GAMETIC RECOGNITION: LACK OF ENHANCED GLYCOSYLTRANSFERASE ECTOENZYME SYSTEM ACTIVITY ON NONSEXUAL CELLS AND SEXUALLY INCOMPATIBLE GAMETES OF CHLAMYDOMONAS

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SUMMARY: Glycosyltransferase ectoenzyme system activity was found on (+) and (-) gametes of Chlamydomonas moewusii and membrane vesicles derived therefrom; upon mixing of (+) and (-) gametes or vesicles the activity was enhanced. Mixing of (+) gametes of C. moewusii with the sexually noncompatible (-) gametes of C. reinhardtii resulted in no enhancement of glycosyltransferase ectoenzyme system activity. Mixing of the sexually incompetent (+) and (-) vegetative stage vesicles of C. moewusii also resulted in no enhancement of glycosyltransferase ectoenzyme system activity. These results further substantiate the fact that glycosyltransferase ectoenzyme systems may be important in gametic recognition.

It is of interest that despite the striking advances made of late in our understanding of cellular membranes, it still remains unclear how two cells adhere to each other or how cells in general adhere to substratum. Furthermore, molecular mechanisms by which cells "recognize" each other in functions such as "sorting out," gametic recognition, or cellular aggregation, etc., have been advanced but certainly have not been conclusively proven. Such mechanisms usually fall into one of two groups: (a) mechanisms in which a cellular substance is elaborated and secreted by the cell and is postulated to act as a "glue" or mediator in the adhesion or recognition phenomena or (b) mechanisms in which components of the external surface of the plasma membranes actually act in a lock-and-key manner to mediate recognition or adhesion.

An interesting mechanism falling into group (b) above is the glycoconjugate:glycosyl ectoenzyme system initially postulated by Roseman (1). In this mechanism cell external glycoconjugate:glycosyltransferases and their complementary acceptor glycoconjugates would be located on the external cell surface. Formation of the activated intermediate (enzyme:substrate complex) in the ectoenzyme transferase reaction is postulated to mediate recognition or actually to hold one cell to another (2). Since the initial postulation (1), numerous reports have appeared confirming the existence of glycosyl ectoenzyme systems (2-6) although a report (7) has appeared

repudiating such ectoenzymes. In any case, it has been extremely difficult to definitively establish a cause-and-effect relationship between the surface glycosyltransferase systems and actual biological adhesion, recognition, agglutination, etc., phenomena.

Recently (8) it was determined that in the algae Chlamydomonas the active factor in the mating reaction between (+) and (-) gametes was actually in the growth medium as a membrane vesicle and not as a single macromolecule. Even more recently (9,10) it has been shown that these (+) and (-) gametes from Chlamydomonas moewusii and active membrane vesicles from these (+) and (-) gametes have glycosyltransferase ectoenzyme system activity, and that upon mixing (mating) of appropriate (+) and (-) gametes or vesicles this glycosyltransferase ectoenzyme system activity is significantly enhanced. In the present report experiments showing that mixing of sexually incompetent vegetative (+) and (-) vesicles and mixing of (+) gametes of C. moewusii with (-) gametes from the sexually incompatible species of Chlamydomonas, C. reinhardtii, resulted in no enhancement of glycosyltransferase ectoenzyme system activity are presented.

MATERIALS AND METHODS

Cells and culture. The (+) and (-) mating types of the unicellular flagellate Chlamydomonas moewusii and the (-) mating type of Chlamydomonas reinhardtii were grown on growth medium agar (8-10) for 8-10 days at 25 ± 1°C in north light. Cells were then suspended in liquid induction medium to induce gametogenesis (8-10). Vegetative cells (cells not responsive in the mating reaction) were obtained by supplementing the liquid growth medium with 0.1% NH_4Cl (11). All cultures were axenic, and aseptic techniques were used during induction. Vegetative cells are not distinguishable from gametes except that gametes of one mating type will adhere to gametes of the opposite mating type; i.e., (+) gametes will adhere to (-) gametes, but (+) gametes will not adhere to (-) vegetative cells. Additionally, (+) and (-) vegetative cells have no affinity for each other (11); C. reinhardtii is sexually incompatible with C. moewusii, i.e. (+) C. moewusii gametes will not adhere to (-) C. reinhardtii gametes.

Isolation of vesicles. Membrane vesicles were isolated by differential centrifugation from the induction medium as described (8-10) after gametes were incubated 24-48 hr.

Analytic procedures. Glycosyltransferase assay procedures and particulars concerning substrates (preparation, specific activity, etc.) followed those previously reported (2,5,9). Gametes were washed in induction medium,

Table 1. Glycosyltransferase ectoenzyme system activity of various active *Chlamydomonas* gametes

Substrate	*C. moewusii*			*C. reinhardtii*	*C. moewusii* (+) plus *C. reinhardtii* (-)*
	(+)	(-)	(+) plus (-)*	(-)	
UDP-galactose	1400	1300	3400	900	1200
UDP-glucose	1300	1300	2800	800	800
UDP-N-acetyl-glucosamine	700	1400	2800	600	700
CMP-N-acetyl-neuraminic acid	600	900	2300	400	700
GDP-mannose	900	1300	2300	800	900
GDP-fucose	1000	1100	2200	1000	900

The surface activity system contained 10 µl of the ^{14}C-monosaccharide nucleotide phosphate (approx. 10^{-9} mole), 10 µl of 0.1 M $MgCl_2$, 0.1 M $MnCl_2$, 10 µl of 0.1 M Tris, pH 7.6, and 50 µl of the active gametes (about 1 mg protein) suspended in 0.145 M NaCl. The final pH of the reaction was 6.9. After 30 min incubation macromolecular-bound radioactivity was determined (2,9). Data are cpm per mg protein. All data have background subtracted. Data are means from 5 determinations and are rounded to nearest hundred.

