

The Influence of Borate and Calcium on the Gel Formation of a Sulfated Polysaccharide from *Ulva lactuca*

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The water-soluble, sulfated polysaccharide isolated from *Ulva lactuca*, forms a soft gel when dialysed against sea-water, provided the polysaccharide concentration is 1 % or higher and the pH above 7.5. The components of sea-water needed for this gel formation are boric acid and calcium ions. At concentration normally found in sea-water, neither borate nor calcium ions alone produce a gel.

It is assumed that the gel formation involves the formation of borate-polysaccharide complexes leading to intermolecular linkages which are stabilized by calcium ions in an unknown manner. This assumption is supported by experiments with extraction of this polysaccharide from the dry seaweed and by the results obtained with the isolated polysaccharide. Agreement between these experiments leads to the conclusion that the sulfated polysaccharide (*in vivo*) most probably occurs as a gel stabilized by the borate and calcium ions in sea-water.

The chemical structure of sulfated polysaccharides of green algae has been extensively investigated.¹ Little is known, however, about the form in which they occur in the plant. These polysaccharides are usually described as water-soluble, as they can be extracted by distilled water, and they are apparently located outside the cell membrane, possibly as the matrix substance of the cell wall. However, their solubility in sea-water does not seem to have been investigated, in spite of the fact that this might give some indications about their physical state in the plant and, thus, their biological function.

This work is an investigation of the solubility properties and gel formation of the sulfated

polysaccharide of *Ulva lactuca*. This polysaccharide represents one of the main groups of sulfated polysaccharides of green algae, containing rhamnose, xylose, glucose, and glucuronic acid in a highly branched molecule without any well-defined backbone structure.¹

EXPERIMENTAL

Ulva lactuca was collected at Drøbak in the Oslofjord in September. The polysaccharide was prepared by dialysing the dried weed contained in a dialysis bag against a solution of EDTA (0.1 M) at pH 7, followed by dialysis against water, centrifugation and freeze-drying of the clear supernatant. The solubility of the polysaccharide was investigated by dissolving it in distilled water and dialysing against salt solutions of the desired composition. The polysaccharide solution (2.5 ml) was dialysed against four batches of 50 ml salt solution, and each period of dialysis lasted for at least 3 h. After dialysis, the contents of the bags were centrifuged, the precipitate washed with salt solution and dissolved in distilled water. The amounts of soluble and insoluble polysaccharide were determined by the phenol-sulfuric acid method using glucose as a standard.

Extraction experiments were carried out by suspending dry algal material in water and dialysing against the salt solution. After thorough dialysis, the content of the dialysis bag was centrifuged and the amount of carbohydrate in the supernatant determined by the phenol-sulfuric acid reaction.

Optical rotation was measured in a Zeiss polarimeter, using a 10 cm cell.

RESULTS

Polysaccharide solutions of different concentrations were dialysed against natural sea-water of a salinity of 31.5. When the concentra-

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Table 1. Gel formation by dialysis against sea-water at different polysaccharide concentrations, (C).

C % (W/V)	Soluble % (W/W)	Insoluble % (W/W)
0.5	100	0
1.0	10	90
2.0	3	97
3.0	3	97

tion of polysaccharide was 1 % or more, the solution became a soft gel which could be washed with sea-water without dissolving. At a concentration of 0.5 % the polysaccharide remained completely soluble and no signs of gel formation was observed. (Table 1). In all subsequent experiments, solutions containing 2 % polysaccharide have been used.

In order to investigate whether the gelling of this polysaccharide from *Ulva* was caused by the presence of specific ions, or the high ionic strength of sea-water, dialysis against some simple salt solutions were carried out. As shown in Table 2, none of these salt solutions led to gel formation.

These results indicated that components of sea-water other than the main cations were essential for gel formation, and experiments with artificial sea-water or components thereof were carried out. The composition of the artificial sea-water (a slightly modified Kester medium²) is given in Table 3. Some results are given in Table 4 suggesting that the presence of boric acid is essential for gel formation. Other experiments using artificial sea-water buffered at pH 8.0 with Tris were carried out as shown in Table 5. These clearly indicate that boric acid and calcium ions are the only

components in sea-water necessary for gelation. Boric acid alone or calcium ions alone do not lead to gel formation.

