Functional Role of Laminin Carbohydrate

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Previous work showed that tunicamycin suppresses glycosylation of laminin. In the present work, the role of glycosylation in the secretion of laminin and in the disulfide bonding of laminin subunits was studied, using tunicamycin to inhibit glycosylation. Tunicamycin inhibited extensively the secretion of laminin into culture medium and extracellular matrix even though the treated cells contained higher concentrations of laminin than the control cells. The laminin subunits synthesized in the presence of tunicamycin were disulfide bonded. Thus, suppression of glycosylation did not adversely affect disulfide bonding of the subunits, but did decrease the secretion of laminin. Glycosidases were also used to remove the carbohydrate of laminin to study the role of carbohydrate in the stability of laminin and in its interaction with another extracellular matrix component, heparin. The glycosidases removed about 73% of [³H]glucosamine. Both glycosidase-treated and untreated laminin were stable when incubated with cell lysate or culture medium. The glycosidase-treated laminin bound as efficiently as the untreated laminin to heparin. These results suggest that the presence of a carbohydrate moiety, at least at the level found in untreated laminin, is not essential in binding to heparin or in protecting laminin from proteolytic degradation in the cell or culture medium.

The basement membrane is a complex of extracellular macromolecules that separates epithelial and endothelial cells from the mesenchyme. In early postimplantation mouse embryos, the basement membrane appears as Reichert's membrane between the parietal endoderm and trophoblast and, at a later stage, between the ectoderm and endoderm of postimplantation embryos (20). The extracellular macromolecules of basement membrane include type IV collagen, noncollagenous glycoproteins, and proteoglycans (13). Laminin is a noncollagenous glycoprotein (3, 27) consisting of three distinct subunits (4, 10) with 11 to 14 oligosaccharide chains in each subunit (10). Usually localized close to epithelial cells in the basement membrane (7, 17), laminin not only interacts with other extracellular matrix components such as glycosaminoglycan and type IV collagen (19, 21, 25), but also promotes cell adhesion (2, 6, 12).

It has been suggested that the carbohydrate moieties of glycoproteins play a role in glycoprotein secretion (8, 9) and also in cell surface interactions that mediate embryonic development and differentiation (1, 24). We have found that tunicamycin (TM) efficiently inhibits glycosylation of laminin and that the resulting underglycosylated laminin contains little glucosamine and has a faster mobility than the glycosylated form (10). Thus, TM may be expected to inhibit the secretion of laminin, which is first detectable in murine embryos at the 16-cell stage (16), and to account for the reported inhibition of compaction of 8-cell embryos (1, 24). We report here the functional role of laminin carbohydrate in secretion, disulfide bonding, stability, and interaction with other ligands.

MATERIALS AND METHODS

Cell culture and radiolabeling. Murine teratocarcinoma-derived parietal yolk sac (PYS-2) cells (15) were incubated with or without TM (1 µg/ml; Calbiochem-Behring) for 4 h in Eagle minimum essential medium containing one-tenth the normal amount of methionine and 10% dialyzed fetal bovine serum and then labeled in the same medium with [³⁵S]methionine (50 µCi/ml; >400 Ci/mmol; New England Nuclear Corp.). TM was solubilized in dimethyl sulfoxide as 100×

stock solution. At the end of the labeling period, culture medium was removed, adjusted to 2 mM phenylmethylsulfonyl fluoride, and centrifuged to remove dead cells. The cell layers were washed three times with Dulbecco modified phosphate-buffered saline (PBS), scraped from the plates, suspended in small volumes of 0.15 M NaCl–0.05 M Tris-hydrochloride (pH 7.5), and lysed with 0.5% sodium dodecyl sulfate (SDS). The lysates were sonicated with ultrasonics (Heat Systems), and 4 volumes of 0.15 M NaCl–0.05 M Tris-hydrochloride (pH 7.5) was added to yield a final concentration of 0.1% SDS. Protein was precipitated from a 50-µl sample of the cell lysate or culture medium by the addition of 2 ml of 10% trichloroacetic acid (TCA) and collected by filtration onto nitrocellulose filters. The radioactivity was then determined with a Packard liquid scintillation spectrometer, and from this, the total radioactivity for the culture medium or cell extract was calculated. The total protein in the 50-µl sample of the cell extract was determined by a Bio-Rad protein assay.

