Aggregation of Sponge Cells

A NOVEL MECHANISM OF CONTROLLED INTERCELLULAR ADHESION, BASING ON THE INTERRELATION BETWEEN GLYCOSYLTRANSFERASES AND GLYCOSIDASES*

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The biochemical and functional properties of β-glucuronidase and β-galactosidase, isolated and partially purified from the sponge Geodia cydonium, were studied. The two glycosidases are not only localized in the cytoplasm but are also associated at a high activity with the cell membrane.

The aggregation receptor, a low molecular weight cell surface-bound glycoprotein, is deglucuronylated by the action of the Geodia β-glucuronidase both in the state at which the receptor is bound to the cell surface and in its isolated form. The deglucuronylated aggregation receptor can be reglucuronylated enzymatically by the extracellularly occurring homologous glucuronosyltransferase.

Untreated cells lose their aggregation potency if they are incubated under conditions optimal for β-glucuronidase activity. Cells, depleted of membrane-bound β-glucuronidase, do not show any reduction of their aggregation potency under identical conditions. Cells that carry on their cell surface deglucuronylated molecules and are biologically characterized by only a low aggregation potency can be reglucuronylated in the presence of glucuronosyltransferase and UDP-glucuronic acid. These restored cells show again the original high aggregation potency.

From the results presented it is assumed that cell aggregation can occur after glucuronosylation of the aggregation receptor by the glucuronosyltransferase via a linkage of the aggregation factor with the aggregation receptor. Some evidence is presented, indicating that cell separation is the consequence of an activation of the cell membrane bound β-glucuronidase, which results in a deglucuronylation of the aggregation receptor.

Among the different biological models studied to understand cell-cell aggregation processes both on the cellular and biochemical level, the retina system (for a survey, see Refs. 1 and 2) and the sponge cell system (for a survey, see Ref. 3) are the most widely used. It is owing to Moscona (4), who succeeded to isolate an “extracellularly functioning material” from the sponge Microciona prolifera which promotes reaggregation of single cells. This “material” was later termed aggregation factor; in 1973 the factor was purified and characterized from the sponge Geodia cydonium (5, 6).

The aggregation factor of Geodia is associated with high molecular weight particles with a sedimentation coefficient (s20w) of 90 (6a); in the presence of minute amounts of Ca++, the particles form complexes which have a sedimentation coefficient of 3,000 (6). The Geodia particles occur in the intercellular space and are found to act multifunctionally. The first subunit identified was the aggregation factor (5, 7); later a series of glycosyltransferases was identified as subunits of the Geodia particles: sialyltransferase (8), glucuronosyltransferase (9), and galactosyltransferase (9). Hence, the Geodia particles represent a multiglycosyltransferase system associated with an aggregation promoting activity.

The Geodia particles, in particular the aggregation factor associated with them, bind to specific cell surface molecules, the so-called aggregation receptors, which are of low molecular weight (MW = approximately 20,000; s20w = 2.6) and consist chemically of glycoproteins (10). The termini of the aggregation receptor which are involved in the binding with the aggregation factor were determined to consist of glucuronic acid (11). A further cell membrane component, the antiaggregation receptor (MW = 180,000), a glycoprotein with D-galactose termini (12), modulates the activity of the aggregation factor; the antiaggregation receptor is most likely involved in a controlled segregation and subsequent reaggregation of the cells in randomly aggregated cell clumps.

Stimulated by the study of Aoyagi et al. (13), demonstrating the presence of a variety of glycosidasases on the surface of mammalian cells, we searched for the cell surface enzymes neuraminidase, β-glucuronidase, and β-galactosidase. Only the latter two enzymes could be detected, partially purified, and characterized. In addition, attempts were made to determine the role of both the β-galactosidase and the β-glucuronidase in the control of the reaggregation process, with the main emphasis on the latter enzyme. From our studies, we hypothesized that the β-glucuronidase and the glucuronosyltransferase might control the initial events of cell aggregation, mediated by the aggregation factor and the aggregation receptor, while the β-galactosidase and the galactosyltransferase might play a role in the secondary event the “sorting out.”

