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BINDING OF HEAVY METALS BY ALGAL POLYSACCHARIDES

D. Kaplan

The Jacob Blaustein Desert Research Institute
Ben-Gurion University of the Negev, Sede Boqer Campus, 84993 Israel

D. Christiaen* and S. (Malis) Arad

The Institutes for Applied Research
Ben-Gurion University of the Negev, Beer-Sheva,84110,Israel

ABSTRACT

The capacity of dissolved microalgal polysaccharides to bind copper, lead, cadmium and zinc was studied by anodic stripping voltammetry (ASV). Two marine species of the unicellular alga Chlorella were grown in artificial seawater (ASW), lacking EDTA, under controlled conditions. Cultures were harvested after reaching the stationary phase of growth. The sugar content of the growth medium was analysed. Preliminary characterization of the dissolved polysaccharides and their metal- complexing capacities are presented. The relationship between the chemical structure of the polysaccharides and their metal-complexing properties is discussed.

INTRODUCTION

Some industrial processes result in the release of heavy metals such as cadmium, copper, lead, mercury, and nickel, into natural water systems. This has lead to increasing concern about the effects of toxic heavy metals as environmental contaminants. In the aquatic environment metals are generally found in sediments, as suspended particulates (>0.45um) in association with organic and inorganic colloids and in solution. Metals in solution may be present as free species, as inorganic complexes or as organic complexes (1). Since the initial report by Fogg and Westlake (2) extensive information on the release of metal-complexing organic materials by fresh-water and marine algae has been published (3,4,5,6). Among the organic compounds that has been identified are humic and fulvic acids (7,8) and polysaccharides (9). It has been suggested that polysaccharides exert their complexing action via

*Permanent address: Equipe Polysaccharides Parietaux des Vegetaux Universite de Lille, Flandres Artois, 59655, Villeneuve d'Ascq Cedex, France

interaction between their negative charges and metal cations (10). Association of metals with organic materials can affect the chemical state and availability of the metals in natural waters and hence their toxicity to biota (11,12,13). Therefore, information on the total concentration of a particular metal in a water sample, as measured by atomic absorption spectrophotometry for instance, can be misleading. Thus, analytical methods that facilitate measurement of the free cations and determination of metal complexation are required. An electrochemical method such as anodic stripping voltammetry (ASV), has the advantages of being extremely sensitive, allowing multi-element analysis, and requiring minimal sample manipulation (14). Essentially ASV is a two step electroanalytical technique. In the first step: plating or preconcentration step, the metals are deposited from solution into a small-volume mercury electrode under constant potential. In the second step, the metals are electrooxidized from the mercury. The method relies on the assumption, which is supported by numerous studies (15, 16), that the ASV response is related only to labile ionic species. The metal complexing capacity can thus be determined by plotting the ASV peak current (Ip) versus the concentration of the added metal.

In the present work, the ability of dissolved polysaccharides obtained from two different marine Chlorella species (C. stigmatophora and C. salina) to bind zinc, cadmium, lead and copper, was studied, as well as their preliminary chemical composition.

MATERIALS AND METHODS

Chlorella stigmatophora LB 993 and Chlorella salina 211-25 were grown in batch culture using artificial seawater (ASW) prepa red in 1-liter columns, 6 cm in diameter, at 24^{o}C and maintained under continuous illumination with fluorescent cool-white light at 150 uE $m^{-2}s^{-1}$. The medium was aerated with sterile air containing 2-3% CO_2.

Growth was followed by counting cells with a hemocytometer. Cultures at the stationary phase of growth were harvested by centrifugation (10,000g for 15 min). The supernatant was collected, dialyzed and used for analyses.

