Plant Cell Physiol. 40(9): 973–977 (1999)

Secretion of Lectin-Binding Material in Rhizoid Differentiation of *Spirogyra*

Naoko Inoue, Seiji Sonobe, Yoko Nagata and Teruo Shimmen

Department of Life Science, Faculty of Science, Himeji Institute of Technology, Harima Science Park City, Hyogo, 678-1297 Japan

Some species of *Spirogyra* (a green alga) anchor to the substratum by differentiating the rhizoid. The transparent material secreted at the tip of the terminal cell or rhizoid is speculated to be glycoprotein (Nagata 1977). To examine this, we treated *Spirogyra* filaments with differentiating rhizoid with fluorescently labeled lectins. Among the nineteen lectins examined, only *Bandeiraea (Griffonia) simplicifolia* lectin I (BSL-I) strongly stained the transparent material, suggesting that the transparent material contains α-D-galactose and methyl-α-D-galactopyranoside. Since the lectin stained the transparent material very strongly, we could detect the secretion at the very early stages of rhizoid differentiation.

Key words: Adhesion — *Bandeiraea (Griffonia) simplicifolia* lectin I (BSL-I) — Lectin — Rhizoid — *Spirogyra*.

Anchoring to the substratum enables algae to stay in their habitats, including seashores and streams. Some algae differentiate rhizoids and secrete adhesive materials to anchor strongly to the substratum. Glycoprotein has been suggested to be involved in adhesion of various algae, including *Enteromorpha, Ectocarpus, Hormoseira, Laminaria, Nereocystis*, and *Ceramium*. However, no adhesive compounds have yet been identified (Vreeland and Epstein 1996). *Spirogyra*, a popular fresh water alga, is classified into two groups depending on habitat. One group floats in still water without forming a rhizoid. In the other group living in streams, a filament differentiates a rhizoid to attach to the substratum; otherwise they can not remain in their habitat.

By differentiating a rhizoid, *Spirogyra* can adhere to various substrata, such as glass, polystyrene, polyethylene, aluminum, wood, filter paper, Teflon, and agar (Nagata 1977). Nagata (1973a, b, 1977, 1979) extensively studied the process of rhizoid differentiation in *Spirogyra*. When a filament of *Spirogyra* is cut with a razor blade, the terminal cell differentiates a rhizoid. It seems that a terminal cell senses its position. Since the differentiation occurs without cell division, the rhizoid is composed of a single cell. This rhizoid can be formed irrespective of the stage of the cell cycle (Nagata 1973a). Light is the most important factor in rhizoid differentiation, and involvement of phytochrome has been clearly demonstrated (Nagata 1973a, b, 1977, 1979).

Secretion of transparent material during rhizoid differentiation was indicated by using Indian ink (Nagata 1977). The transparent material was found to be secreted around the tip of the terminal cell and rhizoids. This transparent material was apparently sticky, because dust particles in the medium adhered to it (Nagata 1977). Although it was speculated that the transparent adhesive was glycoprotein, the chemical entities involved have not yet been elucidated.

In the present study, we succeeded in detecting secretion using an FITC labeled lectin, *Bandeiraea (Griffonia) simplicifolia* lectin I (BSL-I), during rhizoid differentiation. Because the present method is very sensitive, we could detect secretion in the very early stages of rhizoid differentiation.

Material and Methods

Plant materials—Filaments of *Spirogyra* sp. anchored to the substratum were collected from a stream near our laboratory. Preliminary experiments showed that this *Spirogyra* sp. differentiates rhizoids. In most experiments, *Spirogyra* filaments collected from the stream were used. In others, *Spirogyra* cultured in the laboratory was used. Axenic culture was established according to Ohiwa (1977). A slightly modified Reichart's medium was used for culture (Nagata 1973a).

