The Extracellular Region of CD147 Does Not Self-Associate in the Absence of Another Mediator

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Introduction
CD147 (also known as Extracellular MatrixMetalloproteinase) is a type I transmembrane protein that stimulates the secretion of Matrix MetalloProteinases (MMPs) and cytokines integrally involved in numerous cancers, inflammatory disorders, and viral infection. Previous reports have suggested that CD147 on one cell targets itself on neighboring cells and initiates the first step of a signal transduction pathway. However, there have been many recent studies that have suggested CD147 does not act alone and that CD147 is often associated with multiple proteins. To confuse matters even more, the recently determined X-ray crystal structure of CD147 exhibits four intermolecular contacts that were suggested to correspond to the biological interactions, yet the predominant soluble species was found to be a monomer (1). Thus, to determine whether CD147 self-associates and whether the observed crystal contacts persist in solution, we conducted NMR studies on the ecodomain of CD147. Our results indicate that CD147 does not self-associate in solution at millimolar concentrations and thus, suggests that other mediators are critical for CD147 activity.

Experimental
HNCACB and CBCAcoNH spectra were collected at 600 MHz (NHMFL) on the ectodomain of $^{13}$C,$^{15}$N-labeled CD147 (residues 22-205). Nearly complete backbone assignments were determined from these spectra allowing for the subsequent collection of both transverse relaxation rates (R2) and longitudinal relaxation rates (R1) at 900 MHz on $^2$H,$^{15}$Nlabeled CD147 (the Rocky Mountain 900).

Results and Discussion
We have discovered that CD147 does not self-associate as previously predicted using native gel analysis, size-exclusion chromatography and NMR investigations (2). The high resolution $^{15}$N-HSQC is already indicative of a monomeric protein (Fig. 1, top right) and there is no concentration-dependent chemical shift changes observed. Even at concentrations as high as 2 mM there were no changes in chemical shifts, suggesting that if CD147 does self-associate then the self-association constant is higher than these concentrations. Finally, the ectodomain of CD147 comprises two immunoglobulin-like (Ig-like) domains that each has very different correlation times (tumbling times), as determined by their R1 and R2 relaxation rates (Fig. 1, bottom). These correlation times are consistent with independently folded Ig-domains that neither associate with each other nor self-associate.

Conclusions
While the ectodomain of CD147 (residues 22-205) exhibits four crystal contact, our data here indicates that these contacts do not persist in solution (Fig. 1). Biologically, this would suggest that if CD147 targets itself on neighboring cells then other molecules mediate such an interaction.

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References