8 Abstract

The 395-residue proteolytic fragment E3, which comprises the two most C-terminal LG modules of the mouse laminin α1 chain, was previously shown to contain major binding sites for heparin, α-dystroglycan and sulfatides. The same fragment (α1LG4-5) and its individual α1LG4 and α1LG5 modules have now been obtained by recombinant production in mammalian cells. These fragments were apparently folded into a native form, as shown by circular dichroism, electron microscopy and immunological assays. Fragment α1LG4-5 bound about five- to tenfold better to heparin, α-dystroglycan and sulfatides than E3. These binding activities could be exclusively localized to the α1LG4 module. Side-chain modifications and proteolysis demonstrated that lysine and arginine residues in the C-terminal region of α1LG4 are essential for heparin binding. This was confirmed by 14 single to triple point mutations, which identified three non-contiguous basic regions (positons 101, 103, 105, 126-128, 154, 155) as contributing to both heparin and sulfatide binding. Two of these regions were also recognized by monoclonal antibodies which have previously been shown to inhibit heparin binding. The same three regions and a few additional basic residues also make major contributions to the binding of the cellular receptor α-dystroglycan, indicating a larger binding epitope. The data are also consistent with previous findings that heparin competes for α-dystroglycan binding.