

Ion Exchange Properties of Alginate Fractions

ARNE HAUG

*Norwegian Institute of Seaweed Research,
N.T.H., Trondheim, Norway*

Recently, two methods for the separation of alginates into chemically different fractions have been published. McDowell¹ separated alginate from *Laminaria hyperborea* (= *L. Cloustoni*) and *Ascophyllum nodosum* into fractions with different ion exchange properties, by precipitation with manganous salts. Haug² fractionated alginates from *Laminaria digitata* f. *stenophylla* by means of precipitation with potassium chloride, and the fractions differed in uronic acid composition. The present note reports an investigation of the ion exchange properties and uronic acid composition of the fractions obtained by the two methods.

Materials and methods. The sampling of seaweed and the preparation of alginate is described earlier². The precipitation with potassium chloride was carried out by mixing equal volumes of saturated aqueous potassium chloride and a solution of 0.3 % sodium alginate in water. The amounts of alginate precipitated with manganous salts were found to depend on the pH and the concentration of potassium and sodium ions present. In this investigation, one part of a solution of 0.09 M manganous sulphate and 0.3 M potassium chloride was added to two parts of a solution containing approximately 0.3 % sodium alginate, adjusted to pH 5. After one hour the gellike precipitate was separated from the solution by centrifugation. The alginate was converted to the sodium salt by washing with a dilute solution of sulphur dioxide in water and dissolving in dilute sodium carbonate solution.

A procedure similar to that of Mongar and Wassermann³ was used for the determination of ion exchange properties. Alginic acid fibers were made by forcing a viscous sodium alginate solution from a pipette with a narrow opening (0.5 mm) into 1 N hydrochloric acid, stirred with a magnetic stirrer. The alginic acid fibers were transferred to a 0.1 M calcium acetate solution. After thorough washing with water, the calcium alginate fibers were placed in 20–35 ml 0.2 M potassium chloride solution and allowed to stand overnight. The calcium concentration in the solution was determined by oxalate precipitation and titration. The cal-

cium bound to the alginate was liberated by 1 N hydrochloric acid and determined.

The methods used for hydrolysis of alginates and chromatography of uronic acids were those given by Fischer and Dörfel⁴.

Alginates from *Laminaria digitata* f. *stenophylla*, collected at Tarva, outside the Trondheimsfjord, 3/7 1957, and from *L. hyperborea*, stipes, collected at Munkholmen in the Trondheimsfjord, 26/8 1957, were investigated. The alginates were fractionated according to the two methods, and the ion exchange properties and uronic acid composition of the fractions investigated. The results are shown in Table 1. No attempts have been made to determine the amounts of uronic acids quantitatively, and the results given in the table are estimates based on the size and strength of the spots. The ion exchange properties are characterized by the selectivity coefficient

$$k = \frac{[Ca_g][Na_s]^2}{[Na_g]^2[Ca_s]}$$

where subscripts g and s refer to gel and solution and the concentrations are given as equivalent fractions and normalities, respectively. No attempt has been made to determine the true equilibrium constant involving activities. All figures given in the table are an average of several determinations, and the conditions were chosen so that the equivalent fraction of calcium in the gel in all cases was between 0.4 and 0.7.

The results show that there is a marked difference in ion exchange properties and uronic acid composition between alginate from *Laminaria digitata* and *L. hyperborea* stipes. Preliminary experiments have shown that alginate from *L. hyperborea* fronds resembles that from *L. digitata*. In the latter plant, no large difference between alginate from stipes and fronds have been observed.

The results also show that for both alginates the soluble fraction contains more guluronic acid and has a higher selectivity coefficient than the precipitated fraction when fractionated with potassium chloride, while the opposite is the case when manganous ions are used as precipitating agent. This result indicates that the same type of fractions are produced by both methods, but that the fraction precipitated by potassium chloride corresponds to the fraction that is not precipitated by manganous sulphate. Combining the two methods

Table 1. Uronic acid composition and selectivity coefficients of alginate fractions.

| Fractionation method | Fraction | <i>Laminaria digitata</i> | | | <i>L. hyperborea</i> , stipes | | |
|----------------------|---|---------------------------|-----------------|----------------|-------------------------------|-----------------|----------------|
| | | k | Mannuronic acid | Guluronic acid | k | Mannuronic acid | Guluronic acid |
| No fractionation | | 7.5 | ++ | + | 21.1 | + | +++ |
| Potassium chloride | Insoluble (K _i) | 6.7 | +++ | + | 15.5 | + | ++ |
| » | Soluble (K _s) | 9.2 | ++ | ++ | 20.5 | (+) | +++ |
| Manganous sulphate | Insoluble (Mn _i) | 8.4 | ++ | ++ | 22.9 | (+) | +++ |
| » | Soluble (Mn _s) | 7.4 | +++ | + | 10.2 | + | ++ |
| | Mn _s K _i | 6.5 | +++ | (+) | 8.6 | ++ | ++ |
| | Mn _i K _s | 11.2 | + | ++ | 20.3 | (+) | +++ |
| | Mn _s K _i Mn _s K _i | 5.1 | +++ | traces | | | |
| | Mn _i K _s Mn _i K _s | 13.1 | + | ++ | | | |

The number of +'s indicates approximately the proportion between the uronic acids in each fraction.

(+): One fifth or less of the total amount of uronic acids.

Traces: One tenth or less of the total amount of uronic acids.

should therefore offer a good opportunity for purifying the fractions. The fractions from the precipitation with manganous sulphate were subjected to fractionation with potassium chloride, and the selectivity coefficient and uronic acid composition were determined for the fraction soluble in manganous sulphate and insoluble in potassium chloride (Mn_sK_i) and the fraction insoluble in manganous sulphate and soluble in potassium chloride (Mn_iK_s). To obtain a further purification the procedure was repeated on the two purified fractions of *L. digitata* alginate. The results are given in Table 1.

The results confirm that alginate from *L. digitata* and *L. hyperborea* stipes is a mixture of macromolecules with different uronic acid composition and different ion exchange properties. Molecules containing a large proportion of guluronic acid residues are accumulated in the fractions which are soluble in potassium chloride solution and precipitated by manganous ions, while molecules rich in mannuronic acid residues are accumulated in the opposite fractions.

The fractions containing molecules rich in mannuronic acid residues have a lower affinity for calcium in a calcium-potassium ion exchange reaction than fractions with molecules with predominately guluronic acid residues. Alginates from different sources may have different uronic acid composition, and, consequently, different ion exchange properties, a fact which stresses the necessity of defining the source of the alginate in investigations of these polyuronides.

Further investigations of the alginate fractions are in progress.

1. McDowell, R. H. *Chem. & Ind. London* **1958** 1401.
2. Haug, A. *Acta Chem. Scand.* **13** (1959) 601.
3. Mongar, I. L. and Wassermann, A. *J. Chem. Soc.* **1952** 492.
4. Fischer, F. G. and Dörfel, H. *Z. physiol. Chem. Hoppe-Seyler* **302** (1955) 186.

Received June 9, 1959.