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Article

Mercury Adsorption of Chemically Modified Polysaccharide from *Methylobacterium organophilum*

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Methylan, a polysaccharide produced from *Methylobacterium organophilum*, was chemically modified by adding diethylaminoethyl (DEAE) group to the backbone of methylan. The structure of DEAE-methylan was determined by measuring its nitrogen content obtained from an elemental analysis. From the analysis of mass spectrum, the DEAE group in DEAE-methylan was also confirmed by determining diethylaminoethene as a separate form of DEAE. Mercury adsorption of DEAE-methylan was higher than that of native methylan. This fact was valid for a variety of pH, reaction times, metal concentrations, and polysaccharide concentrations. In particular, native methylan and DEAE-methylan adsorbed 16% (w/w) and 18% (w/w) for mercury after 30 min at pH 7, respectively. The increase in mercury adsorption of DEAE-methylan may be resulted from mercury adsorption by the lone pair electron of nitrogen atom in DEAE group.

Key words: Methylobacterium organophilum, *chemically modified polysaccharide, methylan, mercury adsorption.*

In view of new and increasingly sophisticated performance requirements for polysaccharide-based materials and the limitations of conventional modification procedures, considerable research efforts have been directed in recent years at the development of new methodology for the chemical derivation of industrial polysaccharides. Novel classes of polysaccharide products have been introduced, such as amino derivatives, hydrophobically-modified ether and ether derivatives, acyclic products, oragnomercurylic conjugates, and various branched derivatives. In addition, a growing trend towards the application of selective modification techniques has emerged.[1,2)]

Chemical modification of polysaccharides have been carried out in order to give polysaccharides good physical and chemical properties for industrial applications. In particular, chemically modified polysaccharides as amino derivatives have some advantages such as heat stability, mechanical stability, viscosity, gelling ability, and various chemical reactions due to amino group. Amino derivatives of polysaccharides can apply to anion exchange resin, enzyme immobilization, chelating agent for heavy mercury, hypocholesteolemic agent, and artificial kidney membrane.[3)]

Microbial polysaccharides assist in the removal of metal ions from aqueous solutions. Interaction between metal ions and polysaccharides can occur through the adsorption of metal ions to polysaccharides and the absorbed metal ions are removed as metal complexes with polysaccharides.[4)] Polysaccharides especially can be recommended as good adsorbing agents for heavy metals because, for examples, microbial polysaccharides in activated sludge have an extensive adsorbing capacity for heavy metals[5)] and acidic polysaccharides can function as polyelectrolytes.[6)]

It was found in our laboratory that *Methylobacterium organophilum* could produce a new acidic polysaccharide, methylan, from methanol under specific culture conditions,[7)] methylan production could be increased by optimizing the culture conditions,[8)] controlling the concentration of ammonium ion,[9,10)] and using a novel bioreactor,[11)] and methylan non-specifically adsorbed mercury ions.[12)] In this study, chemical modification of polysaccharide was performed to enhance the adsorbing capacity for mercury ions and the mercury adsorption of chemical modified polysaccharide was investigated.

Materials and Methods

Microorganism and culture media. A facultative meth-

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Abbreviations: DEAE, diethylaminoethyl; DO, dissolved oxygen.

ylotropic bacterium, *Methylobacterium organophilum* NCIB 11278 KC-1 was used in this study. The microorganism was maintained on frozen state at -20°C. The growth medium consisted of 1.0% (v/v) methanol, 1.0 g/*l* $(NH_4)_2SO_4$, 1.31 g/*l* KH_2PO_4, 2.13 g/*l* Na_2HPO_4, 0.45 g/*l* $MgSO_4 \cdot 7H_2O$, and 0.2% (v/v) metal solution which was contained 3.3 mg/*l* $CaCl_2 \cdot 2H_2O$, 1.3 mg/*l* $FeSO_4 \cdot 7H_2O$, 130 mg/*l* $MnSO_4 \cdot 4H_2O$, 130 mg/*l* $ZnSO_4 \cdot 4H_2O$, 40 mg/*l* $CuSO_4$ $4H_2O$, 40 mg/*l* $Na_2MoO_4 \cdot 2H_2O$, 40 mg/*l* $CoCl_2 \cdot 6H_2O$, and 30 mg/*l* H_3BO_3. The fermentation medium was composed of methanol, 0.3 g/*l* $(NH_4)_2SO_4$, 0.63 g/*l* KH_2PO_4, 1.06 g/*l* Na_2- HPO_4, 0.45 g/*l* $MgSO_4 \cdot 7H_2O$, and 0.2% (v/v) metal solution. Methanol was initially added at the concentration of 1.0 % (v/v) and then intermittently fed at 0.5% (v/v) by DO-stat technique.[13]

