A New Cell Surface, Detergent-insoluble Glycoprotein Matrix of Human
and Hamster Fibroblasts

THE ROLE OF DISULFIDE BONDS IN STABILIZATION OF THE MATRIX*

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William G. Carter | Sen-itiroh Hakomori
From the Department of Biochemical Oncology, Fred Hutchinson Cancer Research Center, and Department of Pathobiology, School of Public Health and Department of Microbiology and Immunology, School of Medicine, University of Washington, Seattle, Washington 98104

Extraction of cell surface labeled human fibroblasts with a zwitterionic detergent, Empigen BB, solubilizes most of the cytoplasmic and membrane components. The remaining insoluble cell matrix, with numerous fibrillar meshwork structures, is composed of prominent nuclei, cytoskeletal components, and four major cell surface glycoprotein: fibronectin (FN), GP250, GP170, and GP140. Fibronectin and GP170 are rapidly released from the cell surface and the Empigen BB-insoluble cell matrix by mild trypsin digestion. In contrast, both GP250 and GP140 are resistant to trypsin digestion. None of the glycoproteins are sensitive to digestion with collagenase. Neither GP250, GP170, nor GP140 cross-reacts with antisera prepared against fibronectin and are therefore not derived from fibronectin by proteolytic processing.

Extraction of the Empigen BB-insoluble matrix with sodium dodecyl sulfate solubilizes all of the GP250, GP170, GP140, and approximately half of the fibronectin. Chromatography of the sodium dodecyl sulfate extract on Sepharose 2B under nonreducing conditions resolved a high molecular weight protein matrix that was subsequently resolved into the subunits: GP140, GP250, and fibronectin under reducing conditions. This new type of disulfide-dependent glycoprotein matrix at the cell surface is composed of fibronectin multimers, GP250 multimers, and GP140 multimers. The GP140 and GP250 are not disulfide bonded to fibronectin since immune precipitation of the sodium dodecyl sulfate extract with anti-fibronectin antibodies specifically precipitates only fibronectin. (However, evidence is presented suggesting that GP250 and GP140 may form disulfide-dependent heteromultimers.) Since extraction with hot sodium dodecyl sulfate would destroy all noncovalent interactions, the possibility that GP140 and GP250 may form noncovalent interactions with fibronectin in the intact cell is discussed.

In studies of the mechanisms and components involved in cell attachment and spreading (for reviews see Refs. 1–3), as well as cell-cell interactions (4–6), FN, a major transforma-
tion-sensitive cell surface glycoprotein, has been of particular interest (for reviews see Refs. 7–9). FN stimulates the cell attachment and spreading process of both normal and transformed cells when coated on plastic surfaces or when attached to collagen-coated surfaces (for reviews see Refs. 2 and 9). Studies (3, 10) have also indicated that the attachment of cells to FN layers requires the involvement of the cytoskeleton as well as membrane receptors of FN. Recent studies (10, 11) have also suggested that the formation of a stable interaction of FN with the cell surface during cell attachment requires a polyvalent FN surface, as well as polyvalent receptors functioning in a cooperative manner.

Attempts have been initiated to define the cell surface components involved in the interaction with FN. Inhibition studies (12) have indicated that polyasialogangliosides may be involved in the interaction of the cell surface with FN-coated surfaces. However, the fact that polyasialogangliosides inhibit cell attachment nonspecifically on various surfaces, and the fact that polyasialogangliosides are absent from many cells that are stimulated to attach to FN-coated surfaces (13), have suggested that alternative receptors may be involved in the interaction of FN with the cell surface. Cross-linking studies have suggested that soluble FN may interact with sulfated proteoglycan (14) on the cell surface.

Additional information concerning the components involved in the cell attachment process have been gained by differential extraction experiments. Characteristically, extraction of cultured fibroblasts with nonionic or zwitterionic detergents does not disrupt the FN, cytoskeletal, or nuclear matrix organization (15–21), suggesting that these components may play a cooperative role in maintaining the organization of the attached cells. The pericellular matrix of human fibroblasts has also been reported to contain hyaluronic acid, heparan sulfate, and procollagen (22) in addition to myosin and actin. The possibility that FN may be associated with the cytoskeleton was suggested by the co-isolation of FN and actin from hamster fibroblasts (15) and by the observation that FN will bind to insolubilized actin in vitro (23). Double label immunofluorescence studies utilizing antibodies to both FN and actin detected coincident staining of the two fibrillar structures and suggested a transmembrane relationship between the cell surface and the actin cytoskeleton.

