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Author(s)	Seki, Hideshi; Suzuki, Akira; Maruyama, Hideo
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**Biosorption of chromium (VI) and arsenic (V) onto
methylated yeast biomass.**

Hideshi Seki*, Akira Suzuki and Hideo Maruyama

Division of Marine Biosciences, Graduate School of Fisheries Sciences, Hokkaido University, Minato-cho

3-1-1 Hakodate 041-8611, Japan

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Biosorption of Cr (VI) and As (V) onto methylated yeast

Corresponding author

Hideshi Seki

Division of Marine Biosciences, Graduate School of Fisheries Sciences, Hokkaido University,

Minato-cho 3-1-1 Hakodate 041-8611, Japan

E-mail address: seki@elsie.fish.hokudai.ac.jp.

Abstract

Yeast biomass was methylated in a 0.1 M HCl methyl alcohol solution at room temperature and the methylated yeast (MeYE) was applied to the adsorptive separation of Cr(VI) and As(V) anions from aqueous solutions. At near neutral pH, while Cr(VI) and As(V) anions were scarcely adsorbed onto unmethylated yeast biomass, their adsorbed amounts increased with increasing methylation degree. The amount of Cr(VI) adsorbed onto MeYE was almost constant at pH 4 - 6 and decreased with increasing pH above pH 6. The adsorbed amount of As(V) onto MeYE was rather lower than that of Cr(VI) and it had a peak at about pH 7. A metal-binding model was used to describe the adsorption characteristics of Cr(VI) and As(V) onto MeYE. The results showed that MeYE has two different types of adsorption sites. The saturated adsorbed amount of Cr(VI) and As(V) onto MeYE having methylation degree 0.94 was 0.55 mmol g⁻¹.

Keywords: Biosorption; Chromium; Arsenic; Methylated yeast biomass

1. Introduction

The pollution of surface and ground water by Cr(VI) and As(V) leaching from contaminated soils has become one of the most serious problems today. Both Cr(VI) and As(V) cause serious threat to the environment, animals, and humans. The recovery of Cr(VI) and As(V) from dilute aqueous solutions by biosorption is an emerging field of interest, from both a resource conservation standpoint and an environmental remediation standpoint. Unlike conventional sorptive resins or organic solvents, biosorption employs inexhaustible, inexpensive, nonhazardous materials. Secondary waste from the biosorption-based process is readily treated and can be easily disposed by incineration. Thus, the adsorptive separation of Cr(VI) by microorganisms [1-6], algae [7], and plant biomass [8, 9] have been investigated. Many of these studies focused on the adsorption of Cr(VI) under strongly acidic conditions (pH 1-3) because the adsorption amount of Cr(VI) onto these biomaterials steeply decreased above pH 3. It may be possible to control the water's pH in the industrial waste water treatment process, however, it is impossible to control the natural water's pH in the environmental remediation process.

Thus, the information about the biosorption behavior of Cr(VI) at near-neutral pH is much important, and moreover, the biosorption of As(V) has scarcely been investigated.

As most microorganisms have an isoelectric point around pH 2, their surface charge should be negative at near-neutral pH. On the other hand, Cr(VI) and As(V) also exist as anions in water. Therefore, it is not expected that these metals can adsorb onto microorganisms at near-neutral pH. In our previous study [10], we prepared a new biosorbent, methylated yeast (MeYE), for the adsorptive separation of negatively charged proteins. At near-neutral pH, egg albumin which has negative charge was scarcely adsorbed onto unmethylated yeast, but its adsorbed amount remarkably increased with the increase of methylation degree. The saturated adsorbed amount of egg albumin onto MeYE having methylation degree 0.77 was 0.38 g g^{-1} at pH6.5. In the present study, MeYE having a methylation degree of 0.94 was applied to the adsorptive separation of Cr(VI) and As(V) at pH 4 - 8. The dissociation characteristics of acidic and basic sites of MeYE were determined from the results of potentiometric titration. Then, based on a simple metal-binding model, the biosorption mechanism of

Cr(VI) and As(V) onto MeYE will be discussed.

2. Materials

2. 1. Chemicals

Sodium arsenate dibasic heptahydrate was purchased from Wako Pure Chemical Industries (Japan). Potassium chromate and sodium nitrate were obtained from Kanto Chemical Co. Inc. (Japan). These chemicals were of reagent-grade quality. They were used with no further purification. Distilled water, boiled for 15 min and cooled under nitrogen, was used in all experiments.