Laminin secretion. To determine the relative concentration of laminin, the culture medium or cell extract was first microfuged to remove any insoluble material. Samples containing equal amounts of radioactivity were taken from control and experimental culture media (5.6×10^5 cpm and 1.5×10^6 cpm from 4-h and 18-h labeled cells, respectively) or cell extracts (7×10^6 cpm and 1.3×10^7 cpm from 4-h and 18-h labeled cells, respectively). From each sample, the laminin was immunoprecipitated and the radioactivity was measured, and from this, the total amount of laminin in the medium or cell extract was calculated. The relative concentration of laminin is the ratio of the total laminin radioactivity to the total radioactivity in the medium or cell extract. Immunoprecipitation of laminin with antilaminin serum prepared in our laboratory was performed as described previously (11), and its electrophoresis on one-dimensional 5% SDS-polyacrylamide gels was performed by the method of Laemmli (14).

Immunofluorescence microscopy. PYS-2 cells were grown on glass cover slips with or without 1 µg of TM per ml for 18 h. Cells were then fixed and made permeable in acetone-

methanol (1:1) at -20°C for 10 min and then treated with antilaminin serum (1:10 dilution) and then with fluorescein-conjugated goat anti-rabbit immunoglobulin G (heavy and light chain specific, 1:10 dilution, Cappel Laboratories).

Electron microscopy. Cells were grown in 60-mm plastic petri dishes with or without $1\text{ }\mu\text{g}$ of TM per ml for 18 h, washed twice with PBS at room temperature, and fixed with ice-cold 3% glutaraldehyde in 0.1 M PIPES [piperazine-*N*, *N'*-bis(2-ethanesulfonic acid)] (pH 7.2). The cells were then postfixed in 1% OsO_4 in 0.1 M PIPES (pH 7.2), stained with 1% uranyl acetate (wt/vol) at 60°C overnight, dehydrated with alcohol, and embedded in Epon. Sections were cut perpendicular to the plastic surface with a diamond knife on a Sorvall ultramicrotome, stained with lead citrate, and examined in a Hitachi HU 11-E electron microscope.

Iodination of laminin. Purified EHS tumor laminin (Bethesda Research Laboratories) was labeled with ^{125}I by the procedure of Markwell and Fox (18). 1,3,4,6-Tetrachloro-3 α ,6 α -diphenylglycouril (Iodogen; Pierce Chemical Co.) solubilized in chloroform was added to the test tubes and dried under a stream of N_2 gas. Laminin ($5\text{ }\mu\text{g}$) and Na^{125}I (400 μCi ; Amersham Corp.) were added to the Iodogen-coated test tube, and the reaction was allowed to proceed for 10 min with gentle mixing. The iodinated laminin was dialyzed to remove free ^{125}I .

Digestion of carbohydrate by glycosidases. The mixture of endo- and exoglycosidases purified from *Streptococcus*

FIG. 1. Immunoprecipitation of laminin from cell lysate or culture medium of cells incubated with or without TM. Confluent cultures (6-cm petri dish per treatment) were incubated for 4 h in the presence or absence of $1\text{ }\mu\text{g}$ of TM per ml and labeled for 18 h with [^{35}S]methionine (50 $\mu\text{Ci}/\text{ml}$) in the same medium. At the end of the labeling period, the medium was removed, and cells were lysed with 0.15 M NaCl–0.05 M Tris-hydrochloride (pH 7.5)–0.5% SDS. Samples of control and experimental media or cell extracts containing equal amounts of radioactivity were adjusted to contain final concentrations of 0.1% SDS and 1% Nonidet P-40, and laminin was immunoprecipitated and analyzed by SDS-polyacrylamide gel electrophoresis. The autoradiogram was exposed for 4 h, except lane 5, which was exposed for 2 days. Lanes 1 and 2, Laminins immunoprecipitated from culture media (1.5×10^6 cpm) of control and TM-treated cells, respectively. Lanes 3 and 4, Intracellular laminins immunoprecipitated from lysates (1.3×10^7 cpm) of control and TM-treated cells, respectively. Lane 5, Same as lane 2 but exposed for 2 days. Lanes 6 and 7, The laminins shown in lanes 1 and 2, respectively, analyzed under nonreducing conditions (without dithiothreitol). The numbers show the molecular weight ($\times 10^3$). Similar analysis was conducted on laminin from the culture medium or lysate of cells labeled for 4 h with [^{35}S]methionine. The results were quantitated and are shown in Table 2.

