Light-induced Adhesion of *Spirogyra* Cells to Glass

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**ABSTRACT**

Adhesion of *Spirogyra* (tentatively, *Spirogyra fluviatilis*) cells to glass is described. The cells of an algal filament can adhere to a substrate only when they are located at the end of the filament. Rapid adhesion is induced by blue-violet light (blue adhesion) as well as by temperature shift (about 6 C to about 22 C) or shaking (dark adhesion). Adherent cells detach in 1 hour in the absence of one of these stimuli. Slow adhesion is induced by red light (red adhesion) 1 hour after irradiation, and may be controlled by phytochrome. A cell once caused to adhere by red light does not release from the glass.

Adhesion seems to be maintained by a cementing substance, probably a mucoprotein. A transparent material which appears around the tip of the cell may be the cementing substance.

Light has a great influence on the life of most organisms and adhesion is another important factor concerned in differentiation. Only one example of light-induced adhesion has been known, that reported by Tanada (13, 14). Phytochrome controls the rapid adhesion of excised root tips of barley or mung bean to a glass surface.

We isolated a species of *Spirogyra*, a green alga, which grows in small streams attached to the beds. The filament can adhere to various kinds of substrates. This adhesion which can be induced by light is also a rapid phenomenon. We wondered by what mechanism the adhesion is caused and maintained.

**MATERIALS AND METHODS**

Axenic filaments of *Spirogyra* (tentatively, *Spirogyra fluviatilis*) were cultured at 20 C under a 12 hr-12 hr light-dark cycle with fluorescent light in Darden's medium (3).2

The vessel used for these experiments consisted of a glass ring (1.6 cm in diameter and 1 cm high) sealed above a soda glass slide. It was carefully cleaned with acetone, soap, tap water, and distilled H2O.

Colored light was obtained by placing interference and glass filters between the light source and the stage of the microscope. *Spirogyra* filaments in the vessel were irradiated on the stage.

*Spirogyra* filaments were cut into about 1 mm length, containing four to nine cells. Immediately after cutting, 10 to 20 short pieces were placed in 1 ml of culture medium in a vessel and incubated at 20 C in the dark for 24 hr. After the dark incubation, they were subjected to various treatments, e.g. colored light irradiation, temperature shift, and shaking. The number of adherent cells was then counted under an inverted type microscope with green (500 nm) safelight, shaking the vessel by hand in order to judge whether a cell had adhered to the slide glass or not. Cut filaments were also used to investigate the effects of enzymes and salts. Dark-incubated filaments were scooped up with a stainless steel mesh, washed with distilled H2O, and put into a new vessel which contained the test solution, under dim green light. Then they were irradiated or chilled to induce adhesion. To examine the effects of solutions on adherent cells, the culture medium in the vessel was removed by inverting the vessel, and filaments remaining on the bottom of the vessel were washed with distilled H2O. Then enzyme or salt solutions were added to the filaments.

The adhesion rate was calculated as the percentage of end cells which were not released from the substrate by the shaking (under "Results" for reasons for using this method of calculation).

All experiments were carried out in a temperature-controlled room kept between 20 and 24 C, ± 1 C except where otherwise noted.

Further details were previously reported including the isolation (11, 12).

All enzymes used here were supplied commercially: pronase from Kaken Chemicals (Japan); trypsin type I and hyaluronidase type I from bovine testes from Sigma Chemical; cellulase from Kyowa Hakko (Japan); RNase from Worthington Biochemical; α-amylase from bacteria from Nagase (Japan). Other chemicals used were reagent grade.

**RESULTS**

*Spirogyra* cells attached to all of the substrates examined: glass, polystyrene, polyethylene, aluminum, wood, filter paper, Teflon, and agar. In the present experiment, a glass slide was chosen as the substrate.

