Assignment of laminin heavy chains using the lectin *Ricinus communis* agglutinin-1

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Using high-resolution PAGE and Western-blotting techniques the lectin *Ricinus communis* agglutinin-1 (RCA-1) was tested for its ability to recognize laminin subunits from the mouse Engelbreth–Holm–Swarm (EHS) tumour and from bovine cardiac and skeletal muscle. Biotinylated RCA-1 recognized both the A and B chains of purified EHS-tumour laminin with a sensitivity comparable to anti-(EHS laminin) antibodies. In cardiac and skeletal muscle RCA-1 also recognized the B chains of laminin, together with a ~ 330 kDa RCA-1-binding glycoprotein that was undetectable in smooth muscle. This glycoprotein was not recognized by antibodies raised to laminin from the EHS tumour. Purification of the 330 kDa binding

glycoprotein from skeletal muscle, using ion-exchange and lectin-affinity chromatography, revealed that in its native form, this glycoprotein is disulphide-bonded to the B chains of laminin. The demonstrated properties of the ~ 330 kDa RCA-1-binding glycoprotein are identical to those reported for the variant M chain of merosin which is known to replace the A chain in laminin from the extrasynaptic regions of skeletal muscle. These results establish that biotinylated RCA-1 can recognize A-, B- and M-chain subunits of laminin isoforms, and that, when used in conjunction with other techniques, they provide a useful method for the assignment of laminin heavy chains.

INTRODUCTION

Laminins are high-molecular-mass (800–900 kDa) heterotrimeric glycoproteins that are ubiquitously involved in the formation and function of mammalian basement membranes (reviewed by Martin et al., 1988; Timpl, 1989; Beck et al., 1990). Five genetically related polypeptide subunits of laminin [designated A, B₁, B₂, M and S (see below)] have been identified which assemble into isoforms that differ in both tissue specificity and biological activity.

Most fully documented of these isoforms is 'classical' laminin isolated from the mouse Engelbreth–Holm–Swarm (EHS) tumour (Timpl, 1989). EHS laminin consists of an A chain (400–440 kDa) together with a B₁ and a B₂ chain (each ~ 200–220 kDa) arranged through disulphide and non-covalent linkage into a characteristic cruciform structure (Timpl, 1989; Beck et al., 1990). Multiple N-glycosylation sites are distributed throughout EHS laminin, resulting in a carbohydrate content ranging from 13–15% to 25–30% of the total molecular mass (Beck et al., 1990). This glycosylation pattern represents one of the most heterogeneous arrays of oligosaccharides reported for any glycoprotein (Knibbs et al., 1989) and provides specific recognition sites for a variety of lectins (Dean et al., 1990; Cooper et al., 1991; Sato and Hughes, 1992). Overall, laminin exhibits a diverse array of molecular interactions, that include binding to numerous basement-membrane components, an ability to self-polymerize in a Ca²⁺-dependent fashion (Timpl, 1989; Beck et al., 1990), and the capacity to recognize several classes of cell-surface receptors (Mecham, 1991). Through concerted interactions laminin is involved in a wide variety of biological activities, including promotion of cell growth, migration, differentiation, and tumour metastases (Beck et al., 1990; Mecham, 1991).

In recent years, two variant polypeptide subunits of laminin have been described. S-laminin (Hunter et al., 1989a) is a

~ 190 kDa polypeptide highly homologous to the B₁ chain and is concentrated in a subset of basement membranes, including that of the synaptic cleft at the neuromuscular junction (Hunter et al., 1989a; Sanes et al., 1990) and the neural retina (Hunter et al., 1992). S-laminin possesses a motor-neuron-selective adhesive site (Hunter et al., 1989b), and laminin containing this subunit, rather than B₁, appears to play a key role in the formation of motor end plates and in retinal differentiation.

