Con A- and WGA-Binding Glycoproteins of Stationary and Migratory Corneal Epithelium

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When stratified corneal rat epithelium becomes migratory in response to a wound, an increase in binding by the lectins concanavalin A (Con A) and wheat germ agglutinin (WGA) is seen. These lectins bind the membranes of the cells of the leading edge of the migrating sheet more intensely than normal epithelium and epithelium behind the leading edge, suggesting a change in cell surface properties during migration. In the present study, analysis of cell surface proteins using lactoperoxidase-catalyzed iodination followed by SDS-PAGE indicates the appearance of a 70 K protein in epithelium migrating to cover a wound. Con A-affinity chromatography shows that two bands, 70 K and 155 K, increase 4.0- and 2.9-fold, respectively, in epithelium that is migrating. WGA-binding glycoproteins increased 1.61-fold following wounding with the major band present at 155 K in SDS-PAGE. The data suggest that these glycoproteins are responsible for the increase in Con A and WGA binding to cell membranes in migratory corneal epithelium. Invest Ophthalmol Vis Sci 27:1205-1210, 1986

Cell surface glycoproteins are altered in processes involving cell movement, such as differentiation, development, and migration in response to a wound. These alterations may have physiological significance, since cell surface glycoconjugates have been suggested as influencing various processes such as adhesion and cell sorting.

Following a wound, the cell membranes of the leading edge of the migrating sheet of rat corneal epithelium binds more Con A and WGA than does stationary epithelium, and protein and glycoprotein syntheses increase dramatically. In addition, tunicamycin, Con A, and WGA block or slow epithelial motility. These reports suggest that cell surface glycoproteins are altered during epithelial migration and that these glycoproteins may play a role in the migratory process. Changes in cell surface lectin-binding properties could be the result of the synthesis of new migration-specific glycoproteins or the result of the alteration of the carbohydrate moieties of glycoproteins normally present on the cell surface. To determine whether the change was the result of new biosynthesis, cell surface proteins of migrating and stationary stratified epithelium were compared using lactoperoxidase-catalyzed iodination followed by SDS-PAGE, lectin-affinity chromatography, and lectin staining of Western blots. The results indicate that: (1) a 70 K protein is present on the cell surface of migrating corneal epithelium in a much greater amount than on stratified epithelium, (2) Con A-affinity columns bind two glycoproteins, 155 K and 70 K, present at a 2.9- and 4.0-fold higher level in epithelium migrating to cover a wound, (3) biotinylated-Con A reaction with Western blots indicates four glycoproteins (155 K, 110 K, 90 K, and 70 K) present in a greater amount in migratory tissue and a 48 K glycoprotein present only in stratified control epithelium, and (4) the major WGA-binding protein migrates to 155 K in both migratory and stationary control epithelium; this glycoprotein appears in a greater amount, 1.61-fold higher, in migratory tissue. These results suggest that the change in lectin-binding properties are the result of the presence of these molecules.

Materials and Methods

Male Sprague-Dawley rats with clear corneas were used. Rats were killed with an overdose of sodium pentobarbital, a 3 mm circular area was demarcated with a trephine, the epithelium was removed with a small scalpel, and the corneas were excised and pinned over paraffin posts as previously described (Fig. 1). The corneas were cultured, four per dish, in a completely defined medium at 35°C in 5% CO2. After 18 hr of incubation, the remaining defect was detected with Richardson's stain, the original 3 mm area concentric to the defect was marked with the trephine, and the epithelium within the 3 mm area was removed with a small scalpel. This epithelium migrating to cover a wound was designated as wound epithelium (W); the
epithelium outside the 3 mm area was also harvested and designated as wound periphery. This type of wound leaves the basement membrane intact.

Cell surface proteins were radiolabeled using lactoperoxidase-catalyzed iodination.22 The epithelial sheets were placed in 0.5 ml of phosphate-buffered saline (PBS). The following additions were then made: lactoperoxidase (40 U/ml), glucose oxidase (20 U/ml), glucose (5 mM), and Na125I (400 μCi/ml) (ICN Radiochemical, Irvine, CA). The tissues were incubated for 30 min on ice with occasional shaking. The reaction was stopped by the addition of phosphate-buffered iodine (PBI). The tissues were washed five times in PBI and then pelleted through PBI containing 15% glycerol and 0.1 mM phenylmethyl sulfonyl fluoride. Typical specific activities obtained were 100,000 DPM/μg of protein. Omission of lactoperoxidase from the incubation mixture resulted in less than 5% as much incorporation of 125I as when the enzyme was included.

