Amide-Modified RNA: Synthesis, Structure and RNA Interference Activity

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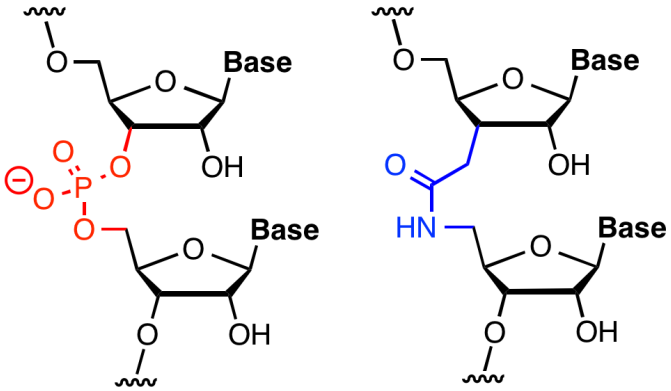
Discovery of RNA interference (RNAi) has reinvigorated interest in chemical modifications of RNA for in vivo applications. Improved enzymatic stability, delivery, cellular uptake and sequence specificity have been the focus of optimization using chemical modifications. While numerous good solutions for improving enzymatic stability have emerged, optimization of the latter properties remains challenging. Our current work develops novel nonionic analogues of RNA that have the phosphodiesters replaced by amide linkages (**AM1** in Figure).



We hypothesize that the reduced negative charge and hydrophobic nature of such modifications will not only increase the enzymatic stability, but also have the potential to optimize potency, cellular uptake, and suppress unwanted off-target effects of small interfering RNAs (siRNAs). Structural studies by our collaborators, NMR spectroscopy by Dr. Scott Kennedy (University of Rochester) [1] and x-ray crystallography by Dr. Martin Egli (Vanderbilt University) [2,3], show that amides are excellent mimics of the phosphodiester linkages in RNA. The local conformational changes caused by the amide linkages are easily accommodated by small adjustments in RNA structure. The NMR and crystal structures reveal that the amide carbonyl group assumes an orientation that is similar to one of the non-bridging P-O bonds.

RNAi activity assays show that amides are well tolerated at internal positions in both strands of siRNAs [3-4]. Surprisingly, amide modifications in the middle of the guide strand and at the 5´-end of the passenger strand increase the RNAi activity compared to unmodified siRNA [3,4]. Our hypothesis that amide may also mimic phosphate in forming hydrogen bonding interactions with proteins is further confirmed by a crystal structure of a short amide-modified DNA-RNA hybrid in complex with RNase H [3]. Taken together, our results suggest that amides are excellent mimics of phosphate backbone in RNA and may have potential to optimize biological and pharmacological properties of siRNAs. These findings are unexpected and raise the possibility that RNAi may tolerate and benefit from even more substantial modifications than the ones tried so far.

References

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