A second variant polypeptide subunit was discovered in the laminin-like protein merosin, first identified in human placenta (Leivo and Engvall, 1988). Merosin possesses an immunologically distinct ~ 300 kDa polypeptide (M chain) that is homologous to the A chain of EHS-tumour laminin and is disulphide-bonded to the B₁ and B₂ chains (Ehrig et al., 1990). Detailed analysis of laminin isoforms in placental tissue have identified four heterotrimeric proteins A–B₁–B₂, A–S–B₂, M–B₁–B₂, and M–S–B₂ (Engvall et al., 1990). Similarly, it has been discovered that laminin from mouse and bovine heart both contain ~ 300 kDa polypeptides that are immunologically similar to each other but immunologically distinct from the A and B chains of EHS-tumour laminin (Paulsson and Saledin, 1989; Paulsson et al., 1991). These ~ 300 kDa components are now known to be structurally and immunologically closely related to the merosin M chain (Paulsson et al., 1991). So far, laminin isoforms containing the M chain or its homologues have been immunologically identified in the basement membranes of placenta, cardiac muscle, skeletal muscle, lung and Schwann cells (Leivo and Engvall, 1988; Paulsson and Saledin, 1989; Sanes et al., 1990; Engvall et al., 1990). In contrast, laminin isoforms containing the A chain exhibit a more restricted distribution, being found mainly in epithelial, endothelial, smooth muscle and neuromuscular synaptic cleft basement membranes (Engvall et al., 1990; Sanes et al., 1990). The functional reasons underlying the differential expression of structurally related but immunologically distinct laminin heavy chains is unknown. However, in

Abbreviations used: EHS, Engelbreth–Holm–Swarm; RCA-1, *Ricinus communis* agglutinin-1; CAPS, 3-(cyclohexylamino)-1-propanesulphonic acid.

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this context, it has been noted that the M chain is more resistant than the A chain to proteolytic degradation (Paulsson et al., 1991).

At present the identification of laminin isoforms which contain the M chain relies upon the use of specific antisera raised against the M chain (not yet readily available) or upon a purification strategy that provides sufficient yield to allow subunit assignment through conventional protein staining. In the present study, we have exploited the glycoprotein properties of laminin and demonstrate that the D-galactose-specific lectin *Ricinus communis* agglutinin 1 (RCA-1) can be used as a sensitive probe for the assignment of laminin heavy chains. We also show that this lectin may be utilized in the isolation of laminin from skeletal muscle.

MATERIALS AND METHODS

Detection of laminin subunits by SDS/PAGE and electroblotting

Samples for electrophoretic analysis were prepared by heating (95 °C for 2 min) in 25 mM Tris buffer, pH 6.8, containing 2% (w/v) SDS, 10% (v/v) glycerol, 2 mM EDTA and a cocktail of protease inhibitors (0.25 mM phenylmethanesulphonyl fluoride; 1 mM benzemethonium chloride; 1 mM iodoacetamide and 0.5 mM EGTA). For analysis of intact tissues, samples (~100 mg) were frozen in liquid N₂ and crushed to powder between steel blocks before extraction as described above. Following removal of insoluble material by centrifugation (14000 g; 5 min), protein content was assessed using BSA as a standard (Lowry et al., 1951). Electrophoresis of appropriate aliquots was performed in the presence or absence (reducing or non-reducing conditions respectively) of 5% (v/v) 2-mercaptoethanol, as detailed in the legends of the figures. SDS/PAGE was performed using gradient [4–20% (w/v) polyacrylamide] gels for 20 h at 20 mA (10 °C) using an electrode buffer composed of 192 mM glycine/25 mM Tris, pH 8.6, containing 0.1% (w/v) SDS. Bromophenol Blue was used as a tracking dye. Sequential electrophoretic transfer of protein from SDS/polyacrylamide gels to nitrocellulose was performed for 20 h at 250 mA (10 °C) in 150 mM glycine/20 mM Tris, pH 8.3. Electroblots were washed (1 h at 20 °C) with PBS, pH 7.3 (PBS; Dulbecco A; Unipath), containing 0.05% (v/v) Tween-20 (PBS/Tween). Subsequently, proteins were detected by incubation (1 h at 20 °C in PBS/Tween) with either 10 µg/ml biotinylated RCA-1 lectin (Vector Laboratories), or affinity-purified rabbit antibodies (1:1000 dilution) raised to purified mouse EHS-tumour laminin (Sigma Immunochemicals). Bound lectin was visualized (after three 5 min washes with PBS/Tween) using streptavidin horseradish peroxidase (Dako Chemicals) in conjunction with diaminobenzidine. Bound anti-laminin antibodies were similarly visualized after incubation (1 h) with the appropriate biotinylated secondary antibody [1:400 dilution of biotinylated anti-(rabbit IgG) (Amersham)] as described previously (Sherratt et al., 1992). Purified mouse EHS-tumour laminin was obtained from Sigma.