MATERIALS AND METHODS

Chemicals

p-Nitrophenyl-o-D-galactopyranoside, p-nitrophenyl-β-D-galactopyranoside, p-nitrophenyl-o-D-glucoside, p-nitrophenyl-β-D-glucoside, p-nitrophenyl-β-D-glucuronide, p-nitrophenyl-α-D-mannoside, p-nitrophenyl, UDP-glucuronic acid, ethylene glycol bis(β-aminoethyl...
glycosyltransferases (sialyltransferase, Ref. 8; glucuronosyltransferase, Ref. 9; galactosyltransferase, Ref. 9) as subunits, was used for the glycosyltransferase reactions. The specific activities of the enzymes were as follows: sialyltransferase 9.1 pkat/mg of protein, glucuronosyltransferase 18.3 pkat/mg of protein, and galactosyltransferase 27.7 pkat/mg of protein.

**Glycosyltransferase Assays**

The β-glucuronosyltransferase was measured in a reaction mixture containing 10 mM MgCl₂, 300 mM NaCl, 50 mM Tris-HCl (pH 8.2), 0.1 mM UDP-[U-¹⁴C]glucuronic acid (4 × 10⁸ dpm/mmol), 20 μl of deglucuronylated aggregation receptor (2.6 μg of protein/assay), and 30 μl of the enzyme sample in a final volume of 80 μl. The incubation was carried out for 60 min at 22°C. The acid-insoluble radioactivity was determined as described (9).

The β-galactosyltransferase assay was composed and performed as described for the glycosyltransferase assay with the exception that 0.1 mM UDP-[U-¹⁴C]galactose (5 × 10⁶ dpm/mmol) instead of UDP-glucuronic acid was used and degalactosylated aggregation receptor served as the acceptor.

**Isolation and Purification of β-Glucuronidase and β-Galactosidase**

All procedures were carried out at 0–4°C. A typical preparation (starting with 50 g of material) is summarized in Table I.

**Step 1: Initial Extract—**The sponge was cut into 2-mm cubes and suspended 1:3 (w/v) in 100 mM Tris-HCl buffer (pH 7.5) containing 5 mM 2-mercaptoethanol and 0.1% Nonidet NP-40 and homogenized with 10 strokes of a Dounce homogenizer. The homogenate was centrifuged at 20,000 × g for 30 min at 2°C. The supernatant (120 ml) was collected and contained 8.3 mg of protein/ml; it was concentrated in a dialysis tubing with polyethylene glycol to 25 ml (Fraction 1).

**Step 2: Sepharose 4B—**Fraction 1 was passed through a Sepharose 4B column (4.5 × 20 cm) to remove the high molecular weight aggregation factor (5). The column was previously equilibrated with 100 mM Tris-HCl buffer (pH 7.5; 100 mM NaCl and 5 mM 2-mercaptoethanol) and, after application of the sample, it was eluted with the same buffer. Three-milliliter fractions were collected. Both the β-glucuronidase- and the β-galactosidase activity appeared within the Kₑₒ (16) range 0.23 to 0.30 (Fraction 33-40). The pooled fractions (24 ml; Fraction 9) were directly subjected to a DEAE-Sephadex column.

**Step 3: DEAE-Sephadex—**Fraction 2 was applied to a DEAE-Sephadex column as described in Fig. 1. The two glycosidases (β-glucuronidase and β galactosidase) were obtained by stepwise gradient; they appeared after elution with 0.2 M NaCl. The enzyme-containing fractions (Fraction 24–28) were pooled; they contained 6.1 nkat of β-glucuronidase and 2.0 nkat of β-galactosidase in a total volume of 25 ml (0.88 mg of protein/ml). The enzyme fractions were concentrated in a dialysis tubing with polyethylene glycol to 2 ml (Fraction 3).

**Step 4: Sephadex G-100—**Fraction 4 was passed over a column of Sephadex G-100 (Fig. 2). The β-glucuronidase activity appeared as an asymmetric peak near the void volume (V₀/𝑉ₑ value: 1.3). The β-galactosidase activity was completely separated from the β-glucuronidase one; maximal activity was determined around a V₀/𝑉ₑ value of 1.9. The β-glucuronidase Fractions 14 to 19 were pooled (Fraction 4); this preparation contained the enzyme with a specific activity of 0.857 nkat/mg of protein (0.53 mg of protein/ml). Fractions 22 to 26 were discarded.