Rhizoid differentiation—Incubation of *Spirogyra* filaments for observation of rhizoid differentiation was carried out using a chamber made of slide glass and silicon rubber sheet. A sheet of silicon rubber 2 mm thick (25 mm × 25 mm) was prepared with a square hole (10 mm × 10 mm). When the silicon sheet was pressed on a slide glass, it strongly attached to the glass. The silicon sheet remained attached during incubation of at least 22 d. The chamber thus prepared was filled with incubation medium (see above). Filaments of *Spirogyra* were cut into 1–3 mm fragments with scissors, and the fragments were transferred into the incubation chamber. Hereafter, fragments of filament thus prepared are simply called filaments. The chamber was placed in a transparent polyacrylate box; the bottom was covered with a sheet of wet paper, and a lid was put on the box to avoid evaporation of the

Abbreviations: FITC, fluorescein isothiocyanate; LBM, lectin-binding material; BSL-I, *Bandeiraea (Griffonia) simplicifolia* lectin I; BSL-II, *Bandeiraea (Griffonia) simplicifolia* lectin II; DBA, *Dolichos biflorus* agglutinin; DSL, *Datura stramonium* lectin; ECL, *Erythrina cristagalli* lectin; LCA, *Lens culinaris* agglutinin; LEL, *Lycopersicon esculentum* lectin; PHA-E, *Phaseolus vulgaris* erythroagglutinin; PHA-L, *Phaseolus vulgaris* leucoagglutinin; PNA, *peanut* agglutinin; PSA, *Pisum sativum* agglutinin; RCA-I, *Ricinus communis* agglutinin I; S-WGA, succinylated *wheat germ* agglutinin; SBA, *soybean* agglutinin; SJA, *Sophora japonica* agglutinin; STL, *Solanum tuberosum* lectin; UEA-I, *Ulex europaeus* agglutinin I; VVL, *Vicia villosa* lectin; WGA, *wheat germ* agglutinin.

incubation medium. The filaments were incubated at 23°C under a 12 h light–12 h dark cycle with fluorescent light. The light intensity at the surface of the polyacrylate box was 90 μE m^{-2} s^{-1}. To observe the filaments, most of the incubation medium and the silicon rubber sheet were removed, and the filaments were subjected to microscopic observation after covering with a cover glass.

Lectin-binding analysis—To observe binding of lectin, filaments were treated with lectins labeled with either fluorescein or rhodamine (Vector Laboratories, Inc., Burlingame, U.S.A.). All fluorescently labeled lectins were diluted to 1/100 with the incubation medium (final concentration 20 μg ml^{-1}). Filaments on slides were treated with a lectin solution for 1 min at room temperature. After the lectin solution was removed, the filaments were washed with the incubation medium and observed with a fluorescence microscope (Olympus, BH2-RFCA, Japan).

Pectin staining—To observe pectin, filaments were treated with 1 mM ruthenium red in the incubation medium for 1 min at room temperature. After removing the solution, filaments were observed with a differential interference microscope (Zeiss, Axiophot, Germany).

Results and Discussion

First, the time course of rhizoid differentiation in the present material was analyzed. When filaments were cut with scissors, the cell wall of the cut cell remained attached to the terminal cell (Fig. 1a). Before cutting, the cross wall between two cells is usually planar (not shown). Upon cutting a cell, the cross wall of the neighboring (terminal) cell became convex (Fig. 1a, arrowhead), due to the loss of the turgor pressure from the cut cell.

The next day, the cell wall of the cut cell fell away from the terminal cell (Fig. 1b). Then, a protuberance began to grow from the end of the terminal cell (Fig. 1c). It has been suggested that this protuberance grows via tip growth (Nagata 1973a). The protuberance elongated with time and looked like a rod (Fig. 1d, e). When filaments were further incubated, it branched and finally formed a rosette (Fig. 1f). These changes in morphology were very similar to those reported by Nagata (1973a). When the incubation time was prolonged, the number of rosette type rhizoids increased, indicating that this is the last stage of differentiation.

