Production, Isolation, and Characterization of Stable Isotope-Labeled Standards for Mass Spectrometric Measurements of Oxidatively-Damaged Nucleosides in RNA

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RNA undergoes oxidatively-induced damage in living organisms in analogy to DNA. RNA is even more exposed to damage than DNA due to more abundance, single-strandedness, lack of repair and chromatin proteins shield, instability among other effects. RNA damage can adversely affect gene expression, leading to alterations in protein synthesis, and to cell death and other detrimental biological consequences. Mounting evidence suggests the involvement of oxidatively-induced RNA damage in the pathogenesis of a variety of human diseases, aging and age-related diseases. Oxidative damage causes modifications to all four heterocyclic bases in RNA. Accurate measurement of such modifications in RNA is essential for the understanding of the biological effects of oxidatively-induced RNA damage. In the past, mass spectrometry has been used for this purpose. In mass spectrometric measurements, the use of stable isotope-labeled analogues of analytes is essential for accurate quantifications. Past work utilized a stable isotope-labeled analogue of 8-hydroxyguanosine only as an internal standard. Thus far, no stable isotope-labeled analogues of other modified RNA nucleosides were available. In the present work, we report on the preparation, isolation, and characterization of the 13C- and 15N-labeled analogues of a number of modified pyrimidine- and purine-derived RNA nucleosides. We also show the application of these internal standards for the measurement of oxidatively-induced RNA damage in several commercially available RNA samples and in DNA along with DNA damage.