TABLE I

<table>
<thead>
<tr>
<th>Purification of β-glucuronidase and β-galactosidase</th>
<th>β-Glucuronidase</th>
<th>β-Galactosidase</th>
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<tr>
<td>Step and fraction</td>
<td>Specific activity</td>
<td>Total enzyme activity</td>
</tr>
<tr>
<td>nkat/mg</td>
<td>nkat</td>
<td>%</td>
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<td>1. Initial extract</td>
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<td>11.9</td>
</tr>
<tr>
<td>2. Sepharose 4B</td>
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<td>9.4</td>
</tr>
<tr>
<td>3. DEAE-Sephadex</td>
<td>0.277</td>
<td>6.1</td>
</tr>
<tr>
<td>4. Sephadex G-100</td>
<td>0.857</td>
<td>5.4</td>
</tr>
</tbody>
</table>

The abbreviations used are: CMF, calcium- and magnesium-free artificial sea water; ASW, calcium- and magnesium-containing artificial sea water; EGTA, ethylene glycol bis(β-aminoethyl ether)N,N′-tetraacetic acid; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid).
buffer; 2-n-J fractions were collected. The arrow marks the position was loaded with 2 ml of Fraction 3 and eluted with the equilibration and assayed for enzyme activity. X, A~80 nm; O---O, β-glucuronidase activity; O- - -0, β-galactosidase activity.

Tris-HCl buffer (pH 7.5; 200 mM NaCl and 5 mM 2-mercaptoethanol), 0.25 M and 0.5 M NaCl (----). Five-milliliter fractions were collected with 100 mM Tris-HCl buffer (pH 7.5; 100 mM NaCl and 5 mM 2-mercaptoethanol). For elution, the Tris buffer was stepped up with 100 mM Tris-HCl buffer (pH 7.5; 100 mM NaCl and 5 mM 2-mercaptoethanol). The purification scheme is summarized in Table I. A 71-fold increase in the specific activity of the β-n-glucuronidase was achieved, the one observed in the case of β-glucuronidase; the optimum activity was determined to be 0.652 nkat/mg of protein (0.23 mg of protein/ml).

The purification scheme is summarized in Table I. A 71-fold increase in the specific activity of the β-n-glucuronidase was achieved; in the case of β-d-galactosidase, a 217-fold enrichment was obtained. Under the standard incubation conditions used, the two enzyme preparations were free of the following glycosidases: α-d-galactosidase, α-d-glucosidase, β-d-glucuronidase, and α-d-mannosidase.

**Assay of Glycosidases**

The amount of a given glycosidase activity was determined using the p-nitrophenyl derivatives (17). The standard assay (1 ml) contained the following constituents: 10 mM sodium phosphate (pH 5.0), 200 mM NaCl, 5 mM 2-mercaptoethanol, 1 mM concentration of the p-nitrophenyl derivative and routinely 50 μl of the enzyme preparation. The incubation was usually carried out for 60 min at 22°C. The reaction was terminated with 1 ml of 0.4 M glycine/NaOH buffer (pH 10.5) and the absorbance of the released p-nitrophenol measured at 490 nm. The substrates used were the p-nitrophenyl derivatives from α-d-galactoside, β-d-galactoside, α-d-glucoside, β-d-glucuronide, and α-d-mannoside. p-Nitrophenol was used as standard.

For protein determination the method of Lowry et al. (18) was used. Neutral carbohydrates were determined according to the method of Dubois et al. (19); lipids, according to Hinsberg et al. (20); and nucleic acids, according to Mägdefrau et al. (21). The molecular weight, based on the V0/Vs value, was determined according to Determann (16).

The experiment revealed that the aggregation factor is devoid of α-d-glucosidase, β-d-galactoside, α-d-glucoside, β-d-glucuronide, and α-d-mannoside. p-Nitrophenol was used as standard.

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Polyacrylamide gel electrophoresis in dodecyl sulfate was performed according to Laemmli (22) with an 8% polyacrylamide gel. The glycoprotein was located by the periodic acid-Schiff reaction (23) and densitometric scanning was performed using an ISCO model UA4 absorbance monitor.