Total cellular proteins were radiolabeled by adding [3H]-leucine (New England Nuclear, North Billerica, MA) to the culture medium 3 hr before harvesting of the epithelium. Radiolabeled proteins were then detected following SDS-PAGE using ENHANCE (New England Nuclear) and fluorography as previously described.18

Con A-binding glycoproteins were isolated using Con A-Sepharose 4B (Sigma Chemical Co., St. Louis, MO). Radiolabeled proteins were solubilized in PBS containing 0.5% Nonidet P-40 for 1 hr at 4°C with occasional shaking. The samples were centrifuged at 12,000 xg and the supernatants containing 90–95% of the total 125I were applied to the Con A-Sepharose 4B column (0.7 × 5 cm). The column was then washed with 20 column volumes of PBS—0.5% Nonidet P-40. Specifically bound glycoproteins were eluted with 0.5 M methyl-α-mannose in the same buffer. Flow rate was approximately 10 ml/hr. WGA binding glycoproteins were isolated in an identical method with a WGA-Sepharose 4B column. Bound proteins were eluted with 0.2 M N-acetylglucosamine. No radioactivity was eluted when 0.2 M N-acetylglucosamine was added to the Con A column or 0.5 M methyl-α-mannose to the WGA column. Also, no radioactivity was bound upon reapplication of the wash material. SDS-polyacrylamide gel electrophoresis was performed as described by the manufacturer.23 Radioactivity was quantified by slicing individual lanes with a Hoeffer (San Francisco, CA) model 220 gel slicer. Slices (1 mm) were quantified in a Beckman (Palo Alto, CA) Gamma 4000 counter. For autoradiography, gels were dried and allowed to expose Kodak (Rochester, NY) K-Ormat film.

Results

Stratified rat corneal epithelium normally consists of 5–7 layers of cells with a single basal cell layer, 2–3 cell layers of wing cells, and 2–3 layers of flattened superficial cells. Following a wound where the basement membrane is left intact, the epithelium migrates...
as a unified sheet with the leading edge tapering down to a single cell layer. Cell elongation and flattening allows the wound area to be covered. Following wound closure, cell mitosis increases and the tissue regains its normal stratification.

Cell surface proteins of epithelium migrating to cover a wound and control, stationary epithelium were compared following lactoperoxidase-catalyzed iodination and SDS-PAGE. At least 12 bands were seen following autoradiography with 2 bands, 70 K and 65 K, present to a much greater extent in epithelium migrating to cover a wound (Fig. 2). The increased presence of the 70 K band was also detected when newly synthesized proteins that incorporate $[^3H]$-leucine were analyzed with SDS-PAGE and fluorography (Fig. 3). The increased synthesis of this 70 K polypeptide was the major change in the radioactivity profiles of the control (C) and wound (W) epithelium.

Con A-binding glycoproteins were identified by reacting biotinylated-Con A with Western blots of wounded migrating and control stationary tissue. Using this technique, four bands, 155 K, 110 K, 90 K, and 70 K, were detected that appeared in a greater amount in epithelium migrating to cover a wound (Fig. 4). One band at 48 K was seen in control epithelium only. No difference was seen between wound periphery tissue and the epithelium from the original scrape to create the 3 mm wound.

Affinity chromatography was used to determine if the total amount of Con A and WGA-binding glycoproteins increased during migration. When radiolabeled proteins from wound and control epithelium were applied to the Con A column, the column bound

1.82 ± 0.40 (N = 5) times more of the total radioactivity from epithelium migrating to cover a wound than control epithelium. SDS-PAGE of the glycoproteins bound
to the column indicated that the major protein bands in control and wound periphery tissue were present at 20–30 K (Fig. 5). The migrating epithelium contained this broad band and also two additional peaks at 155 K and 70 K (Fig. 5). The 155 K band made up 8% of the total radioactivity in migrating epithelium and 2.8% in control; the 70 K band represented 9.3% in migrating and 2.3% in control.