Concentration of total sugars was determined by the phenol-sulfuric acid method (18). Uronic acids content was estimated by the meta-hydroxydiphenyl method (19). Sulfate content was determined after hydrolysis of the polysaccharide sample in 2 M HCl for 2 h at 100^{0}C by means of the rodisonate method (20). Anion-exchange chromatography was performed on a DE-52 (Whatman) column. Elution was performed by successively passing through the column: doubly distilled water (Fraction 1), then 0.5 M NaCl (Fraction 2), 5 M NaCl (Fraction 3), 6 M urea (Fraction 4), and 0.5 M HCl (Fraction 5). The elution was monitored by the phenol-sulfuric acid method (18). Contents of carbohydrate, sulfate and uronic acids, as well as metal-binding capacity, were determined for each fraction.

Metal ion concentration and metal-complexing capacity were measured by ASV in the differential pulse mode (DPASV) as previously described (21,22). The metal-complexing capacity of the tested solutions was determined by addition of known amounts of the different cations and monitoring changes in the ASV calibration curve. The total amount of cation required to reach the break point represents the complexing capacity of the original solution (14). All chemicals used were of analytical grade; appropriate blanks were run for all of them, and only corrected values are presented.

RESULTS

When grown in batch culture under the conditions mentioned above, the two Chlorella species reached maximal cell concentrations of 150 10^6 cell ml^{-1} for C. stigmatophora and 60 10^6 cell ml^{-1} for C. salina. The concentrations of total dissolved polysaccharides detected in the growth medium after harvesting cultures at the stationary phase of growth were similar (60 and 65 mg l^{-1}glucose eqv., respectively).

The metal-complexing capacity of the dissolved polysaccharides produced by the two alga was measured using DPASV. ASV peak heights vs. concentrations of Zn^{2+}, Cd^{2+}, Pb^{2+} and Cu^{2+}, added simultaneously to 30 ug l^{-1} glucose eqv. of dissolved polysaccharide from C. stigmatophora are presented in Fig. 1. The straight line obtained in response to Pb^{2+} addition indicates that the polysaccharide did not complex this metal. The break in the titration curves observed for Zn^{2+} and Cd^{2+} indicate that 30ug l^{-1} glucose eqv. of the C. stigmatophora polysaccharide exhibited complexing capacities of 200 and 150 ug l^{-1}, respectively. The highest complexing capacity was observed for Cu^{2+} (400 ug l^{-1}). The sigmoidal shape of the titration curve obtain for Cu^{+2} indicate the existance of at list two binding sites for this metal.

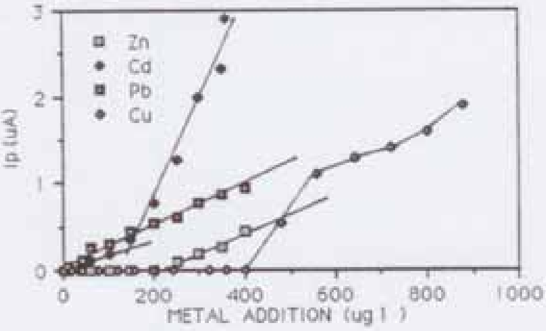

Figure 1. ASV titration curves of current peak heights vs. concentrations of Zn^{2+}, Cd^{2+},Pb^{2+} and Cu^{2+}, added to dissolved polysaccharide of C. stigmatophora

ASV titration curves for simultaneous addition of Zn^{2+}, Cd^{2+} and Pb^{2+} to 30 ug l^{-1} of dissolved polysaccharide from C. salina are presented in Fig. 2.

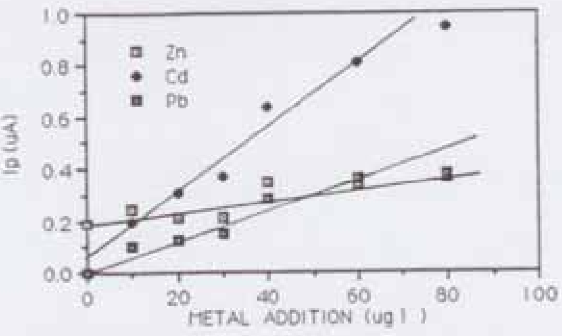

Figure 2. ASV titration curves of current peak height vs. concentrations of Zn^{2+}, Cd^{2+} and Pb^{2+}, added to C. salina dissolved polysaccharide.

