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METAL BINDING POLYSACCHARIDE FROM THE ALGA,
KLEBSHORMIDIUM FLUITANS

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SUMMARY. *An alkali soluble polymer was prepared from the metal tolerant alga* Klebshormidium fluitans *which had a recombining capacity of 0.41 mg Cu^{++}/mg carbohydrate. Chemical preparation and properties, ion exchange behaviour, infra-red spectroscopy and acid hydrolysis indicate a polysaccharide containing uronic acid residues.*

INTRODUCTION. Lotic plants of the *Hormidium* species have been noted frequently in water polluted by heavy metal ions (Say *et al.*, 1977). The acid tolerant *Klebshormidium fluitans,* found in minewater, accumulates copper in excess of concentrations in surrounding solutions (e.g. 4.3 grams/ kilogram wet alga from 0.3 grams/litre at pH 3) (Madgwick and Ralph, 1977). This communication describes identification and preliminary characterisation of a metal ion binding polysaccharide, extracted from *K. fluitans* with dilute alkali.

METHODS AND MATERIALS. The alga *K. fluitans* was harvested from minewater drainage at the Bougainville Copper Limited mine at Panguna, Bougainville Island, Papua and New Guinea. It grew as luxurious green filaments in vigorously flowing streamlets containing soluble copper (ca pH3 and 0.3 grams per litre Cu^{++}). The samples were air-freighted to Sydney and stored at 4°C until used. Filaments were examined by phase contrast microscopy and were judged to be uni-algal and without significant bacterial or fungal contamination.

Scanning Electron Microscopy (SEM) with Energy Dispersive Analysis of X-Rays (EDAX) was carried out using S-4-10 Stereoscan(R) Cambridge Ltd., Instruments. Semi-quantitative emission spectroscopy was performed with a Baird Atomic 3M grating spectrograph, model GX. Quantitative mineral analyses were determined with a 'Varian - Techtron'(R) Type AA5 Atomic Absorption Spectrophotometer.

Acid digestions for metal analysis were made with concentrated $HCl:HNO_3$, 1:1(v/v) at 200°C for 5 hr (10 ml acid/0.5 g dry algae). Loosely bound ionic species were removed from the dry algae by treatment with 0.5 M HCl at room temperature.

Cells were chemically fractionated to determine the polymeric counter-ion for heavy metal attachment. Algae were first lysed and de-fatted at 20°C, overnight, in 90% acetone/water (v/v) (50 ml/gram dry weight). Solids were drained, washed with diethyl ether, and air dried. Powders were then extracted with dilute acid (0.5M HCl, 40 ml/gram) at 20°C for two hours, and centrifuged at 12,000 g for ten minutes. The pellets were treated with phenol:acetic acid:water (2:1:1, w/v/v) (100 ml/3 grams dry weight at 25°C, with agitation, overnight) to remove protein (Bagdasarian *et al.*, 1964). After washing with diethyl ether, remaining solids were extracted with dilute alkali (0.2 M NaOH, 5 ml/gram) at 25°C, with agitation, overnight. Alkaline solutions were treated with two volumes of ethanol, and resulting precipitates re-suspended in 0.1 M NaCl and reprecipitated with alcohol. The capacity to recombine with copper ion was only found in the fractions containing alkali soluble polymers. Carbohydrate was estimated by the phenol-sulphuric acid method of Dubois *et al.*,(1956) using glucose as a standard sugar. Uronic acid detection employed the colorimetric technique of Knutson & Jeanes (1968). Acid hydrolysis of purified polysaccharide was by the method of Jeffrey & Rienits (1967) and the resulting sugars were separated by thin layer chromatography (Stahl & Kaltenbach, 1965).

Infra-red spectra of semi-pure algal polymer were recorded on a 'Perkin Elmer-337'(R) grating spectrophotometer using freeze-dried polymer embedded in KBr discs.

RESULTS. The elemental composition of *K. fluitans* was determined from semi-quantitative emission spectroscopic analysis of ashed algal residues (average of three determinations), and showed the following elements (% dry weight in parentheses; fresh algae, 35% dry weight): Al(2.04), Fe (1.90), Cu (1.23), Mg (0.56), P (0.15), Ca (0.10), Ti (0.02), B (0.01).

SEM-EDAX on whole cells independently verified the identity and relative abundance of the major elements. Dilute acid triturates of organic solvent extracted dry algae yielded the same amounts of copper and iron as were found in acid digests. This showed that these elements were bound mainly by ionic association with insoluble polymeric cellular material. After removing protein from the dry lipid-free insoluble cell material, with the acetic acid: phenol solvent, and treating the residue with dilute alkali, a soluble polymeric fraction was obtained which was responsible for most of the copper binding capacity. The polymer represented 0.7 - 1.0% algal dry weight and gave pronounced phenol-sulphuric acid, carbazole and napthoresorcinol reactions for polysaccharide. The ratio of carbohydrate to nitrogen was greater than 30:1. Semi-quantitative spectral analysis showed small quantities of metal ions remained in the final polysaccharide product (Ag, 0.004% dry polysaccharide; Al, 2%; Cu, 0.1%; Fe, 1.2%; Mg, 1.0% and Ti, 0.05%). Exhaustive dialysis against disodium ethylene diamine tetracetic acid reduced the iron content of the polymer by some 25% but the amount of polysaccharide remained unchanged.