Nagata (1977) reported that a transparent material was secreted at the surface of terminal cells and rhizoids. Although the transparent material was speculated to be a glycoprotein, no further analysis has been carried out. Ridge et al. (1998) examined the secretion of glycoproteins at the root hair tips of various plants using fluorescently labeled lectins. In most cases, only the tip was stained. Based on the specificity of lectins, it is possible to tentatively identify the sugar residue of such glycoproteins. In the present study, we examined the binding of fluorescently labeled lectin to the transparent material secreted by *Spirogyra* during rhizoid differentiation. Nineteen kinds of fluorescently labeled lectins were examined using fully differentiated rhizoids (Table 1). As a result, five kinds of lectins were found to bind to the transparent material, but there were great differences in their efficiency of binding. Among these five lectins, BSL-I bound to the transparent material most strongly. BSL-I is contained in the seed extract of *Bandeiraea (Griffonia) simplicifolia*. In fully differentiated rhizoids, lectin-binding material(s) (LBM) distributed over the entire surface of the terminal cell. The

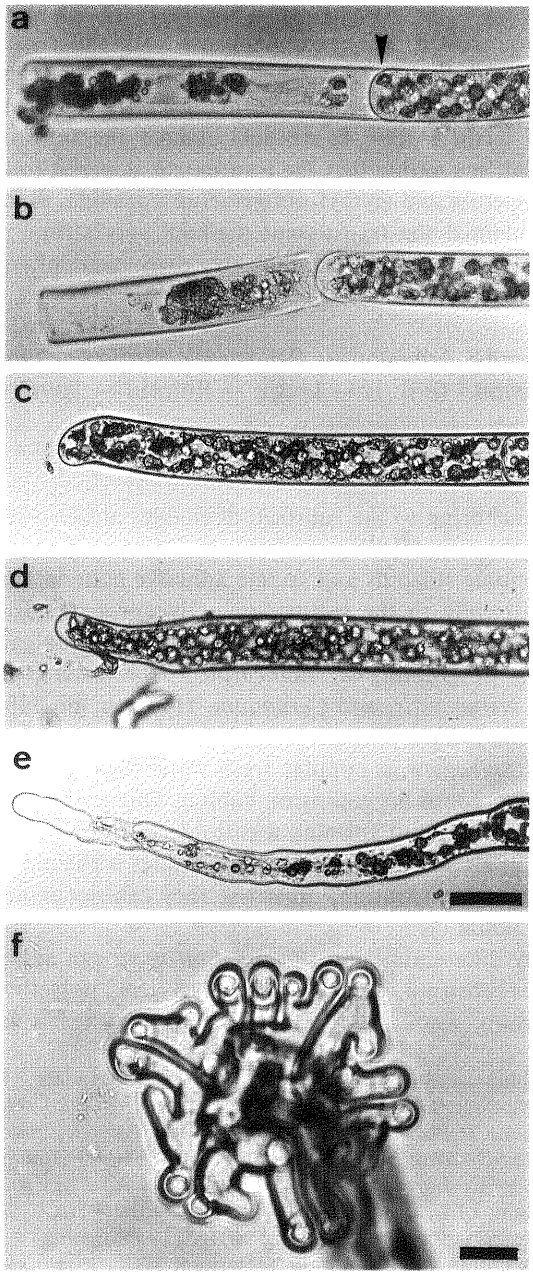

Fig. 1 Process of rhizoid differentiation, 18 h (a), 24 h (b), 27 h (c), 50 h (d), 94 h (e) and 132 h (f) after cutting the filament. An arrowhead indicates the convex cross wall of the terminal cell. Bar (a–e), 50 μm. Bar (f), 20 μm.

