Unravelling RNA chemical reactivity: Multiscale simulation and calculation of SHAPE probe/RNA interactions for better insight into experimental results

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RNA molecules are involved in most steps of genetic expression, including catalysis of central cellular functions. RNA functions crucially rely on both the specific 3D folding of the molecule, which in turn depends on the sequence and on how nucleobases pair through hydrogen bonds (secondary structure), and its conformation.[1] To overcome the lack of 3D structures, in the last decades, the number of chemical probing data has been largely increased and integrated into the prediction of 2D and 3D RNA structures with different levels of detail.[2] In particular, the SHAPE technology provides quantitative reactivity information for each nucleotide and has become the most popular among these techniques since it does not depend on the nature of the nucleotide, unlike other chemical probing techniques, and is amenable to high-throughput protocols. The probes are small-molecule electrophiles that acylate the 2'-hydroxyl group to form a 2'-O-adduct.[3-4] Although this approach is very popular and it is known that the SHAPE reaction is dependent on the local structural properties of each nucleotide, it has not yet been understood why different reactivities can be obtained for the same nucleotide depending on the probe used, and several questions associated with the relationship between structure, conformation, flexibility, and reactivity are still open. To overcome this, with the aim of using SHAPE data to predict bound and unbound RNA structures, we have first performed classical biased all-atom molecular dynamics simulations, using two different SHAPE probes, on a stable tetraloop of *Bacillus subtilis* yitJ S-box (SAM-I) riboswitch. SHAPE data for this system were experimentally obtained in our wet lab, allowing us to analyze the correlations between various geometric parameters and chemical reactivity. Moreover, to better understand our results and elucidate the SHAPE acylation reaction, we also performed QM/MM calculations on the same systems. Our investigations confirm that SHAPE reactivity is guided by the local flexibility of the different chemical moieties and the ribose, but also by the attack orientation of the SHAPE probe. Finally, we carried out QM calculations in the presence of a probe and a nucleotide, which reveal and give the first initial assessment of the potential reaction mechanism in the context of SHAPE reactivity. These results confirm the importance of the attack angle and emphasize the need for a more comprehensive understanding of the reaction mechanism underlying the SHAPE reaction.

KEYWORDS: RNA structures; Multiscale simulation; Reactivity; SHAPE technique

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