SULFATION OF FUCOIDIN IN *FUCUS* EMBRYOS

III. Required for Localization in the Rhizoid Wall

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ABSTRACT

Zygotes of the brown alga *Fucus distichus* L. Powell accumulate a sulfated polysaccharide (fucoidin) in the cell wall at the site of rhizoid formation. Previous work indicated that zygotes grown in seawater minus sulfate do not sulfate the preformed fucan (an unsulfated fucoidin) but form rhizoids. Under these conditions, we determined whether sulfation of the fucan is required for its localization in the rhizoid wall. This was accomplished by developing a specific stain for both the fucan and fucoidin. Using a precipitin assay, we demonstrated in vitro that the lectin ricin (RCA1) specifically complexes with both the sulfated and desulfated polysaccharide. No precipitate is observed when either is incubated in 0.1 M D-galactose or when RCA1 is mixed with laminarin or alginic acid, the other major polysaccharides in *Fucus*. RCA1 conjugated with fluorescein isothiocyanate (FITC) is also shown to bind specifically to fucoidin using a filter paper (DE81) assay. When added to zygotes, RCA1-FITC binds only to the site of fucoidin localization, i.e., the rhizoid cell wall. However, RCA1-FITC is not observed in the rhizoid wall of zygotes grown in the absence of sulfate. This observation is not due to the inability of RCA1-FITC to bind to the fucan in vivo. Chemically desulfated cell walls that contained fucoidin in the rhizoid wall bind RCA1-FITC only in the rhizoid region. Also, the concentration of fucose-containing polymers and polysaccharides that form precipitates with RCA1 is the same in embryos grown in the presence or absence of sulfate. If sulfate is added back to cultures of zygotes grown without sulfate, fucoidin is detected at the rhizoid tip by RCA1-FITC several hours later. These results support the conclusion that the enzymatic sulfation of the fucan is a modification of the polysaccharide required for its localization and/or assembly into a specific region of the cell wall.

KEY WORDS *Fucus distichus* · ricin · fluorescence microscopy · fucoidin · sulfation · cell wall · cytoplasmic localization · rhizoid

The localization of subcellular components into specific regions of a variety of plant and animal cells is a common and important aspect of cellular differentiation (c.f. reference 17). However, the mechanism by which macromolecules are sequestered into localized regions or structures of cells is not well understood. The rhizoid of the *Fucus* zygote provides a model system to investigate the regulation of intracellular localization. Previous reports indicated that a new and different sulfated polysaccharide is deposited in only that region of the zygote cell wall that forms the rhizoid (4, 20).
The localized polysaccharide is an α-1,2-linked fucan characterized by an ester-sulfate bond to the C-4 of the fucose residues. Although fucose is the predominant sugar, xylose, mannose, glucose, galactose, and glucuronic acid are also found, but in an unsulfated form. At least a portion of the galactose is found as the terminal sugar in the chain(s) (11), and various chemical fractionation schemes have described a range of polymers with differing amounts of these sugars and uronic acid residues (10). However, by electrophoresis in two different buffers, the sulfated polysaccharides from each of the three major chemical fractions displayed essentially the same two subfractions (20). This group of polysaccharides containing fucose sulfate as the predominant monomer unit will be referred to as fucoidin, while the same sugar chains that lack sulfate will be termed fucan.

The two subfractions observed upon electrophoresis are fucoidin 1 and 2 (F1 and F2). F1 has a lower electrophoretic mobility, is not so heavily sulfated and contains less fucose and more uronic acid moieties than F2. The early cell wall contains only F1 while F2 is incorporated into the wall at the time of rhizoid formation (20).

The fucan which is localized in the rhizoid is synthesized either during the first 4–6 h after fertilization or during oogenesis (18). Just before rhizoid formation (8–10 h after fertilization), the fucan becomes sulfated within Golgi bodies located at random throughout the cytoplasm (19). The Golgi bodies and vesicles containing the fucoidin (predominantly F2) are transported to the rhizoid pole and their contents are discharged into the newly formed, expanding rhizoid wall (1, 19). Only the rhizoid wall exhibits an intense metachromatic color when stained with toluidine blue O (TBO) at pH 1.5. Under these conditions, only F1 while F2 is incorporated into the wall at the time of rhizoid formation (20).

