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# Effects of tunicamycin on sexual reproduction in heterothallic strains of *Closterium*

**Atsushi KATO* and Kimiko SASAKI**

The effect of tunicamycin on the sexual reproduction of heterothallic strains of *Closterinm peracerosum-strigosum-littorale*, KAS-4-29 (mating type-minus) and KAS-4-30 (mating type-plus), was investigated. At a concentration of 0.5 $\mu$g/m*l* of liquid mating medium, tunicamycin inhibited the sexual interaction between the opposite mating types almost completely, but the cell division about 45%. When the mating type-minus cells were treated with 0.5 $\mu$g tunicamycin/m*l* agar-mating medium, the ability to produce protoplast-releasing substance which induces protoplasts from the mating type-plus cells was lost. When the mating type-plus cells were treated as the same as above, the response to the substance disappeared significantly. These results suggest that the protoplast-releasing substance is a glycoprotein.

Heterothallic strains of *Closterium peracerosum-strigosum-littorale*, KAS-4-29 (mating type-minus, $mt^-$) and KAS-4-30 (mating type-plus, $mt^+$), seemed to be convenient materials for the study of mating process which is controlled by changing culture conditions. The morphological and biochemical properties of the cells change synchronously during the process (KATO *et al.* 1981). When the $mt^-$- and $mt^+$-cells were mixed in a nitrogen-depleted mating medium, they were sexually activated and form zygote within 60 hr. During the process, most cells paired and a few of the $mt^+$-cells unpaired: protoplasts were released from the paired cells and fused, whereas they were also released from the unpaired $mt^+$-cells and yet soon disrupted. A factor which was produced by the $mt^-$-cells and made the $mt^+$-gametangial cells to produce the protoplasts was presumed to be a glycoprotein (KATO *et al.* 1981). In this connection, effect of tunicamycin, an inhinitor of glycosylation of proteins containing asparagine linked sugar chains, on the gametic differentiation of *Chlamydomonas reinhardtii* has been studied (MATSUDA *et al.* 1981).

In this paper, we report that tunicamycin inhibits the sexual cell division and sexual activation of the $mt^-$ and $mt^+$-cells of *Closterium* and discuss on the involvement of some mating-specific glycoproteins in cell-cell recognition and interaction during the sexual reproduction process.

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## Materials and Methods

*Algal materials and culture conditions*

*Closterium peracerosum-strigosum littorale,* KAS-4-29 (mating type-minus, $mt^-$) and KAS-4-30 (mating type-plus, $mt^+$) were used in this study. Cells were grown vegetatively and sexual reproduction was induced as described previously (ICHIMURA 1971, KATO *et al.* 1981).