Table 1 Survey of various lectins for binding to the rhizoid differentiated cells

Lectins	Staining
BSL-I	◎
BSL-II	×
DBA	×
DSL	×
ECL	○
LCA	×
LEL	×
PHA-E	×
PHA-L	×
PNA	△
PSA	×
RCA-I	△
S-WGA	×
SBA	×
SJA	×
STL	×
UEA-I	×
VVL	△
WGA	×

BSL-I, *Bandeiraea (Griffonia) simplicifolia* lectin I; BSL-II, *Bandeiraea (Griffonia) simplicifolia* lectin II; DBA, *Dolichos biflorus* agglutinin; DSL, *Datura stramonium* lectin; ECL, *Erythrina cristagalli* lectin; LCA, *Lens culinaris* agglutinin; LEL, *Lycopersicon esculentum* lectin; PHA-E, *Phaseolus vulgaris* erythroagglutinin; PHA-L, *Phaseolus vulgaris* leucoagglutinin; PNA, *peanut* agglutinin; PSA, *Pisum sativum* agglutinin; RCA-I, *Ricinus communis* agglutinin I; S-WGA, succinylated *wheat germ* agglutinin; SBA, *soybean* agglutinin; SJA, *Sophora japonica* agglutinin; STL, *Solanum tuberosum* lectin; UEA-I, *Ulex europaeus* agglutinin I; VVL, *Vicia villosa* lectin; WGA, *wheat germ* agglutinin.
Rhizoid was stained strongly (◎), weakly (○), very weakly (△), not stained at all (×).

intensity of fluorescence was strongest at the area of the rhizoid (Fig. 2e). LBM was observed only on the surface of the terminal cell differentiating a protuberance or rosette. Terminal cells differentiating neither protuberance nor rosette did not secrete LBM (Fig. 3c right). The red fluorescence in Figs. 2 and 3 is from chlorophyll.

Since this analysis using fluorescence is very sensitive, the secretion of LBM in the early stages of rhizoid differentiation could be examined (Fig. 2a–d). Immediately after cutting the filaments, no LBM was observed (Fig. 2a). LBM appeared at the tip of the terminal cell soon after (12–24 h) the wall of the cut cell detached from the terminal cell (Fig. 2b). LBM was also observed on the cell wall of the cut cell fallen away from the terminal cell (Fig. 2b arrowhead). This was observed in all filaments examined. However, it is difficult to speculate the mechanism of secretion from a dead cut cell. One possibility is that LBM secreted from a terminal cell was absorbed to a limited area of the dead cut cell. The area of LBM distribution increased with time (Fig. 2c). When the protuberance grew, the surface of the whole protuberance was stained strongly. Weak staining began to be observed in the basal region of the terminal cell (Fig. 2d). When the rhizoid was fully developed, the whole surface of the terminal cell was stained with BSL-I, and the staining was stronger in the area of the rhizoid (Fig. 2e). If the incubation time was prolonged up to 14 d, fluorescence was also observed over the whole surface of the neighboring cell in some filaments (data not shown). However, further proximal cells so far examined were not stained.

When filaments of *Spirogyra* were cut with scissors, shorter filaments composed of a few cells were occasionally prepared (Fig. 3). In such small filaments, both ends of the single filament can be observed. Filaments incubated for 3–4 d after cutting were examined. Even in a single cell, rhizoid differentiation can be demonstrated with a strong fluorescence of labeled BSL-I. Both ends of the two cell fragment stained strongly. The whole cell surface was stained in the left filament, but not in the right filament in Fig. 3a. Sometimes, such single cells differentiated two rod type rhizoids at both ends (data not shown). Figure 3b shows a filament composed of three cells. Both ends were stained strongly, indicating the start of differentiation at both ends. The filament in Fig. 3c was composed of four cells. Differentiation started only at one end, as indicated by BSL-I staining.

