GLYCOSYLTRANSFERASE ACTIVITY IN DEVELOPING SEA-URCHIN EMBRYOS

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SUMMARY
The developmental course of activity changes of 4 endogenous membrane glycosyltransferases: UDP-glucose : glycosyltransferase, UDP-galactose : glycosyltransferase, GDP-mannose : glycosyltransferase, and UD-PN-acetylglucosamine : glycosyltransferase was determined in various stages of developing sea-urchin embryos from unfertilized eggs up to the 16-cell stage. The results show that there is a 2-fold increase in glycosyltransferase activity after fertilization. These results are discussed in relation to the postulated role of glycosyltransferases in mediating adhesion, recognition, and agglutination of cells and embryos.

INTRODUCTION
It is now well documented that glycoconjugates, including glycoproteins and glycolipids, are present in a variety of cells and tissues where they function as, for example, hormones, enzymes, immunoglobins, and cellular receptors. Recent evidence has suggested that glycoprotein synthesis may occur both intracellularly and at the external surface of the plasma membrane (Cook, Laico & Eylar, 1965; Roseman, 1970; Bosmann, 1972). Intracellularly, many glycoproteins are a structural component of the internal cell membrane, while extracellularly glycoprotein-glycosyl ectoenzyme systems have been postulated to function as mediators of a variety of cellular recognition and adhesion phenomena.

The formation of glycosylated macromolecules has been studied extensively by Roseman (1970), who found that glycosylation of molecules was carried out by specific glycosyltransferases. These enzymes catalyse the transfer of specific monosaccharides from an activated monosaccharide nucleotide to a specific glycoprotein, glycolipid, or other acceptor macromolecule. The existence of glycosyltransferases on the surface of cells has also been reported (Bosmann, 1972; for review see Shur & Roth, 1975). Roseman (1970) has suggested that such surface glycosylating enzymes and their acceptors may have the important function of mediating and regulating cell-cell recognition and adhesion. Evidence for this hypothesis has come from studies of platelet-collagen adhesion (Jamieson, Urban & Barber, 1971), amoeboid aggregation (Hoover, 1974), and embryonic retinal cell adhesion (Roth, McGuire & Roseman, 1971), where it has been shown that inhibitors that interfere with transferase activity also prevent cell-cell adhesion. Studies on gamete recognition in the unicellular green algae *Chlamydomonas* have also suggested that cell surface glycosyltransferases function in mediating successful mating behaviour in this species (McLean & Bosmann, 1975).
Cell dissociation and reaggregation experiments in sea-urchin embryos have been useful for understanding the role of cell-cell interactions in regulating morphogenesis and development. These experiments (reviewed in Giudice, 1973, chapter 6) show that embryos dissociated and reaggregated at an early (16-cell) stage seem to lose spatial organization and do not develop into normal larvae, whereas embryos dissociated and reaggregated at the blastula and gastrula stages are able to form successful larvae. The nature of the extracellular factors necessary for successful reaggregation in sea-urchin embryos is unknown.

If glycosyltransferases do play an important and obligatory role in mediating cell recognition and adhesion, it is to be expected that these enzymes will be detected in a variety of developing systems. We report here a study of glycosyltransferase activity in developing sea-urchin embryos, in which we found that levels of transferase activity varied with the different stages of embryo development. The results are discussed in relation to a regulatory role for glycosyltransferases in controlling development.

MATERIALS AND METHODS

Embryo culture conditions

Sea urchins of the species *Lytechinus pictus* were obtained from Pacific Biomarine (Venice, California). Shedding of gametes and sperm was obtained by intercoelomic injection of 0.55 M KCl. Eggs were filtered through gauze, washed 3 times by centrifugation at 100 g, resuspended at 1 x 10^6 embryos/ml seawater and fertilized with a minimum of sperm. After fertilization embryos were cultured at 16 °C in artificial seawater (Harvey, 1956) in aerated spinner flasks or in a shallow layer of seawater (25 ml) in glass culture dishes. Only cultures showing more than 95 % fertilization and successful first cleavage were used.