Culture conditions. A single colony on agar plate was inoculated into a 250-m*l* Erlenmeyer flask containing 50 ml growth medium and cultivated in a rotary shaker (New Braunswick Scientic, U.S.A.) at 30°C, 200 rpm, for 24 h. The culture broth was transferred to the new growth medium of 50 m*l* and cultivated for 20 h under the same conditions. This seed culture was used as the inoculum of fermentor. A 5 *l* fermentor was used in this study. The working volume of the fermentor was 3 *l* and the temperature and pH of the culture with 3.0 N NaOH were controlled at 30°C and 7.0, respectively, during the fermentation. The DO level was controlled above 20% of the air saturation by adjusting aeration volume and agitation speed.

Purification of polysaccharide. In order to remove the bacterial cells, the culture broth was diluted to the concentration below 1.0 g/*l* polysaccharide. The bacterial cells were completely removed from the culture broth by centrifugation (10,000×g for 2 h) and membrane filtration (0.45 μm, Millipore). The cell free polysaccharide was precipitated by adding two volume of ethanol. The polysaccharide precipitated was washed twice with ethanol and dissolved in distilled water. The polysaccharide solution was dialyzed with distilled water for desalting. The partial purified polysaccharide was obtained by freeze-drying the desalted polysaccharide solution.

Synthesis and hydrolysis of DEAE-methylan. Fifteen m*l* of 3M 2-(diethylamino)ethylchroride and 15 m*l* of 3M NaOH were added to 300 mg methylan with stirring at 60°C for 18 h. Reaction mixture was washed sequentially with 0.1 M HCl and 0.001 M HCl solutions and was ultrafiltrated (Molecular Weight 100,000) with methanol, diethylether, and water. The ultrafiltrated product was freeze-dried to obtain DEAE-methylan. DEAE-methylan was hydrolysed to DEAE and methylan after 2 hours treatment with 2.0 N HCl.

Determination of methylan and DEAE-methylan. Elemental analysis was conducted with 1.0 g purified methylan or 1.0 g purified DEAE-methylan using a Perkin-Elmer 240-C elemental analyzer. The structure of DEAE-methylan was determined by a mass spectrum (JMS-SX102A spectrometer). The substitution degree of DEAE in DEAE-methylan was determined by carbon and nitrogen analyses.

Mercury adsorption. The purified polysaccharide or DEAE-methylan solution was added to the mercury solution at 25°C. In pH experiment, the pH of the mixture was adjusted with 10 N acetic acid or 2.0 N NaOH and in the others experiments, the pH was adjusted to 7.0 with 10 N acetic acid. Mixing was achieved with the magnetic stirrer. The stirring rate was 200 rpm. In time course experiment, the stirring time was varied from 10 minutes to 50 minutes at 10 minutes intervals and the other experiments, the mixture was equilibrated for 30 min. After stirring, two volumes of ethanol were added to precipitate the polysaccharide solution. The polysaccharide precipitated were filtered through Whatman No. 1 of filter paper. Amount of mercury adsorbed to the polysaccharide was determined by measuring the residual mercury concentration in the filtrate. The analyses were performed by an atomic adsorption spectrometer (Perkin Elmer and PYE UNICAM SPS).

Results and Discussion

Structure confirmation of methylan and DEAE-methylan. Native methylan consists of carbohydrate (glucose, galactose, mannose), protein, and negative charged groups such as uronic, pyruvic, and acetic acids.[7] The chemically modified methylan to which DEAE group was attached showed the substitution degree of 0.70 by measuring its nitrogen content obtained from an elemental analysis. According to Katsura *et al.*,[14] it was suggested that DEAE was attached mainly to sixth carbon of hexose in polysaccharide after the amination reaction. In order to investigate the structural difference between the native methylan and DEAE-methylan, elemental analyses were performed. As shown in Table 1, the nitrogen content in DEAE-methylan was found to be 20 times higher than that in methylan due to the nitrogen content of the DEAE group. The structure of DEAE-methylan was also confirmed by determining diethylaminoethane as a separated form of DEAE from the analysis of mass spectrum after acid hydrolysis of DEAE-methylan, compared with Wiley's Library (Hewlett Packard, USA) (Fig. 1).