The straight lines observed for the three metals indicate that the C. salina polysaccharide do not exhibite metal-complexing capacity for these metals. The titration was continued up to 1000 ug l^{-1} of each metal, including Cu^{2+} (not shown), but no change in the pattern of the curves was observed.

Chemical analyses of the two polymers revealed some differences in their composition.

TABLE 1

Uronic acid and sulfate contents of the dissolved polysacchrides obtained from C. stigmatopora and C. salina.

	Uronic acid %	Sulfate %
C. stigmatophira	30	9
C. salina	6	8

The polysaccharide of C. stigmatophora contains about three times more uronic acids than that of C. salina while the sulfate content of both polymers is similar. Thus, a relationship between a high content of uronic acids i.e. high negative surface charge,
and metal-complexing capacity was observed.

Comparison between the elution patterns of the two polysaccharides through DE-52 anion-exchange columns indicates that the C. salina polysaccharide is mostly neutral or weakly charged (90% was eluted in the first two fractions), while that from C. stigmatophora is highly charged (only 12% was eluted with doubly distilled water and 0.5 M NaCl) (Fig. 3).

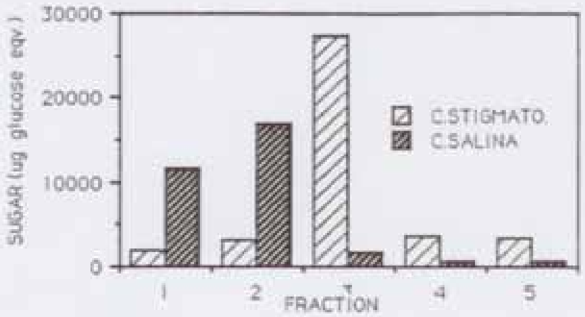

Figure 3. Elution profiles of the polysaccharides of C. stigmatophora, and C. salina on a DE-52 anion-exchange chromatography column. (1= doubly distilled water; 2= 0.5 M NaCl; 3= 5 M NaCl; 4= 6 M urea; 5= 0.5 M HCl).

The uronic acids and sulfate content of each fraction eluted from the anion-exchange column loaded with polysaccharide of C. stigmatophora were also analyzed (Fig. 4).

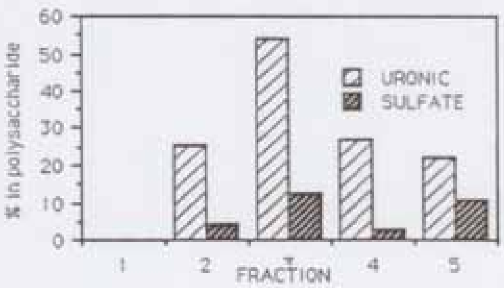

Figure 4. Uronic acids and sulfate contents of each fraction eluted from the anion-exchange column loaded with C. stigmatophora polysaccharide.

The highest contents of uronic acids and sulfate were measured in fraction 3, which was also the fraction where most of the polysaccharide of C. stigmatophora was obtained.

The metal-complexing capacity of the polysaccharide eluted from each fraction (chromatography of C. stigmatophora polysaccharide) was measured. The ASV peak heights vs. concentrations of Cu^{2+} added to each fraction is shown in Fig. 5.

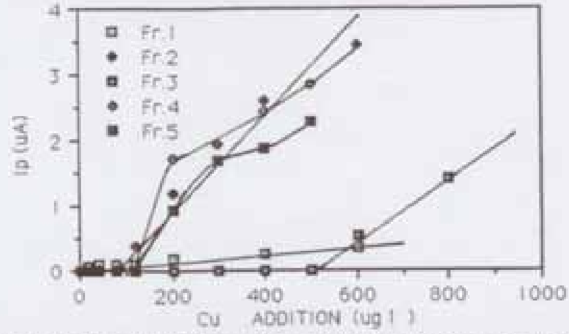

Figure 5. ASV titration curves of current peak heights vs. concentrations of Cu^{2+} added to each fraction eluted from the anion-exchange column, loaded with polysaccharide from C. stigmatophora. [The sugar content in each fraction in ug glucose eqv. $l^{-1}$was: (1) 74; (2) 76; (3) 652; (4) 105; (5)' 95].