Glycosyltransferase assays

Glycosyltransferase activity was assayed essentially as described previously (Bosmann, 1972). Specific stage embryos were collected by centrifugation at 100 g, washed twice with seawater, and resuspended at about 1 x 10^6 embryos per ml of seawater containing 0.1 % Triton X-100. The embryo suspension was lysed by at least 50 strokes of a tightly fitting Dounce homogenizer and assayed for degree of cell homogenization by observation in a phase-contrast microscope until 100 % breakage was obtained. Homogenates were incubated for specific periods of time (usually 30 min) in an incubation assay (final volume, 0.22 ml) containing the following: 20 μl of 14C-labelled nucleotide monosaccharide (~ 5 x 10^4 cpm), 100 μl of Tris-HCl buffer (0.1 M, pH optimized for individual transferase activity), and 100 μl of 0.1 % Triton X-100 extract of embryos in seawater. When exogenous acceptor molecules were added to the assay, the incubation assay was modified to consist of 20 μl of 14C-labelled nucleotide monosaccharide, 50 μl of Tris HCl (0.2 M, pH previously optimized for specific transferase activity with endogenous acceptors), 50 μl of fetuin minus N-acetylneuraminic acid and galactose (10 mg/ml) or collagen minus glucose (10 mg/ml) and 100 μl of 0.1 % Triton X-100 embryo extract. The incubation temperature was usually 30 or 37 °C. The reaction was terminated by addition of 1 ml of 1 % phosphotungstic acid in 0.5 M HCl. The precipitate from this reaction was collected by filtration on GF A filters (Whatman), washed twice with 10 % trichloroacetic acid and twice with ethanol : ether (2:1, v/v), dried, and counted in a Nuclear Chicago Mark II Liquid Scintillation Counter. Overall counting efficiency for 14C under these conditions was 45 %.

Control values for each of the enzymes studied were obtained by incubating at 0 °C, substituting water for enzymes, substituting boiled enzyme extract, or immediately precipitating the assay. By all of these measurements, background activity and non-specific binding artifacts were negligible (10-40 cpm/mg protein).
**Glycosyltransferase activity**

**Substrate materials**

UDP-[14C]galactose (310 Ci/mol), UDP-[14C]glucose (310 Ci/mol), UDP-N-[14C]acetylglucosamine (269 Ci/mol), and GDP-[14C]mannose (83 Ci/mol) were obtained from Amersham Searle, diluted to 10 μCi/3–3 ml, and stored frozen at –20 °C. Fetuin minus N-acetyleneuraminic acid and galactose, and guinea pig skin collagen minus glucose acceptor fractions were prepared as previously described (Bosmann, 1972).

**Protein determination**

Protein determinations were done by the method of Lowry, Rosebrough, Farr & Randall (1951), with bovine serum albumin serving as a protein standard.

**RESULTS**

Since to our knowledge glycosyltransferase enzyme activity has not been previously described in sea-urchin embryos, it seemed important to characterize the conditions necessary to optimize the enzymic activity present at the various stages of development and to determine both enzyme and acceptor presence. Part of this study, therefore, reports on conditions necessary to optimize glycosyltransferase activity in unfertilized

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![Fig. 1. Effect of pH of incubation assay on incorporation of [14C]glucose, [14C]galactose, [14C]mannose and N-[14C]acetylglucosamine onto endogenous acceptors. Incubation mixtures prepared from 0.1% Triton X-100 homogenates were incubated for 30 min in buffers of various pH. After incubation, samples were precipitated, washed, and assayed as described in Materials and methods. Data show means and ranges of values obtained in typical experiments. Transferase activities are identified by the radioactive substrate used for each assay. A, UDP-[14C]galactose; B, GDP-[14C]-mannose; C, UDP-[14C]glucose; D, UDP-N-[14C]acetylglucosamine.](image-url)
eggs and embryo extracts of *Lytechinus pictus*. Unless otherwise stated, all data are from experiments with embryo extracts from the 2- to 4-cell stage of synchronously developing embryos.

The conditions important for optimizing glycosyltransferase enzyme activity in sea-urchin embryos are (1) the pH of the assay, (2) temperature, (3) time of incubation, and (4) presence or absence of divalent cations. The effect of varying each of these conditions was studied in *Lytechinus pictus* embryos. It should be noted that in the experiments in which measurement with endogenous acceptors is being made, the optimization may reflect characteristics of either enzyme or acceptor macromolecule.

