

## Algal Polysaccharides as Natural Metal Chelators

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Some industrial processes result in the release of heavy metals such as cadmium, copper, lead, mercury, nickel and others, into natural water systems. This has led to increasing concern about the effects of toxic heavy metals as environmental contaminants. Metals in aquatic environments exist as free metal ions and in various combined chemical forms (16, 18). Biological availability of a trace metal as either a required nutrient, e.g. iron, or a toxicant, e.g. copper, is dependent on its chemical form (4). Since the initial report by Fogg and Westlake in 1955 (17), extensive information on the release of metal complexing organic material by algae in fresh water and marine environments has been published (15, 24, 32, 41, 44). It was demonstrated in many studies that the free metal ions are the toxic chemical species (2, 20, 21, 45). Association of metals with organic materials can affect the chemical state and availability of the metals in natural waters and their toxicity to biota (9, 10, 34). The stronger the metal complex the lower the toxicity of a given concentration of total metal (3). Therefore, knowledge of the total metal concentration alone is not always sufficient. To understand the distribution and ecological implications of trace metals, the physical form and chemical speciation of the metal must be elucidated.

This requires an analytical technique which cannot only detect trace metals at the natural concentration levels but also differentiate the various chemical species of a given trace metal. Only potentiometry with specific-ion electrodes and anodic stripping voltammetry (ASV) can presently differentiate, on a routine basis, the chemical forms of a trace metal in natural waters. Potentiometric studies of trace metal complexation in natural waters are limited primarily due to the lack of sensitivity of specific-ion electrodes. ASV has been employed in a number of studies of trace metal complexation (1, 5, 11). For measurement of bioavailable forms in natural samples, ASV seems to offer the best possibility because it is fast and has the required sensitivity (46). 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, supported by numerous studies (11, 47), 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 ( $I_p$ ) versus the concentration of the added metal.

Algae respond to toxic metals in various ways depending on the algal species and the specific metal. Laube *et al.* (29) studied the binding of  $Cd^{2+}$  and  $Cu^{2+}$  by the algae *Anabaena* 7210 and *Ankistrodesmus braunii*, during growth. They found that the interaction of *Anabaena* with  $Cu^{2+}$  follows a different pattern than that of *Ankistrodesmus*, and that the two species seem to have different modes of dealing with  $Cd^{2+}$  and  $Cu^{2+}$ . In some cases, algal growth rate is reduced and in others the lag phase is extended followed by regular growth rates (6, 42, 43).

Some algal species are more tolerant to toxic metals than others. However, the relation between metal sorption to algal cells and toxicity is still not clear. In order to affect growth or physiological activities, the toxic metals have to enter the cell and reach the sensitive site. Therefore, any mechanism which will prevent the interaction of toxic metals with their target sites will reduce their

toxicity. This can be achieved by complexing the metal to extracellular organic matter produced by the algal cells, or by sorption of the metal to the algal cell wall. If uptake of toxic metal occurs, it can be detoxified by a mechanism of internal sequestration resulting in the formation of metal complexes which are either accumulated in the cell or excluded from the cell (9, 19, 39). Among the organic compounds interacting with cations; amino-acids, polypeptides, organic-acids (8) and polysaccharides (30, 31), were identified. A common feature of all of these organics is their negative surface charge. It was suggested that the negative charges are the sites for binding of the positive charged metals (12, 31). The relative large surface area of microalgal and bacterial cells and the chemical structure of their cell walls make them potentially good "scavengers" of free metal ions. Some algal species, which are capable of releasing of metal binding organic compounds, are most likely to increase metal complexing capacity in water systems and thus reduce metal toxicity.