The formation of borate complexes with carbohydrates (and diols in general) depends upon the presence of borate ions, *i.e.* the pH of the solution. Dialysis experiments were carried out against solutions containing boric acid, a simplified sea-water medium and tris buffer with different pH values. The results are given in Table 6.

Some extraction experiments were carried out by dialysing a suspension of dry weed against the extraction medium. The results are given in Table 7, showing that distilled water and unbuffered salt solutions removed roughly the same amount of material, while at pH 8 the extraction was incomplete without the presence of a calcium complexing agent. In natural sea-water and in a simplified Kester medium containing borate, an amount of carbohydrate corresponding to only 5 % of the dry weed was removed.

In order to determine whether the amount of borate bound to the polymer was sufficient to cause an appreciable change in the optical rotation of the polymer, $[\alpha]_D$ was determined for the polysaccharide, using a 2 % solution in tris buffer without borate and in the presence of 260 mg boric acid/l. The observed values of $[\alpha]_D$ were -61 and -59.5, respectively. Thus, no effect of the presence of borate was observed.

As shown in Table 5, calcium ions are needed in addition to borate to obtain a gel. No gel formation took place when a polysaccharide solution was dialysed against a solution of borate in tris buffer pH 8, followed by tris buffer solution without borate, and finally tris buffer containing calcium ions. Both borate and calcium ions were present in sea-water

Table 2. Dialysis against some simple salt solutions.

Composition of solution	Soluble %	Insoluble %
1 M KCl	100	0
0.3 M CaCl ₂	100	0
1 M NaCl	100	0
0.3 M MgCl ₂	100	0
0.055 M MgCl ₂ , 0.012 M CaCl ₂ , 0.55 M NaCl	100	0
Sea-water	5	95

Table 3. Composition of solutions and of artificial sea-water.

Solution	Compound	Conc. g/l	Artificial sea-water (amount in 1 l)
A I	NaCl	22.4	22.4 g
	Na ₂ SO ₄	41.00	
A II	KCl	6.93	91.4 ml
	KBr	1.00	
A III	NaF	0.030	
	H ₃ BO ₃	0.273	
B	MgCl ₂ ·6H ₂ O	203	49.8 ml
C	CaCl ₂ ·2H ₂ O	191	9.7 ml
D	SrCl ₂ ·6H ₂ O	26.7	0.82 ml
E	NaHCO ₃	33.6	5.00 ml
F	NaHPO ₄ ·12H ₂ O	10	0.5 ml
G	NaNO ₃	50	0.5 ml
H	Trace element solution ²		1.0 ml
I	Vitamin solution ²		1.0 ml
	Distilled water to 1 l		

Table 4. Dialysis against components of synthetic sea-water medium.

	Soluble %	Insoluble %
Complete medium	4	96
NaCl, Solution A I, B, C, E	100	0
NaCl, Solution A I, II, III, B, C, D, E	3	97
NaCl, Solution A I, III, B, C, E	4	96

concentrations. A similar experiment with glycine buffer pH 9 replacing the tris buffer gave the same result.

DISCUSSION

The results clearly show that the water-soluble sulfated polysaccharide isolated from *Ulva lactuca* forms a gel in sea-water and that only two of the components of the sea-water

are needed for the gel formation; boric acid and calcium ions. The gel is very soft and does not retain any fixed shape. It is thus very different from both a typical alginate and carrageenan gel.¹ Even if little is known about the correlation between gel properties and the molecular structure of the gel, it seems probable that the *Ulva*-polysaccharide gel only contains very few intermolecular linkages per molecule, *i.e.* that it is near to the critically branched state.²

Table 5. Dialysis against tris buffer (pH 8) containing various sea-water components.

	Soluble %	Insoluble %
A III	100	0
A III, NaCl	100	0
A I, II, III, NaCl	100	0
A III, B	100	0
A III, C	1.5	98.5
C	100	0

Table 6. Dialysis against a simplified sea-water medium (NaCl, Solution A I, A III, B and C) in tris-buffers with varying pH.

pH	Soluble %	Insoluble %
8.02	2	98
7.86	2	98
7.50	2	98 ^a
7.25	100	0

^a Gel softer than usual.