All of the cut filaments in the vessel sedimented easily toward the glass bottom to lie there at full length. A cut filament adheres to the glass substrate by its end cell(s), especially by the end cell tip(s), with very few exceptions (Fig. 1). The end cells become adhesive 24 hr after the filaments are cut and shed residual broken cells. They remain adhesive for a few days. Under optimal conditions, almost all filaments (up to 97% of adhesion rate) attached by both their end cells. This shows that all cells of the filaments have the potential for adhesion. Therefore, the adhesion rate was calculated as the percentage of end cells which were not released from the substrate by the shaking.

**FACTORS INDUCING ADHESION**

Once adhesion began, the adhesion rate increased within a few minutes. What factors induced the adhesion? The effect of light was investigated. As shown in Figure 2, the effect was not the same with different colors of light, i.e. blue (480 nm) and violet (440 nm) light caused a rapid adhesion (blue adhesion) while red light (645 nm) manifested its influence 30 to 60 min.
after a brief irradiation (red adhesion). Curve A, which expresses the effects of different colors just after the irradiation, resembles the visible part of the absorption spectrum of carotin or riboflavin. This shows that a carotenoid or flavonoid may be a light receptor for the blue adhesion. The effect of red light on red adhesion could be reversed by subsequent exposure to far red light (720 nm) (Table I). The repeated reversibility strongly suggests that the phenomenon is controlled by the phytochrome system.

It was also found that the Spirogyra cell could attach to the glass surface without light (dark adhesion). Cut filaments incubated in the dark were then chilled at a temperature between 4 and 8°C for 1 hr in a dark refrigerator and observed with green safelight after varying time intervals of darkness at room temperature. The adhesion rate increased 10 min after chilling (Fig. 3). Cells kept at low temperature did not become adherent even if irradiated with light effective at room temperature. A rise in the temperature of the surrounding culture medium is likely to be a stimulus for adhesion. Another way to induce dark adhesion is to agitate the filaments (Fig. 3). The maximum adhesion rate was obtained at room temperature 5 min after shaking by hand for 1 min. It is possible that repeated contacts of the cell with the glass or flow of the medium around the cell also could be a stimulus to adhesion.

With both the dark adhesion and the blue adhesion, most of attached cells came off the substrate 30 to 60 min after stimulation, and all cells were detached by the next day. This contrasts with the red adhesion in which most adhered cells did not detach. These cells differentiated into rhizoid cells 8 to 16 hr after irradiation (12).

**MECHANISM OF ADHESION**

**Evidence Suggesting Involvement of Cementing Substance in the Adhesion.** What mechanism mediates these adhesions? Coulombic force is unimportant as the major force because: (a) cation (AG 50W-X8, H+ form) and anion (Dowex 2-X, OH− form) exchange resins attached equally well to filament tips when cut filaments and resin were shaken together. Cation exchange resin attached to the filament uniformly both before and after (white light-induced) adhesion of the filament to glass while anion exchange resin attached only to the adherent filament tips. This also implies secretion of a substance during the adhesion process; (b) Tanada's treatment (13) of the soda glass with phosphate had no effect on the adhesion rate induced by white light; (c) many substrates examined, glass, polystyrene, polyethylene, alminum, wood, filter paper, Teflon, and agar, were attached well by cut filaments whether the substrates were polar or not; (d) EDTA (10−3 M) in the culture medium inhibited sharply the adhesion.

**Table I. Reversibility of red light effect on adhesion by far-red light**

Cut filaments, incubated for 24 hr at 20°C in the dark, were irradiated with different colored lights (68 ergs·cm−2·sec−1 for 3 min). Adhesion rates were measured with and without dark incubation at room temperature after the irradiation. A: 0-min; B: 30-min; C: 60-min dark incubation; D: dark control. For different lengths of dark incubation, different vessels, each containing 10 to 20 of the cut filaments, were used. 23°C. Each point represents the mean of three separate experiments.

