PREPARATION OF HIGH MOLECULAR WEIGHT RADIOIODINATED ALGINIC ACID

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SUMMARY

A simple procedure is described for preparing ^{125}I-labelled alginic acid. The reducing terminal of alginic acid is derivatized with tyramine through the formation of Schiff base which is subsequently reduced with sodium cyanoborohydride. By radioiodination of the aromatic ring introduced, ^{125}I-labelled alginic acid was obtained with high specific activity.

Key-words: *^{125}I, Tyramine, glycuronans, alginic acid, labelling.*

INTRODUCTION

Alginic acid, a component of the brown seaweed *Phaeophyceae* (1), is a high molecular weight polymer of a repetitive unit containing D-mannosyluronic acid and L-gulosyluronic acid (2). A major property of alginate is its ability to form gels in the presence of Ca^{2+} and other divalent or polyvalent cations (3, 4).

Alginates of different molecular weight are widely used in food and beverage processing as well as in pharmaceutical formulations. In addition to the use of alginates as stabilizers and gelling agents, more specific applications have been devised, for instance, as the polysaccharide is insoluble in an acid milieu, it can be used as an anti-ulcer agent, because it precipitates as a film of gel on the the stomach lining. Moreover, the buffering capacity of alginate enables its use in dyspepsia as an antiacid (5, 6). At present, relatively few methods for labelling alginate with radioactive isotopes have been proposed to employ in biological and pharmaceutical studies. Steiner and McNeely have synthesized radiolabelled propylene glycol alginate, by esterification under high pressure of alginic acid with ^{14}C-labelled propylene oxide (7). A biological method for preparing [U- ^{14}C]-labelled propylene

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glycol alginate has been reported by Sharrat and Dean (8). More recently, a new method for labelling alginic acid has been proposed by Carre' et al. (9). The overall procedure consists of the periodate oxidation of vicinal diols, followed by reductive amination with [^{3}H]- $NaB(CN)H_3$. The major drawback of this method is the extensive depolymerisation of the high molecular weight glycuronans.

In the present paper an efficient and simple method for labelling alginic acid is described. Alginic acid is derivatized with tyramine by reductive amination and the secondary amine obtained is radioiodinated according to established procedures.

EXPERIMENTAL PROCEDURE

Materials

The alginic acid used in the present work (MW 70.000) was obtained from Fidia Research Laboratories (Abano Terme, Italy). Sodium cyanoborohydride was purchased from Merck (Darmstadt, Germany), as was the Tyramine hydrochloride. Na^{125}I (specific activity, 2090 Ci/matom) and [1-^{14}C]-Tyramine hydrochloride (specific activity, 53.5 Ci/mmol) were purchased from NEN Dupont.

Synthesis of Tyramine-Alginic acid derivative

A preliminary study was carried out to find the best conditions for coupling tyramine to the terminal aldehydic group of the polymer. Alginic acid (0.29 μmols) was solubilized in 3 ml of phosphate buffered saline (PBS, 10 mM, pH 8.0). A fixed amount of 2 μCi of [1-^{14}C]-tyramine- HCl and amounts of unlabelled tyramine ranging from 0.29 to 29 μmols were added to the solution. The mixture was supplemented with 58 μmols of $NaB(CN)H_3$ and stirred for different times at 25°C or 60°C. The reaction mixture was dialyzed for four hours against 150 vol of PBS, with four changes of the dialysis buffer.

The reaction yield was assessed by evaluating the labelling of the tyramine-alginic acid derivative, at any of the experimental conditions tested. The amount of the radioactive alginate derivative formed was determined by liquid scintillation counting, after removal of unreacted ^{14}C-labelled tyramine by dialysis. Alternatively, the absorbance of the dialysate at 276 nm was measured to assess the yield of the reaction in the different experimental conditions. The results shown in Fig. 1 demonstrate that the highest yield was obtained after 3 h of reaction at 60°C and with a hundred times molar excess of tyramine. No reaction was observed in experiments performed at 25°C.

The homogeneity of the purified tyramine-alginic acid derivative was determined by chromatography on 1 x 70 cm column packed with Sephacryl S-400 HR (Pharmacia LKB, Uppsala, Sweden). Samples of the derivative (0.5 ml) were loaded on the column and elution was performed with 10 mM PBS (pH 8.0) at a flow rate of 30 ml/h recording the absorbance of the eluates at 205 and

280 nm. The derivatized alginic acid was eluted as a single peak, with a retention volume of 2.8 times V_o. The elution pattern of the derivative was found to be coincident with that of the native alginic acid, showing the absence of any depolymerisation process during the reductive amination reaction. The alginic acid derivative also showed absorbance at 280 nm, due to the presence of the aromatic ring. (Fig.2).

In all subsequent experiments the conditions described below were used to perform the derivatization reaction. Alginic acid (0.29 μmols) was solubilized in 3 ml of phosphate buffered saline (PBS, 10 mM, pH 8.0) at 60°C. After 10 min, 29 μmols of tyramine and 58 μmols of $NaB(CN)H_3$ were added to the solution. The mixture was stirred for 3 hours at 60°C .The reaction mixture was dialyzed for four hours at 60°C against PBS, 150 vol x 4 changes. The reaction yield was 65%, in terms of percent of alginate molecules derivatized with tyramine.