Mercury adsorptions with pH and time. For metal adsorption experiment, mercury ion was used. Adsorptions of the mercury ion to methylan and DEAE-methylan were carried out for 30 min at various pH (Fig. 2).

Table 1. Elemental analyses of methylan and DEAE-methylan.

Element (w/w)	C (%)	O (%)	N (%)	H (%)	S (%)
Methylan	33.58	60.00	0.305	6.040	0.070
DEAE-methylan	37.14	49.69	5.484	7.632	0.054

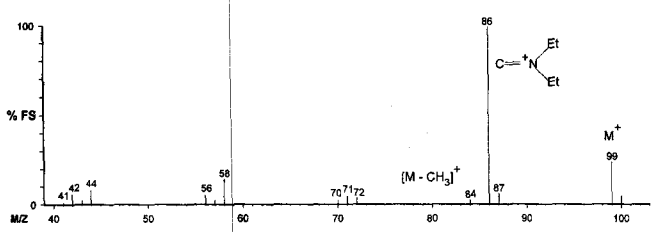

Fig. 1. Mass spectrum [EI, 70eV] of diethylaminoethane.

The mercury adsorption patterns for methylan and DEAE-methylan were similar, but yielding higher adsorption for the chemically modified one. Mercury adsorption was ineffective below pH 5 but increased as pH was increased ranging from 5 to 7. Since, above pH 7, mercury adsorption was constant, the pH for optimum condition of mercury adsorption was chosen as 7. The increase in mercury adsorption at high pH (above pH 7) may be due to the increase of the interaction between mercury cations and negative charges of acidic residues of the polysaccharide such as uronic, pyruvic, and acetic acids which were enhanced at high pH. Mercury adsorption of DEAE-methylan was found to be higher than that of native methylan at the same pH. This may be resulted from mercury adsorption by the lone pair electron of nitrogen atom in DEAE group which can donate electron to mercury.

Under the conditions of pH 7 and 25°C, the time courses of mercury adsorption to methylan and DEAE-methylan were performed (Fig. 3). Mercury adsorption reached equilibrium within 30 min and prolonged exposure did not increase the adsorption of mercury. From this result, the reaction times of mercury adsorptions to methylan and DEAE-methylan were determined at 30 min.

It was previously reported in our labaratory that native

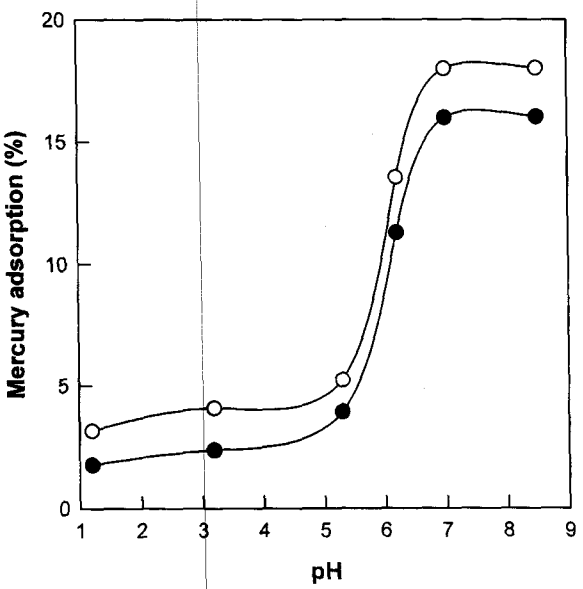

Fig. 2. Mercury adsorption as a function of pH. The concentration of polysaccharides was 0.52 g/*l*. Mercury concentration was in excess. Methylan (●—●) and DEAE-methylan (○—○).

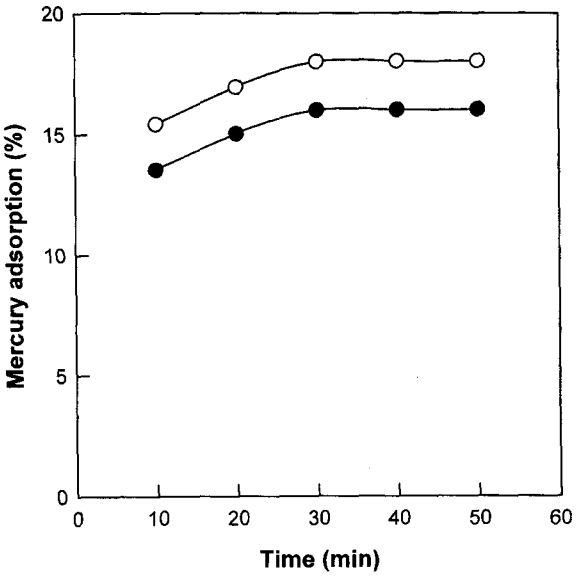

Fig. 3. Time courses of mercury adsorption to methylan and to DEAE-methylan. The concentration of polysaccharides was 0.52 g/*l*. Mercury concentration was in excess. Methylan (●—●) and DEAE-methylan (○—○).