Purification of laminin from skeletal muscle

All extraction, chromatography and dialysis steps were performed at 4 °C in the absence of reducing agents; all chemicals were reagent grade. Bovine quadriceps muscle (50 g) was sliced into thin strips, immersed in liquid N₂ and crushed to powder between metal blocks. Cytosolic and weakly bound membrane proteins were subsequently removed by extraction in 25 mM-Hepes, pH 8.2, containing 1% (v/v) Triton X-100 in the presence of protease inhibitors (0.25 mM phenylmethanesulphonyl fluoride; 1 mM benzemethonium chloride and 1 mM iodoacet-

amide) at a ratio of 5 ml/g of tissue. After centrifugation (30000 g, 15 min) and washing (25 mM Hepes, pH 8.2, containing protease inhibitors), crude membrane pellets were pooled and re-extracted for 1 h with 200 ml of 25 mM 3-(cyclohexylamino)-1-propane sulphonic acid (CAPS; Sigma), pH 10.0, containing protease inhibitors. After centrifugation (as above) the alkaline supernatant was dialysed overnight against 4 litres of 25 mM Tris, pH 8.2, containing 0.01% (v/v) Tween-20. The dialysed material was chromatographed on a column (15 cm × 2.5 cm diam.) of Q-Sepharose Fast Flow (Pharmacia) equilibrated in Tris/Tween buffer. After washing, bound proteins were eluted with a 120 ml linear gradient of 0–0.5 M NaCl in Tris/Tween buffer. Fractions (1 ml) were collected and monitored at A₂₈₀. To detect laminin, aliquots of alkaline muscle extract, together with the Q-Sepharose void and salt eluates, were processed for SDS/PAGE/electroblotting and probed with biotinylated RCA-1 lectin or anti-laminin antibodies. Q-Sepharose salt eluates containing laminin were pooled (to approx. 10–15 ml) and dialysed against 2 litres of PBS, pH 7.3, containing 0.01% (v/v) Tween-20. Dialysed material was circulated overnight on a column (5 cm × 0.75 cm diam.) of RCA-1-agarose (Vector Laboratories) equilibrated in PBS/Tween. After washing (with ~50 ml of equilibration buffer), bound protein was eluted in 0.5 ml fractions with a 40 ml linear gradient of 0–0.5 M D-galactose produced in equilibration buffer. Fractions containing purified laminin were determined by electrophoretic analysis and stored frozen at –80 °C.