2. 2. Preparation of biosorbents

Yeast (dried) of practical grade was purchased from Wako Pure Chemical Industries (Japan) and washed in the following manner. A sample of 100 g of yeast was suspended in a 1 dm³ of 0.01 M NaOH solution and mechanically stirred for 2 h. The yeast was separated in a centrifuge at 3300 rpm for 30 min and washed repeatedly with distilled water. Then it was suspended in 1 dm³ of methyl alcohol and stirred at room temperature. After 24 h of stirring, yeast was separated in a centrifuge at 3300 rpm for

30 min, freeze-dried and ground to a fine powder. The ground yeast was sieved through a 120-mesh (0.125-mm) sieve and the undersized fraction was used. Hereafter, the yeast will be abbreviated as YE.

Methylated yeast was prepared according to the method reported by Fraenkel-Conrat and Olcott [11]. A sample of 10 g of YE was dispersed in 1 dm³ of methyl alcohol containing HCl (0.1 mol dm⁻³) as a catalyst and mechanically stirred at room temperature. The methylated yeast was collected in a centrifuge at 3300 rpm for 20 min and washed repeatedly with distilled water. Then it was freeze-dried and stored in a desiccator. Hereafter, the methylated yeast will be abbreviated as MeYE. In this study, we used two types of MeYE that had a methylation degree of 0.64 and 0.94. They were prepared by changing the methylation time 6 and 24 h, respectively.

3. Experimental Methods

3. 1. Potentiometric titration of MeYE and YE

The degree of methylation was determined from the change in the number of carboxylic groups before and after methylation. The number of carboxylic groups was determined by potentiometric titration [12-14]. A solution (0.5 dm^3) containing a certain amount of MeYE/YE was mechanically stirred at 30°C . The ionic strength of the solution was adjusted to 0.01 mol dm^{-3} by the addition of NaNO_3 . After reaching thermal equilibrium, the solution was titrated with a volumetric standard solution of HNO_3 or NaOH (0.01 mol dm^{-3}). The pH of the solution was measured with a pH meter (Mettler Toledo MP225). To eliminate CO_2 , the titration was performed under nitrogen. The number of protonated acidic/basic groups was determined from the difference between the bulk proton concentrations in the presence of MeYE/YE and those in the absence of MeYE/YE.

3. 2. Adsorption experiments of Cr(VI) and As(V) onto MeYE and YE

A solution of NaNO_3 (0.01 mol dm^{-3}) containing a certain amount of MeYE/YE was prepared. The pH of the solution was adjusted to the desired value with HNO_3 or NaOH . After thermal equilibrium was reached at 30°C , a certain amount of K_2CrO_4 or Na_2HAsO_4 solution was added to the suspension. The suspension was stirred for the time necessary to attain the adsorption equilibrium, and then MeYE/YE was separated from the liquid phase in a centrifuge at 3300 rpm for 20 min. The pH and metal concentration of the supernatant were measured. The amount of metal adsorbed onto MeYE/YE was determined from the difference between the metal concentrations in the initial and equilibrium states. The concentration of Cr(VI) was determined with a Ubest-30 spectrophotometer (Japan Spectroscopic Co., Ltd.), using 1,5-diphenylcarbonohydrazide as a chromogenic reagent. The concentration of As(V) was determined with the spectrophotometer using hexaammonium heptamolybdate as a chromogenic reagent.

4. Results and Discussions

4. 1. Proton binding sites of MeYE

Figure 1 shows the proton adsorption isotherm of MeYE (methylated for 24 h) obtained from potentiometric titration (open circles). The result with YE is also presented in Fig. 1 (solid circles). The proton adsorption isotherms of microorganisms are usually ill-defined, reflecting the diversity in the proton binding sites. The proton binding sites of microorganisms are divided into three main types and the proton adsorption isotherm can be expressed by the following equation [15, 16]:

$$X_H = \frac{N_1[\text{H}^+]}{K_1 + [\text{H}^+]} + \frac{N_2[\text{H}^+]}{K_2 + [\text{H}^+]} + \frac{N_3[\text{H}^+]}{K_3 + [\text{H}^+]} \quad (1)$$

where X_H represents the equilibrium amount of protons adsorbed to 1 dry g of microorganisms. K and N (mol g^{-1}) represent the dissociation constants of proton binding sites and the number of binding sites on 1 dry g of microorganisms, respectively.