It was demonstrated that bacterial species with polysaccharide capsular coatings were very efficient adsorbents of heavy metals compared with similar non capsular varieties. Since metal adsorbance by the polysaccharide-producing bacteria is very efficient, it has been suggested that they be used as biosorbents (33, 35, 36, 37). Biosorption of metals by the biomass of filamentous fungi is also associated with the cell wall. Similarly, interaction of algal cell walls with metallic ions was reported (22, 25, 27). Algal cell walls are generally composed of polysaccharides and small amounts of proteins. It has been suggested that metal binding capacity is a function of surface ionic charge resulting from carboxylic and hydroxy groups as well as sulfate (12).

In a recent study, Kaplan *et al.* (1987) (26) compared the chemical composition and metal complexing capacity of polysaccharides produced by *Chlorella stigmatophora* and *C. salina*, grown under controlled laboratory conditions.

The dissolved polysaccharides produced by the two *Chlorella* species differ in their chemical composition and metal complexing capacity (Table I). The native

Table I. Uronic acid and sulfate content in the dissolved polysaccharides of *C. stigmatophora* and *C. salina* and their metals complexing capacity.

	Uronic acid %	Sulfate %	Complexing capacity			
			Cd <sup>2+</sup>	Cu <sup>2+</sup>	Pb <sup>2+</sup>	Zn <sup>2+</sup>
<i>C. stigmatophora</i> <sup>a</sup>	30	9	150	400	ND <sup>b</sup>	200
<i>C. salina</i> <sup>a</sup>	6	8	ND	ND	ND	ND

<sup>a</sup>30 µg glucose eqv. l<sup>-1</sup> of each polysaccharide

<sup>b</sup>Not detected

polysaccharide from *C. stigmatophora*, which is rich in uronic acid, exhibited high metal complexing capacity, whereas the uronic acid content of the *C. salina* polysaccharide is low and it did not bind metals under the same experimental conditions. Since both polysaccharides contain similar amounts of sulfate, it seems that the metal-complexing capacity of the *C. stigmatophora* polysaccharide is due to its uronic acid content.

Both polysaccharides are heterogeneous polymers, however they are significantly different in their surface anionic charge. The polymer of *C. salina* is mostly neutral and weakly charged while that of *C. stigmatophora* is highly charged. All the charged fractions of the *C. stigmatophora* polysaccharide exhibit

Table II. Fractionation of *C. stigmatophora* and *C. salina* dissolved polysaccharides on a DE-52 anion exchange chromatography column.

	Polysaccharide $\mu\text{g gluc.}$ $\text{eqv l}^{-1}$	Uronic acid %	Sulfate %	$\text{Cu}^{2+}$ complexing capacity $\mu\text{g l}^{-1}$
I. <i>C. stigmatophora</i>				
DDW <sup>a</sup>	1850	0	0	0
0.5M NaCl	3040	26	4	15
5.0M NaCl	27384	54	13	340
6.0M Urea	3570	27	3	76
0.5M HCl	3337	22	11	89
II. <i>C. salina</i>				
DDW <sup>a</sup>	11704	3	0	NT <sup>b</sup>
0.5M NaCl	16860	4	6	NT <sup>b</sup>
5.0M NaCl	1680	6	7	NT <sup>b</sup>
6.0M Urea	815	ND <sup>c</sup>	27	NT <sup>b</sup>
0.5M HCl	779	ND <sup>c</sup>	23	NT <sup>b</sup>

<sup>a</sup>Double distilled water

<sup>b</sup>Not determined

<sup>c</sup>Not detected

copper-complexing capacity (Table II). Indeed, as expected, a relation was found between highly ionic charged polymers and metal complexing capacity. It is also supported by the observation that alginates extracted from seaweeds and bacteria exhibit  $\text{Cu}^{2+}$  binding ability as does pectate (23, 28, 31, 40). Similarly, bacterial extracellular polysaccharides with negative surface charge, containing uronic acids and hexosamines, also bind metals (7, 13, 14).

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 chelators. An indication that algal polymers and algal cell wall components contribute to metal detoxification in the environment is found in the literature (22, 25, 26, 28, 30, 31). Whether these processes can be enhanced in polluted bodies of water by introducing algae that produce highly charged polymers is still to be tested.

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