Abbreviations used: FN, fibronectin; BSA, bovine serum albumin; DME, Dulbecco's modified Eagle's medium; EDTA, ethylenediaminetetraacetic acid; M, relative molecular mass as estimated by electrophoresis on polyacrylamide gels in the presence of sodium dodecyl sulfate; NIL, hamster embryo fibroblasts; PMSF, phenylmethylsulfonyl fluoride; salt/P, 137 mM NaCl, 2.7 mM KCl, 0.7 mM CaCl₂, 0.5 mM MgCl₂, 8.1 mM NaH₂PO₄, 1.5 mM KH₂PO₄, SDS, sodium dodecyl sulfate.

buffer, pH 7.8 containing 25 mM NaCl; BSA, bovine serum albumin; DME, Dulbecco's modified Eagle's medium; EDTA, ethylenediaminetetraacetic acid; M, relative molecular mass as estimated by electrophoresis on polyacrylamide gels in the presence of sodium dodecyl sulfate; NIL, hamster embryo fibroblasts; PMSF, phenylmethylsulfonyl fluoride; salt/P, 137 mM NaCl, 2.7 mM KCl, 0.7 mM CaCl₂, 0.5 mM MgCl₂, 8.1 mM NaH₂PO₄, 1.5 mM KH₂PO₄, SDS, sodium dodecyl sulfate.

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Disulfide Bonds in Glycoprotein Matrix of Fibroblast Surface

We have investigated the cell surface glycoprotein components present in the detergent-insoluble matrix of cultured human and hamster fibroblasts. Our experiments were undertaken to examine the possibility that new glycoproteins may be involved in localizing FN at the cell surface and possibly connecting the predominantly pericellular FN matrix (28) to the cell surface or the cytoskeleton. Our results indicate that FN and an additional major trypsin-resistant, noncollagenous cell surface glycoprotein, termed GP140, are the major components of the detergent-insoluble matrix. Like FN, GP140 can form an extensive disulfide-cross-linked structure possibly with GP250 a second glycoprotein component of the detergent-insoluble matrix. The possibility that the GP140-GP250 matrix may involve FN through covalent and/or noncovalent bonds has been investigated and discussed.

EXPERIMENTAL PROCEDURES

Cells and Cell Cultures—Primary cultures of human fibroblasts were obtained from skin biopsies of normal donors in the laboratory of C. Ronald Scott (Dept. of Pediatrics, University of Washington). Routinely, stock cells were frozen at the fifth passage and then used for experimentation up to passage 12. Cells were grown in Dulbecco's modified Eagle's medium supplemented with penicillin and streptomycin (100 μg/ml) and fetal calf serum (10%).

Hamster embryo fibroblasts (NIL cells) were maintained in culture as previously described (17).

Materials—Dithiothreitol, soybean trypsin inhibitor, phenylmethylsulfonyl fluoride, N-ethylmaleimide, sodium dodecyl sulfate, bovine serum albumin (crystallized), ethylenediaminetetraacetic acid, and collagen (calfskin) were purchased from Sigma Chemical Co., St. Louis, MO. Sodium periodate (sodium metaperiodate) was from Fisher Scientific Company, Fair Lawn, NJ. 2-Mercaptoethanol was from Polysciences, Inc., Warrington, PA. Empigen BB, a zwitterionic detergent was a generous gift from Alibright & Wilson, Ltd., Whitehaven, England. The detergent was supplied as a stock solution containing 0.1% SDS and 0.5% 2-mercaptoethanol and was diluted for subsequent experiments. Highly purified bacterial collagenase (Form III) was purchased from Advance Biofactures Corp., Lynbrook, NY. Protein A-Sepharose CL-4B (2 mg of protein A/ml of resin) and Sepharose CL-2B-200 were from Pharmacia, Sweden. IgG sorb (attenuated Staphylococcus aureus) was purchased from The Enzyme Center, Inc., Boston, MA. Lyophilized trypsin was obtained from Worthington Biochemicals. Galactose oxidase was from Kabi, Aktebalaget, Fack, Stockholm, Sweden. IgGsorb (attenuated Staphylococcus aureus) was purchased from the University of Washington. Cell surface form FN was purified from cultured hamster fibroblasts as previously described (16, 17). Alternatively, released form human FN was purified from conditioned medium of human fibroblasts by affinity chromatography on gelatin-Sepharose (29).