**pH optimization**

The pH optima for endogenous enzyme activity were established for 4 transferases: UDP-glucose: glycosyltransferase, UDP-galactose: glycosyltransferase, UDP-N-acetylglucosamine: glycosyltransferase, and GDP-mannose: glycosyltransferase. Using assay conditions described in Materials and methods, the pH was varied from 5.0 to 7.0 using 0.1 M citrate-phosphate buffer and from 7.0 to 9.2 using Tris-HCl buffer (0.1 M). The pH activity profiles in Fig. 1 indicate a pH optimum of 6.6 for the
Glycosyltransferase activity

UDP-glucose:glycosyltransferase, 6-2 for the UDP-galactose:glycosyltransferase, 7-0 for UDP-N-acetylglucosamine:glycosyltransferase, and 6-6 for GDP-mannose:glycosyltransferase.

Temperature

All transferases utilizing endogenous acceptors except GDP-mannose:glycosyltransferase showed optimal enzyme activity at 37 °C (Fig. 2). GDP-mannose:glycosyltransferase had a temperature optimum of 30 °C.

Fig. 3. The effect of concentration of activated monosaccharides on incorporation into acid-precipitable endogenous acceptor molecules. Incubation assays were prepared as described in the text and incubated with the indicated concentrations of labelled activated monosaccharide substrates. After 30 min at 30 or 37 °C, assays were precipitated and processed as described in the text. Each point is the mean and standard error obtained from 3 experiments, each on a separate embryo population. Transferase activities are identified by the radioactive substrate used for each assay. A, UDP-galactose; B, GDP-mannose; C, UDP-glucose; D, UDP-N-acetylglucosamine.

Effect of ion concentration

None of the 4 enzyme activities exhibited dependence on added divalent cations when the assays were performed on 0.1% Triton X-100 homogenates suspended in artificial seawater. Enzyme activity for UDP-glucose:glycosyl- and UDP-galactose:glycosyltransferase was decreased by 55 and 76%, respectively, however, when the assay was made 0.1 M in EDTA. The activities of GDP-mannose:glycosyltransferase and UDP-N-acetylglucosamine:glycosyltransferase in the presence of EDTA were
not tested. (This result suggests that divalent cations may be necessary for maximum enzyme activity and that Mg$^{2+}$ (shown in other systems, e.g., Roseman, 1970, to be important for activity) and trace amounts of other cations may be supplied by the seawater in the incubation assay. The seawater contained 0.05 M Mg$^{2+}$ and 0.009 M Ca$^{2+}$.

Fig. 4. Effect of increasing concentrations of enzyme on incorporation of activated nucleotide monosaccharide into acid-precipitable endogenous acceptor molecules. Incubation assays were prepared as described in the text with various dilutions of sea-urchin embryo extract and incubated and processed as described in Fig. 2. Data are the means and ranges of typical experiments. Transferase activity are identified by the radioactive substrate used for each assay, A; UDP-$[^{14}C]$galactose; B; GDP-$[^{14}C]$mannose; C; UDP-$[^{14}C]$glucose; D, UDP-$[^{14}C]$acetylglucosamine.

**Effect of substrate concentration**

The effect of increasing the substrate concentration of the nucleotide monosaccharide on the rate of incorporation of $^{14}$C-activated monosaccharides is shown in Fig. 3. The activities of all transferases were linear with increased substrate concentrations. It was calculated that the 20 μl of each substrate used contained 0.2 to 0.4 nmol of substrate. Enzyme activity was proportionally increased and not saturated when substrate was increased to 0.5–1.0 nmol. Assays were performed with 20 μl of added substrate, a concentration below substrate saturation of enzyme present but in a linear portion of the substrate curve.
Effect of varying enzyme and acceptor concentrations

The activity of all transferases was linear with added enzyme extracts up to 0.5 mg protein of added enzyme extract (Fig. 4). This suggests that most assays (performed at 100 µl of added enzyme, or about 300 µg of protein per assay) were carried out below saturation but in a linear portion of the enzyme curve.