RESULTS

Recognition of laminin subunits by RCA-1 lectin

Aliquots (100 ng) of purified EHS laminin were analysed by reducing SDS/PAGE, electroblotted, and probed with biotinylated RCA-1. This revealed that relatively low concentrations of RCA-1 (optimal at 10 µg/ml) recognized both the A and B chains of EHS-tumour laminin (Figure 1; track 1), producing an intensity of labelling comparable with that observed using a 1:1000 dilution of affinity-purified antibody raised against laminin from the mouse EHS tumour (Figure 1; track 2). At these concentrations, both biotinylated RCA-1 and anti-laminin antibodies exhibited a similar limit of detection of ~5–10 ng of purified mouse EHS laminin (not shown). Labelling of laminin subunits by RCA-1 was completely abolished by simultaneous incubation of the lectin with 0.1 M D-galactose, whereas labelling by anti-laminin antibodies was not discernibly affected by the presence of this sugar (results not shown). In view of these findings, we were interested to know whether biotinylated RCA-1 could similarly recognize the variant ~300 kDa M chain of laminin, previously identified immunochemically on blots of skeletal and cardiac muscle (Paulsson and Saledin, 1989; Paulsson et al., 1991). Accordingly, bovine quadriceps and cardiac muscle were separated by SDS/PAGE, blotted and probed with biotinylated RCA-1. Such analysis revealed labelling of many glycoproteins (particularly those of molecular mass < 200 kDa); however, a prominent diffuse band of ~330 kDa was observed to migrate identically in both skeletal and cardiac muscle extracts (Figure 1; tracks 3 and 4). This glycoprotein was not detected in similar extracts of aorta and bladder (Figure 1; tracks 5 and 6), a finding in accord with the documented absence of the M chain in smooth muscle (Paulsson and Saledin, 1989; Engvall et al., 1990). Importantly, the ~330 kDa RCA-1-binding glycoprotein was not recognized by antibodies to EHS laminin in extracts of skeletal or cardiac muscle, even when blots were over-developed to produce intense labelling of the laminin B chains (Figure 1; tracks 7 and 8). The latter finding strongly suggests that the

Figure 1 Lectin- or immuno-blotting of mouse EHS-tumour laminin and SDS extracts from different bovine muscles

Aliquots (100 ng of protein) of purified mouse EHS laminin (tracks 1 and 2) or aliquots (60 μ g of protein) of SDS extracts of bovine skeletal muscle (tracks 3 and 7), heart (tracks 4 and 8), aorta (track 5) and bladder (track 6) were separated by gradient-pore [4–20% (w/v) polyacrylamide] SDS/PAGE run under reducing conditions. After electrophoretic transfer to nitrocellulose, proteins were probed with either 10 μ g/ml biotinylated RCA-1 (tracks 1, 3, 4, 5 and 6) or a 1:1000 dilution of anti-(EHS-tumour laminin) antibodies (tracks 2, 7 and 8). Molecular masses were calculated from the position (shown in kDa) of known molecular-mass standards. Note the ability of biotinylated RCA-1 to not only recognize both the A and B chains of EHS laminin, but also a $\sim$ 330 kDa glycoprotein (arrow) in bovine skeletal muscle and heart. This glycoprotein is undetectable in smooth muscle (aorta and bladder) and is not recognized by anti-(EHS laminin) antibodies.

$\sim$ 330 kDa RCA-1-binding glycoprotein does not represent a truncated form of the laminin A chain.

Purification of laminin from skeletal muscle

To establish whether the $\sim$ 330 kDa RCA-1-binding glycoprotein detected in bovine cardiac and skeletal muscle corresponds to the laminin M chain, we sought to follow its association with laminin B chains during enrichment from skeletal muscle. Although a detailed strategy for the preparative purification of laminin from skeletal muscle has not been reported, it is known that extraction of laminin from other sources is specifically facilitated by chelating agents which disrupt its Ca^{2+} -dependent polymerization (Timpl, 1989). Low concentrations of EDTA selectively extract high proportions of laminin from the EHS tumour (Yurchenco et al., 1992) and from mouse or bovine heart (Paulsson and Saledin, 1989; Paulsson et al., 1991). Likewise, we found that EDTA (10 mM in PBS) extracted $>$ 50 % of total laminin from skeletal muscle. However, for the purpose of rapid laminin subunit analysis this preparation contained unacceptably high levels of other contaminating glycoproteins. Therefore, we sought alternative methods of extraction,

Figure 2 SDS/PAGE and lectin blotting of samples obtained during the isolation of laminin from bovine skeletal muscle