Table 7. Extraction experiments.

Extraction medium	Carbohydrate extracted, % of dry algae
Distilled water	18.2
0.1 M EDTA followed by distilled water	19.5
0.1 M CaCl ₂	16.7
0.7 M NaCl	16.8
Tris-buffer, pH 8	9.9
Tris-buffer, pH 8, containing 0.1 M EDTA	18.1
Tris-buffer, pH 8, NaCl, Solution A I, II, B, C	9.0
Tris-buffer, pH 8, NaCl, Solution A I, II, III, B, C	4.5
Natural sea-water	4.7

The *Ulva*-polysaccharide gel dissolves in distilled water in contrast to alginate gels formed by dialysis of sodium alginate against sea-water, which needs exchange of calcium with monovalent ions to dissolve. This agrees well with the extraction results. *Ulva*-polysaccharide may be extracted by distilled water, while in order to extract alginate from brown algae, calcium ions must be replaced with monovalent ions, e.g. by acid pre-extraction and extraction with sodium hydroxide or sodium carbonate.⁴ In solutions buffered at pH 8 only incomplete extraction of *Ulva*-polysaccharide was obtained. Gels formed by isolated polysaccharide, however, dissolved slowly upon dialysis against media buffered at pH 8 only if the media did not contain borate ions.

The results thus indicate that the formation of gel depends upon an equilibrium reaction between borate and polysaccharide, and it is likely that the intermolecular linkages leading to gel formation involve the formation of borate-polysaccharide complexes. In agreement with this is also the observation that no gel formation took place at pH 7.25 and a markedly softer gel than normal was formed at pH 7.50 (Table 6). That no change in optical rotation in presence of borate was observed, indicates that only small amounts of borate are bound, as should be expected from the gel structure. It is likely that the borate complex is formed with the vicinal hydroxyl groups of the 1,4-linked rhamnose units. These units, however, carry usually half-ester sulfate at C(2) (Ref. 1) and it is probably only a few units in the molecule with the free vicinal hydroxyl groups necessary for formation of a borate complex.

The effect of calcium ions is less clear. No gel is formed without borate and calcium ions being present simultaneously. The calcium ions may form bridges between borate complexes of the type shown in Fig. 1A, or they may be necessary for stabilizing borate linkages of the type shown in Fig. 1B. Both explanations are in agreement with the observation that complete extraction of the polysaccharide takes place also at pH 8 when calcium ions are removed by EDTA (Table 7).

The structures in Fig. 1 are only meant to indicate two possibilities, and neither of them gives a satisfactory explanation for the observation that the presence of magnesium ions do not give gel formation, i.e. that the formation of junctions is specific with respect to the cation.

If we assume, as the present results strongly suggest, that the sulfated polysaccharide from *Ulva lactuca* occurs in the plant as a gel stabilized by borate linkages, this is, as far as the author is aware, the first example of a borate-polysaccharide complex in Nature, and further investigation into the nature of the gels obtained with the polysaccharide from *Ulva*

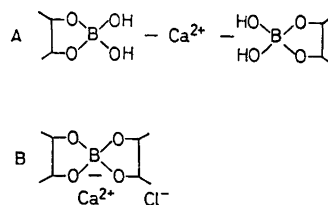


Fig. 1. Possible interactions between borate-polysaccharide complexes and calcium ions.

lactuca seems to be of direct importance for the understanding of the biological function of this complex.

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REFERENCES

1. Percival, E. and McDowell, R. H. *Chemistry and Enzymology of Marine Algal Polysaccharides*, Academic, London-New York 1967.
2. Kester, D. R., Duedall, I. W., Connors, D. N. and Pytkowicz, R. M. *Limnol. Oceanogr.* 12 (1974) 176.
3. Peniche-Covas, C. A. L., Dev, S. B., Gordon, M., Judd, M. and Kajiwara, K. *Faraday Discuss. Chem. Soc.* 57 (1974) 183.
4. Haug, A. *Report No. 30*, Norwegian Institute of Seaweed Research, Trondheim 1964.

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