*Treatment with tunicamycin*

To see involvement of glycoprotein in mating process, equal number of

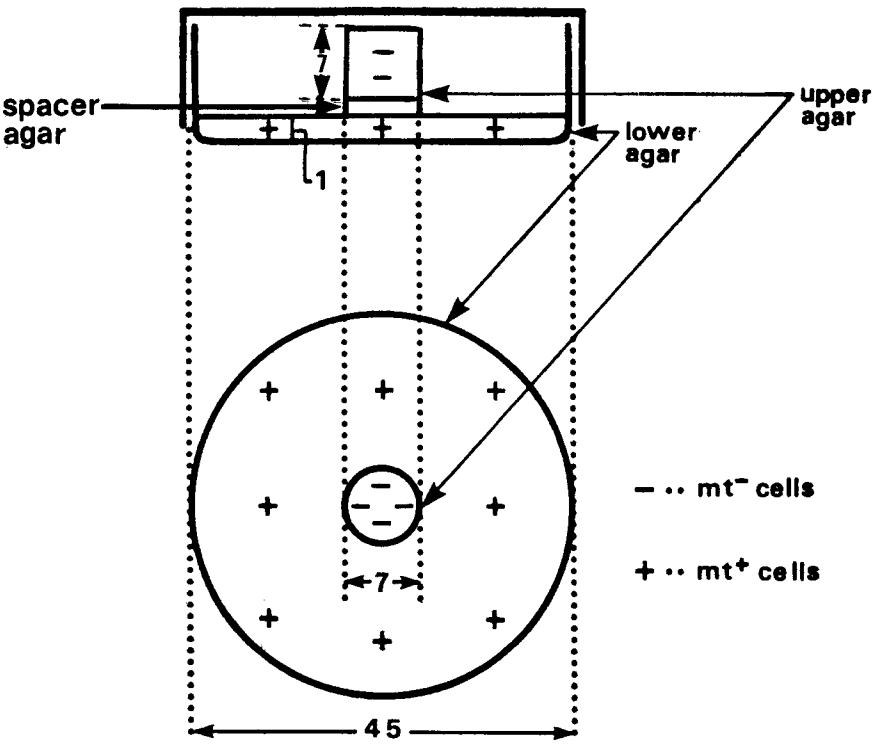


**Fig. 1.** Illustration of procedures for estimation of effect of PRS on the $mt^+$-cells. Agar disk (7 mm diameter and 3 or 7 mm height) containing the $mt^-$-cells, "upper agar", was placed on agar plate (1 mm thickness) containing the $mt^+$-cells, "lower agar", in a Petri dish (45 mm diameter). "Spacer agar" (1 mm thickness) was placed between the agar disk and agar plate to prevent diret contact between the $mt^-$- and $mt^+$-cells. By the procedure, PRS excreted from the $mt^-$-cells was diffused from "upper agar" to "lower agar" and reached to the $mt^+$-cells. As an indicator of the PRS effect, protoplast production from the $mt^+$-cells was used.

the $mt^-$ and $mt^+$-cells, $3\times10^3$ cells/ml, were mixed and cultured in liquid mating medium with or without tunicamycin, 0.1 to 100 $\mu$g/ml, and the degrees of cell division and sexual activation were measured. The sexual activity was expressed as the percentage of sexually activated cells, zygotes and protoplast-releasing $mt^+$-cells, in the total cells (KATO *et al.* 1981). Using tunicamycin, the sexual activity was expressed as % of control without the drug. Under the mating conditions, mating-specific "protoplasts-releasing substance (PRS)" was produced from the $mt^-$-cells and interacted with the $mt^+$-cells (KATO *et al.* 1981). To determine whether PRS is glycoprotein, either the $mt^-$ or $mt^+$-cells, $3\times10^3$ cells/m*l*, were treated with tunicamycin (0.5 or 1.0 $\mu$g/m*l* of 1% (disk) or 0.6% (plate) agar-containing mating medium) according to the procedure illustrated in Fig. 1. Sexual activity with the drug was expressed as % of control without the drug.

*Chemicals*

Tunicamycin was purchased from Sigma Chemical Co.

## Results and Discussion

In liquid mating medium, sexual activation (formations of paired cells, zygotes, and protoplasts from $mt^+$-cells) was inhibited completely with 0.5 $\mu$g/m*l* tunicamycin, and sexual cell division only about 53% (Table 1). These results indicate that cell-cell recognition and adhesion are mediated by mating-

TABLE 1. Effects of tunicamycin on the sexual cell division and sexual activation of *Closterium* in liquid mating medium

| Exp. (No.) | Tunicamycin ($\mu$g/m*l*) | Cell division (% of control) | Sexual activity (%)[a] | Sexual activity (% of control) |
|---|---|---|---|---|
| 1 | 0 | 100 | 74 | 100 |
| | 0.1 | 65 | 69 | 93 |
| | 0.5 | 53 | 1.2 | 1.6 |
| | 1.0 | 12 | 0 | 0 |
| 2 | 0 | 100 | 72 | 100 |
| | 1.0 | 21 | 0 | 0 |
| | 10.0 | 17 | 0 | 0 |
| | 100.0 | 0 | 0 | 0 |

Tunicamycin was added to the mixture of the $mt^-$- and $mt^+$-cells ($3\times10^3$ cells/m*l*). Cell number and sexually activated cells were measured after 60 hr of the mating culture. Cells divided once. The values without the drug was used as control.

a Percentage of sexually activated cells in total cells.

specific glycoprotein. A factor produced and excreted from the $mt^-$ cells, which acted on the $mt^+$-cells so as to let them release protoplasts and was designated as PRS (protoplast-releasing substance), was reported to seem likely to be a glycoprotein having molecular weight of 200 to 500 kdal (KATO *et al.* 1981). Therefore, it was proved by the experimental results with tunicamycin that the above presumption was not unreasonable.