The formation of the rhizoid itself, i.e., a polar cell, is not dependent upon this sulfation, however. Crayton et al. (2) found that zygotes grown in seawater lacking sulfate but containing methionine (necessary for protein synthesis) form rhizoids and two-celled embryos that do not stain metachromatically with TBO. Apparently, there are no endogenous pools of SO4−−− and hence, fucan sulfation can be controlled by the amount of exogenously added SO4−−−. Concerning the role of the localized fucoidin, zygotes with rhizoids normally adhere tenaciously to the substratum, but in the absence of sulfate in the seawater, zygotes and embryos are free floating. Within 6 h after addition of sulfate to such cultures, two-celled embryos adhere by means of the rhizoid which now stains metachromatically with TBO. One function of the localized fucoidin might be related to the necessity for the embryo to adhere to the substratum for survival in the intertidal region.

Although fucoidin is not needed for rhizoid formation, we were in a position to ask whether the enzymatic sulfation is required for localization of the polymer, i.e., its assembly into the rhizoid cell wall. The approach needed to answer this question depended upon our ability to detect both fucan and fucoidin in the rhizoid wall. TBO, which depends on the presence of the ester-linked SO4 in the polymer for metachromatic staining, cannot distinguish between a rhizoid wall lacking fucoidin or a wall containing fucoidin. This paper demonstrates that the lectin (8, 22), ricin (RCA2), specifically binds to both fucoidin and fucan in vivo and in vitro. When a fluorescein-conjugated RCA2 is placed in contact with zygotes grown in the presence or absence of sulfate, localization of the polymer in the rhizoid cell is evident only if sulfation occurred.

MATERIALS AND METHODS

Isolation and Purification of Ricin

The procedure of Nicolson and Blaustein (12) was utilized to isolate and purify RCA1. A 10% (wt/vol) extract of Ricinus seeds (mixed variety; W. Atlee Burpee Co., Riverside, Calif.) in 0.2 M NaCl-0.05 M sodium phosphate buffer, pH 7.2 (PBS) was adjusted to 60% saturation with (NH4)2SO4 (Schwartz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.; Ultrapure-enzyme grade). After centrifugation, the pellet was dissolved in PBS, dialyzed, and applied to an affinity column of Agarose A-0.5 M (5 x 30 cm) supplied by Bio-Rad Laboratories (Richmond, Calif.). After washing with 2 liters of PBS to remove unbound protein, RCA1 was eluted from the column with 0.2 Mα-galactose in PBS. After concentration by ultrafiltration, the eluate was applied on a Bio-Gel P-150 column (Bio-Rad Laboratories) (1.5 x 40 cm) for separation of RCA1 and RCA2 by elution with PBS. Only RCA1 was employed in this study because of its specificity for β-D-galactosyl residues (13, 23). Protein concentration was determined by ultraviolet absorption (7). RCA1 was also obtained from Miles Laboratories Inc., Elkhart, Ind.) (RCA-120), and the identical results were obtained in duplicate experiments.

Specificity of Binding

In vitro binding specificity of RCA1 toward polysac-
charides found in *Fucus* embryos was determined by a precipitin method (3) and a filter paper assay. The turbidity caused by the interaction of RCA1 and polysaccharide was measured at 450 nm with a Zeiss PMQ III spectrophotometer (Carl Zeiss, Inc., New York) exactly as described by Goldstein et al (3). Solutions of fucoidin (a commercial preparation of fucoidin from ICN-K & K Laboratories Inc., Plainview, N. Y.), laminarin (ICN-K & K Laboratories), as well as alginic acid (Sigma Chemical Co., St. Louis, Mo.) were prepared in distilled water. Fucan was prepared chemically by treating fucoidin with 0.09 N HCl in anhydrous methanol for 18 h at room temperature, resulting in ~80% removal of ester sulfate groups (18). No TBO staining of the desulfated material was evident when spotted on filter paper and assayed by the TBO spot test (18).