pneumoniae and shown to be essentially protease-free was a generous gift of G. Ashwell, National Institutes of Health. The preparation contains at least seven glycosidases: neuraminidase, β -galactosidase, β -*N*-acetylglucosaminidase, α -fucosidase, endo- β -galactosidase, endo- β -*N*-acetylglucosaminidase D, and endo- α -*N*-acetylglucosaminidase. The ^{125}I -labeled EHS tumor laminin (2.6×10^6 cpm; 150 ng) was digested overnight at 37°C with the glycosidases in 0.1 M phosphate-citrate buffer (pH 6.0) in a reaction volume of 400 μl . The [^3H]glucosamine-labeled laminin was also digested with or without glycosidases and, immunoprecipitated with antilaminin serum, and the precipitates were fractionated on 5% SDS-polyacrylamide gel. The amount of [^3H]glucosamine label removed by the enzyme was calculated by comparing the densitometry tracings of the individual bands in the autoradiograms. About 73% of the [^3H]glucosamine label was removed from the laminin.

Binding of laminin to heparin-Sepharose. Heparin-Sepharose (Pharmacia) was swollen and washed with PBS at 4°C . The ^{125}I -labeled EHS tumor laminin (3.5×10^5 cpm; 20 ng) treated with or without glycosidases in 200 μl of PBS was added to 20 μl of heparin-Sepharose (50% suspension in PBS) and incubated in the presence of 1% bovine serum albumin at 4°C overnight with gentle shaking. As controls, binding of laminin to gelatin-Sepharose or Sepharose was also performed. The microfuge tubes used in the binding assays were preincubated with 10% bovine serum albumin to saturate any nonspecific binding sites for laminin. The heparin-Sepharose was microfuged, and the unbound material in the supernatant was removed. The beads were then washed four times with PBS and transferred to new microfuge tubes. The bound material was quantitated in a gamma counter and then eluted twice by boiling with 25- μl volumes of electrophoresis buffer. The eluents were pooled and analyzed by 5% SDS-polyacrylamide gel electrophoresis.

To inhibit binding to heparin-Sepharose, 20, 50, or 100 μg of cold unlabeled laminin treated with or without glycosidases was preincubated with heparin-Sepharose for 1 h at 4°C with gentle shaking and then ^{125}I -labeled laminin (glycosidases treated or untreated; 3.5×10^5 cpm; 20 ng) was added into the mixture, which was incubated overnight at 4°C and washed as described above. Inhibition of binding with soluble heparin was performed similarly.

To test binding of underglycosylated laminin from culture medium of TM-treated cells to heparin-Sepharose, 0.5 ml of medium from TM-treated cells labeled for 4 h with [^{35}S]methionine was incubated with 40 μl of heparin-Sepharose or gelatin-Sepharose at 4°C overnight. The bound material was then either eluted twice with electrophoresis buffer and analyzed directly by SDS-polyacrylamide gel electrophoresis or eluted twice with a small volume of 1 M NaCl, adjusted to 0.15 M NaCl, 0.05 M Tris-hydrochloride (pH 7.4), 1% Nonidet P-40, and 0.1% SDS, immunoprecipitated with antilaminin serum, and then analyzed electrophoretically.

RESULTS

Effect of TM on secretion of laminin. PYS-2 cells were preincubated for 4 h in the presence of TM and then labeled for 4 or 18 h with [^{35}S]methionine, which was added to the same medium. Based on the TCA-precipitable radioactivity in the medium and in the cell extract, TM inhibited both secretion and synthesis of total proteins by only 10 to 25% during the total 8- or 22-h period of treatment (Table 1). The autoradiogram (Fig. 1) of laminin immunoprecipitated from culture medium shows that the relative concentration of

TABLE 1. Effect of TM on synthesis and secretion of total proteins^a

Treatment	[³⁵ S]methionine (cpm/μg of cell protein) incorporated into TCA-precipitable materials in:			
	Medium		Cell extract	
	4 h (8 h)	18 h (22 h)	4 h (8 h)	18 h (22 h)
Control	2,843 (100%) ^b	22,744 (100%)	35,875 (100%)	125,128 (100%)
TM	2,122 (74.6%)	18,755 (82.5%)	28,080 (78.3%)	110,074 (88.0%)

^a Cells were preincubated for 4 h in TM and then labeled with [³⁵S]methionine in the presence of TM for 4 or 18 h. Total time of TM treatment is shown in parentheses. Cells were lysed with 0.15 M NaCl–0.05 M Tris-hydrochloride (pH 7.5)–0.5% SDS, sonicated, and adjusted to 0.1% SDS, and 50 μl samples of cytoplasmic extracts were precipitated with TCA as described in the text. Samples (50 μl) of media were also TCA precipitated.