No binding of Cu2+ was observed in the neutral fraction. All the charged fractions exhibited Cu2+-complexing capacity. However, the break point in fractions 2 and 3 was followed by a linear response while that in fractions 4 and 5 (containing very highly charged polymers) was followed by a sigmoidal curve.

DISCUSSION

The dissolved polysaccharides produced by the two Chlorella species differ in their chemical composition and metal-binding capacity. The native polysaccharide from C. stigmatophora, which was found to be highly charged, exhibited high metal-complexing capacity, whereas that of C. salina, which was found to be mostly neutral and only weakly charged, did not bind metals under the same experimental conditions. Moreover, the fraction from C. stigmatophora containing the higest amount of uronic acids was the fraction exhibiting the higest copper-complexing capacity. Since the polysaccharides from the two Chlorella species contain similar amounts of sulfate, it seems that metal-complexing capacity of the C. stigmatophora polysaccharide is mainly due to its content of uronic acids. Our results are thus in agreement with previous studies suggesting that metal-complexing capacity is a function of surface ionic charge, resulting from carboxylic and hydroxylic groups (23,24,25). It has been reported that extracellular polysaccharides, such as alginates of algal and bacterial origin and pectates from plants, which are negayivly charged and rich in uronic acids exhibit high metal-complexing capacity (10). Similarly, species of fresh the fresh water alga Mesotaenium have an extracellular polysaccharide, rich in uronic acids, which is capable of binding copper, and thus protecting the alga against toxic copper cations (9). Since metal complexing capacity also depends on the concentration of the polysaccharide, it is possible that the weakly charged fraction of the C. salina because of the low concentration.The differences in the shapes of the curves- sigmoidal for copper and linear for the other three metals tested -indicate that the interaction between each metal and the polysaccharide is specific. It seems that copper is bound by more than one polymer and/or by more than one site on the same polymer. This is also supported by the results showing the binding of copper ions by various fractions obtained by fractionation the C. stigmatophora polysaccharide. Polymers of four out of the five fractions collected bind copper ions, indicating that, indeed, polymers differing in their content of uronic acids and surface ionic charges are able to bind copper ions. Moreover, the sigmoidal shape of the curves obtained for fractions 4 and 5 indicates that these polymers contain more than one binding site for copper ions, while these of fractions 2 and 3 have one bindig site for this metal. Additional information concerning the distribution of the ionic charges along the polymers and their secondary and tertiary structure are required for better understanding of the mechanism(s) involved in metal binding by these polymers.

It should be noted that native polysaccharides were used for all ASV measurements. Thus, cations such as Ca^{2+}, Mg^{2+}, Na^{+} and others were probably attached to the polysaccharides when the complexing capacity for heavy metals was tested. This implies that the heavy metals tested were either interacting with free sites on the polysaccharide or competing with other cations on the available binding sites on the polymer and perhaps be replacing some of them.

The observation that some native algal polysaccharides exhibit metal-complexing capacity might be of ecological importance, i.e. polysaccharides that are capable of binding heavy metals under natural conditions may thus serve as natural chelates. The dissolved polysaccharides studied in this work constitute only a small portion of all the extracellular polysaccharides produced by algal cells. Most of the polysaccharide is attached to the cells. We still do not know if the chemical composition of the attached fraction is similar to that of the dissolved one. If so, whole algal cells could serve as heavy metals "scavangers". Thus, cultivation of algae in water bodies contaminated by heavy metals could be used as a technique for ameliorating metal toxicity of polluted waters.

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