**RESULTS**

**Properties of β-D-Glucuronidase**—The partially purified sponge β-glucuronidase has a broad pH optimum between 6.5 and 4.0; at pH above 7.5 and below 3.0, its activity is lower than 20%. The divalent cations, Mg2+ as well as Ca2+ (at a molarity of 5 mM), have no effect on the enzyme activity; addition of 20 mM EDTA to the assay was also without influence on the enzyme reaction. For full enzyme activity, 200 mM NaCl must be added to the assay; in the absence of NaCl and in the presence of 500 mM NaCl, the enzyme activity is inhibited by more than 70%.

The temperature optimum was determined to be around 20°C. The temperature coefficient Q10 (24) at 20°C:32°C is 2.6 and at 22°C:32°C is 2.2. The molecular weight of the enzyme estimated from the elution behavior through Sephadex G-100 is approximately 80,000.

**Properties of β-D-Galactosidase**—The pH dependence of the partially purified β-galactosidase is almost identical with the one observed in the case of β-glucuronidase; the optimum is at 5.0. Addition of 5 mM Ca2+, 5 mM Mg2+, and 20 mM EDTA to the galactosidase assay is without influence on the enzyme activity. Estimations gave a salt optimum at 200 mM NaCl; at a low ionic strength (absence of NaCl), the activity decreased by 90% and, in the presence of higher salt concentrations (500 mM NaCl), the enzyme reaction is reduced by 35%.

The temperature optimum lies around 22°C. The Q10 value of 22°C:32°C is 2.9 and that of 22°C:32°C is 1.8. The apparent molecular weight of the β-galactosidase is about 25,000, as found by gel filtration with Sephadex G-100.

**Localization of β-Glucuronidase**—The β-glucuronidase activity was determined in the standard assay using either intact cells or subcellular fractions (cell-free extract, cell membrane fraction, and purified aggregation factor) as source (Table II). After incubation of intact cells in the standard glycosidase assay a β-glucuronidase activity of 10.4 pkat/107 cells was determined. From this finding alone, it was not possible to draw the conclusion, that a considerable amount of the enzyme is localized on the outer cell surface because of possible leakage phenomena which might occur during the incubation at pH 5.0 in the standard assay. Therefore, the main subcellular fractions, the cell-free extract, the cell membranes, and the aggregation factor were tested for their enzyme activity. The experiment revealed that the aggregation factor is devoid...
of detectable β-glucuronidase activity. The enzyme is predominantly located in the cell-free fraction; however, the membrane fraction (which has been pretreated (1 h; 0°C) with 0.1% Nonidet NP-40 to obtain full enzyme activity; Ref. 25) contains β-glucuronidase at a level of 3.7 pkat/mg of protein. From these data, we have strong indications that the β-glucuronidase is not only located in the cytoplasm but also in the cell membrane.

Localization of β-Galactosidase—In analogous studies, as outlined for β-glucuronidase, the localization of β-galactosidase was determined (Table II). Again, the enzyme is absent in the aggregation factor complex. β-Galactosidase activity was detected not only after incubation of intact cells and a cell-free extract in the standard assay but also after testing the cell membrane fraction. Therefore, it seems to be obvious that also the β-galactosidase is associated with cell membranes from Geodia.

Alterations of β-Glucuronidase- and β-Galactosidase Activity during Reaggregation—As a first approach to the question whether the two glycosidases, studied in the present work, might be involved in the aggregation process, the amount of membrane-associated enzymes was determined during the early phase of formation of aggregates from single cells in the presence of the aggregation factor. Under the incubation conditions used, the kinetics of aggregation was almost identical with the one described previously (26). Aggregation factor-mediated reaggregation process starts after a lag phase of about 60 min and is completed 4 h after addition of the factor (formation of aggregates with a diameter of 2 to 3 mm).