When the $mt^-$-cells were treated with 0.5 $\mu$g/m*l* tunicamycin in agar disk containing mating medium, sexual activation of the $mt^+$-cells (release and disruption of protoplasts) was completely inhibited, whereas in the $mt^+$-cells treated by the same procedure, the activation was not inhibited completely (Table 2). These results suggest that PRS is a glycoprotein and produced in the $mt^-$-cells only under the mating conditions, and that receptor for PRS on the $mt^+$-cells is a glycoprotein. PRS-synthesizing system in the $mt^-$-cells seemed to be more sensitive to tunicamycin than the receptor-synthesizing system which has not be identified in the $mt^+$-cells. In *Clamydomonas reinhardtii*, it has been reported that the gametic flagellar membrane of the $mt^+$-cells may consists of glycoprotein with tunicamycin-sensitive chain, the half life of which is about 6 hr (MATSUDA *et al.* 1981). In the $mt^+$-cells of *Closterium* used here, receptor of PRS seemed to be present in an inactive state for mating unless they associate with PRS. The receptor has not been characterized yet. As the result of receptor-PRS interaction,

TABLE 2. Effects of tunicamycin on the sexual activation of *Closterium* gametes in agar containing mating medium

| Mating time (hr) | Sexual activity[a] Tunicamycin ($\mu$g/m*l*) | | | | |
|---|---|---|---|---|---|
| | 0/0 | 0/0.5 | 0/1.0 | 0.5/0 | 0.5/0.5[b] |
| 24 | 0 | 0 | 0 | 0 | 0 |
| 32 | 6 | 0 | 0 | 0 | 0 |
| 48 | 6 | 0 | 0 | 0 | 0 |
| 50 | 20 | 5 | 0 | 0 | 0 |
| 52 | 35 | 7 | 0 | 0 | 0 |
| 56 | 65 | 10 | 0 | 0 | 0 |
| 58 | 65 | 10 | 0 | 0 | 0 |
| 72 | 65 | 10 | 0 | 0 | 0 |

a Sexually activated $mt^+$-cells in "lower agar" were counted.

b Numerator and denominator represent tunicamycin contents in "upper agar ($mt^-$-cells)" and "lower agar ($mt^+$-cells)", respectively. Each of the values in the Table was average of three or four assays.

protoplast-releasing system may be activated and induce mating-specific cell-wall lytic enzyme. In paired cells, the enzyme degrades the cell walls of the $mt^-$- and $mt^+$-cells, thereby their protoplasts are released and fused to form zygospore. The enzyme seemed to be synthesized in the $mt^+$-cells by the contact with PRS. Since the $mt^-$-cells could not release their protoplasts without the direct contact with the activated $mt^+$-cells (KATO *et al.* 1981), the enzyme may be able to act on the $mt^-$-cells after the direct contact with the $mt^+$-cells. In unpaired $mt^+$-cells, the enzyme may act on themselves to release and degrade the protoplasts. In *Clamydomonas reinhardtii*, it has been reported that cell-wall lytic enzyme is induced in $mt^+$-cells by the contact with $mt^-$-cell flagellar substance (MATSUDA *et al.* 1978; TAMAKI *et al.* 1981). Induction of cell-wall lytic enzyme in the $mt^+$-cells by the contact with $mt^-$-cell substance may be mediated by a similar way as in *Clamydomonas* and *Closterium*.

Tunicamycin (TAKATSUKI *et al.* 1971) has been used as a specific inhibitor of the glycosylation of proteins which have important role in biological processes. Important role of glycorprotein in sexual interactions in yeast (MANNEY *et al.* 1981) and in *Volvox* (KOCHERT 1981) has been reported.

Further experiments on isolation, purification, and characterization of PRS and PRS-receptor in *Closterium* should be need to elucidate the mechanism of cell-cell recognition and interaction for the mating.

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