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GLYCOPROTEINS FROM *ULVA LACTUCA*

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Key Word Index—*Ulva lactuca*; chlorophyta; glycoproteins; glucuronic acid; xylose; rhamnose; amino acids.

Abstract—*Ulva lactuca* yielded glycoprotein materials which differed significantly in their carbohydrate and protein moieties. The electrophoretic patterns of the isolated materials showed the presence in each of glycoprotein and polysaccharide components. All the glycoprotein components moved towards the cathode.

The glycoprotein material isolated by extraction with NaOH was fractionated on a DEAE-cellulose column into four glycoprotein components and one protein component. All the glycoprotein components contained glucuronic acid, xylose, and rhamnose in different proportions, and in addition two of them contained glucose.

INTRODUCTION

In previous studies [1–4], *Ulva lactuca* was shown to contain a polysaccharide composed of high proportions of glucuronic acid and rhamnose, and low proportions of xylose and glucose. Abdel-Fattah and Edrees [5] reported on the seasonal changes in the constituents of *U. lactuca* and found that its protein content reached a maximum (32.7%) in August and a minimum (8.7%) in April. Studies on algal glycoproteins are, however, scanty.

This paper deals with the isolation, by sequential extraction, of glycoprotein materials and their characterization from a single sample of the local alga *U. lactuca*. We also report the fractionation of one of the isolated glycoprotein materials into four different glycoprotein components and one protein component.

RESULTS AND DISCUSSION

U. lactuca was found to contain protein (12.5%) and residues of glucuronic acid (12.5%), xylose (15%), rhamnose (3.8%) and glucose (1.4%). The qualitative amino acid composition of the algal material included: alanine, arginine, aspartic acid, cysteic acid, glycine, glutamic acid, histidine, isoleucine, leucine, methionine, phenylalanine, serine, threonine, tyrosine, and valine.

A sample of the algal material was extracted in succession with water (A), 0.9% NaCl (B), 1% ammonium oxalate (C), 0.2 M acetate buffer (pH 5) (D), M/15 Sörensen phosphate buffer (pH 6) (E), McIlvaine citrate–phosphate buffer (pH 8) (F), and 1 M NaOH (G). Each of these extracts was shown by electrophoresis to contain a mixture of polysaccharides and glycoproteins together with a protein in B and G. But no attempt was made to determine the proportion of each constituent. Table 1 gives the composition of each of these extracts.

Due to the high recovery of carbohydrate and protein in G, it was selected for further fractionation by column chromatography on DEAE-cellulose (D-52). This led to the isolation of one fraction consisting of only protein and four other fractions containing different proportions of carbohydrate and protein but no consistent trends were observed. The results of the present work establish the presence in *U. lactuca* of a variety of glycoproteins.

EXPERIMENTAL

U. lactuca was collected in Dec. 1978 from Roushdy at Alexandria. The alga was washed with water to remove foreign substances, air-dried and milled.

General. Chromatography on Whatman no. 1 paper was

Table 1. Composition of the various extracts of *U. lactuca*

Extract	Alga (%)	Carbohydrate (%)	Protein (%)	Monosaccharide composition (% total)			
				GlcUA	Glc	Xyl	Rha
A	10.9	31	14	56	5	12.5	26.5
B	5.2	7	14	63	12	5	20
C	5.2	30	12	84	—	10	6
D	4.2	30	15	86	4	2	8
E	5.8	17	10	93	—	5	2
F	6.3	28	14	61	6	8	30
G	12.3	42	29	69	9	5	17

performed with the following solvent systems: (A) *n*-BuOH–C_5H_5N–H_2O [3] (6:4:3), (B) *n*-BuOH–HOAc–H_2O [6] (4:1:5, upper layer), and (C) PhOH–H_2O [7] (4:1). Sugars and amino acids were detected with aniline hydrogen phthalate reagent and ninhydrin reagent respectively [8]. Paper electrophoresis was performed (4 hr, room temp.) in an Elphor apparatus: HOAc–acetate buffer (0.01 M, pH 3.42). 300 V, 0.2 mamp/strip. Staining was effected with Amido Black dye and with Toluidine Blue reagents [9].

The protein content of the algal material was determined by the micro-Kjeldahl method and that of the algal extracts by the method of ref. [10]. Total carbohydrate of the fractions eluted from the column was determined as glucose by the method of ref. [11]. Complete acid hydrolysis of the algal material and algal polysaccharides was performed with H_2SO_4 [12]. Sugars in the hydrolysates were determined, after descending PC (solvent A) and elution from the chromatograms, by the method of ref. [11] with appropriate graphs for each of the sugars. Amino acids were determined by the method of ref. [13] after hydrolysis of the sample with 6 M HCl, in a sealed tube for 16 hr at 105°, followed by 2D-PC (solvents B and C).

Sequential extraction. The algal material (100 g) was extracted with distilled H_2O (1 l) with stirring for 1 hr at room temperature and then filtered. The residue was re-extracted with H_2O (400 ml) in a shaker at 40° for 16 hr and then centrifuged. The aq. extracts were combined, dialysed against distilled H_2O in a refrigerator for 72 hr and lyophilized. The algal residue was extracted (1 × 700 ml and 1 × 400 ml), as described above, sequentially with 0.9% NaCl, 0.1% ammonium oxalate, 0.2 M acetate buffer (pH 5), M/15 Sörensen phosphate buffer (pH 6) and McIlvaine citrate–phosphate buffer (pH 8). Each extract was dialysed against distilled H_2O in a refrigerator for 72 hr and lyophilized. The final algal residue was extracted with 1 M NaOH (385 ml) in a boiling water bath for 30 min. After centrifugation, the supernatant extract was dialysed as before and lyophilized.

Electrophoretic pattern of the algal extracts. Electrophoresis was performed as described above. The developed chromatograms were stained for proteins and anionic polysaccharides with Amido Black and Toluidine Blue respectively. Glycoproteins reacted positively to both stains.

Fractionation of the algal NaOH extract. A sample of the NaOH extract, containing 304 mg protein and 450 mg carbohydrate, in 5 ml 0.03 M phosphate buffer (pH 7) was applied to a DEAE-cellulose (D-52) column (2 × 38 cm) which had been equilibrated with 0.03 M phosphate buffer, pH 7. Stepwise elution was carried out in succession at pH 7 with 0.03 M phosphate buffer (552 ml), 0.06 M phosphate buffer (128 ml), 0.1 M NaCl in 0.06 M phosphate buffer (112 ml), 0.5 M NaCl in 0.06 M phosphate buffer (440 ml), and with 0.05 M NaOH (88 ml), 0.1 M NaOH (304 ml), and 0.2 M NaOH (176 ml). 8-ml fractions were collected at a flow rate of 24 ml/hr. The protein and total carbohydrate contents of each fraction were determined. The fractions which contained protein and/or carbohydrate were thereafter pooled, dialysed against distilled H_2O and lyophilized.

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