After incubation for different periods of time, both the single cells and the cell aggregates were harvested (total, 25 ± 5 x 10⁷ cells) and then treated to isolate the cell membranes. Subsequently, the membrane-associated β-glucuronidase- and β-galactosidase activity was determined. The results, plotted in Fig. 3, show for the two enzymes a strong increase of the glycosidase activities during the reaggregation process. From previous studies (3) it is known that the glycosyltransferases, as well as the aggregation factor, carrying high molecular weight particles remain integer and stable throughout the aggregation process. The particles can be reisolated after reaggregation by incubation of the aggregates in EGTA with a yield greater than 94%. Due to the fact that EGTA chelates selectively Ca²⁺, the activation ion for the transferases, magnesium remains in the ASW which is used as aggregation medium. Therefore, a determination of the transferase activity can be performed without a possible interference by the chelating agent. The experiments revealed (Fig. 4) that both the β-glucuronosyltransferase and the β-galactosyltransferase activity remains constant during the early aggregation phase.

Influence of β-Glucuronidase on Aggregation Receptor—From cytological experiments, it is known that the α-glucuronic acid moieties of the aggregation receptor play an important role in its binding to the aggregation factor (11). Analytical studies revealed that 8.9% of the aggregation receptor consists of α-glucuronic acid (11). Hence, it seemed promising to check whether the β-glucuronidase, partially purified from the sponge Geodia, can use the aggregation receptor isolated from the same source as a substrate. Therefore, the aggregation receptor was labeled in vivo with [1-¹⁴C]glucuronic acid and subsequently partially purified (see “Materials and Methods”). Analysis of the aggregation receptor by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate revealed that the main radioactivity was observed at a relative
treated with 20 mM EGTA (final concentration). After 20 min, the aggregates were completely redissociated into single cells. The suspension was centrifuged (20,000 × g; 10 min; 2°C) and the cell-free supernatant was dialyzed (12 h, 2°C) and the cell-free supernatant was dialyzed (12 h, 2°C) against saline followed by subsequent lyophilization. The residue was dissolved in 50 μl of distilled water and a 30-μl aliquot was assayed for both β-glucuronosyltransferase activity (△△△△A) and β-galactosyltransferase activity (△△△△△).

Fig. 4. Influence of the aggregation process on the activity of the glycosyltransferases, associated with the extracellular high molecular weight particle. Dissociated single cells were incubated in the presence of the high molecular weight particle, also carrying the aggregation factor, as described in Fig. 3 and under "Materials and Methods." At different time points, the assays, containing the aggregates, were treated with 20 mM EGTA (final concentration). After 20 min, the aggregates were completely redissociated into single cells. The suspension was centrifuged (20,000 × g; 10 min; 2°C) and the cell-free supernatant was dialyzed (12 h; 2°C) against saline followed by subsequent lyophilization. The residue was dissolved in 50 μl of distilled water and a 30-μl aliquot was assayed for both β-glucuronosyltransferase activity (△△△△A) and β-galactosyltransferase activity (△△△△△). mobility, characteristic for the authentic, almost homogeneous, aggregation receptor (Fig. 5, A and B). The [14C]glucuronic acid-labeled aggregation receptor was then treated with the homotypic β-glucuronidase (see legend to Fig. 5) and subsequently analyzed by polyacrylamide gel electrophoresis (Fig. 5C). It was found that only 65% of that radioactivity, determined for the aggregation receptor band before the digestion, is located at the position of the aggregation receptor. The main amount of radioactivity migrates with a relative mobility of around 1.0, indicating that the radioactivity, released after enzymatic treatment of the aggregation receptor preparation with β-glucuronidase, is of low molecular weight. In addition, it is obvious that both the radioactivity band (Fig. 5C) and the periodic acid-Schiff-positive band (Fig. 6B), which are detected after digestion of the preparation with β-glucuronidase, are identical with the band of the authentic, untreated aggregation receptor.

Reglucuronylation of the Aggregation Receptor—From the previous section we have to conclude that the aggregation receptor is accessible to an enzymatic digestion with the homotypic enzyme β-glucuronidase. As a further step, experiments were performed to determine whether the deglucuronylated aggregation receptor acts as acceptor for glucuronic acid in the assay with the glucuronyltransferase from Geodia cydonium. This enzyme could be detected extracellularly as a subunit of the high molecular weight particle, which carries also the aggregation factor (9).