Inhibition of binding of RCA1 to fucoidin and fucan was determined by including the following compounds in the precipitation assay: d-galactose, l-fucose, or d-glucose at 0.1 M, or NaCl at 1.0 M final concentration. Percentage of inhibition was calculated from the expression \( \frac{(A - B)}{A} \times 100 \) where \( A \) and \( B \) represent the absorbance obtained in the absence and presence of inhibitor, respectively.

Binding of RCA1 to various polysaccharides was also determined by a filter paper assay utilizing fluorescein-conjugated RCA1 (fluorescein-isothiocyanate [FITC]-RCA1, see below). A 100-μl solution of alginic acid or fucoidin at 0.5 mg/ml was applied to a circular 25-mm RCA1, see below). A 100-μl solution of alginic acid or fucoidin, respectively. Determined by a filter paper assay utilizing fluorescein-inhibitor, respectively.

Percentage of inhibition was calculated from the expression \( \frac{(A - B)}{A} \times 100 \) where \( A \) and \( B \) represent the absorbance obtained in the absence and presence of inhibitor, respectively.

In Vivo Localization of FITC-RCA1

RCA1 (1 mg) was coupled with 3 mg of FITC-isomer I (Sigma Chemical Co.) in 0.05 M Na2CO3 buffer, pH 8.5 (21). After mixing for 10 min at room temperature, the conjugated RCA1 was separated from free FITC by chromatography on a Bio-Gel P-10 column (1.5 × 15 cm) using PBS. The FITC-RCA1 was assayed for activity by using a hemagglutinin assay with a 3% suspension of human type O erythrocytes. Introduction of the FITC label did not alter the agglutinin activity of the RCA1. An inhibitor titre was determined using d-galactose. FITC-RCA1 was also obtained from Miles Laboratories, and the same results were observed in duplicate experiments.

Zygotes of the brown alga *Fucus distichus* L. Powell were obtained according to Quatrano (16) and grown in artificial seawater (ASW) with sulfate (0.1 mM), or without sulfate but containing methionine (10 mM). Zygotes were grown on glass microscope slides at 15°C in diffuse light, and after 24 h were washed with ASW and either used directly with the FITC-RCA1 or fixed in formalin:ASW (1:4) and washed with distilled water before treatment. To chemically desulfate, fixed embryos were treated with methanolic-HCl as described above.

Whole untreated embryos, chemically desulfated embryos, or isolated cell walls were treated with 100 μl of FITC-RCA1 (1 mg/ml) while control slides were treated with the same solution except that 0.1 M d-galactose was added. This concentration of d-galactose in vitro inhibited binding of the lectin to fucoidin. Both control and treated slides were placed in a humid atmosphere at room temperature in the dark for 8–12 h. Slides with attached embryos were then washed three times with PBS and mounted in PBS for microscope observation.

Slides were examined with a Leitz Laborlux fluorescence microscope equipped with a UGI exciter filter, a GB38 red suppression filter, and a K430 barrier filter which removed light below 410 nm. Pictures were taken with a Leica 4 × 5 camera with Tri-X-orthochrome film (Eastman Kodak).