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Fig. 2. Time course of adhesion induced by light of different wavelengths. Cut filaments, incubated at 20°C for 24 hr in the dark, were irradiated with different colored lights (68 ergs·cm−2·sec−1 for 3 min). Adhesion rates were measured with and without dark incubation at room temperature after the irradiation. A: 0-min; B: 30-min; C: 60-min dark incubation; D: dark control. For different lengths of dark incubation, different vessels, each containing 10 to 20 of the cut filaments, were used. 23°C. Each point represents the mean of three separate experiments.
hibited cell adhesion (by 85%) but it did not detach already adherent cells from the substrate.

However, some salts seem to play an important role in the adhesion. In distilled H₂O, no attachment could take place. In the presence of Ca(NO₃)₂, CaCl₂, MgCl₂, MgSO₄, NaCl, NaNO₃, KCl, or KNO₃ (10⁻⁴, 10⁻³, 10⁻², or 10⁻¹ M), the blue adhesion (also the temperature shift-induced dark adhesion) was restored more or less. An adhesion rate comparable to that in the culture medium was obtained in 10⁻² M Ca(NO₃)₂ (95% of the rate in the culture medium control), 10⁻² M Ca(NO₃)₂ (91%), 10⁻² M CaCl₂ (91%), and 10⁻² M MgSO₄ (78%). The concentrations seem high, but they are roughly the same as the ionic strength of the culture medium (cf. footnote 2). The effect of salts is not due to osmotic pressure of their solutions because polyethylene glycol (mol wt 500–600), sucrose, and sorbitol (10⁻⁴, 10⁻³, 10⁻², and 10⁻¹ M) were not effective; no adhesion took place in these solutions. These results together with the above result of EDTA effect suggest that these salts may act either as stabilizers buried in some adhesive (described below) rather than bridges between glass and the cell or as stimuli which induce an adhesive exudation.

Hence, involvement of some adhesive, cementing substance in the adhesion between the tip and the glass substrate was investigated. To search for such a substance, cells which had just adhered under white light were treated with various digestive enzymes, in concentrations of 0.01% to 0.1%. Pronase, trypsin, and hyaluronidase detached significant numbers of cells from the substrate. The effects of cellulase and RNase were equivocal and that of α-amylase was not appreciable. Thus, adhesion may be mediated by a substance which is proteinaceous in character, probably mucoprotein.

**Transparent Material on the Tip of the Adhered Cell.** Nothing could be detected on the outer surface of the tip of the adhered cell by staining with toluidine blue, alician blue, or ruthenium red, but a transparent layer was found by placing carbon granules of India ink around the cell (Fig. 4). The material forming the transparent layer was adhesive judging from the fact that dust was often easily trapped by it. The material was observed in blue and red adhesion as well as dark adhesion but not in controls. It was observable 3 to 10 min after attachment, and then increased in mass. It remained on the rhizoids developed from red adhered cells, but it disappeared soon, if the cell did not form a rhizoid and was released from the substrate. The substance on blue adhered cells was examined with an electron microscope (at ×40,000 magnification). Negative stained material exhibited filamentous structure. Treatment with cellulase (1%) caused the filaments to shortened or disappear, and trypsin (0.01%) appeared to break down the matrix surrounding the filaments.

These results suggest that the transparent material is a proteinaceous matrix within which are cellulose filaments. The filaments might be part of the cell wall secreted from the cell together with the proteinaceous material. It is probable that the transparent material is the cementing substance and that the

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**FIG. 3.** Time course of dark adhesion. ●—●: dark adhesion induced by temperature shift. Cut filaments, incubated at 20°C for 24 hr in the dark, were chilled at a temperature between 4 and 8°C for 1 hr and then were left at room temperature for 0, 30, and 60 min in the dark before counting. C: control, counted after 10 min of dark incubation at the room temperature without chilling. ○—○: dark adhesion induced by shaking. Cut filaments, incubated at 20°C for 24 hr in the dark, were shaken by hand for 1 min in the dark at room temperature and then treated the same as the filaments that had been temperature shifted. C: control, counted after 10 min of dark incubation at the room temperature without shaking.

