Membrane Protein Labeling

Endothelial cell membrane proteins were biotin-labeled with EZ-Link Sulfo-NHS-Biotin (Pierce). Confluent dishes containing approximately $3x10^6$ endothelial cells were rinsed five times with cold PBS and incubated at 4°C with 4mg of Sulfo-NHS-Biotin. The biotin reagent was neutralized with tris-buffer saline. Cells were scraped off the dish, pelleted by centrifugation and lysed with 0.5% Nonidet P-40 in PBS (NP/PBS). Soluble labeled membrane proteins were collected after centrifugation at 11,000 x g at 4°C.

Affinity Chromatography and Immunoprecipitation

Affinity chromatography was performed with A13 peptide linked to Affi-gel 10. A13 (4mg) was coupled overnight to Affi-gel resin in 100 mmol/L sodium carbonate buffer, pH 8.5. Remaining active groups were blocked with 100 mmol/L diethanolamine, and the resin was equilibrated with NP/PBS. Beads blocked with diethanolamine were used as control. Biotin-labeled membrane proteins were passed through the column and allowed to incubate for 30min. After washing with 50ml of NP/PBS, bound proteins were eluted successively with 5mmol/L EDTA, 1 mol/L NaCl and 4 mol/L urea. Fractions were collected (500 µl) and screened for the presence of biotin-labeled proteins. Biotin-containing fractions were pooled and dialyzed against water. Aliquots from each fraction were electrophoresed on 10% SDS-PAGE, transferred to nitrocellulose membranes, and biotin-labeled proteins were detected with streptavidin-horseradish peroxidase and enhanced chemiluminescence (ECL, Amersham Life Science). Isolated proteins were immunoprecipitated with antibodies to different integrin chains as previously described, run on SDS-PAGE and visualized by ECL as above