^b Percent activity relative to control as 100% is shown in parentheses.

laminin in medium from TM-treated cells is markedly lower than that from control cells. In contrast, the relative concentration of laminin in cell extract from TM-treated cells is, if anything, higher than that from control cells (Fig. 1). Measurement of the radioactivity of the immunoprecipitate reveals that the relative concentration of laminin in TM-treated culture medium is only 7 to 11.5% of that found in control medium during 8 to 22 h of experimental time (Table 2). However, the relative concentration of laminin in TM-treated cell lysate is 133.3 to 191.7% of that in control cell lysate, indicating selective enrichment of laminin in TM-treated cells. Thus, the results show that the secretion of laminin relative to other proteins is specifically and markedly inhibited by TM even though the cells have a higher concentration of laminin.

Analysis of the laminin immunoprecipitated from the medium of TM-treated cells in SDS-polyacrylamide gel under nonreducing conditions shows that the underglycosylated laminin subunits, like the glycosylated ones, are disulfide bonded (Fig. 1, cf. lane 1 with lane 6 and lane 5 with lane 7). The underglycosylated laminin in the cell lysate is also disulfide bonded (not shown).

Since it is not known whether extracellular matrix laminin is soluble in 0.5% SDS under isotonic conditions, the following experiments were performed to determine whether TM inhibits secretion and incorporation of laminin into the extracellular matrix. In the presence of TM, newly trypsinized PYS-2 cells do not adhere to the substrate. However, cells allowed to attach before TM treatment remain attached and continue to incorporate radioactive amino acid during the 22-h treatment (Table 1). By indirect immunofluorescence microscopy, these cells exhibit no detectable cell-associated extracellular matrix laminin, but clearly exhibit the presence of cytoplasmic laminin (Fig. 2A). By contrast, extracellular matrix laminin predominates over intracellular cytoplasmic laminin in control cells (Fig. 2B).

Electron micrographs of cells incubated for 18 h with or

without TM revealed that the rough endoplasmic reticulum and Golgi complex, organelles involved in glycosylation (22, 23), were more distended in TM-treated cells (Fig. 3), as previously observed (5). However, no apparent differences were observed in cells treated with TM for 8 h (not shown).

Stability of underglycosylated laminin. Purified laminin from the EHS tumor was labeled with ¹²⁵I and incubated with or without a mixture of glycosidases to remove carbohydrates. The treated laminin migrated faster than the untreated laminin and showed the same mobility as the underglycosylated laminin from the TM-treated PYS-2 cells (cf. Fig. 4, lane 1, with Fig. 4, lane 3, and with Fig. 1). In addition, the glycosidase-treated laminin yielded three distinct bands, indicating that there are three subunits in the EHS tumor laminin, two of which migrate together as a diffuse band, similar to the migration of the glycosylated laminin from PYS-2 cells. The stabilities of the glycosidase-treated and untreated laminins were tested by incubating them with the cell lysate or with the culture medium of the TM-treated cells. No detergents or protease inhibitors were added to the cell lysate or the culture medium. Figure 4 shows that the underglycosylated laminin, like the glycosylated form, is not susceptible to proteolytic digestion in the cell lysate. The same results were obtained when the glycosidase-treated or untreated laminin was incubated with culture medium of TM-treated cells or with cell lysate or culture medium of control cells (not shown).

Interaction of glycosidase-treated laminin with heparin. Both glycosidase-treated and untreated laminins bound equally well to heparin, and the interactions of both laminins with heparin were specific, the binding to heparin being much more efficient than to gelatin or Sepharose (Table 3). The electrophoretic profiles of heparin-bound materials show that these materials were indeed the glycosylated and underglycosylated laminins (Fig. 5). Similarly, underglycosylated laminin in the culture medium of TM-treated cells binds specifically to heparin-Sepharose, but not to gelatin-

TABLE 2. Secretion of laminin in the presence of TM^a

Treatment	Total radioactivity (× 10 ³ cpm) in:				Total laminin radioactivity (× 10 ³ cpm) in:			
	Medium		Cell extract		Medium		Cell extract	
	4 h (8 h)	18 h (22 h)	4 h (8 h)	18 h (22 h)	4 h (8 h)	18 h (22 h)	4 h (8 h)	18 h (22 h)
Control	2,912	9,552	36,772	52,553	375 (12.9) ^b	1,330 (13.9)	1,088 (3.0)	605 (1.2)
TM	1,394	4,651	16,008	27,298	13 (0.9)	75 (1.6)	647 (4.0)	616 (2.3)

^a The total incorporated radioactivity in medium or cell extract was calculated from the data shown in Table 1. To determine laminin radioactivity, samples containing equal amounts of radioactivity were taken from control and experimental media or cell extracts shown in Table 1 and laminin precipitated, and radioactivities were measured. The total laminin radioactivity in the medium or cell extract was calculated from those measurements.

