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Title: Structural and Functional Investigation of an Antagonistic Heterodimeric VEGF
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Vascular endothelial growth factor (VEGF) is a dimeric protein that controls much of vascular development through binding, dimerization and activation of its receptor (KDR), resulting in activation of angiogenic cell signaling pathways. Based upon this mechanism, we constructed a heterodimeric variant of VEGF (HD-VEGF) that contained one functional and one non-functional site. HD-VEGF can only bind to monomeric receptors and act as VEGF antagonist. In our previous studies the KDR binding sites of VEGF were precisely determined and replaced by some suitable segments of other proteins. After building a 3-D model of the mutant form and MD simulation, the binding of this variant to KDR was investigated using docking energy landscapes. Based on the constructed model, the modified encoding gene of VEGF receptor binding domain was synthesized. The modified and native VEGF genes were then overexpressed as inclusion bodies in E. coli and refolded together to produce HD-VEGF variant. VEGF heterodimer was purified from VEGF homodimers through two-step affinity chromatography using Ni-NTA agarose and Strep-Tactin columns. Far-UV CD and fluorescence spectroscopy studies showed no significant structural changes in the HD-VEGF in comparison with homodimer variants and confirmed that the formation of heterodimer and its purification were successfully carried out. This variant can significantly inhibit the proliferation and capillary tube formation of endothelial cells in vitro (with IC$_{50}$ values 33 ng/ml and 24 ng/ml, respectively). Based on these studies, it can be concluded that the HD-VEGF will compete with the native VEGF for receptor binding and antagonizes its action.