Samples taken during the enrichment of laminin from bovine quadriceps were separated by gradient-pore [4–20% (w/v) acrylamide] SDS/PAGE run under reducing conditions. After electrophoretic transfer of protein to nitrocellulose, the blot was probed with 10 μ g/ml biotinylated RCA-1. Lane 1, alkaline extract (50 μ g of protein) of crude skeletal muscle membranes. Lane 2, pooled 0.10–0.15 M NaCl fraction (2 μ g of protein) obtained from Q-Sepharose ion-exchange chromatography. Lane 3, pooled 0.05–0.10 M D-galactose fraction (0.5 μ g of protein) from RCA-1-agarose-affinity chromatography. The position of the 330 kDa RCA-1-binding glycoprotein is indicated by the arrow, arrowheads denote the position of molecular-mass markers as in Figure 1. The blot presented is representative of samples obtained from three independent purifications. Most of the lower-molecular-mass ($<$ 100 kDa) glycoproteins observed in the lectin-purified material appear to be degradation products of laminin; their relative amounts varied between preparations and they were not detected under non-reducing conditions.

and found that after the removal of cytosolic and weakly associated membrane proteins with non-ionic detergent [1 % (v/v) Triton X-100] approx. 20 % of total skeletal muscle laminin (on the basis of B-chain content) could be extracted from crude skeletal muscle membranes using a low-ionic-strength buffer of alkaline pH (25 mM CAPS, pH 10). At higher pH values (pH 11–12) up to 50 % of skeletal muscle laminin could be extracted; however, this material was heavily contaminated with contractile proteins. Analysis of alkaline extracts of bovine skeletal muscle with biotinylated RCA-1 revealed the presence of the $\sim$ 330 kDa RCA-1-binding glycoprotein together with laminin B chains, as well as numerous other glycoproteins (Figure 2; track 1). Additionally, small amounts of two other high-molecular-mass glycoproteins ($\sim$ 440 kDa and 600 kDa)

Figure 3 Comparative analysis of purified skeletal muscle laminin with biotinylated RCA-1 or antibodies raised to EHS-tumour laminin

Aliquots (100 ng of protein) of RCA-1-agarose-purified skeletal muscle laminin (tracks 2, 3, 6 and 7) or purified mouse EHS-tumour laminin (tracks 1, 4 and 5) were analysed by gradient-pore [4–20% (w/v) polyacrylamide] SDS/PAGE run under reducing (tracks 1 to 3) or non-reducing (tracks 4 to 7) conditions. After electrophoretic transfer to nitrocellulose, proteins were detected with either 10 μ g/ml biotinylated RCA-1 (tracks 1, 2, 4 and 6) or a 1:1000 dilution of anti-(EHS-tumour laminin) antibody (tracks 3, 5 and 7). The position of the 330 kDa RCA-1-binding glycoprotein is indicated by the arrow, arrowheads show the position of the 200 and 97 kDa molecular-mass markers.

were detected in the alkali-extracted material (Figure 2; track 1). These were immunochemically identified as the laminin A chain and a high-molecular-mass precursor of the laminin A and B chains (see below, and Figure 3). Although alkaline extraction results in a lower recovery of laminin from skeletal muscle than found using EDTA, purification is simplified by lower levels of contaminating glycoproteins.