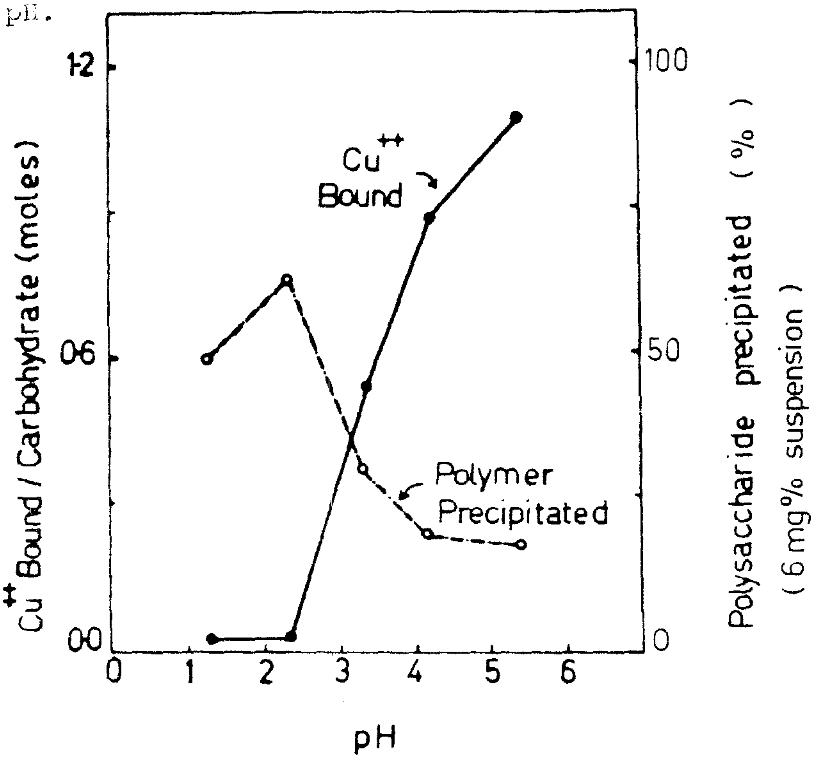
Metal ions in solution (100 µg/ml) treated with polysaccharide suspensions, at pH 3, were taken up according to their affinity for the polymer. Table I sets out results of ionic uptake tests with some common minewater elements. The binding order of divalent ions by the polymer resembles the ionotropic series for alginate (Haug and Smidsrod, 1965), and the amount of ion uptake was also of a similar order to various alginate preparations.

TABLE I Metal Ion Binding by *K. fluitans* alkali soluble polysaccharide

Ion	Ion Bound/Carbohydrate* (mg/mg) * calculated as glucose
Ni^{++}	0.24
Zn^{++}	0.26
Co^{++}	0.30
Cu^{++}	0.41
Pb^{++}	0.90
Fe^{++}	1.06

Figure I shows the interaction of copper ion and polysaccharide at various pH. More copper was bound at lower hydrogen ion concentrations with less precipitate, while more precipitate formed at high acidity with less copper. This ion-exchange behaviour is typical of a polysaccharide counterion binding a divalent heavy metal ion such as copper.

Figure I Copper bound and *K. fluitans* polysaccharide precipitated during recombination at different pH.



Infra-red spectra of the alkali soluble polymer had a broad band at 3500 cm^{-1} a peak at 2900 cm^{-1}, a weak band at 2550 cm^{-1}, a shoulder at 1740 cm^{-1} and a peak at 1670 cm^{-1} which indicate the presence of carboxylic acid groups. No evidence of sulphate groups was found. A sharp peak at 830 cm^{-1} may be ascribed to linkages between sugar monomers.

TLC separation of acid hydrolysis products of the semi-purified polymer showed reducing sugars ($AgNO_3$ -positive) with similar mobility to mannuronic acid standards. A chromatographic region approximately coincident with uronolactone was present in all analyses. At least three unidentified sugars showed up in separations of the polysaccharide hydrolysate.

The method of preparation, ion-exchange behaviour, thin-layer chromatography of acid hydrolysis products and infra-red spectra show that the alkali soluble polymer of *K. fluitans* was a polysaccharide with significant numbers of uronic acid residues. From histological studies for heavy metal ion (Madgwick and Ralph, 1977) and the above analytical data it is evident the alga, in nature, is able to tolerate high soluble heavy metal ion by forming insoluble ionic complexes with its extracellular mucilaginous cell-wall material. Ion binding was virtually instantaneous which was consistent with the 'adsorption with no growth' model proposed by Khummonkol *et al.*, 1981).

Efforts are being made, in this laboratory, to cultivate this alga on a large scale to facilitate preparation and detailed identification of the copper binding polysaccharide.

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