Fig. 6A shows the pattern of the untreated, highly purified aggregation receptor on a polyacrylamide gel in the presence of sodium dodecyl sulfate. After enzymic deglucuronylation of the aggregation receptor with the Geodia β-glucuronidase, the pattern is not altered. This indicates that the molecular weight of the deglucuronylated aggregation receptor is not changed detectably. Using this preparation of deglucuronylated aggregation receptor as acceptor for the Geodia glucuronyltransferase in an assay containing UDP-[14C]glucuronic acid as activated substrate, a radioactivity peak appears at the position of the gel which is characteristic for the aggregation receptor (Fig. 6C). This finding is a strong evidence that reglucuronylation of the deglucuronylated aggregation receptor, mediated by the Geodia glucuronosyltransferase does occur. In a control experiment, no radioactivity was detected in the gel, if untreated aggregation receptor, instead of β-glucuronidase digested material, was added to the assay (data not shown).

Inhibition of Cell Aggregation by β-Glucuronidase—From our previous experiments, it is known (11) that after preincubation of dissociated cells from Geodia with soluble β-glucuronidase, isolated from Escherichia coli, the aggregation potency of the cells in the assay with the soluble aggregation factor is strongly reduced. Due to this finding, we performed experiments to determine whether the β-glucuronidase, associated with the cell membrane from Geodia, exerts the same effect (Table III). In the first step, the cells were either pretreated with CMF alone (Assays a and b) or with CMFpretreated with CMF alone (Assays a and b) or with CMF pretreated with CMF alone (Assays a and b) or with CMF.
plus Nonidet (Assay c). The mild, nonionic surface active agent, Nonidet, was used to remove the β-glucuronidase from the cell membrane (25), without destroying its lipid layer (28). Subsequently, the cells were transferred into media which are either optimal for β-glucuronidase reaction (phosphate buffer, pH 5.0) or which suppress the action of β-glucuronidase (CMF). The determination of the cell membrane-bound β-glucuronidase activity of the cells after the pretreatment and incubation procedures revealed that those cells, which had been pretreated with Nonidet (Assay c), have lost 80% of their β-glucuronidase activity.

In the crucial set of experiments, the aggregation potency of cells, either containing (Table III, Assay a and b) or lacking (Assay c) β-glucuronidase activity, was determined. β-Glucuronidase-containing cells, which had been pretreated and incubated under assay conditions (CMF, Assay a), at which this glycosidase is inactive, have not lost any aggregation potency during the incubation period. Cells, which are lacking cell surface-bound β-glucuronidase, (pretreated with CMF plus Nonidet) show the same aggregation potency as the controls (Assay a), even though they are incubated under assay conditions, found to be optimal for β-glucuronidase (Assay c). In contrast, those cells which have not lost their membrane-bound β-glucuronidase during the pretreatment period (in CMF) but were incubated in the phosphate buffer (pH 5.0, NaCl and 2-mercaptoethanol), which allows optimal β-glucuronidase reactivity, show a strongly reduced aggregation potency (Assay b). The latter result led us to assume that the cell membrane-bound β-glucuronidase reduces under optimal assay conditions the extent of the aggregation potency of the cells. The next section presents the evidence that the reason for this reduced ability of the cells to reaggregate is indeed due to a β-glucuronidase-mediated deglucuronolysis of a cell membrane component.

### Table III

<table>
<thead>
<tr>
<th>Assay</th>
<th>Pretreatment of the cells</th>
<th>Incubation of the cells</th>
<th>β-Glucuronidase activity after incubation of the cells</th>
<th>Diameter of aggregates (μm)</th>
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<td></td>
<td>Before incubation</td>
<td>After incubation</td>
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<td>CMF</td>
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<tr>
<td>b</td>
<td>CMF</td>
<td>Phosphate buffer (pH 5.0)</td>
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<td>1,100 350</td>
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<tr>
<td>c</td>
<td>CMF plus Nonidet</td>
<td>Phosphate buffer (pH 5.0)</td>
<td>1.7</td>
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### Table IV

<table>
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<th>Diameter of aggregates (μm)</th>
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<td></td>
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<td>Before incubation After incubation</td>
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### Role of Glucuronosyltransferase during Cell Aggregation