*For the mixed experiments, 25 µl of the (+) and 25 µl of the (-) were the enzyme/acceptor fraction in the assay. In all experiments whole active gametes were used for the assay. The gametes were always kept separate for the experiments and incubated in the above assay solution for 30 min except for the mixed experiments, in which the (+) and (-) gametes were mixed just before the 30-min incubation.

and vegetative cells and derived vesicles were washed in growth medium. The surface activity assay contained 10 µl of the nucleotide phosphate ^{14}C-monosaccharide (approx. 10^{-9} mole), 10 µl of 0.1 M $MgCl_2$, 10 µl of 0.1 M $MnCl_2$, 10 µl of 0.1 M Tris, pH 7.6, and 50 µl of the active vesicles (about 10 µg protein) or cells (about 1 mg protein) suspended in 0.145 M NaCl. The final pH of the reaction was 6.9. After 30 min incubation at either 25° or 37°C, 3 vol of 1% phosphotungstic acid in 0.5 N HCl were added, and the precipitate was centrifuged out of solution. The precipitate was washed twice with 10% trichloroacetic acid, once with ethanol:diethyl ether (2:1, v/v), and the resultant precipitate was dissolved in 1 N NaOH and radioactivity determined in a liquid scintillation counter. Protein was determined by the method of Lowry et al. using serum albumin as standard (12).

RESULTS AND DISCUSSION

The results in Table 1 indicate that when active (+) and (-) gametes of

Table 2. Surface and total glycosyltransferase ectoenzyme system activity of vesicles prepared from gametes and vegetative cells of Chlamydomonas moewusii

Substrate	Vesicles from gametes			Vesicles from vegetative cells		
	(+)	(-)	(+)plus(-)*	(+)	(-)	(+)plus(-)*
UDP-galactose	3800	4400	17,000	1700	1800	1900
UDP-glucose	5200	4700	19,000	2100	2200	2000
UDP-N-acetyl-glucosamine	5600	5000	22,000	2600	2900	2500
CMP-N-acetylneuraminic acid	6800	6100	49,000	3400	2700	3100
GDP-mannose	5300	5900	29,000	2700	2700	2900
GDP-fucose	3500	4100	19,000	1400	2400	2200

Systems of assay were exactly as given in Table 1 except that vesicles from active gametes or vegetative cells (about 10 µg protein) replaced the gametes in the assay. Data are cpm per mg protein, rounded to the nearest hundred, and are means from 5 independent determinations. All data had background subtracted.

* For the mixed experiments, 25 µl of the (+) and 25 µl of the (-) were the enzyme/acceptor fraction in the assay.

C. moewusii are mixed, there is a 2- to 3-fold enhancement of glycosyltransferase ectoenzyme system activity, as described previously (9,10). This indicates that during mating an elevation in surface glycosyltransferase system activity occurs, perhaps related directly to holding the two gametes together. Furthermore, it implies that a threshold phenomenon is operative; i.e., both the (+) and the (-) gametes have substantial glycosyltransferase ectoenzyme system activity; yet they do not adhere (mate)--it is only when mixing and mating occur that an enhancement in activity is observed. Most importantly, in Table 1, even though the C. reinhardtii gametes (-) have measurable surface glycosyltransferase system activity, there is no enhancement of activity upon mixing with the C. moewusii gametes (+). This implies that elevations in activity occur only when sexually compatible gametes are mixed (and only when mating/adhesion occurs). These data strongly suggest that the glycosyltransferase ectoenzyme systems may play some role, either primary or secondary (or unrelated; see below) in the phenomena of gametic recognition and/or gametic adhesion.

The data in Table 2 demonstrate that the (+) and (-) membrane vesicles previously shown (8) to contain the mating/adhesion factors when prepared from active (mating) gametes (vesicles were prepared separately from the

gametes and then mixed) show greatly enhanced activity of the glycosyltransferase ectoenzyme systems (9,10) when mixed. However, the data of Table 2 clearly show that when similar (+) and (-) vesicles are prepared from vegetative cells (i.e., sexually incompetent; unable to adhere) there is no elevation in glycosyltransferase ectoenzyme system activity. Thus only sexually competent complementary vesicles prepared from gametes capable of adhesion demonstrate the enhancement of glycosyltransferase ectoenzyme system activity.

The above results strongly implicate the glycosyltransferase ectoenzyme systems in the mating reaction. It is tempting to speculate that the enhancement of activity occurs during the mating reaction by membrane rearrangement and the systems function for gametic recognition or even gametic bonding by a lock-and-key (enzyme/substrate) mechanism. However, it is also possible that the enhancement of system activity is incidental to the mating reaction and merely represents increased membrane synthesis required for elaboration and secretion of a "glue" type of macromolecule necessary for adhesion. Finally, it is possible that these enzymes are actually responsible for synthesis of a glycoprotein adhesion substance and that the increased activity at the time of mating represents increased total cellular production of this glycoprotein.

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