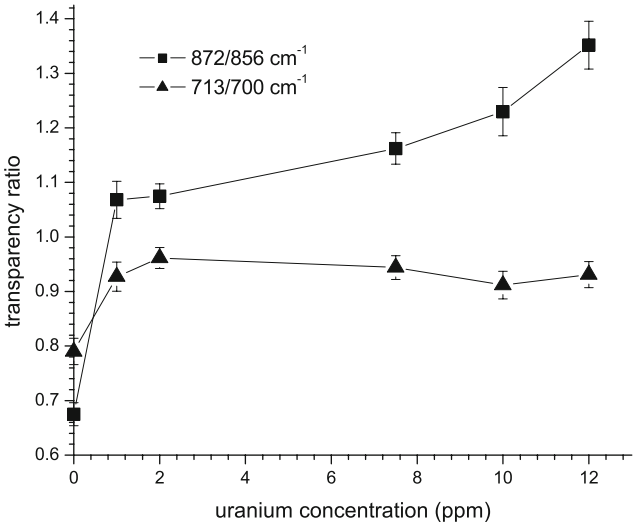

Fig. 6 The transparency ratios of IR bands 872/856 and 713/700 cm^{-1} for live algae treated with various concentrations of uranium

spectra of live algae to trace changes induced by different uranium concentrations (Fig. 6). The first ratio (872/856 shows clear dependence of uranium concentration, which indicates increasing precipitation of UO_2CO_3 in the form of rutherfordine and decreased amount of calcite (see also Fig. 5b). The second ratio shows that incorporation of UO_2^{2+} in aragonite shows saturation on a rather low uranium concentration consistent with low solubility of UO_2^{2+} in aragonite (Chernorukov et al. 2002).

pH dependence of uranium binding as measured by UV-VIS spectrometry for dry and live algae is shown in Fig. 7. pH profiles of uranium sorption for dry algae follow the

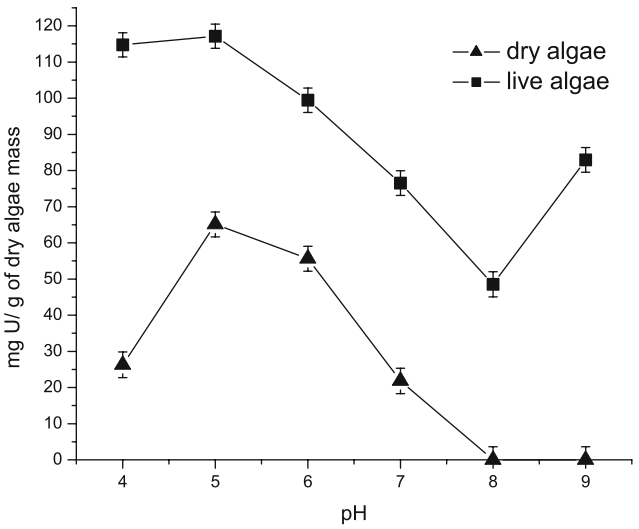

Fig. 7 The pH dependence of total uranium uptake as measured using the Arsenazo III method and UV–VIS spectroscopy by live and dry algae treated with solution containing 12 ppm of uranium

general profile for UO_2CO_3 concentration in solution (see Fig. 2), which implies that its co-precipitation is the main mechanism of uranium sequestration. The process of Ca^{2+}–UO_2^{2+} exchange should also be present in live algae at very low pH (observe the difference at pH 4 in Fig. 7), but it seems to have a small role in overall uranium uptake by both specimens. The overall quantitative difference in the uptake between dry and live algae could be the consequence of the UO_2CO_3 role in complex processes of calcite deposits forming in live algae (McConnaughey and Falk 1991). Increase of uptake for alive algae above pH 8 (Fig. 7) could arise from the involvement of other present carbonate species such as $UO_2(CO_3)_2^{2-}$ or $UO_2(CO_3)_3^{4-}$ whose contribution in the total dissolved uranium becomes significant at high pHs (Fig. 2). Since these anion complexes are too large to pass through the pores of the cell wall, increased uptake above pH 8 suggests their chemical transformation prior to binding to the cell wall. The formation of rutherfordine from negatively charged carbonate complexes of uranyl in solution could be explained by the following reactions:

$$UO_2(CO_3)_2^{2-} + H_3O^+ = UO_2CO_3 + HCO_3^- + H_2O$$

$$UO_2(CO_3)_3^{4-} + 2H_3O^+ = UO_2CO_3 + 2HCO_3^- + 2H_2O$$

When bicarbonate concentration in the solution is low (such as in our experiments), the equilibriums of these reactions are shifted to favor the formation of UO_2CO_3 and HCO_3^-. Incorporation of UO_2CO_3 in the cell wall can result in further $CaCO_3$ deposition in aragonite form, because of the fact that the orthorhombic structure of rutherfordine is more similar to the structure of aragonite (Russel et al. 1993).

pH dependence of transparency ratios 872/856 and 713/700 cm^{-1} for live algae is shown in Fig. 8. Transparency ratios do not show a similar profile as pH dependence of uranium uptake. It should be noted that these ratios reflect a rather complex equilibrium of rutherfordine versus calcite and aragonite versus calcite formation; hence ratios should be considered as a qualitative measure of different crystalline forms present in the cell wall during formation of $CaCO_3$ deposits. Experiments using confocal and atomic force microscopy should be used to further clarify uranium interactions with the plant cell wall.

In conclusion, our experiments showed that coprecipitation of uranyl species with $CaCO_3$ is the major binding mechanism in uranium sequestration by *C. fragilis* while the direct exchange of Ca^{2+} with UO_2^{2+} has a minor role. Different crystalline forms such as aragonite and rutherfordine are formed in the presence of uranyl species in solution, which was confirmed by both FTIR and X-ray diffraction. The contribution of these forms relative to the formation of calcite (a native form of calcium deposits) depends on various conditions such as pH and bicarbonate concentration. Uptake of uranium by live and dry algae shows remarkable differences both in terms of overall uptake and the mechanisms involved. This is only to be expected since the micro-environment of the cell wall of live algae differs from that of the bulk solution in terms of lower pH (regulated through photosynthesis) and the higher local concentrations of ions (McConnaughey and Falk 1991; Carpita 2007). Nevertheless, it is clear that understanding of the metabolic control of uranium uptake is needed to improve planning of remediation of this element by plants.

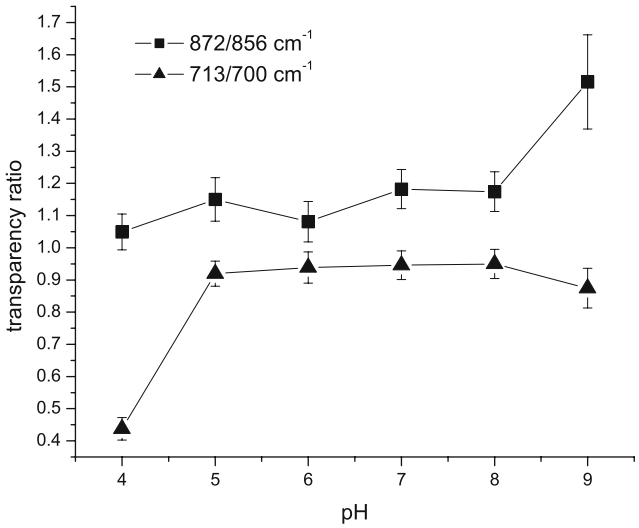

Fig. 8 The pH dependence of transparency ratios of IR bands of the cell walls of live algae treated under the same conditions as in Fig. 7

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