For this series of experiments (Table IV), the dissociated single cells were pretreated in the phosphate buffer (pH 5.0) for 2 h at 20°C to deglucuronylate cell membrane components by the membrane-bound β-glucuronidase (see previous chapter). Subsequently, the cells were transferred into the Tris buffer (pH 8.2) which has been shown to be optimal for glucuronosyltransferase (9) but which suppresses the activity of β-glucuronidase (this work). The cells were incubated for 2 h at 22°C either in the absence (Assay a) or presence of the glucuronosyltransferase (Assays b and c). Due to the fact that the transferase is associated with the aggregation factor, the biological activity of the latter component was suppressed by addition of the selective calcium-chelating agent EGTA. In a previous study, it was established that, only in the presence of Ca²⁺, can the aggregation factor exert its aggregating properties (29). In this reaction, Mg²⁺ was found to be inactive.

The results, summarized in Table IV, show that the aggregation potency of cells, carrying on their cell surface deglucuronylated molecules, can be restored after incubation with glucuronosyltransferase and UDP-glucuronic acid (Assay b). Cells incubated in the absence of the transferase (Assay a) or in the presence of DTNB-inactivated glucuronosyltransferase (Assay c) do not show a marked increase in their ability to form large aggregates in the presence of the aggregation factor. Due to the finding that the aggregation receptor, a glycoprotein with glucuronic acid termini, is a prerequisite for aggregation factor-mediated reaggregation (10, 11, 30), we have to assume that the aggregation receptor molecules have been reglucuronylated during the incubation of the cells in the presence of the glucuronosytransferase and UDP-glucuronic acid.
DISCUSSION

The studies with embryonic retina (1, 2), brain (31), and sponge cells (4, 5) have provided experimental evidence that molecules with ligand activity (aggregation factors) cross-link cells and thereby enhance cell attachment and cell aggregation. The data available indicate that they may be glycoproteins which the aggregation factors attach to the cell membrane (10, 30, 32). Actually, three hypotheses are offered explaining the involvement of carbohydrates in the intercellular adhesion: antibody-antigen hypothesis (33), the hydro- gen bond concept (34), and the substrate-enzyme mechanism proposed by Roseman (35). In the latter interesting theory, it is suggested that a glycosyltransferase on one cell surface binds to its receptor molecule on the surface of another cell. If the sugar nucleotide appropriate for the enzyme is then provided, glycosylation takes place, and this sugar is added to the growing chain. When no sugar nucleotide is present, the reaction does not complete and the enzyme on one cell surface is supposed to remain bound to the substrate on the other cell surface. Thus, the addition of the nucleotide sugar allows completion of the enzymatic reaction, with the subsequent loss of the bond between the two cells. Objections have arisen over Roseman’s theory because of the lack of direct enzymatic data demonstrating the existence of glycosyltransferases on the outer cell surface of mammalian cells (36). In addition, the hypothesis does not take into account the presence of glycosidases on the cell surface. At least since the studies of Bosmann (37, 38), it seems to be established that the glycosidase activity alters with the growth state and possibly with the aggregation potency of the cell. The data of Aoyagi et al. (13) conclusively demonstrate that some glycosidases are also associated with the cell membrane.