RESULTS

RCA1 formed a precipitate with both fucan and fucoidin, but no precipitin reaction was detected with equal concentrations of laminarin or alginate (Fig. 1). Fucoidin fractions F1 and F2 (c.f. references 4 and 20) isolated from *Fucus* embryos exhibited the same results. Cellulose, alginate, and fucoidin, the major components of *Fucus* cell walls (4, 20), were also tested in the filter-paper binding assay with FITC-RCA1. Only those filter papers containing fucoidin exhibited a fluorescence (Fig. 2). When equal concentrations of alginate and fucoidin were bound to the same diethylaminoethyl impregnated filter, or when fucoidin was fixed to a cellulose filter (Whatman 3MM), the binding of the FITC-RCA1 was the same as fucoidin alone. Therefore, the presence of two other cell wall components, cellulose or alginate, did not interfere with binding. These results also demonstrated that the attachment of FITC to the RCA1 did not alter its specificity for fucoidin. The fluorescence and precipitation reactions between fucoidin and RCA1 were completely inhibited when 0.1 M d-galactose was included in the reaction mixture. l-fucose (0.1 M) was 50%
vesicles and particles are clearly visible within the extruded cytoplasm, indicating an intracellular binding of the RCA₁, as well. In all of the above cases, complete removal of the fluorescence could be achieved by incubation with 0.1 M D-galactose (Fig. 8).

Since we showed in vitro that RCA₁ complexed with the unsulfated fucan (see Fig. 1), does the FITC-RCA₁ recognize and bind to the fucan in vivo? Intact embryos with a sulfated fucoidin localized in the rhizoid were treated with methanolic-HCl to remove ester-linked sulfate from the polymer. After this treatment, no TBO metachromasy was observed in the rhizoid region. However, when these desulfated embryos are incubated with FITC-RCA₁, a localized fluorescence is clearly present (Fig. 5). Hence, the FITC-RCA₁ was able to bind to a localized fucan in vivo. One can conclude from these data that FITC-RCA₁ can be used as a specific in vivo marker for both fucan and fucoidin. With this probe, one can now ask whether the fucan is localized when embryos are formed in the absence of exogenous sulfate, i.e., when sulfation is prevented. Two-celled embryos less effective than galactose in blocking the precipitation, while D-glucose (0.1 M) was without effect. NaCl (0.5–1.0 M) decreased the amount of precipitation by 33%, but did not alter the specificity of RCA₁ binding.

Both autoradiographic and cytochemical results from several labs (9, 14) including our own (19) clearly showed the localization of a sulfated fucoidin in the rhizoid cell wall of intact embryos and isolated walls (14, 20). As a check for the specificity of FITC-RCA₁ in vivo, we incubated two-celled Fucus embryos (grown in ASW containing sulfate) with FITC-RCA₁. An intense fluorescence was localized over the rhizoid in precisely the same region as the metachromatic stain of TBO (Figs. 3 and 4). The localized, intense fluorescence most probably represents the accumulated F₂. The very weak fluorescence throughout the rest of the wall probably corresponds to F₁ which is deposited uniformly in the new forming wall in response to fertilization (20). If unfixed embryos are placed in 5 mM phosphate buffer (pH 7.2), the rhizoid tip bursts and the contents in the tip region are extruded onto the substratum. If these embryos are treated with FITC-RCA₁, fluorescent vesicles and particles are clearly visible within the extruded cytoplasm, indicating an intracellular binding of the RCA₁, as well. In all of the above cases, complete removal of the fluorescence could be achieved by incubation with 0.1 M D-galactose (Fig. 8).
Two-celled *F. distichus* embryos after various treatments with FITC-RCA. Figs. 3 (× 60) and 4 (× 240) represent embryos grown in the presence of sulfate. Fig. 5 (× 70) contains embryos grown in sulfate that were chemically desulfated with methanolic-HCl. In all of these Figures (3–5), a localized concentration of the FITC-RCA is observed in the region of expected fucoidin deposition, i.e., the rhizoid cell wall. Figs. 6 (× 60) and 7 (× 240) represent embryos grown in the absence of sulfate, and no localization of FITC-RCA in the rhizoid area is observed. Fig. 8 (× 60) shows the lack of FITC-RCA binding when embryos grown in the presence of sulfate are incubated in 0.1 M galactose.

