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Cellular proteins of *Microcystis aeruginosa* inhibiting coagulation with polyaluminum chloride

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ABSTRACT

Cyanobacterial growth in semi-closed water areas such as reservoirs brings about a coagulation inhibition in a drinking water treatment system, but the inhibitory substances and mechanisms involved have yet to be elucidated. In this study, proteins having a high affinity with polyaluminum chloride (PACl) were isolated from organic substances produced by *Microcystis aeruginosa* with the affinity chromatography technique. Both extracellular organic matter (EOM) and cellular organic matter (COM) disturbed the flocculation of suspended kaolin with PACl, but it was likely that nonproteinous substances in EOM cause the reduction of coagulation effciency. In contrast, proteins in COM were obtained as possible inhibitory substances for the coagulation with PACl. These proteins could consume PACl in the coagulation process due to the formation of chelate complexes between these inhibitory proteins and the coagulant. The consumption of PACl by cyanobacterial proteins could be one of the important causes of the increase in coagulant demand.

1. Introduction

Semi-closed water areas supply about 45% of the total amount of water intake in Japan (Japan Ministry of Health, Labour and Welfare, 2005). Eutrophication in semi-closed water areas causes seasonal growth of cyanobacteria such as *Microcystis aeruginosa* (*M. aeruginosa*), which interferes with drinking water treatment processes (Chow et al., 1999; Alam et al., 2001). In particular, excess growth of cyanobacteria and needle-like diatoms affects the coagulation efficiency in flocculation and sedimentation processes (Zhang et al., 2006; Jun et al., 2001). A dose increase of coagulant is a tentative way to improve the coagulation efficiency, but this creates subsidiary problems, including an increased cost for the coagulant and sludge treatment.

Some researchers have reported that algogenic organic matter (AOM) is involved in the reduction of coagulation efficiency (Cheng and Chi, 2003), and one of the inhibitory mechanisms is that AOM can form complexes with cations in coagulant, which deteriorates the coagulation ability of the coagulant (Bernhardt et al., 1991). It has also been reported that the formation of polynuclear mixed ligand complexes between AOM and metals in the coagulant gives rise to an increase in the dissolved and colloidal coagulants that cannot be effective in the coagulation process (Bernhardt et al., 1987). However, AOM that can chelate metals in the coagulant and contribute to the reduction of coagulation efficiency have yet to be identified. The identification of constituents of AOM forming complexes with coagulant could lead to the elucidation of the inhibition mechanism, which makes it possible to establish alternatives to overcome the interferences of the coagulation process caused by the excess growth of cyanobacteria.

In this study, AOM that has an affinity with the coagulant were isolated as a plausible inhibitory matter of the coagulation process. In particular, we paid attention to cyanobacterial proteins as the possible inhibitory organic substances,

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because it was suggested that proteins produced by *M. aeruginosa* induced the reduction of coagulation efficiency in our previous work (Takaara et al., 2005). It is known that cyanobacteria produce diverse proteins as main components of cells, and some proteins such as metalloenzymes are known for having a high affinity with metal ions (Schwartz et al., 2002; Tamagnini et al., 2002). Proteins in cyanobacterial cells could be released when the cells are destroyed by lysis or prechlorination in the drinking water treatment plant, and the released cyanobacterial proteins could interfere with the coagulation in drinking water treatment processes.

The purposes of this study are to isolate and characterize cyanobacterial proteins that have a high affinity with polyaluminum chloride (PACl), a coagulant used in many countries. The coagulation of the kaolin suspension using PACl was performed, and the contribution of AOM to the reduction of coagulation efficiency was evaluated by the difference in optical absorbance of the supernatants after the sedimentation of coagulated kaolin in the presence or absence of AOM from *M. aeruginosa*. Organic components in the supernatant after the precipitation of coagulated kaolin were analyzed by ultraviolet (UV) spectrophotometry and three dimensional-excitation emission matrix (3D-EEM) spectroscopy. The residual AOM in the supernatant that has a high affinity with the coagulant was isolated by the affinity chromatography using PACl as a ligand. The molecular weights of isolated proteins were analyzed by SDS-PAGE, and the contribution of the isolated cyanobacterial proteins to the reduction of coagulation efficiency is discussed.