methylan non-specifically absorbed 21% (w/w) for copper and 18% for lead of the polysaccharide used after 30 minutes of reaction time at pH 7.[7] Other polysaccharides such as chitin and chitosan can also absorb metal ions and the amounts of cupper adsorbed to biomass, *Zoogloea ramigera* was 17% (w/w).[4,5]

Mercury adsorptions with mercury and methylan concentrations. The effect of mercury concentration on mercury adsorption to the polysaccharides was studied at the same polysaccharides concentration with different mercury concentrations (Fig. 4). Mercury adsorptions to methy-

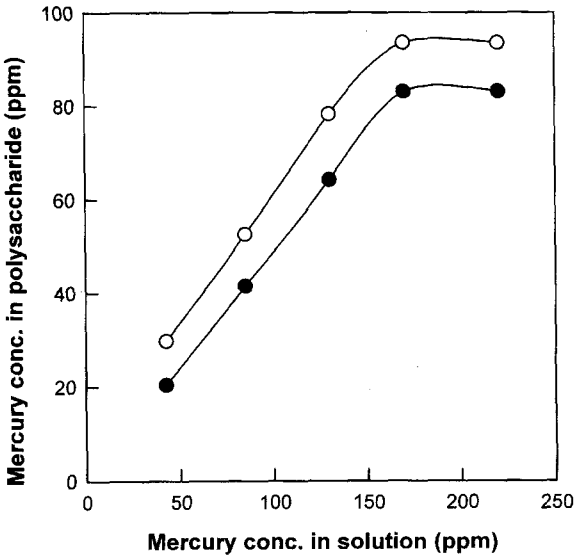

Fig. 4. Effect of mercury concentration on mercury adsorptions to methylan and to DEAE-methylan. The concentration of polysaccharides was 0.52 g/*l*. Mercury concentration was in excess. Methylan (●—●) and DEAE-methylan (○—○).

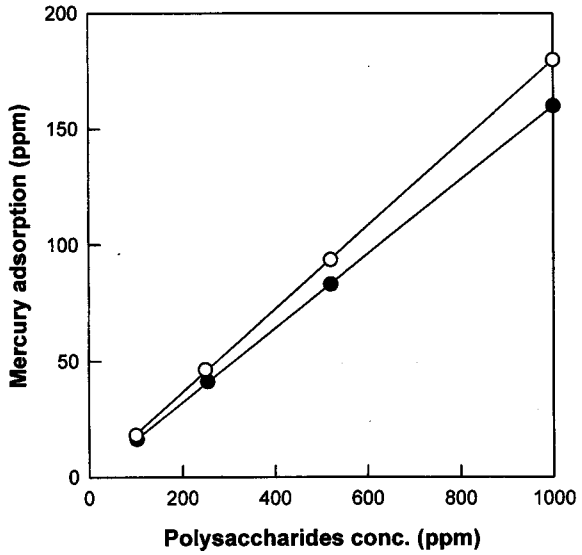

Fig. 5. Effect of polysaccharide concentration on mercury adsorptions to methylan and to DEAE-methylan. Mercury concentration was in excess. Methylan (●—●) and DEAE-methylan (○—○).

lan and to DEAE-methylan increased as the mercury concentration in solution was increased within the concentrations of mercury saturation to methylan and DEAE-methylan. The concentrations of mercury saturations to methylan and DEAE-methylan were 83 ppm and 94 ppm for mercury which corresponded to 16% (w/w) and 18% (w/w), respectively. However, above the concentrations of mercury saturations, mercury adsorptions did not increase.

The effect of the concentration of polysaccharides on mercury adsorption was also investigated at the same mercury concentration by varying the concentrations of polysaccharides (Fig. 5). Mercury adsorption proportionally increased as the concentrations of methylan and DEAE-methylan were increased. The amounts of mercury adsorbed to methylan and DEAE-methylan were 16% (w/w) or 18% (w/w), respectively.

In conclusion, the adsorbing capacity for mercury ion can increase with using the chemically defined polysaccharide.

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