Short consensus repeat domains extend the E-selectin structure in order to grab cells out of flow

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ABSTRACT

Selectins are key adhesion molecules responsible for initiating a multistep process that leads a cell out of the blood circulation and into a tissue or organ. They are composed of an N-terminal extracellular C-type lectin like domain, followed by an Endothelial Growth Factor-like domain (EGF), a defined number of short consensus repeats SCR (also called “sushi” domains), a transmembrane domain and a C-terminal cytoplasmic tail. The adhesion of cells (expressing ligands) to the endothelium (expressing the selectin i.e., E-selectin) occurs through the interaction between the lectin domain of selectins and the presenting ligands. Structural/function studies to date have mainly focused on investigating the influence of the lectin domain of E-selectin on its ability to bind its ligands while other domains received less attention. We prepared a number of different recombinant E-selectin proteins with changes in the SCR units. Specifically, we generated wild-type E-selectin proteins as monomeric or dimeric structures, mutant proteins with varied numbers of SCRs as well as proteins where strategic residues were mutated to change the conformation of the selectin. Using a novel real time immunoprecipitation surface plasmon resonance (SPR)-based in vitro binding study developed in our lab, the interaction of recombinant E-selectin proteins with immunoprecipitated endogenous ligands (i.e., CD44) captured on a CM5 chip was assessed. These studies provided quantitative binding kinetics with on and off rates of selectin-ligand interactions and suggested that robust binding is dependent on the presence of the SCRs and oligomerization. These results provide significant implications on the functional mechanism of E-selectin binding to its ligands.

OBJECTIVE

Characterizing the structural influence of Short Consensus Repeats (SCR) in the binding functionality of E-selectin to bind its ligands.

MATERIALS AND METHODS

In this study, PCR construction of four different constructs of E-selectin with varying lengths of the SCR units (Figs. 1) were designed and expressed in silkworm. The resulting recombinant proteins were assayed for its binding to known E-selectin ligands (PSGL-1 and CD44) using various biochemical approaches (Figs. 2 to 5).

RESULTS

1. Construction and expression of various forms of recombinant E-selectin (rE-selectin)

   a) Western Blot

   b) Flow Cytometry

   c) Blotting

   d) Immunoprecipitation

2. Determining the ability of the purified recombinant proteins to bind E-selectin ligands under static conditions

   a) Western Blot

3. Functional characterization of recombinant E-selectin proteins determined under flow

4. Rolling assay

5. Concluding

CONCLUSIONS

1. SCR domains are important in extending the lectin domain of E-selectin a sufficient length to assess mediating interaction with ligands on cells travelling through the blood circulation.

2. E-selectin plays a clear functional role in improving the binding affinity of E-selectin in addition to the lectin and EGF domains.

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