Antisera—Antisera against FN were from two sources: goat anti-human plasma FN was purchased from Calbiochem-Behring Corp., La Jolla, CA, and formed a single precipitin line in an Ouchterlony test against human plasma. Rabbit anti-hamster plasma FN was prepared by Dr. Kiyotoshi Sekiguchi against affinity-purified (29) hamster plasma FN and formed a single precipitin line against both hamster and human plasma in an Ouchterlony test.

Cell Surface and Metabolic Labeling—Human fibroblasts were metabolically labeled with [3H]glucosamine by culturing in DME (0.25 mM glucose, 0.1 mg of pyruvate/ml supplemented with 10% fetal calf serum, and [3H]glucosamine (91 mCi/mg), New England Nuclear, 2 μCi/ml of media) for 48 h (30). Cells were washed with salt/F, scraped with a rubber policeman, and utilized as described under "Results."

For cell surface labeling, cells were washed, scraped with a rubber policeman, and labeled by the galactose oxidase-NaB[3H], method (31), or the sodium periodate-NaB[3H] method (32).

Polyacrylamide Gel Electrophoresis—Polyacrylamide slab gels containing 0.1% SDS were prepared following the basic stacking SDS gel technique of Laemmli (33). Samples were dissolved in a sample buffer containing 2% w/v SDS, 5% v/v 2-mercaptoethanol and heated in a boiling water bath for 5 min. Where indicated, nonreduced samples were treated as above without 2-mercaptoethanol. Slab gels were stained with Coomassie blue R-250 (34). Fluorography of slab gels followed the procedure of Bonner and Laskey (35). Protein standards for relative molecular mass (Mr) estimation in polyacrylamide gels are as follows: hamster skeletal muscle myosin, 200,000; bovine serum albumin, 68,000; hamster skeletal muscle actin, 43,000, Dolichos biflorus lectin subunit, 27,000. Protein was determined by the fluorescamine method (36).

Scanning Electron Microscopy—NIL cells were grown on glass cover slips, then incubated with 2% v/v Empigen BB plus 0.1% w/v SDS as described in Fig. 2. The adherent cells and extracted cell matrix were fixed in Karnovsky solution for 1.5 h at room temperature, dehydrated with increasing concentrations of ethanol, and then critical point dried. The samples were then shadow cast and examined with a Joel scanning electron microscope.

RESULTS

Extraction of Adherent Cultures of Human and Hamster Fibroblasts with Empigen BB Detergent—Extraction of confluent cultures of human or hamster embryo fibroblasts with the zwitterionic detergent, Empigen BB, did not detach the cells from the culture plates and did not disrupt the adhesion mechanisms or components. The attached Empigen BB-insoluble matrix (Fig. 1) possessed numerous fibrillar meshwork structures that crossed over and under the prominent convoluted nuclei. These results suggest that the components present in the Empigen BB-insoluble matrix may be interacting with each other.

Glycoprotein and Cytoskeletal Components of the Empigen BB-insoluble Matrix of Human Fibroblasts—Confluent cultures of human fibroblasts were cell surface labeled utilizing the sodium periodate-NaB[3H] method (32) in order to label glycoconjugates containing terminal sialic acid residues. The labeled cells were subject to sequential extraction with buffer containing the following: 1) Empigen BB (2%, v/v, a zwitterionic detergent) and SDS (0.1%, w/v). The addition of 0.1% w/v SDS to the Empigen BB solution decreased the quantity of minor proteins detected in the Empigen BB-insoluble matrix without affecting the major components. 2) SDS (0.5%, w/v), 3) SDS (0.5%, w/v) and dithiothreitol (25 mM). Routinely, the first extraction with Empigen BB solubilized 70 to 80% of the cell protein and 60 to 70% of the radioactivity. As seen in Fig. 2B, the Empigen BB-insoluble matrix contained three major labeled glycoproteins with relative molecular masses of 250,000 to 230,000, 170,000 (GP170), and 230,000 (GP230) (see later results). FN and GP170 found in human fibroblasts and human and hamster plasma in an Ouchterlony test. Human and Hamster plasma FN was purchased from Calbiochem-Behring Corp., La Jolla, CA, and formed a single precipitin line in an Ouchterlony test against human plasma.