Staining with BSL-I of differentiated rhizoid is shown at higher magnification in Fig. 4. The refractive index of the transparent material seems to be close to that of incubation medium, so it was hard to observe under differential interference optics (Fig. 4a). The cell wall showed strong contrast against the background, indicating high refractive index. The same area was observed with a fluorescence microscope (Fig. 4b). It is evident from the figure that the labeled material is deposited outside of the cell wall and, therefore, must have been excreted through the cell wall. In this photomicrograph, fluorescence from chlorophyll was eliminated using a cut-filter. After the fluorescence observation, the same fragment was stained with ruthenium red (Fig. 4c). As reported by Nagata (1977), the cell wall was stained, indicating that pectic mucilage is contained in the cell wall. The transparent material was weakly stained (Fig. 4d), probably due to non-specific adsorption.

We thus successfully stained the transparent material secreted during rhizoid differentiation. The specificity of BSL-I suggests that the transparent material contains α-D-galactose and methyl-α-D-galactopyranoside (Ridge et al. 1998). From other kinds of lectin, ECL, PNA, RCA-I, and VVL, also bound to the transparent material, albeit only weakly (Table 1). These lectins are classified as D-galactose

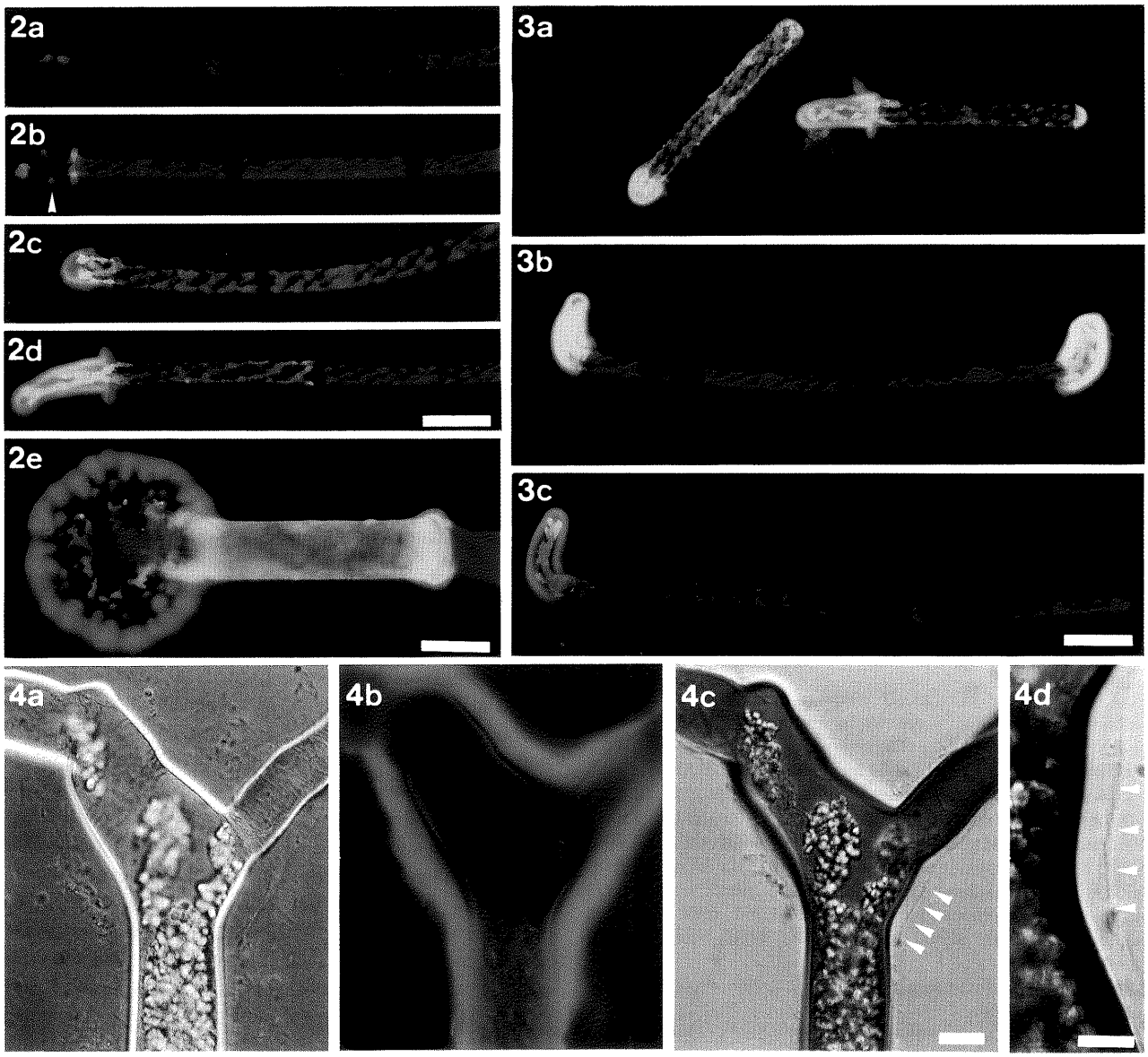

Fig. 2 Time course of LBM secretion. Immediately after cutting (a), 12 h (b), 19 h (c), 3 d (d) and 7 d (e) after cutting. Bar, 100 μm for a–d, 50 μm for e.

Fig. 3 LBM secretion in short filaments. a, Two filaments composed of single cells (3 d). b, A filament composed of three cells (4 d). d, A filament composed of four cells (4 d). Bar, 100 μm.

Fig. 4 Higher magnification of branched area of a rhizoid observed 22 d after cutting. a, Differential interference image. b, Fluorescence image of BSL-I staining. c, Differential interference image after staining with ruthenium red. d, a part of c indicated by arrowheads was magnified. Arrowheads indicate transparent material weakly stained with ruthenium red. Bar, 20 μm for a–c, 10 μm for d.