The acid-catalyzed methylation reaction is a specific one involving

only the carboxylic groups [11]. Therefore, the proton adsorption isotherm of MeYE can be expressed by the following equation:

$$X_H = \frac{(1-d_m)N_1[\text{H}^+]}{K_1 + [\text{H}^+]} + \frac{N_2[\text{H}^+]}{K_2 + [\text{H}^+]} + \frac{N_3[\text{H}^+]}{K_3 + [\text{H}^+]} \quad (2)$$

where d_m represents the degree of methylation of MeYE.

A nonlinear least-squares method was applied to find the constants, d_m , K and N , in Eq. (2). The constants which gave the best fit with the experimental data are listed in Table 1. The type 1 ($pK_1 = 3.46$) and type 3 ($pK_3 = 9.78$) sites can be considered as the carboxylic groups and amino groups, respectively. The major part of the type 2 site ($pK_2 = 6.53$) can be considered as the phosphatic groups [15, 16]. According to the data supplied by the company, yeast protein contains about 3 wt % histidine. Therefore, it can be considered that the type 2 site includes a small amount of the histidine imidazol groups. The methylation degree, d_m , of MeYE methylated for 24 h was obtained to be 0.94. The solid lines in Fig. 1 represent the theoretical curves calculated from Eq. (2). The correlation coefficients between the experimental and the predicted values are 0.994

and 0.999 for MeYE and YE, respectively.

4. 2. Effect of methylation on Cr(VI) and As(V) adsorption

Figure 2 shows the adsorption isotherm of (a) Cr(VI) and (b) As(V) to MeYE at pH 6.5. MeYE methylation degrees of 0.64 (open triangles) and 0.94 (open circles) were used in the experiment. For comparison, the results of YE (open squares) are also presented. The concentrations of MeYE/YE in Figs. 2a and b were 0.7 - 5.0 and 4.5 - 6.4 g dm⁻³, respectively. The initial concentrations of Cr(VI) and As(V) in Figs. 2a and b were 0.5 - 2.0 and 0.5 - 2.5 mM, respectively. Preliminary kinetic experiments on the Cr(VI)-MeYE/YE and As(V)-MeYE/YE systems were performed for 12 h. The adsorption reaction proceeded rapidly and 10 min or so was enough to attain the equilibrium. The adsorption curve showed a plateau from 0.5 to 12 h. From the results, we determined the contact time for the adsorption experiments to be 1 h. The adsorbed amount of both Cr(VI) and As(V) to MeYE increased with increasing methylation degree, while they were scarcely adsorbed to YE. Urrutia and Beveridge [17, 18] reported that silicate anions (SiO₄⁴⁻) can adsorb to the positively charged amino groups

present within the cell wall of a bacterium, *Bacillus subtilis*. Bai and Abraham [2] also reported that the amino groups within the cell wall of *Rhizopus nigricans* play a contributory role in Cr(VI) adsorption based on the results of FTIR analysis. The present methylation reaction is a specific reaction with only the carboxylic groups and the other functional groups are unaffected. Thus, the results in Fig. 2 suggest that the negatively charged carboxylic groups within the cell wall of MeYE and YE inhibit the adsorption/access of Cr(VI) and As(V) to amino groups.

The yeast cell wall is composed of several layers bearing anionic groups. Up to 90% of the yeast cell wall is polysaccharide complexed with proteins, lipids, and other substances. The role of phosphomannans and carboxylic groups of cell wall protein of the yeast *Saccharomyces cerevisiae* for the binding of metal cations has been identified by Strandberg et al. [19]. Strouhal et al. [20] reported that the carboxylic groups, which are dominant functional groups in the cell wall, played an important role in the biosorption of Cd and Ni to the yeast *Yarrowia lipolytica*. On the basis of the results, they suggested that the cell wall is the first protective barrier to prevent penetration of heavy metals into the cell.

Furthermore, it is known that the yeast *Rhodotorula glutinis* produces an extracellular polysaccharide composed of neutral sugars (85%) and uronic acid (15%). Uronic acids confer a net negative charge to the polymer and play an important role in the binding of metal cations [21]. Therefore, it can be supposed that the negatively charged carboxylic groups in the yeast cell wall prevent the permeation of As(V) and Cr(VI) anions into the cell while the neutralization of the carboxylic groups by methylation allows the permeation of As(V) and Cr(VI) anions into the cell.