The similarity and dissimilarities of GP170 found in human fibroblasts to the transformation sensitive 170,000 dalton glycoprotein previously described (Carter, W. G., and Hakomori, S. (1976) J. Biol. Chem. 251, 2867-2874 (37) will be the subject of a detailed report to be presented elsewhere.
Essentially all of the cell surface-labeled glycoproteins, GP250, GP170, and GP140 were extracted from the Empigen BB-insoluble matrix with SDS solution (Fig. 2B4). In contrast, 60% of the labeled FN was solubilized under the same conditions. Complete solubilization of FN required both SDS and reducing agents (Fig. 2B5).

In the absence of 2-mercaptoethanol (Fig. 2B6), the glycoprotein components extracted with SDS exhibited much reduced mobility on the polyacrylamide gel, suggesting extensive disulfide-dependent subunit interactions. Treatment of the cells with 2 mM N-ethylmaleimide, prior to and during the detergent extractions, failed to reduce the extent of the inter-subunit disulfide bonding (results not shown). This indicated that the disulfide-dependent interaction of SDS-extractable glycoproteins was not an artifact induced by the gel electrophoresis or extraction conditions. In addition, when cells were metabolically labeled with [3H]glucosamine and subsequently extracted, the extensive disulfide-dependent interaction was still present, indicating that the cell surface labeling procedure did not induce the disulfide bonding (results not shown) and that the glycoproteins were synthesized by the cells.

Digestion of the Empigen BB-insoluble matrix (Fig. 4) with highly purified bacterial collagenase in the presence of N-ethylmaleimide and PMSF did not affect any of the glycoproteins. In parallel control experiments, collagen was completely digested by the collagenase (Fig. 4). The results indicate that none of the cell surface labeled glycoproteins are collagen-like by the criterion of collagenase sensitivity. However, purification and further analysis of the components will be required to confirm this point.

Digestion of the Empigen BB-insoluble matrix (Fig. 4) with thrombin cleaved the FN, resulting in formation of a major 205,000 molecular mass glycopeptide (Fig. 4). Thrombin has previously been reported to cleave plasma fibronectin (38), resulting in the formation of high molecular mass fragments. Similarly, GP170 was degraded by thrombin. In contrast, neither GP250 or GP140 were affected by the incubation with thrombin.

Digestion of the Empigen BB-insoluble matrix (Fig. 4) with thrombin resulted in the formation of high molecular mass glycopeptide (Fig. 4). Thrombin has previously been reported to cleave plasma fibronectin (38), resulting in the formation of high molecular mass fragments. Similarly, GP170 was degraded by thrombin. In contrast, neither GP250 or GP140 were affected by the incubation with thrombin.

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GP170, or GP140 might hold antigenic determinants in common with FN and that any of these glycoproteins might be disulfide-bonded to FN. The SDS extract of the Empigen BB-insoluble residue was analyzed by electrophoresis on polyacrylamide gels (7%) followed by fluorography. Gel 1, no trypsin; 30 min incubation; 2, 0.1 µg/ml; 5 min; 3, 0.1 µg/ml; 15 min; 4, 0.1 µg/ml; 30 min; 5, 1.0 µg/ml; 5 min; 6, fraction run on gel 1, under nonreducing conditions; 7, fraction run on gel 5, under nonreducing conditions. The cells were incubated for 5, 15, or 30 min at room temperature, then washed twice with PBS containing soybean trypsin inhibitor (50 µg/ml). The washed cells were extracted with Empigen BB detergent as described in Fig. 2 and dissolved in SDS. Equal volumes of each solution of Empigen BB-insoluble residue was analyzed by electrophoresis on polyacrylamide gels (7%) followed by fluorography. Gel 1, no trypsin; 30 min incubation; 2, 0.1 µg/ml; 5 min; 3, 0.1 µg/ml; 15 min; 4, 0.1 µg/ml; 30 min; 5, 1.0 µg/ml; 5 min; 6, fraction run on gel 1, under nonreducing conditions; 7, fraction run on gel 5, under nonreducing conditions.