or *N*-acetyl-D-galactosamine specific lectins. The presence of galactose in LBM was supported by the following two results. (1) In the presence of 10 mM D(+)-galactose, the transparent material was not stained by fluorescently labeled BSL-I. (2) When rhizoids were pretreated with unlabeled BSL-I (20 μg ml^{-1}) for 2 h, they were not subsequently stained by fluorescently labeled BSL-I (data not shown). Although DBA, SBA and SJA are also D-galactose or *N*-acetyl-D-galactosamine binding lectins, these lectins didn't bind to the transparent material. The possible involvement of a higher-order structure in recognition of LBM by lectins is suggested.

It is still an open question whether the LBM contained in the transparent material, per se, is responsible for

adhesion of filaments to the substratum. However, since BSL-I stained very sensitively and the staining was observed only in the differentiated terminal cells, this fluorescently labeled lectin can be used as a probe to detect the early events of rhizoid differentiation in *Spirogyra*.

References

Nagata, Y. (1973a) Rhizoid differentiation in *Spirogyra*. I. Basic features of rhizoid formation. *Plant Cell Physiol.* 14: 531–541.

Nagata, Y. (1973b) Rhizoid differentiation in *Spirogyra*. II. Photoreversibility of rhizoid induction by red and far-red light. *Plant Cell Physiol.* 14: 543–554.

Nagata, Y. (1977) Light-induced adhesion of *Spirogyra* cells to glass. *Plant Physiol.* 59: 680–683.

Nagata, Y. (1979) Rhizoid differentiation in *Spirogyra*. III. Intracellular localization of phytochrome. *Plant Physiol.* 64: 9–12.

Ohiwa, T. (1977) Preparation and culture of *Spirogyra* and *Zygnema* protoplasts. *Cell Struct. Funct.* 2: 249–255.

Ridge, W.R., Kim, R. and Yosida, F. (1998) The diversity of lectin-detectable sugar residues on root hair tips of selected legumes correlates with the diversity of their host ranges for rhizobia. *Protoplasma* 202: 84–90.

Vreeland, V. and Epstein, L. (1996) Analysis of plant-substratum adhesives. *Modern Methods Plant Anal.* 17: 95–116.

(Received March 3, 1999; Accepted July 12, 1999)