4. 3. pH dependence of Cr(VI) and As(V) adsorption onto MeYE

Figure 3 shows the pH dependence of (a) Cr(VI) and (b) As(V) adsorption to MeYE having a methylation degree of 0.94 at 30°C. The initial concentrations of metal ions were 1.0 mmol dm⁻³ (open symbols) and 0.5 mmol dm⁻³ (solid symbols). The concentrations of MeYE were (a) 1.0 and (b) 4.9 g dm⁻³. Adsorption of Cr(VI) and As(V) showed very different pH dependence. The adsorbed amount of Cr(VI) was almost constant up to pH 6 and gradually decreased with increasing pH, while the adsorbed amount of As(V) had a peak at about pH 7.

The interaction between the metal ions and the functional groups on/in microorganisms depends not only on the nature of the microorganisms but also on the solution chemistry of the metal to be adsorbed. In aqueous solutions, Cr(VI) and As(V) exhibit different types of pH dependent equilibria. The possible ionic species of Cr(VI) and As(V) and the corresponding stability constants, K_s , are listed in Table 2 [22]. The mole fraction of ionic species in total Cr(VI) and As(V) at the concentration of 1.0 mmol dm⁻³ was calculated as a function of pH using the stability constants in Table 2. The results for Cr(VI) and As(V) are shown in Figs.

4(a) and (b), respectively. Both Cr(VI) and As(V) are present in monovalent and bivalent form in the pH range from 4 to 9.

We reported that the biosorption of bivalent metal ions (Cd^{2+} and Pb^{2+}) onto microorganisms (bacteria and microalgae) was due to the monodentate binding of metal ions to the acidic groups on/in the microorganisms [15, 16]. Assuming that a similar relation holds in the present biosorption system, the binding reactions of monovalent and bivalent anions to the amino groups (type 3 site) can be written as



where $-\text{S}_A^+$ represents the positively charged amino groups on MeYE, and M^- and M^{2-} respectively represent the Cr(VI) and As(V) anions in monovalent and bivalent form.

The metal-binding constants, β_{A1} and β_{A2} , are defined as

$$\beta_{A1} = [-S_A M] / [-S_A^+][M^-] \quad (5)$$

$$\beta_{A2} = [-S_A M^-] / [-S_A^+][M^{2-}] \quad (6)$$

The adsorbed amount of the monovalent anions, X_{A1} (mol g⁻¹), and the bivalent metal anions, X_{A2} (mol g⁻¹), to amino groups can be expressed as

$$X_{A1} = \frac{N_3 \beta_{A1} (1 - \alpha_A) [M^-]}{1 + \beta_{A1} (1 - \alpha_A) [M^-] + \beta_{A2} (1 - \alpha_A) [M^{2-}]} \quad (7)$$

$$X_{A2} = \frac{N_3 \beta_{A2} (1 - \alpha_A) [M^{2-}]}{1 + \beta_{A1} (1 - \alpha_A) [M^-] + \beta_{A2} (1 - \alpha_A) [M^{2-}]}, \quad (8)$$

where α_A represents the degree of dissociation of amino groups (type 3 site);

$$\alpha_A = K_3 / (K_3 + [H^+]). \quad (9)$$

In this study, we assumed that there is another positively charged

adsorption site, histidine imidazol groups, on/in MeYE. In the same manner as above, the adsorbed amount of the monovalent anions, X_{I1} (mol g⁻¹), and the bivalent metal anions, X_{I2} (mol g⁻¹), to imidazol groups can be expressed as

$$X_{I1} = \frac{fN_2\beta_{I1}(1-\alpha_I)[M^-]}{1 + \beta_{I1}(1-\alpha_I)[M^-] + \beta_{I2}(1-\alpha_I)[M^{2-}]} \quad (10)$$

$$X_{I2} = \frac{fN_2\beta_{I2}(1-\alpha_I)[M^{2-}]}{1 + \beta_{I1}(1-\alpha_I)[M^-] + \beta_{I2}(1-\alpha_I)[M^{2-}]}, \quad (11)$$

$$\alpha_I = K_2 / (K_2 + [H^+]). \quad (12)$$

where f represents the fraction of histidine imidazol groups in total type 2 site. The subscript I represents the parameters for histidine imidazol groups. Thus the amount of adsorbed Cr(VI) and As(V) anions onto 1 dry g of MeYE, X_M , can be obtained by the following equation.

$$X_M = X_{A1} + X_{A2} + X_{I1} + X_{I2}. \quad (13)$$

A nonlinear least-squares method was applied to find the five constants, f , β_{A1} , β_{A2} , β_{I1} , and β_{I2} . In this calculation, the same f value was used for both Cr(VI)-MeYE and As(V)-MeYE systems. The equilibrium concentrations of monovalent and bivalent anions were calculated using the stability constants in Table 2. The constants that gave the best fit with the experimental data (Fig. 3) are listed in Table 3. The solid lines in Fig. 3 represent the theoretical curve calculated from Eqs. (7) - (13). The experimental data in Fig. 2 were also compared with the predicted values and the results were shown in Fig. 5. The experimental data agreed well with the predicted results (solid lines).