Fig. 2 (left). Differential extraction of cell surface-labeled human fibroblasts, followed by electrophoresis on polyacrylamide gels in the presence of SDS. Cells were surface-labeled with sodium periodate-NaB\(^{[3]H}\), and then suspended in 10 volumes of 50 mM sodium borate buffer, pH 7.8, containing 25 mM NaCl (BNS buffer) and 2%, v/v, Empigen BB, a zwitterionic detergent, 0.1% w/v SDS, and 1 mM PMSF. The cell suspension was incubated on ice for 30 min and then centrifuged at 35,000 x g for 20 min. The supernatant was removed and labeled “Empigen BB extract” and the insoluble residue was labeled “Empigen BB-insoluble matrix.” The matrix was dissolved in the same volume of 0.5%, w/v, SDS in 25 mM sodium borate buffer, pH 7.8, containing 1 mM PMSF. The suspension was heated in a boiling water bath for 5 min and then recentrifuged as above. The supernatant was removed and labeled “SDS extract” and the insoluble pellet was re-extracted with 0.5% w/v SDS in 25 mM sodium borate buffer, pH 7.8, containing 25 mM dithiothreitol (100 °C, 5 min). The supernatant was labeled “SDS-dithiothreitol extract.” A, protein stains of cells and cell extracts electrophoresed on a polyacrylamide gradient gel (5-14%) in the presence of SDS. B, fluorography of gels in A. Gel 1 in A and B contained 60 µg of protein and 69 x 10^4 cpm, respectively. Gels 2-6 contained extracts from a corresponding quantity of cells. A, 1, human fibroblasts (60 µg of protein); 2, Empigen BB extract; 3, Empigen BB-insoluble matrix; 4, SDS extract; 5, SDS-dithiothreitol extract; 6, SDS extract seen in gel 4 run under nonreducing conditions. Direction of migration is from top to bottom. Numbers in left margin indicated migration of proteins of known molecular weight: myosin (M, 200,000) 200, etc.

Fig. 3 (center). Time course trypsinization of cell surface-labeled human fibroblasts. Human fibroblasts were labeled with the periodate-NaB\(^{[3]H}\), method and suspended in PBS (pH 7.0). Equal aliquots of the labeled cell suspension were mixed with PBS containing trypsin (Worthington 2X crystalized, 189 units/mg) at a final concentration of 0, 0.1, and 1 µg/ml. The cells were incubated for 5, 15, or 30 min at room temperature, then washed twice with PBS containing soybean trypsin inhibitor (50 µg/ml). The washed cells were extracted with Empigen BB detergent as described in Fig. 2 and dissolved in SDS. Equal volumes of each solution of Empigen BB-insoluble residue was analyzed by electrophoresis on polyacrylamide gels (7%) followed by fluorography. Gel 1, no trypsin; 30 min incubation; 2, 0.1 µg/ml; 5 min; 3, 0.1 µg/ml; 15 min; 4, 0.1 µg/ml; 30 min; 5, 1.0 µg/ml; 5 min; 6, fraction run on gel 1, under nonreducing conditions; 7, fraction run on gel 5, under nonreducing conditions.

Disulfide Bonds in Glycoprotein Matrix of Fibroblast Surface
ternatively, FN, GP250, GP170, and GP140 were solubilized from the Empigen BB-insoluble matrix by extraction with 8 M urea containing 20 mM dithiothreitol, followed by dialysis to remove the urea and reducing agent. As seen in Fig. 6, gels 6, 7, and 8, immune precipitation of the prereduced, soluble glycoproteins with anti-FN serum precipitated only FN. No nonspecific trapping of prereduced GP250 or GP140 was detected in the preimmune fraction even in the absence of reducing agent (Fig. 6, gel 7).

These results indicate that neither GP250, GP170, nor GP140 have any antigenic determinants in common with FN.

In addition, the stickiness exhibited by GP250 and GP140 for the Protein A-immunoglobulin complex or Staphylococcus aureus alone can be eliminated by the reduction of the high molecular mass complex. These results also suggest that the disulfide-dependent matrix of GP140-GP250 does not include covalently attached FN, although noncovalent interactions are possible.