Table 1. Transfer of nucleotide monosaccharides on to endogenous and exogenous acceptor molecules

<table>
<thead>
<tr>
<th>Transferase, A</th>
<th>Activity, cpm/mg protein</th>
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<tr>
<td></td>
<td>Endogenous acceptor</td>
</tr>
<tr>
<td>Collagen: glucosyl</td>
<td>1008</td>
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<tr>
<td></td>
<td>1025</td>
</tr>
<tr>
<td></td>
<td>1065</td>
</tr>
<tr>
<td>Fetuin: galactosyl</td>
<td>283</td>
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<td></td>
<td>300</td>
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<td>338</td>
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All assays were performed using optimized reaction conditions, as described in the text. Data are means of duplicate determinations; each value is a separate experiment. Endogenous assays were performed without the addition of exogenous acceptor; exogenous assays were performed with added acceptor molecules, either collagen minus glucose or fetuin minus N-acetyleneuraminic acid and galactose.

Since activity of these endogenous assays depends not only on levels of enzyme present but also on levels of appropriate acceptor molecules, an attempt was made to investigate whether the amounts of endogenous acceptors were rate-limiting by adding exogenous acceptors to the assay mixture. Fetuin minus galactose and guinea-pig skin collagen minus glucose were used as acceptors. The data from these experiments (Table 1) show no significant change in radioactivity per assay detected. If the fetuin minus N-acetyleneuraminic acid and galactose, and collagen minus glucose molecules are able to function as exogenous acceptor molecules in the sea urchin as they do in other systems, this result suggests that it is the level of transferase present in the embryos at different stages which is rate limiting.

Time course of transferase activity

Fig. 5 shows the incorporation of 14C-labelled monosaccharides by embryo extracts as a function of time. All transferases showed linear incorporation at optimum conditions of temperature and pH for up to 45–60 min. To ensure that transferase activity was linear in all assays performed, enzyme assays were terminated after 30 min and activity was assayed as described in Materials and methods.

Changes in transferase enzyme activity with changes in developmental stage of the embryo

Using conditions optimized for the glycosyltransferase assays, as described above, changes in the various transferase activities with changes in developmental stage were
ascertained. The results (Fig. 6) show that the activity of all transferases almost doubles after fertilization. UDP-glucose:glycosyltransferase reaches maximum activity in the fertilized egg followed by a decrease in activity. UDP-galactose: glycosyltransferase shows essentially constant activity up to the 8- to 16-cell stage. UDP-\(N\)-acetylglucosamine:glycosyltransferase shows a constant activity up to the

![Graphs showing enzyme activities over time]

**Fig. 5. Effect of time of incubation on incorporation of activated monosaccharides on to endogenous acceptor molecules.** Incubation mixtures were obtained from embryos as described in the text and incubated for various time periods after which incorporation was terminated by acid precipitation. The precipitated molecules were washed in ethanol-ether and collected on glass fibre filters and processed as described in the text. Data are the means and ranges of values obtained in typical experiments. Transferase activities are identified by the radioactive substrates used for each assay: A, UDP-galactose; B, GDP-mannose; c, UDP-glucose; D, UDP-\(N\)-acetylglucosamine.

2- to 4-cell stage and then a slight increase at the 8- to 16-cell stage. GDP-mannose: glycosyltransferase, on the other hand, shows a steady increase in activity from fertilization up to the 8- to 16-cell stage. Since the amount of protein per stage remains fairly constant in the developing sea-urchin embryo (see discussion in Giudice, 1973), the changes in glycosyl enzyme activity per mg protein should reflect true increases or decreases in activity for a particular embryo stage.
Affinity of enzyme for substrate

In order to determine whether the affinity of the enzyme for substrate varied with developmental stage, the apparent $K_m$ of each enzyme at different developmental stages was studied (Fig. 7). The results demonstrate no significant changes in enzyme substrate affinity for any of the glycosyltransferases studied up to the 16-cell stage. The apparent $K_m$ of each enzyme is as follows: UDP-glucose:glycosyltransferase, 10 μM; UDP-galactose:glycosyltransferase, 4 μM; GDP-mannose:glycosyltransferase, 6.7 μM; UDP-N-acetylglucosamine:glycosyltransferase, 2.9 μM.

