Uptake of Uranium by Various Cell Fractions of *Chlorella regularis*[†]

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To know what kinds of the cell components of *Chlorella regularis* are concerned with uranium binding, uptake of uranium by various cell fractions was examined. The uptake value ($\mu\text{gU}/\text{mg}$ starting dry cells) of the hot water-treated cells was almost the same as that of the starting dry *Chlorella* cells, showing that the cell components extracted with hot water were not so concerned with uranium binding. The cell components extracted with dilute alkali seemed to play an important role in uranium binding, and those extracted with chloroform-methanol seemed to be partly concerned with uranium binding. The cellulose fraction of the cells was scarcely concerned with uranium binding. In the dry cells, 34% of uranium taken up existed in the cell walls. However, in the living cells, 85% existed in the cell walls. The above results showed that the dry or the hot water-treated cells are the most convenient for uranium recovery from the aqueous systems.

Key Words: uranium uptake, cell fractions, *Chlorella*

1. Introduction

We have been studying the recovery of uranium from the aqueous systems by microalgae⁽¹⁻⁴⁾: (1) *Chlorella regularis*, one of the fresh-water green microalgae, could take up a relatively large amount of uranium from the solution containing uranium only; (2) scalded (treated in boiling water for 5 min) *Chlorella* cells could take up 3 times more uranium than the living cells; (3) the most part of the uptake of uranium was not directly mediated by metabolic processes, but depended upon physico-chemical adsorption on the cell surface. However, little is known about the cell components which are concerned with uranium binding. In this paper, to know what kinds of the cell components are concerned with uranium binding, the uranium uptake by the cells treated with hot water, dilute alkali, concentrated alkali, or chloroform-methanol and by the cell wall material was examined.

2. Materials and Methods

2.1 Cultivation of *Chlorella*

Chlorella regularis S-50⁽¹⁾ was grown in a 20 l-bottle containing 20 l mineral salts-urea medium⁽⁵⁾ with aeration at 30°C. The bottle was illuminated continuously by white fluorescent lamps giving a light intensity of 4,400-4,800 lux at the surface of the bottle. After 6 days, the cells in the linearly growing phase were collected by centrifugation at $6,000\times g$ for 5 min, washed thoroughly with deionized water, and then freeze-dried.

2.2 Preparation of the chemically treated cells and the cell wall materials

(i) Hot water-treated cells. One gram dry cells of *Chlorella* was treated with 100 ml of deionized water at 100°C under reflux. After 2 hr, the cells were collected by centrifugation, washed thoroughly with deionized water, and freeze-dried. The yield was 670 mg on a dry weight basis. (ii) Dilute alkali-treated cells. The dry cells (1 g) were treated with 100 ml of

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0.2% NaOH solution at room temperature with continuously stirring. After 20 hr, the green residue was collected by centrifugation, washed thoroughly with deionized water, and then freeze-dried (yield on a dry weight basis, 470.8 mg). (iii) Chloroform-methanol treated cells. The dry cells (2 g) were treated with 100 ml of aq. 96%(v/v) methanol at 65°C under reflux. After 10 min, the solution was cooled to room temperature, and 200 ml chloroform was added. The chloroform-methanol mixture was stirred at room temperature for 20 min. After centrifugation, the cells treated with chloroform-methanol was retreated with further 100 ml of chloroform-methanol (2:1) for 20 min, centrifuged and dried. The yield of the pale-green residue was 1,413.7 mg. (iv) Chloroform-methanol/concentrated alkali-treated cells. *Chlorella* cells (700.5 mg dry wt.) treated with chloroform-methanol as in (iii) was treated with 100 ml of 24% (w/v) KOH at room temperature under N₂ for 2 hr with sometimes shaking. Then the cells were centrifuged and re-extracted with further 10 ml of KOH for 2 hr. The residue was thoroughly washed with deionized water by suspension and centrifuging, and then freeze-dried. The yield of white preparation was 137.3 mg. (v) The cell wall materials. The dry cells (15.0 g dry wt.) were disrupted using the X-press (LKB Co., Sweden) at 2,700 kg/cm². The resultant cell homogenate was heated at 100°C for 10 min and centrifuged at 1,000×g for 10 min. The 1,000×g precipitate was washed thoroughly with deionized water until the supernatant solution became clear, and freeze-dried. The yield, 4.637 g. The optical microscope examination showed that few unbroken cells existed in the preparation.