Density-dependent Accumulation of GP140 and GP170 in NIL Cells—It is possible that GP250, GP170, and GP140 are unique proteins present only in primary human fibroblasts. In order to eliminate this possibility, we examined hamster embryo fibroblasts (NIL cells) for the presence of similar glycoproteins. NIL cells were cultured at sparse, touching, and confluent cell densities, labeled by the galactose oxidase-NaB[3H] method (31), and extracted with Empigen BB as described above. As seen in Fig. 7, increasing cell density resulted in a corresponding increase in the quantity of GP170, GP140, and FN present in the Empigen BB-insoluble matrix. We were unable to detect any increase in GP250. GP250 may have been masked by the accumulation of FN. It should also be pointed out that in contrast to the results obtained with human fibroblasts, the major quantities of glycoproteins co-migrating with GP140 and GP170, in NIL cells, were efficiently extracted with Empigen BB. This may be due to the presence of additional glycoproteins with similar molecular masses or

![Figure 5](image-url)
incomplete formation of intermolecular disulfide bonds. The accumulation of both GP170 and GP140 in the Empigen BB-insoluble form in NIL cells after cell-cell contact suggests that these components may play a role in an undefined cell-cell contact response. The accumulated GP140 and FN of NIL cells also exhibited extensive intermolecular disulfide bonding on polyacrylamide gels (results not shown).

3 W. G. Carter, manuscript in preparation.
Disulfide Bonds in Glycoprotein Matrix of Fibroblast Surface

...ments of the detergent-insoluble matrix of chicken fibroblasts (42) or the substrate adhesion sites of murine fibroblasts (43), suggesting that collagen may not be required for stabilization of the detergent-insoluble matrix. Studies utilizing scanning and transmission electron microscopy or immunofluorescence microscopy have suggested that actin is present in the matrix in the form of bundles of microfilaments (18-22, 42, 44, 46). The FN is also filamentous (22, 42, 46) while procollagen appeared to co-distribute with FN (22). Intermediate filaments also appear as a fine fibrillar network (19, 44) in the nuclear monolayers. In studies where nuclei have been removed from the nuclear monolayer by extraction with sodium deoxycholate (22, 42), subunits of intermediate filaments have also been lost from the matrix, supporting the report by Lehto et al. (19) that intermediate filaments may be instrumental in anchoring the nuclei in nuclear monolayers.

Gonen et al. (20) concluded that the insolubility of the adherent cytoskeleton in Triton X-100 was acquired either concurrently with cell adhesion or very closely with it. The authors also concluded with cell adhesion or very closely with it. The authors also concluded that the majority (90%) of the cell-surface FN could be removed by digestion with trypsin, without affecting the relative amount or composition of the anchored cytoskeletal proteins. Apparently most of the cell surface FN and procollagens present in the matrix may not be necessary for maintenance of nuclear or cytoskeletal adhesion.

FN and collagen have been the only cell surface glycoproteins previously detected in the detergent-insoluble matrices (15-22, 42, 45). We have examined the Empigen BB-insoluble matrix of confluent cell monolayers and suspended human and hamster fibroblasts with the specific aim of detecting other cell surface glycoproteins in addition to FN or procollagen and defining the basis for their insolubility in Empigen BB. After extraction of cultured human fibroblasts in suspension, or in monolayer cultures with Empigen BB, an insoluble matrix composed of nuclei, cytoskeletal components, and four major cell surface glycoproteins remains. The four glycoproteins termed FN, GP250, GP170, and GP140, are all insensitive to digestion by purified bacterial collagenase suggesting that they are not procollagens. Both GP170 and FN in human fibroblasts were readily digested with trypsin or bovine thrombin. In contrast, GP250 was slowly released by trypsin digestion and GP140 was resistant to prolonged incubation with trypsin. The resistance of GP140 and GP250 to digestion by trypsin is of interest since removal of the major portion of cell surface FN with trypsin does not alter cell attachment to FN-coated surfaces (13) or the composition of the anchored cytoskeleton (20). These reports suggest that a cell surface receptor for FN can rapidly reappear on the cell surface after initial removal by trypsin, or that it is resistant to digestion by trypsin.