Fig. 6. Incorporation of activated monosaccharide substrates during various developmental stages of the sea urchin. Triton X-100 homogenates were obtained from embryos at specific stages and incubated for 30 min at 30 or 37 °C in the optimized assay mixture described in Materials and methods. The incubation mixtures were then processed as described previously. Each point is the mean with standard error from 3 to 7 experiments (depending on the stage assayed), each from a separate sea-urchin embryo population. Transferase activities are identified by the radioactive substrate used for each assay: A, UDP-galactose; B, GDP-mannose; C, UDP-glucose; D, UDP-N-acetylglucosamine. UE, unfertilized egg; FE, fertilized egg.

DISCUSSION

Several laboratories have characterized glycosyltransferase and acceptor activity on cell surface membranes. It has been suggested that such enzymes function in cellular recognition and adhesion, possibly performing the important function of holding together various groups of cells with differing determinative fates, or allowing them to migrate to particular embryological sites where differentiation is induced.
It has been shown that sea-urchin eggs and embryos possess glycoproteins (Aketa, Tsuzuki & Onitake, 1968; Ishihara, 1968; Brown & Bosmann, 1976). Kean & Bruner (1971) reported that sea-urchin nuclei contain CMP-sialic acid synthetase, an enzyme necessary for producing an activated CMP sialic acid molecule used in other systems for synthesizing the sialic acid-containing glycoproteins (Roseman, 1970). Glycoprotein differences on the surfaces of transformed versus non-transformed cells, and differentiated versus stem cells, have been detected (Bosmann, 1972; Bosmann, Hagopian & Eylar, 1969; Patt & Grimes, 1974; Isselbacher, 1974), and the suggestion has been made that such molecular differences of the cellular surface may play a role in regulating abnormal and cellular differentiation.

In sea-urchin development, changes in components of both the internal and the external membrane may play an important role in regulating development and differentiation of the embryo. Gustafson & Wolpert (1967) have proposed that changes in the characteristics of the cell membrane may explain the differences of movement of particular cells, e.g., micromeres, which lead to establishment of localities of cells.

Fig. 7. Apparent $K_m$ of enzyme-transferase reactions at different developmental stages. Standard incubation assays were prepared as described in the text from embryos of specific stages and incubated with increasing concentrations of labelled activated monosaccharide substrates. Data are plotted using the Lineweaver-Burk plot of $1/V$ versus $1/S$. Each point represents the mean of 3 separate determinations, each on a different embryo population. Transferase activities are identified by the radioactive substrate used for each assay: A, UDP-galactose; B, GDP-mannose; C, UDP-glucose; D, UDP-$N$-acetylglucosamine. Unfertilized egg, ●; fertilized egg, □; 2-4 cell stage, ○; 8-16 cell stage ■. When the curves for 2 developmental stages are extremely close, only one curve is drawn.
destined to give rise to the various digestive, skeletal, and nervous adult functioning systems. Recent evidence showing differences in concanavalin A binding and sulphated mucopolysaccharide surface components between cells of differing determinative fates has given support to this proposal (Karp & Solurch, 1974; Roberson & Oppenheimer, 1975; Neri, Roberson, Connolly & Oppenheimer, 1975).

Our results show that sea-urchin eggs and embryos possess glycosyltransferase activity and that an increase in transferase activity occurs after fertilization. Preliminary studies with mixtures of unfertilized and fertilized egg homogenates have demonstrated that unfertilized egg homogenates inhibit transferase activity in the fertilized egg extract. The nature of this inhibition is currently under investigation.

Glycosyltransferases have now been reported in liver, kidney, blood, and intestinal cells, as well as in amoeba and green algal cells. The demonstration that sea-urchin embryo extracts possess these enzyme activities reinforces the idea that the wide species distribution of such enzymes indicates an important cellular function. Further study should indicate whether the biochemical changes in transferase activity detailed in *Lytechinus* embryos play any role in the morphological and biochemical differentiation occurring later in this developing system.

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REFERENCES


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