In view of the success of anion-exchange chromatography in isolating laminin from EDTA extracts of bovine heart (Paulsson and Saledin, 1989) alkali-extracted protein (~0.4 g) obtained from 50 g wet mass of bovine quadriceps was applied (after dialysis) to a column of Q-Sepharose. Both the 330 kDa RCA-1-binding glycoprotein and laminin B chains were undetectable in the break-through volume and were not removed during prolonged washing with equilibration buffer. Subsequent application of a linear NaCl gradient resulted in co-elution of the 330 kDa RCA-1-binding glycoprotein and laminin B chains as two distinct peaks. The first peak eluted sharply between 0.10 M and 0.15 M NaCl and consisted of approx. 10% of the total bound 330 kDa RCA-1-binding glycoprotein, together with a similar proportion of laminin B chains, the A chain and the 600 kDa laminin precursor (Figure 2, track 2). Although unidentified lower-molecular-mass glycoproteins were also detected in these fractions, the majority of contaminants consisted of lower-molecular-mass polypeptides that were not recognized by biotinylated RCA-1 but could be detected by silver staining (results not shown). The second peak, containing the remainder of the 330 kDa RCA-1-binding glycoprotein and laminin polypeptides, eluted broadly between 0.25 and 0.45 M NaCl and was heavily

contaminated with the bulk of other bound proteins (results not shown). For our purposes sufficient material was recovered in the first peak for subsequent analytical procedures. Attempts to improve the yield in this peak by disruption of laminin complexes through titration to pH 12 before dialysis and ion-exchange chromatography resulted in a shift of the elution profile of the 330 kDa RCA-1-binding glycoprotein and laminin polypeptides to a single peak eluting between 0.10 M and 0.15 M NaCl. However, the marked improvement in recovery of the 330 kDa RCA-1-binding glycoprotein and laminin polypeptides in this fraction was compromised by increased levels of contaminating glycoproteins, the elution profile of which was also shifted (not shown).

Material obtained in the 0.10–0.15 M NaCl fraction from Q-Sepharose chromatography was pooled (typically ~2 mg of protein), dialysed and loaded on to a column of RCA-1-agarose. The 330 kDa RCA-1-binding glycoprotein and laminin polypeptides were retained on the lectin column, while the majority of contaminating proteins (detectable only by silver staining) were removed by washing with equilibration buffer (not shown). Subsequent analysis of eluate fractions from a linear sugar gradient with biotinylated RCA-1 revealed the early elution of a single peak (between 0.05 and 0.10 M D-galactose) containing >95% of total bound 330 kDa RCA-1-binding glycoprotein, laminin B chains, the A chain and the 600 kDa laminin precursor (Figure 2, track 3; see Figure 3). Radio-iodination of this lectin-purified preparation using a conventional chloramine-T method and analysis by SDS/PAGE/autoradiography revealed only residual (<5% of total protein) contamination from polypeptide species not detected by biotinylated RCA-1 (results not shown). Typically, ~25–30 μ g of lectin-purified protein was recovered from 50 g of bovine quadriceps.

Comparative analysis of the lectin-purified preparation with biotinylated RCA-1 (Figure 3, track 2) and anti-laminin antibodies (Figure 3, track 3) demonstrated that proteins migrating at ~600 kDa, ~440 kDa and as a doublet at 200–220 kDa could be recognized by antibodies raised to mouse EHS-tumour laminin. Their respective assignment as a high-molecular-mass laminin precursor and the A and B chains of laminin, is in accord with virtually identical electrophoretic profiles of laminin isolated from mouse and bovine heart (Paulsson and Saledin, 1989; Paulsson et al., 1991). Anti-laminin antibodies failed to recognize the purified 330 kDa RCA-1-binding glycoprotein (compare Figure 3, tracks 2 and 3), in agreement with the lack of cross-reactivity observed in crude SDS extracts of skeletal and cardiac muscle (Figure 1). These results are indicative that the 330 kDa RCA-1-binding glycoprotein represents the variant heavy M chain of laminin, and this was confirmed when the lectin-purified preparation was analysed by non-reducing electrophoresis. Under non-reducing conditions all the protein bands resolved in the presence of 2-mercaptoethanol disappeared, being replaced by a high-molecular-mass species of ~750–800 kDa, that was identically recognized by either biotinylated RCA-1 (Figure 3, track 6) or antibodies to EHS laminin (Figure 3, track 7). This species migrated so as to indicate a lower molecular mass than EHS laminin under non-reducing conditions (Figure 3, tracks 4 and 5), reflecting the presence of the 330 kDa RCA-1-binding glycoprotein in skeletal muscle laminin, rather than the laminin A chain.

