Hydrogen bonding enhances stability of HK nanoplexes

Statement of the Problem: Our laboratory has focused on developing histidine and lysine (HK) peptide carriers with varied complexity to deliver siRNA and plasmids in vitro and in vivo. Positively-charged lysines bind and protect DNA, partially neutralizing its charge, whereas histidines bind DNA, buffer endosomal pH and thus presumably aid in release of nucleic acids from endosomes. In addition to these roles of HK, preliminary data indicate that non-ionic interactions between HK peptides and nucleic acids are essential.

Methods: After synthesizing branched peptides that contained varying amounts of histidines, we determined the influence of serum on the stability and gene silencing activity of these peptides in complex with siRNA. The thermodynamic profiles of siRNA binding to peptides with different histidine and lysine contents were then analyzed by isothermal titration calorimetry (ITC). To explore the presence of histidine-mediated hydrogen bonds, protonation of free and siRNA bound imidazoles was characterized using heteronuclear single quantum coherence (HSQC) NMR at pH 7.3 and 5.0.

Findings: A 4-branched HK peptide siRNA nanoplex maintained silencing activity even with prolonged pre-incubation with serum. In marked contrast, siRNA in complex with 4-branched NK4b, in which histidines were substituted with asparagines, showed a marked decreased in silencing activity. To explore whether histidine forms non-covalent bonds with nucleic acids, we compared the thermodynamic properties of HK with other lysine-analogues. While polylysine with siRNA resulted in an endothermic reaction, branched and linear HK peptides exhibited an exothermic reaction, indicating that non-ionic bond formation between histidines with siRNA. Moreover, the peak of Nδ1-protonated tautomers of imidazole shifted downfield by 0.5 to 1.0 ppm with addition of siRNA, providing direct evidence that uncharged histidines formed hydrogen bonds.

Conclusions: These results establish that histidine-rich peptides form hydrogen bonds with siRNA, thereby enhancing the stability and biological activity of the nanoplex.

Biography

A J Mixson has been working since 1994 in the Pathology Department at the University of Maryland School of Medicine with an initial focus on non-viral delivery of anti-angiogenic “nucleic acids” (plasmids/siRNA/DNAzymes) with liposomes utilizing in vitro or in vivo model systems. Our current research has expanded to developing novel peptides as nucleic acid carriers and as antifungal agents. He has been awarded several US and European patents on gene therapy and anti-angiogenesis. During the last 12 years, our laboratory developed histidine-rich peptides as carriers of nucleic acids. We are particularly interested in the mechanisms that govern the stability and dissociation of histidine-lysine polyplexes.

A J Mixson
University of Maryland School of Medicine, USA

JMixson@som.umaryland.edu