2.3 Uptake experiments

The uptake experiments were carried out using 1 l Erlenmeyer flasks containing 500 ml of the uptake solution containing uranium only. Uranium was supplied as UO₂(CO₃)₃⁴⁻ form[®] to make the final uranium concentration 4 ppm. The pH value of the uptake solution was adjusted to 8 with 0.1 N HCl solution. Twenty mg of the dry cells, the chemically treated cells, and the cell wall materials were suspended

in the uptake solution at 30°C with continuously stirring. After 30 min, the cells and the cell wall materials were collected by filtration through Millipore filter (pore size, 0.22 μm), washed thoroughly with deionized water, and then freeze-dried.

2.4 Determination of uranium in the cells and the cell walls

The amount of uranium taken up was determined by neutron activation analysis utilizing the gamma ray delivered from ²³⁵U. The detailed method of the activation analysis was described previously⁷⁾.

3. Results and Discussion

The uranium uptake values of the chemically treated cells and the cell wall materials were expressed as μg of uranium per mg of the starting dry cells correcting the loss of dry weight by each treatment. The results are shown in Table 1. The uptake value of the hot water-treated cells was almost the same as that of the dry cells. This showed that the cell components extracted with hot water (oligo- and polysaccharides, proteins, and many low molecular weight substances) were not so concerned with uranium binding.

The uptake value of *Chlorella* cells treated with chloroform-methanol was 73% of that of the dry cells, showing that the cell components extracted with chloroform-methanol (mainly lipids) seemed to be partly concerned with uranium binding. But the possibility of the denaturation of the ligands for uranium binding by the chloroform-methanol treatment was present.

In the case of the dilute alkali-treated cells, the uptake value was only 53% of that of the dry cells. This result suggested that the cell components extracted with dilute alkali (proteins, polysaccharides, etc.) played an important role in uranium binding. However, it was possible that the denaturation of the ligands for uranium binding by the dilute alkali treatment seemed to lead to the lowering of the uptake capacity.

The uptake value of the cells treated with chloroform-methanol/concentrated alkali was very small, showing that this fraction (mainly

Table 1 Uptake of uranium by various cell fractions of *Chlorella regularis*

Cell fractions		Yields in treatments to the starting whole dry cells (%)	$\mu\text{gU}/\text{mg}$ of starting dry cells
Whole dry cells		100.0	37.3 (100.0)
Chemically treated cells	Hot water-treated	67.0	37.4 (100.3)
	Chloroform-methanol-treated	70.7	27.2 (72.9)
	Dilute alkali-treated	47.1	19.8 (53.1)
	Chloroform-methanol/concentrated alkali-treated	13.9	0.7 (1.9)
Cell walls		30.9	12.6*(33.8)
Living cells		—	14.9*(39.9)

Cell amounts used were 20 mg except for the living cells (15.6 mg). Values in parentheses are expressed as per cent of the value of the dry cells.

* $\mu\text{gU}/\text{mg}$ dry wt..

cellulose) was scarcely concerned with uranium binding.

The uptake value of the cell wall materials was only 34% of the uptake value of the dry cells. This result indicated that in the dry cells the intracellular components participated in uranium binding and these intracellular components were removed during the preparation of the walls. Furthermore, the uptake value of the cell walls was about 85% of that of the living cells, suggesting that the cell wall materials played an important role in uranium uptake by the living cells. This result was consistent with our previous observations¹⁾ that 75-80% of uranium taken up by the living cells was adsorbed on the cell surface.

From the above results we concluded: (a) in the dry cells the cell components extracted with dilute alkali (proteins, polysaccharides, etc.) seemed to play an important role in uranium binding; (b) in the living cells 85% of uranium taken up existed in the cell walls; (c) the dry or the hot water-treated cells are the most convenient for uranium recovery from the aqueous systems.

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要 旨

種々のクロレラ藻体画分によるウランの取込み†

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クロレラ藻体中のどのような成分がウランの取込みに関与しているかを調べるために, 種々のクロレラ藻体画分のウラン取込み能について検討した。その結果, 希アルカリによって抽出される成分はウラン取込みに重要な役割を果しており, クロロホルム-メタノールによって抽出される成分もウラン取込みに若干関与しているが, 熱水によって抽出される成分およびセルロース画分はウランの取込みにほとんど関与していないことが明らかになった。一方, 細胞壁に取り込まれたウランの量は, 乾燥全藻体の取込み量の34%, 生藻体による全取込み量の85%に相当することがわかった。以上の結果から, 水圏中のウラン回収には乾燥藻体, 熱処理藻体を用いると最も効率がよいことが明らかになった。

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