^b The relative concentration of laminin in the medium or cell extract was calculated as the ratio of total laminin radioactivity to total radioactivity and is shown in parentheses.

FIG. 2. Immunofluorescence micrographs of TM-treated and untreated cells. PYS-2 cells were cultured in the presence or absence of 1 μ g of TM per ml for 18 h, fixed, and stained. Antilaminin serum was used to stain TM-treated cells (A), and untreated cells (B). Cells treated with preimmune serum showed weak fluorescence (not shown).

Sepharese, as shown by analysis of the bound material by electrophoresis or immunoprecipitation (results not shown). About the same amount of glycosidase-treated and untreated laminins bound to heparin-Sepharese when the amounts of heparin-Sepharese were increased two- to fourfold (results not shown), indicating that the laminin-binding site in heparin was never saturated within the range of heparin used in our experiment.

The binding of 125 I-labeled glycosidase-treated or untreated laminin to heparin-Sepharese was not inhibited by a 1,000- to 5,000-fold excess of either glycosidase-treated or untreated laminin. However, the binding was inhibited by 65% by 2.5 μ g of soluble heparin per ml and by 91% by 5 μ g of soluble heparin per ml.

DISCUSSION

The data presented in this paper indicate that TM extensively inhibits the secretion of laminin into the culture medium or extracellular matrix, although the treated cells contain a higher concentration of laminin than the control cells. TM does not, however, markedly inhibit either secretion or synthesis of total proteins. It is interesting to note that the effect of TM on total radioactivity incorporated (Table 2) is much greater than the radioactivity incorporated per micro-

gram of protein (Table 1). We attribute this apparent discrepancy to an adverse effect of TM on cell attachment to the substrate. Indeed, we repeatedly obtained fewer cells in TM-treated cultures than in the control cultures. The small amount of underglycosylated laminin found in the culture medium in the presence of TM during prolonged labeling is, in fact, due to inhibition of secretion by TM and is not indicative of its degradation by proteolytic enzymes in the culture medium since glycosidase-treated laminin is as stable as the undigested laminin. The low level of laminin in the culture medium is also not due to the inability of antilaminin serum to immunoprecipitate underglycosylated laminin since the antibody precipitated laminin from both TM-treated and untreated cells. The inhibition of secretion into the culture medium or extracellular matrix of the underglycosylated intracellular laminin is not due to a lack of disulfide bonding of the subunits or to a lack of interaction with other extracellular matrix components, e.g., heparin. Decreased solubility also does not account for the inhibition, as intracellular underglycosylated laminin is readily extracted by Nonidet P-40 for immunoprecipitation, and we found no evidence to indicate precipitation or aggregation of underglycosylated laminin. Accumulation of intracellular immunoglobulin M has also been reported for TM-treated plasmacytoma cells (8).

The TM-treated cells secrete negligible amounts of laminin to the extracellular matrix. Thus, the relative amount of laminin synthesized by TM-treated cells (2.2 to 3.8%) can be calculated from the total laminin radioactivity in the medium plus cell extract. The untreated control cells, however, secrete laminin into the extracellular matrix. At present, we do not know to what extent 0.5% SDS at isotonic salt concentration solubilizes the extracellular matrix laminin. However, the relative amount of laminin synthesized by the control cells should be at least 3.1 to 3.7%, based on the estimated total radioactivity of medium and cell lysate. The inability to estimate the amounts of newly synthesized laminin in basement membrane precludes a more precise estimate of the effect of TM on laminin synthesis.

The mechanisms by which glycosylation modulates secretion is not yet understood. The detection by immunoelectron microscopy of laminin in distended rough endoplasmic reticulum and Golgi complex should provide clues as to whether migration of underglycosylated laminin through these membranous systems is impaired. The inhibition of laminin secretion by TM has also been detected by immunofluorescence microscopy in dental epithelial cells (26). The extent of inhibition of secretion of different immunoglobulin classes by TM has been correlated with the number of carbohydrate chains present in the glycoproteins (8). Thus, the extensive inhibition of laminin secretion by TM is not surprising in light of the 11 to 14 oligosaccharide chains normally present in each subunit (10).

