

## Gas Phase Dissociation of RNA

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Antisense oligonucleotides are nucleic acids on the order of 12 to 20 nucleobases, which are designed to hybridize to a complementary mRNA sequence, thus, inhibiting gene expression. Therefore, they are of great interest in human cancer therapy and for diagnostic applications. In order to improve affinity, bioavailability, biostability and the binding specificity, oligonucleotide analogs exhibiting chemical modifications are evaluated. Due to the presence of unnatural structural elements, the standard sequencing techniques are likely to fail and alternative approaches for rapid and accurate sequencing of chemically modified oligonucleotides have to be developed. Tandem mass spectrometry is a highly attractive candidate for this task, as it is capable of providing the high degree of structural information required.

The dissociation of DNA has been investigated by several groups over the past decade. However, there is hardly any data available on gas phase dissociation of oligoribonucleotides (RNA) and their analogs. Thus, the fundamental mechanistic aspects still need to be defined in order to develop mass spectrometry based protocols for sequence identification.

Oligoribonucleotides and modified oligonucleotides were subjected to low-energy collision-induced dissociation in a hybrid quadrupole time-of-flight mass spectrometer. In contrast to the dissociation of DNA, dissociation of RNA was found to be independent of nucleobase loss and it is characterized by cleavage of the 5'-P-O bond. To evaluate the influence of different 2'-substituents and nucleobases on the dissociation of RNA a number of modified oligoribonucleotides were analyzed. Experiments demonstrated that the dissociation mechanism of RNA is not influenced by the nucleobase, thus, supporting a mechanism where dissociation is initiated by formation of an intramolecular cyclic transition state with the 2'-hydroxyl proton bridged to the 5'-phosphate oxygen.