Extraction of the Empigen BB-insoluble matrix with SDS solubilizes all of the cell surface-labeled GP170, GP140, GP250, and approximately 60% of the FN. Complete solubilization of FN required the addition of reducing agents. As previously reported (47, 48), FN forms an extensive disulfide-dependent matrix on the cell surface. However, molecular sieve chromatography of the SDS extract of the glycoproteins indicated that both GP250 and GP140 were also able to form an extensive disulfide-dependent matrix. However, immune precipitation of the SDS extract with antisera to FN specifically precipitated only FN. These results indicated that neither GP140 nor GP250 appeared to be disulfide-bonded to the FN matrix and also indicated that neither GP250, GP170, nor GP140 had any antigenic determinants shared with FN. Therefore, GP250, GP170, and GP140 are not derived from FN by proteolytic degradation. This conclusion was also supported by the differential extraction results (Fig. 2) and the differential sensitivity of the glycoproteins to proteolytic digestion (Fig. 3). Although FN was not disulfide-bonded to either GP250 or GP140, the co-elution of GP250 and GP140 from the Sepharose 2BCl column (Fig. 5) suggests that the GP250 and GP140 may be disulfide-bonded to each other in a heteromultimer complex. The formation of a disulfide-dependent heterocomplex would account at least partially for the resistance of both GP140 and GP250 to extraction by Empigen BB. We conclude that GP140 and GP250 exist as a new cell surface matrix, consisting of disulfide stabilized homor heteromultimer(s) that are not covalently associated with the FN matrix.

The possibility that the FN matrix possesses a noncovalent interaction with GP140, GP250 or GP170 is intriguing. However, the complete removal of FN from the cell surface with trypsin did not remove GP250 or GP140, suggesting that GP250 and GP140 are not bound to the outside of the FN matrix. Rather, GP140 and GP250 would have to be located between the FN matrix and some other structure, such as the cell surface or the cytoskeleton. This possibility is currently under investigation.

Empigen BB-insoluble GP140 and GP170 were also detected in confluent cultures of NIL cells, suggesting that these glycoproteins are not unique to human fibroblasts. However, in NIL cells the accumulation of Empigen-insoluble GP140 and GP170 depended on increasing cell density. This suggests that cell-cell contact was instrumental in stimulating the accumulation, either as a result of increased synthesis or increased disulfide bonding which stabilized the glycoproteins to detergent extraction. The quantity of FN detectable on the cell surface has previously been reported to depend both on the cell cycle and cell density (49-52). Conceivably, the disulfide-dependent polymerization of GP140 could depend on the cell density of the culture. GP250 was not clearly detected in NIL cells, either as a result of overlap with FN or its absence. GP170 and its relation to the previously reported transformation-sensitive 170,000-dalton glycoprotein (37) will be reported elsewhere.

The presence of cell surface GP140, in addition to FN as cellular components of the Empigen BB-insoluble matrix of both attached and suspended cells, suggests that GP140 may play some role in locating FN at the cell surface particularly in areas of cell-cell contact, where FN has been reported to accumulate (46, 53). FN exists predominantly as a pericellular glycoprotein (28). The mechanism by which the FN matrix associates with the surface of cells is under intensive investigation. The presence of a trypsin-insensitive, cell surface glycoprotein that is present with the FN matrix after detergent extraction, suggests a possible direct interaction between the two disulfide-stabilized glycoproteins on the cell surface. We have previously examined and discussed the importance of cooperativity in the interaction of polyvalent FN surfaces with the polyvalent cell surface receptors in inducing stable cell attachment and cell spreading (10, 13). It is possible that the presence of polyvalent GP140 may provide sufficient stabilization of even low affinity interaction with FN to resist detergent extraction. This possibility is currently under investigation. After completion of this work, Lehto et al. (54) described a trypsin-resistant, 140,000-dalton glycoprotein present in Triton X-100-extracted cell skeletons. These authors suggested that GP140 may play a significant role in anchorage of the cytoskeleton to the substratum. Also, Wylie et al. (55) prepared antisera to cell surface glycoproteins that will inhibit cell attachment to plastic surfaces. A cell surface glycoprotein

* W. G. Carter and S. Hakomori, manuscript in preparation.
with a molecular weight of 140,000 was the major cell surface antigen for the inhibitory antiserum.

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