**FIG. 4.** Transparent material around the tip of adhered cell (large arrow). Carbon granules of India ink surround the cell. The small arrow shows slime layers. The photograph was taken with an inverted type light microscope. × 200.
adhesion is a consequence of exudation of this material. The material is sticky and it appears only after cells become adherent.

**DISCUSSION**

The rapid adhesion (the blue and the dark adhesion) of *Spirogyra* is a new type of adhesion which has never been reported. Tanada (13, 14) found a red light-induced rapid attachment of root tips of barley and mung bean to glass surface charged with anion. Root apices in a beaker, previously treated with phosphate, attached to the bottom after red light irradiation and detached from it with far red light. The phenomenon is controlled by phytochrome. It may be caused by change of surface charge of the root tips (6, 15, 16). The rapid adhesion of *Spirogyra* differs from that of root tips in two ways: (a) It was induced not by red light but by blue (red adhesion appeared slowly); (b) it seems to be mediated by a cementing substance. Studying the mode of action of the inducing factors of the rapid adhesion, e.g. blue-violet light, temperature shift, and shaking, would be interesting. How can the factors affect on the cell membrane? In plant cells, blue (or blue-violet) light is known to induce change in permeability (8), ion absorption (10), and electrical response (9).

The rapid adhesion seems to be similar in mechanism to that of the zoospore of a ship-fouling alga, *Enteromorpha*. The zoospore can rapidly attach to glass by exudating a mucopolysaccharide-adhesive substance (2, 4). The adhesive production is completed within minutes of initial contact with the glass. However, the nature of the *Spirogyra* material may not be the same as that of *Enteromorpha* because the former was not digested with amylase which had a pronounced effect on *Enteromorpha* adhesion. In *Enteromorpha*, there is considerable electron microscopic and histochemical evidence showing that the adhesive mucopolysaccharide is secreted from certain vesicles in the Golgi region. Participation of the Golgi apparatus in secretion of polysaccharide is also known in maize root cap (7). The same event is a possible mechanism of the rapid adhesion of *Spirogyra*.

Other attachments mediated by mucoprotein are known in *Chlamydomonas* (5), and cyprids (1). The adhesion of many aquatic unicellular organisms, zoospores of algae, etc., may be induced and maintained by the same factor(s) and mechanism(s) as those of *Spirogyra*.

*Spirogyra* has transparent slime layers uniformly surrounding cell wall, but the slime layers are not sticky and, as apparent in Figure 4, are obviously different from the transparent material described here. One species of *Spirogyra* (17) can move by gliding with secretion of pectic mucilage, a clear substance covering the entire surface of most of the cells. The transparent material of the present report, on the contrary, was always at the tip of adherent terminal cells. This suggests different sites for secretion in these two cases. Also, the transparent material differs in nature from the pectic mucilage in that it could not be stained with ruthenium red. However, the mucilage secretion of gliding movement is likely to be a response to light since the movement is reported to start during the light period and stop about 2 hr after the light is turned off.

The physiological significance of the adhesion of *Spirogyra* might be explained as follows. When a filament is severed by some mechanical shock from its bottom portion (which is attached to the stream bed by a rhizoid), it will flow downstream. Blue-violet sunlight or contact stimulation may induce its terminal cells to secrete a cementing substance (the transparent material) and the filament will soon adhere to a solid substrate (blue or dark adhesion induced by shaking). The temporarily attached cell which receives red sunlight will adhere for longer time (red adhesion) and will differentiate into a rhizoid.

Rhizoid formation is induced by red light and controlled by phytochrome (12). The cell can form the rhizoid, a protuberance, without contacting the substrate but cannot differentiate into the rosette type of rhizoid which attaches to the substrate permanently (11). The adhesion may be necessary for the differentiation of the rosette type of rhizoid, unless other (unknown) stimuli can also induce this process. The present experiment suggests that phytochrome is also involved in the adhesion.

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