When radiolabeled proteins from wounded migratory and control epithelium were applied to the WGA column, the column bound 1.61 ± 0.35 (N = 4) times more of the total radioactivity in the wounded tissue than control. SDS-PAGE indicated that the major WGA-binding glycoprotein in both tissues was present at 155 K (Fig. 6), suggesting that this molecule is responsible for the increase in WGA binding.

Since WGA and Con A both bound an 155 K glycoprotein, an attempt was made to determine whether this glycoprotein was the same with two lectin-binding abilities. When the radioactive material from epithelium was passed through the WGA column and then Con A, the 155 K band was not seen in the Con A-bound material in SDS-PAGE. When the columns were run in reverse, the 155 K band was bound to both columns. These results suggest that the protein may be the same with a portion of it becoming Con A-binding in response to a wound.

Discussion

We have previously shown that Con A and WGA bind cell membranes of migratory corneal epithelium...
in a greater amount than those of normal stratified corneal epithelium.\(^9\) We have now found glycoproteins that may be responsible for the change in lectin-binding patterns using lactoperoxidase-catalyzed iodination of the cell surface proteins and lectin affinity chromatography.

Two glycoproteins, 70 K and 155 K, are present on the cell surface of epithelium migrating to cover a wound in a greater amount than normal corneal epithelium (Fig. 5). Several other proteins, possibly not on the cell surface, bind Con A (Fig. 4), but the 70 K and 155 K glycoproteins appear to be the major difference between proteins present on the cell surface of wound and control epithelium. The amount of total radioactivity bound for wound epithelium versus control epithelium is 1.82 times higher and 1.61 times higher for the WGA column. This is in agreement with the findings of Gipson et al\(^9\) that migratory tissue bound between 1.49 and 2.12 times as much \(^{3}\H\) Con A as control tissue.

Changes in lectin-binding properties as the epithelium becomes migratory could be the result of the synthesis of migration-specific glycoproteins or the result of altered glycosylation with the core protein remaining the same. Both mechanisms could explain the change in Con A-binding patterns. The 70 K protein appears to be migration specific, since it is not seen in control tissue (Figs. 2, 3) but is present in a Con A-binding form in wound epithelium (Figs. 4, 5). The 155 K core protein does not appear in a greater amount in migratory tissue (Figs. 2, 3) but it does appear to increase in Con A-binding ability (Figs. 4, 5), suggesting an alteration in the carbohydrate moieties. This type of change could be the result of premature termination of glycosylation leaving mannose residues exposed in a conformation that could be bound by Con A.\(^{15,29,30}\) The change in WGA-binding properties seems to be the result of a general increase in the percentage of WGA-binding glycoproteins in the solubilized radioiodinated material. The 155 K protein, being the major WGA-binding glycoprotein, would seem to be the molecule responsible for the increased WGA-binding in migratory tissue.

Since the Con A form of the 155 K glycoprotein is also bound by WGA, the question arises whether this is the same molecule, which has both lectin-binding properties. WGA has a specificity for terminal N-acetylglucosamine residues, whereas Con A binds terminal glucose and mannose residues as well as internal mannose.\(^31\) Perhaps both binding sites are present in the glycoproteins from migratory epithelium as a result of premature termination of glycosylation.\(^{29,30}\) In the stratified control tissue, the Con A-binding site may be removed by cleavage of mannose units or blocked by the addition of other sugars. Since Con A has a minor affinity for N-acetylglucosamine,\(^31\) it is also possible that the Con A column may be binding this sugar on the 155 K molecule. However, we have not been able to elute any radioactivity from the Con A column with N-acetylglucosamine.

The most intriguing aspect of our data is the increased synthesis of a 70 K protein during migration. The identity of this molecule is unknown, but several basement membrane-binding molecules have been identified with this molecular weight,\(^{32-36}\) suggesting that the 70 K glycoprotein may be involved in the mi-
migration process, as an adhesion molecule. Also of interest is the lack of a 220 K molecule corresponding to fibronectin. This in agreement with the findings of Phan et al.\textsuperscript{37} who reported that fibronectin is not deposited in superficial corneal wounds in vitro.

Key words: Con A-binding glycoproteins, WGA-binding glycoproteins, migration, corneal epithelium, lectins

References

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