To confirm the contribution of imidazol groups in the adsorption of Cr(VI) and As(V), a reduced model, setting $f = 0$, was applied to the experimental data in Figs. 3a and b. The values predicted by the reduced model were shown by the dotted lines in the figures. There was significant disagreement between the predicted values and the experimental data. This result suggests that the imidazol groups take part in the adsorption of Cr(VI) and As(V). The best fit value for f is 0.247 and the result suggests

that the imidazol groups accounts for about 25% (0.09 mmol g^{-1}) of the type 2 site. The binding constant of the amino groups for the monovalent As(V) anion was considerably lower than that for the monovalent Cr(VI) anion. Furthermore, the binding constant between the amino groups and the bivalent As(V) anion was negligible small or zero. The results suggest that the interactions between the imidazol groups and the As(V) anions are relatively strong however the interactions between the amino groups and As(V) anions are very weak. Since the number of amino groups is about 5-fold larger than that of imidazol groups, the adsorbed amount of As(V) is much lower than that of Cr(VI).

5. Conclusions

Yeast biomass was methylated in a 0.1M HCl-methyl alcohol solution at room temperature and applied to the adsorptive separation of Cr(VI) and As(V). More than 90 % of carboxylic groups could be methylated by the methylation for 24 hours. Both Cr(VI) and As(V) were scarcely adsorbed to unmethylated yeast. The adsorbed amount of Cr(VI) and As(V) markedly increased with increasing methylation degree. The adsorbed amount of Cr(VI) onto the methylated yeast (MeYE) decreased above pH 6, while the adsorbed amount of As(V) had a peak at about pH 7. The adsorbed amount of As(V) was rather lower than that of Cr(VI).

An adsorption model based on the binding reactions between two types of adsorption sites (amino and imidazol groups) and two types of metal anions (monovalent and bivalent anions) was applied to determine the biosorption characteristics of Cr(VI) and As(V) onto MeYE. The result showed that the interactions between the imidazol groups and the metal anions were relatively strong however the interactions between the amino groups and As(V) anions were very weak.

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Figure Captions

Fig. 1. Proton adsorption isotherms of yeast (solid circles) and yeast methylated for 24 h (open circles). Ionic strength was adjusted to 0.01 mol dm⁻³ by NaNO₃. The solid lines represent the theoretical curves calculated from Eqs. (1) and (2).

Fig. 2. Adsorption isotherms of (a) Cr(VI) and (b) As(V) onto methylated yeast having methylation degrees of 0.94 (circles) and 0.64 (triangles), and yeast (squares) at 30°C and at pH 6.5. Concentrations of MeYE/YE were (a) 0.7 - 5.0 and (b) 4.5 - 6.4 g dm⁻³. Initial concentrations of Cr(VI) and As(V) were 0.5 - 2.0 and 0.5 - 2.5 mM, respectively. Ionic strength was adjusted to 0.01 mol dm⁻³ by NaNO₃.

Fig. 3. pH dependence of (a) Cr(VI) and (b) As(V) adsorption onto methylated yeast having methylation degree of 0.94 at 30°C. Initial concentrations of metal ions were 1.0×10⁻³ (open symbols) and 0.5×10⁻³ mol dm⁻³ (solid symbols). Concentrations of methylated yeast were (a) 1.0 and (b) 4.9 g dm⁻³. Ionic strength was adjusted to 0.01 mol dm⁻³ by NaNO₃.

The solid lines represent the theoretical curves calculated from Eqs. (7) - (13).

Fig. 4. Calculated mole fractions of (a) Cr(VI) and (b) As(V) anion species as a function of pH at $1.0 \times 10^{-3} \text{ mol dm}^{-3}$.

Fig. 5. Comparison between the experimental data in Fig. 2 and the predicted values. The solid lines represent the theoretical curves calculated from Eqs. (7) - (13).