Collectively, this series of experiments demonstrates that the 330 kDa RCA-1-binding glycoprotein is immunologically distinct from the EHS laminin A chain, is more abundant than the latter in skeletal muscle, and is disulphide-bonded to the B chains of laminin. Clearly the 330 kDa RCA-1-binding glycoprotein exhibits properties identical with previously characterized

laminin M-chain polypeptides (Paulsson and Saledin, 1989; Ehrig et al., 1990; Paulsson et al., 1991).

DISCUSSION

The recognition of the A and B chains of mouse carcinoma laminin by plant lectins has previously been reported (Dean et al., 1990) and lectin-affinity chromatography on immobilized *Griffonia simplicifolia* agglutinin-1 has been used to purify laminin from this source (Shibata et al., 1982; Dean et al., 1990). The present study extends this line of investigation by demonstrating that RCA-1 (a lectin of similar specificity for terminal galactose residues) not only recognizes laminin subunits from the mouse EHS tumour, where the protein is over-expressed, but can also be used for the detection of laminin polypeptides present at typically much lower levels in mature mammalian tissue. In skeletal and cardiac muscle, biotinylated RCA-1 recognizes a ~ 330 kDa glycoprotein that is undetectable using antibodies raised to the A and B chains of mouse EHS-tumour laminin. Purification of this glycoprotein under non-reducing conditions demonstrates that, in native form, this polypeptide is disulphide-bonded to the B chains of laminin. These properties are identical with the characteristics of the variant M chain of laminin found in cardiac muscle and in the extrasynaptic basement membrane of skeletal muscle (Paulsson and Saledin, 1989; Sanes et al., 1990). The demonstrated ability of biotinylated RCA-1 to detect (at nanogram sensitivity) an M-chain subunit of laminin offers an important alternative approach for the detection of this glycosylated polypeptide. This may be of particular use in circumstances in which laminin B chains have been immunohistochemically detected, but where the heavy chain cannot be assigned, either due to a lack of M-chain-specific antisera, or to an inability to use conventional protein stains (see for example, Dickson et al., 1992).

Hitherto, there have been no reports documenting the purification of laminin from skeletal muscle, the major constraining factor being its selective extraction from the basement membrane (see Timpl, 1989). In the present study similar problems were encountered. While laminin could be readily extracted with charged anionic detergents (SDS or deoxycholate), at neutral pH values non-ionic or zwitterionic detergents (Triton X-100, Chaps, Nonidet P40 and octylthioglucoside 308) were ineffective even in the presence of high salt concentrations. EDTA, which has been used widely for the selective extraction of laminin from both the mouse EHS tumour and cardiac tissue (Paulsson et al., 1991; Yurchenco et al., 1992), did facilitate extraction of > 50 % of laminin from skeletal muscle but resulted in a huge contamination from low-molecular-mass glycoproteins. Alternatively, we found that laminin could be extracted in a pH-dependent fashion, which at pH values ≥ 12 results in solubilization of ~ 50 % of total M and B chains. pH 10 was found to be preferable,

however, as, despite lower yields, this pH prevented co-extraction of myosin heavy chains. Purification of alkali-extracted skeletal muscle laminin by two successive chromatography steps yielded microgram quantities of highly purified material that migrated as a single major band under non-reducing electrophoresis.

Despite differences in the primary sequence of their heavy chains both laminin and merosin appear to possess similar biological properties. Both promote neuronal migration in assays *in vitro* (Calof and Lander, 1991; Engvall et al., 1992). Because carbohydrate residues appear to be essentially involved in this process (Dean et al., 1990) one can speculate that other functional properties of laminin may be mediated through oligosaccharide substituents. From this point of view, a comparison of the ability of the A and M chains to bind lectins of differing specificity may provide rapid insights into possible functional differences between these heavy chain subunits.

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