2. Materials and methods

2.1. Cultivation of *M. aeruginosa*

M. aeruginosa strain (NIES-91) was provided by the National Institute for Environmental Studies, Japan. *M. aeruginosa* was cultured in a 500 mL conical flask containing 250 mL MA medium (pH 8.6) (Ichimura, 1979) under illumination of a fluorescent lamp (50 $\mu mol\, m^{-2}\, s^{-1}$) with a cycle of 12 h light and 12 h dark at 25 °C with shaking at 50 rpm. The growth of *M. aeruginosa* was monitored by the amount of chlorophyll-*a* in the culture. Cyanobacterial cells in a steady-state growth were harvested and used in further experiments.

2.2. Preparation of extracellular organic matter (EOM) and cellular organic matter (COM) of *M. aeruginosa*

M. aeruginosa in a steady-state growth was filtered by a membrane filter (HEP09000, MILLIPORE, USA). The filtrate was stored as EOMs at −80 °C until further analysis. Cyanobacterial cells were collected by centrifugation at 1600*g* for 10 min. The pellet was resuspended in MilliQ water and centrifuged at 1600g for 10 min. The collected cells were stored at −80 °C in order to facilitate the destruction of cyanobacterial cells. Frozen sample including cells were thawed at room temperature and suspended in 20 mL of MilliQ water. Suspended cells were sonicated for 10 min in order to destroy cyanobacterial cells. The sonicated sample was stored as COM, which is composed of intracellular organic matter (IOM) and surface-retained organic matter (SOM).

2.3. Coagulation of suspended kaolin using PACl in the presence of AOM

Coagulation of suspended kaolin using PACl in the presence of AOM from *M. aeruginosa* (coagulation test) was performed in order to investigate the contribution of AOM to the reduction of coagulation efficiency. Contributions of AOM to the reduction of coagulation efficiency were assessed by the difference in optical absorbances of the supernatants after the sedimentation of coagulated kaolin in the presence or absence of AOM. Samples used in the coagulation test are indicated in Table 1. The subscript of each sample code in Table 1 indicates the volume of AOM samples used in the coagulation test. Concentrations of dissolved organic carbon in EOM_{150}, EOM_{300}, COM_1 and COM_3 were 112.5, 214.9, 7.7 and 20.6 mg carbon L^{-1}, respectively. Kaolin (23,000-02, Kanto, Tokyo, Japan) was added to each sample at a final concentration of 20 mgL^{-1}, and pH value of each sample was adjusted to 7.0 with 1 N HCl or 1 N NaOH. Two hundred milliliters of each sample were put into a 75 cm^2 cell culture flask (CORNING, USA) and agitated with a shaking apparatus at 80 rpm for 1 min. Then, PACl was added to each sample at a concentration of 10 mg Al L^{-1}, and the sample was mixed at 80 rpm for 20 min and at 60 rpm for 15 min. Samples were left for 20 min, and then, each sample's supernatant (100 mL) was collected by using a U-shaped pipette in order to avoid the suction of precipitated solids. Absorbances at 660 nm (A_{660}) and 260 nm (A_{260}) of the collected supernatants were measured by UV1600 (SHIMADZU, Kyoto, Japan). The composition of AOM in the supernatant was analyzed with 3D-EEM by F-2500 spectrofluorometer (HITACHI, Tokyo, Japan). The 3D-EEM spectra were constructed by scanning emission spectra as a function of excitation wavelength. An excitation wavelengths from 220 to 500 nm in 10 nm steps were used, and emission wavelengths from 220 to 800 nm in 1 nm steps were measured. MilliQ water was used as a blank sample. Dissolved organic carbon (DOC) of the supernatant was measured with TOC-5000 (SHIMADZU, Kyoto, Japan).

Table 1 – Composition of samples used in the coagulation test with polyaluminum chloride

Sample code[a]	Volume (mL)		Concentration of dissolved organic carbon (mg carbon L^{-1})
	AOMs from *M. aeruginosa*	Autoclaved tap water	
EOM_{150}	150	150	112.5
EOM_{300}	300	0	214.9
COM_1	1	299	7.7
COM_3	3	297	20.6

[a] Subscript of each sample code indicates volume of AOM sample used in the coagulation test.