Grown in the absence of sulfate were treated with FITC-RCA. Figs. 6 and 7 show no localization in the rhizoid wall of embryos grown under conditions when sulfation is prevented. No intracellular binding of RCA was evident in the rhizoid cytoplasm when tips burst in the presence of phosphate buffer, indicating that no intracellular localization was evident. If sulfate is added back to
embryos grown in ASW lacking sulfate, localization of FITC-\(\text{RCA}_1\) as well as TBO in the rhizoid tip is evident within 6 h. The fucose content of the total ethanol-insoluble fraction (4) from equal-sized populations of two-celled embryos grown in the presence or absence of sulfate was not significantly different (3.8 compared to 4.2 \(\mu g\) fucose/mg ethanol insoluble dry weight). Also, acid extracts from the same populations showed no significant difference in the amount of polysaccharide precipitated with \(\text{RCA}_1\) in the precipitin assay (1.7 mg compared to 1.9 mg). The turbidity was completely prevented in both cases by the presence of 0.1 M D-galactose. It appears from these data that, although rhizoids and fucan are present in embryos grown in ASW lacking sulfate, the fucan is not localized until sulfation occurs.

**DISCUSSION**

The results of this study demonstrated that the lectin from *Ricinus* seeds (\(\text{RCA}_1\)) can specifically bind in vitro to both fucoidin and the unsulfated fucan. TBO depends upon the presence of sulfate groups for its ability to selectively stain fucoidin. \(\text{RCA}_1\) apparently relies on terminal galactose units of the polysaccharide chains for its specificity, not the sulfate groups. Hence, the presence of the sulfate group is not required for \(\text{RCA}_1\) binding to the fucan. We conjugated \(\text{RCA}_1\) with the fluorescent dye FITC for use as a cytological probe to detect the in vitro localization of the fucan and fucoidin. When incubated with embryos grown in sulfate medium, this probe localized fucoidin in only the rhizoid region. No difference in binding was observed when fucoidin, localized in the rhizoid wall, was chemically desulfated and then incubated with the FITC-\(\text{RCA}_1\) probe. Therefore, the FITC-\(\text{RCA}_1\) conjugate was able to detect both fucoidin and an unsulfated fucan if localized in the rhizoid region. Using this probe on embryos grown with and without sulfate, we concluded that a postsynthetic, enzymatic modification of the fucan, i.e., sulfate addition, is required for its localization in the rhizoid region of the cell wall. This conclusion is based on the following results.

Zygotes grown in ASW lacking sulfate but containing methionine are morphologically similar to zygotes grown in ASW containing sulfate. They have the same rate of protein synthesis, undergo at least one cell division, but do not exhibit sulfation of a preformed fucan (2). We showed in this study that embryos grown in the absence of sulfate contain the same amount of fucose-containing polymers and \(\text{RCA}_1\)-binding polysaccharides as zygotes grown in the presence of sulfate. Therefore, preventing sulfation does not alter the amount of unsulfated fucan or its ability to bind \(\text{RCA}_1\) in vitro. Although the unsulfated fucan is present in embryos grown in the absence of sulfate, fucoidin cannot be detected in the same extracts by electrophoresis, precipitation with quaternary ammonium salts, or use of the stain TBO (2). Using the FITC-\(\text{RCA}_1\) conjugate as a probe to determine the localization of the fucan in embryos grown without sulfate, we were unable to detect localized fluorescence in the rhizoid region until sulfate was added to the medium and fucoidin was biochemically detectable. Our inability to detect localized fluorescence of the FITC-\(\text{RCA}_1\) in the rhizoid region when sulfation is taken is evidence that localization of fucoidin is dependent upon its previous sulfation.

Lack of localized binding cannot be explained by the lack of sulfate in the medium causing a decrease in fucose-containing polymers or inhibiting a modification in the fucan required for \(\text{RCA}_1\) binding. The same amount of \(\text{RCA}_1\)-binding polysaccharide is present and the FITC-\(\text{RCA}_1\) probe binds to a desulfated fucan in vivo. However, if an unsulfated fucan is not localized, yet detectable in vitro by \(\text{RCA}_1\) precipitation, why can we not detect it randomly distributed in the cytoplasm? Two explanations are possible: Unlike the highly localized concentration of fucoidin in the rhizoid cell wall or in the underlying cytoplasm, the random localization of the unsulfated fucan throughout the cytoplasm may not bind sufficient FITC-\(\text{RCA}_1\) within a given area to be detected by UV microscopy. Secondly, the fixative used may selectively extract or redistribute the unsulfated fucan while preserving the localization of fucoidin. Both of these explanations are not mutually exclusive and are presently being tested.