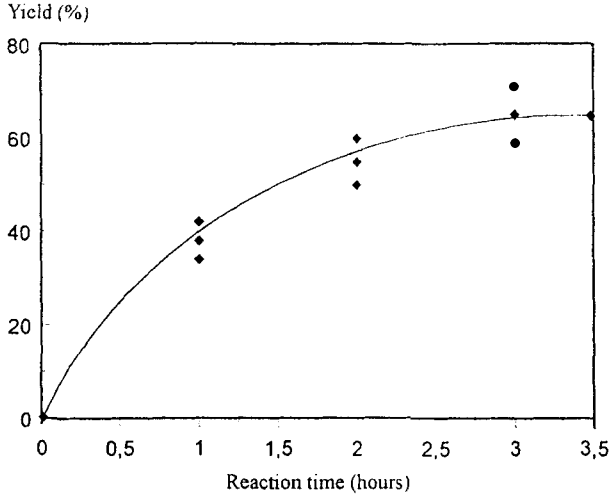

Fig.1. Time course of reductive amination. Alginate/Tyramine molar ratio is 1:100 in the reaction.

Radioiodination of the tyramine-alginic derivative

Alginic acid was coupled with unlabelled tyramine and purified by dialysis as described above. Iodination was carried out with "carrier free" $Na^{125}I$, in the presence of Iodogen (Pierce Chemical Co., Rockford, IL, U.S.A.).

A 2 ml glass vial was coated with iodogen by evaporating with a gentle flow of nitrogen 1 ml of a 10 mg/ml iodogen dissolved in CH_2Cl_2. The coated vial was repeatedly washed with PBS to remove any loose microscopic flakes of Iodogen. The dialyzed solution of the tyramine-alginate derivative (1 ml) was transferred into the vial. After equilibrating the vial at 60°C, 1 mCi of $Na^{125}I$ was added and the reaction mixture was stirred for 10 mins. The solution was dialyzed for four hours at 60°C against

150 vol of 10 mM PBS (pH 8.0), with four changes of the dialysis buffer. The specific radioactivity of the product was 20 Ci/mol, as assessed by solid phase scintillation counting of a small aliquot of solution of known concentration.The yield of the labelling procedure was about 50%.The homogeneity and the radiochemical purity of the iodine-labelled derivative was assessed by thin layer chromathographic analysis on silicic acid plates (Merck, Darmstadt, Germany), using a solution of n-butanol/acetic acid/water (25:4:10, v/v) as developing mixture. The plates were examined by radiochromatoscanning, using a Bioscan System 200 Instrument (Camberra Packard, U.S.A). More than 90% of the radioactivity was found on the iodinated alginic acid spot.

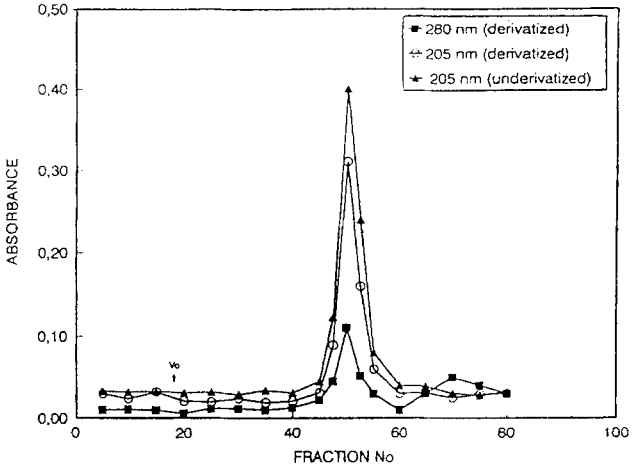

Fig.2. Comparison of elution patterns of derivatized and underivatized alginic acid

CONCLUSION

The present paper describes an easy procedure to prepare a radiolabelled derivative of alginic acid.The first step of the derivatization procedure involves the reaction with tyramine of the free carbonyl of the glycuronan molecule. It is not surprising that this reaction is strongly influenced by the temperature, indeed, intramolecular and intermolecular hydrogen bonds among hydroxylic and carboxylic functional groups of acidic polysaccharides are extremely sensitive to temperature changes.

The viscosity of alginic acid solutions, for example, decreases by about 12% for any increase of temperature of 4 -5°C (13). At 60°C, alginic acid forms in water a suspension of low viscosity and, presumably, at these conditions the aldehydic group is available for the nucleophilic tyramine.The pH chosen for the reaction is 8.0, as previously shown (6,8), at which value the formation of the Schiff base and its reduction to secondary amine with $NaB(CN)H_3$ appears to occur with high yield. Indeed, it is known that at pH 8.0 the reduction of Schiff base is faster than the reduction of an aldehydic function to primary alcohol (14).

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