One important characteristic of laminin is its ability to interact with other macromolecules, e.g., type IV collagen and heparin (19, 21, 25). Such interactions could be the basis for formation of extracellular matrix which, in turn, mediates cellular differentiation. Glycosidase treatment of laminin, which removed more than 70% of the carbohydrate from laminin, yielded a material that bound to heparin as efficiently as untreated laminin. This might suggest that the binding site resides in the polypeptide chain rather than in the carbohydrate moiety. In the laminin-binding assay, the inability of laminin to saturate laminin-binding sites in heparin, regardless of the amount of heparin used, suggests the abundance of laminin-binding sites in heparin. Considering

FIG. 3. Electron micrographs of PYS-2 cells after an 18-h incubation with (B) or without (A) 1 μ g per ml of TM ($\times 10,500$). Prominent distention of the rough endoplasmic reticulum (R) and of Golgi complexes (G), seen as groups of stacked cisternae, is apparent in the TM-treated cells. (C) and (D), Golgi complexes in untreated and TM-treated cells, respectively, at higher magnification ($\times 63,000$); (E) and (F), rough endoplasmic reticulum in untreated and TM-treated cells, respectively, at higher magnification ($\times 63,000$).

FIG. 4. Stability of glycosidase-treated laminin. Purified EHS tumor laminin was labeled with ^{125}I and then digested with or without a mixture of glycosidases. The treated and untreated laminins were incubated at 37°C with the lysate of TM-treated cells. Cell lysate (not labeled) was prepared by disrupting the cells in 0.15 M NaCl–0.05 M Tris-hydrochloride (pH 7.5) by ultrasonics and removing the trace amounts of insoluble material by centrifugation in a microfuge for 1 min. Samples were removed at 0 or 4 h and electrophoresed. Glycosidase-treated laminin was incubated with cell lysate for 0 h (lane 1) or 4 h (lane 2). Untreated laminin was incubated with cell lysate for 0 h (lane 3) or 4 h (lane 4). Numbers on the left show molecular weight ($\times 10^3$).

the repeating disaccharide units of glucosamine and a uronic acid residue in heparin, it seems reasonable that heparin would contain many repeating binding sites for laminin. In fact, we found that the binding to heparin-Sepharose was not inhibited even by a 1,000- to 5,000-fold excess of cold unlabeled laminin. The binding of glycosidase-treated or untreated laminin to heparin-Sepharose appears to be specific since this binding is inhibited by heparin and is more

TABLE 3. Binding of glycosidase-treated laminin to heparin-Sepharose^a

Laminin	Ligand	% Input radioactivity bound ^b
Untreated	Heparin-Sepharose	33.3 (100%)
	Gelatin-Sepharose	1.0 (3.0%)
	Sephacrose	0.4 (1.2%)
Glycosidase treated	Heparin-Sepharose	32.1 (100%)
	Gelatin-Sepharose	3.9 (12.1%)
	Sephacrose	3.7 (11.5%)

^a The same amount of ^{125}I -labeled glycosidase-treated or untreated laminin (3.5×10^5 cpm) was mixed with 20 μl of heparin-Sepharose, gelatin-Sepharose, or Sepharose alone and incubated overnight with shaking at 4°C .

^b Values are the average of two independently performed experiments, each performed in triplicate. The efficiencies of binding to gelatin-Sepharose or Sepharose as compared with heparin-Sepharose are shown in parentheses.

FIG. 5. Electrophoretic analysis of laminin bound to heparin-Sepharose. Lanes 1 and 2, ^{125}I -labeled glycosidase-treated and untreated laminin, respectively, before binding to heparin-Sepharose. Lanes 3 and 4, Heparin-Sepharose-bound glycosidase-treated and untreated material, respectively. Numbers on the left show molecular weight ($\times 10^3$).

efficient than the binding of laminin to gelatin-Sepharose or Sepharose.

The intracellular accumulation of laminin in the TM-treated cells is greater than in the untreated cells, suggesting that the inhibition of secretion of underglycosylated laminin results from an event other than enhanced degradation of intracellular laminin. In fact, the absence of degradation is supported by the incubation of glycosidase-treated or untreated laminin with cell lysates. Other examples of stable underglycosylated proteins have been reported, such as immunoglobulin M, for which stability was demonstrated in pulse-chase experiments (8).

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