In the sponge (Geodia cydonium) model, used for the present study, several glycosyltransferases (sialyltransferase, galactosyltransferase, and glucuronyltransferase; Refs. 8 and 9) could be clearly localized in the extracellular space. In addition, and in contrast to the Roseman theory, the aggregation factor can be separated from glycosyltransferases (8), which indicates that the glycosyltransferases might modulate the aggregation potency of the cells, but that they do not ultimately provoke adhesiveness between two cells. The latter process is, at least in the Geodia system, a result of an interaction between the aggregation factor present in the intercellular space and the aggregation receptor bound to the cell surface (10). In the present study, it could be elucidated for the first time, that cell surface-associated β-glucuronidase can modulate the aggregation potency of sponge cells. It was found that the activity of both the cell surface-associated β-glucuronidase and the cell surface-bound β-galactosidase increase during the reaggregation process. In addition, the soluble β-glucuronidase was found to hydrolyze the terminal glucuronic acid moiety of the aggregation receptor under appropriate incubation conditions. On the other side, incubation of the intact cells under assay conditions, optimal for membrane-bound β-glucuronidase, reduces markedly the aggregation potency of the cells. The strong hint, that this reduction is due to the hydrolyzing action of the bound β-glucuronidase, resulting in a liberation of glucuronic acid from the aggregation receptor, came from the finding that after incubation of these cells with glucuronosyltransferase (isolated from the same sponge) in the presence of UDP-glucuronic acid, the original aggregation potency of the cells is restored. The indirect evidence that it is the aggregation receptor, bound to the cell surface which is deglucuronylated or reglucuronylated, is supported by the presented data, obtained from the experiments with isolated aggregation receptor molecules. These studies showed that the receptor deglucuronylated in the presence of isolated Geodia β-glucuronidase can be reglucuronylated in the presence of Geodia β-glucuronosyltransferase and UDP-glucuronic acid. From the comparison of the mobility pattern of the deglucuronylated aggregation receptor in polyacrylamide gels with those of the untreated or reglucuronylated aggregation receptor preparations, which are almost identical, we have to conclude that the β-glucuronidase is an ectoglucosidase.

As a direct approach to demonstrate that under the influence of β-glucuronidase the aggregation potency of the Geodia cells is abolished, preformed aggregates were treated with heterotypic β-glucuronidase (from E. coli) at assay conditions described elsewhere (11). The results revealed that, at an enzyme concentration of 0.00025 unit/ml, the size of the aggregates is reduced by more than 50% during an incubation period of 30 min (11).

Even if the purity of the membrane fractions was checked by chemical analysis and by the measurement of a marker enzyme (5′-nucleotidase), the possibility cannot be excluded that the membrane preparations used in the present study are free of cytoplasmic contaminations. Already from the studies of Warren et al. (15) it is known that cell membranes, obtained by the Tris method which was applied in our investigation, are associated with vesicles of undetermined origin. Therefore, future studies must answer the question, whether these vesicles contain glycosidases. As suggested by Reutter et al. (39), vesicles which arise by blebbing of the Golgi cisternae or of the lysosomes migrate to the cell periphery carrying glycosyltransferases and glycosidases on their inner surface. When fusing with the plasma membrane, the vesicles might be turned inside out, thus exposing the enzyme molecules. Therefore, the observed increase in membrane-associated enzyme activity during aggregation (Fit. 3) may be the result because membranes prepared from aggregates are “contaminated” with vesicles to a greater extent than membranes from individual cells (15).

From our data, obtained in the Geodia system, we postulate a working hypothesis to explain cell aggregation and cell separation on the level of an interaction between glycosidases and glucuronidases in the following series: (a) activation of the aggregation receptor by its enzyme glucuronosylation; (b) adhesive recognition of two cells, mediated by the aggregation factor and the glucuronylated aggregation receptor; (c) inactivation of the aggregation receptor by its deglucuronylation with the membrane-associated β-glucuronidase; and (d) cell separation due to the loss of the recognition site (glucuronic acid) of the aggregation receptor for the aggregation factor. Steps c and d are consequences of an “activation” of the membrane-associated β-glucuronidase, which is inactive under the pH and ion conditions of the sea water. No data are available which give direct experimental evidence for a pH or ion change of the sponge cell membrane during the process of cell separation. However, from previous studies (40), it is known that the aggregation factor from Geodia alters the cell surface charge after its binding to homologous cells. Due to the fact that an alteration of the cell surface charge is also caused by a pH change at the cell surface (41), it is quite likely that pH fluctuations govern the activity of both the glucosidase and the glycosyltransferase. In addition to the described aggregation-dissociation hypothesis based on the interrelation between glycosyltransferases and glycosidases, a further mechanism (reversible inactivation of the aggregation factor by an antiaggregation receptor) exists which controls cell adhesion (12).

REFERENCES

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33. Tyler, A. (1946) *Growth* 10, 7-19
Aggregation of sponge cells. A novel mechanism of controlled intercellular adhesion, basing on the interrelation between glycosyltransferases and glycosidases.

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