How can sulfation determine where fucoidin will be incorporated into the cell wall? With the demonstration of an intracellular electrical current traversing the zygote cytoplasm with a positive pole at the rhizoid site, a self-electrophoretic mechanism has been proposed to account for the transport of charged macromolecules and particles to various cytoplasmic regions (5, 6). Based on current measurements and the nature of the ions carrying at least a portion of the current, the field generated across the zygote is sufficiently large (even in view of the leveling action of diffusion) to localize such charged components (c.f. reference}
6). We demonstrated above that, to detect fucan localized in the rhizoid cell wall, sulfation is required. Earlier, we showed that enzymatic addition of sulfate to fucoidan results in a net negative charge on the polymer and that the amount of sulfation which occurs in vivo is proportional to its electrophoretic mobility in acrylamide gels (13). Although the electrical potential gradient is sufficient to account for the localization of free fucoidin, most of the sulfated molecules in the cytoplasm are found in Golgi bodies and Golgi-derived vesicles (1, 9, 15). Golgi bodies appear to be redistributed upon rhizoid formation (1, 15). Using autoradiography and transmission electron microscopy, we have recently found that the sulfating sites, i.e., Golgi bodies, are randomly distributed in zygotes grown in the absence of exogenous sulfate (Brawley and Quatrano, unpublished observations, and reference 19). Upon addition of sulfate to the medium, the sulfated fucoidin and Golgi bodies accumulate in the already formed rhizoid structure. No evidence is available yet to indicate whether the vesicles carrying the fucoidin for the rhizoid cell wall have a greater negative charge after sulfation. However, preliminary results indicate that such vesicles can be isolated for determination of surface and electrophoretic properties. Hopefully, these approaches will lead us to test further the hypothesis that the mechanism responsible for localization of fucoidin involves self-electrophoresis (c.f. references 1, 9). The electrical potential gradient is sufficient to account for the localization of free fucoidin, most of the sulfated molecules in the cytoplasm are found in Golgi bodies and Golgi-derived vesicles (1, 9, 15). Golgi bodies appear to be redistributed upon rhizoid formation (1, 15). Using autoradiography and transmission electron microscopy, we have recently found that the sulfating sites, i.e., Golgi bodies, are randomly distributed in zygotes grown in the absence of exogenous sulfate (Brawley and Quatrano, unpublished observations, and reference 19). Upon addition of sulfate to the medium, the sulfated fucoidin and Golgi bodies accumulate in the already formed rhizoid structure. No evidence is available yet to indicate whether the vesicles carrying the fucoidin for the rhizoid cell wall have a greater negative charge after sulfation. However, preliminary results indicate that such vesicles can be isolated for determination of surface and electrophoretic properties. Hopefully, these approaches will lead us to test further the hypothesis that the mechanism responsible for localization of fucoidin involves self-electrophoresis (c.f. reference 17). Other techniques are also being used to determine whether sulfation, in addition to its suggested role in the localization of fucoidin, may also be required for the export and incorporation of fucoidin into the developing cell wall.

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REFERENCES

1. Brawley, S. H., R. S. Quatrano, and R. Weatherbee. 1977. Fine-structural studies of the gametes and embryo of Fucus vesiculosus L. (Phaeophyta). III. Cytokinesis and the multicellular embryo. J. Cell Sci. 24:275-294.

2. Crayton, M. A., E. Wilson, and R. S. Quatrano. 1974. Sulfation of fucoidan in Fucus embryo. II. Separation from initiation of polar growth. Dev. Biol. 39:164-167.

3. Goldstein, I. J., C. E. Holleman, and E. E. Smith. 1965. Protein-carbohydrate interaction. II. Inhibition studies on the interaction of concanavalin A with polysaccharides. Biochemistry. 4:876-883.

4. Hogsett, W. E., and R. S. Quatrano. 1975. Isolation of polysaccharides sulfated during early embryogenesis in Fucus. Plant Physiol. (Bethesda). 58:25-29.

5. Jaffe, L. F. 1966. Electrical currents through the developing Fucus egg. Proc. Natl. Acad. Sci. U. S. A. 56:1102-1109.

6. Jaffe, L. F., and R. Nuccitelli. 1977. Electrical controls of development. Annu. Rev. Biophys. Bioeng. 6:445-476.

7. Layne, E. 1957. Spectrophotometric and turbidimetric methods for measuring proteins. Methods Enzymol. 3:447-454.

8. Lis, H., and N. Sharon. 1973. The biochemistry of plant lectins (phytohemagglutins). Annu. Rev. Biochem. 42:541-574.

9. McCully, M. E. 1970. The histological localization of the structural polysaccharides of seaweeds. Ann. N. Y. Acad. Sci. 175:702-711.

10. Mian, A. J., and E. Percival. 1973. Carbohydrates of the brown seaweeds Himanthalia lorea, Bifurcaria bifurcata and Padina pavonia. 1. Extraction and fractionation. Carbohydr. Res. 26:133-146.

11. Mian, A. J., and E. Percival. 1973. Carbohydrates of the brown seaweeds Himanthalia lorea and Bifurcaria bifurcata. II. Structural studies of the “fucans”. Carbohydr. Res. 26:147-161.

12. Nicolson, G. L., and J. Blausstein. 1972. The interaction of Ricinus communis agglutinin with normal and tumor cell surfaces. Biochem. Biophys. Acta. 266:543-547.

13. Nicolson, G. L., J. Blausstein, and M. E. Etzler. 1974. Characterization of two plant lectins from Ricinus communis and their quantitative interaction with a murine lymphoma. Biochemistry. 13:196-204.

14. Novotny, A. M., and M. Forman. 1975. The composition and development of cell walls of Fucus embryos. Planta (Berl.). 122:67-78.

15. Quatrano, R. S. 1972. An ultrastructural study of the determined site of rhizoid formation in Fucus zygotes. Exp. Cell Res. 70:1-12.

16. Quatrano, R. S. 1974. Developmental biology: Development in marine organisms. Experimental Marine Biology. Richard N. Mariscal, editor. Academic Press Inc., New York. 303-345.

17. Quatrano, R. S. 1978. Development of cell polarity. Annu. Rev. Plant Physiol. 29:487-510.

18. Quatrano, R. S., and M. A. Crayton. 1973. Sulfation of fucoidan in Fucus embryos. I. Possible role in localization. Dev. Biol. 30:29-41.

19. Quatrano, R. S., W. E. Hogsett, and M. Roberts. 1978. Localization of a sulfated polysacchar-
ride in the rhizoid wall of *Fucus distichus* (Phaeophyta) zygotes. Proceedings of the International Seaweed Symposium 9th, 1977. Santa Barbara, Calif. In press.

20. QUATRANO, R. S., and P. T. STEVENS. 1976. Cell wall assembly in *Fucus* zygotes. I. Characterization of the polysaccharide components. *Plant Physiol.* (Bethesda) 58:224-331.

21. RINDERKNECHT, H. 1962. Ultra-rapid fluorescent labelling of proteins. *Nature (Lond.)* 193:167-168.

22. SHARON, N., and H. LIS. 1972. Lectins: Cell-agglutinating and sugar-specific proteins. *Science (Wash. D. C.)* 177:949-959.

23. TOMITA, M., T. KUROKAWA, K. ONOZAKI, N. ICHIKI, T. OSAWA, and T. UKITA. 1972. Purification of galactose-binding phytoagglutinins and phyto-toxin by affinity chromatography using Sepharose. *Experientia (Basel)* 28:84-85.