The importance of being r: greater oxidative stability of RNA compared with DNA

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The 2′-hydroxyl group of ribose imparts hydrolytic lability on RNA, which provides a mechanism for numerous biological functions. Recent evidence from chemical cleavage studies shows that this hydroxyl group also stabilizes the sugar moiety in RNA towards oxidation relative to DNA. Is this just because RNA needs to be distinguishable from DNA or does it have other evolutionary significance?

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Lady Bracknell: Yes, I remember now that the General was called Ernest. I knew I had some particular reason for disliking the name.
Gwendolen: Ernest! My own Ernest! I felt from the first that you could have no other name!
Jack: Gwendolen, it is a terrible thing for a man to find out suddenly that all his life he has been speaking nothing but the truth. Can you forgive me?
Gwendolen: I can. For I feel that you are sure to change.
Lady Bracknell: My nephew, you seem to be displaying signs of triviality.
Jack: On the contrary, Aunt Augusta, I've now realized for the first time in my life the vital Importance of Being Earnest.

Oscar Wilde, The Importance of Being Earnest, Act III, Scene 3

Chemists and biologists have a common view of RNA as a more transient entity than DNA [1]. This notion stems perhaps from the way we work with RNA. When we have RNA in the laboratory, we take great pains to make sure it is not degraded. RNase enzymes are ubiquitous, so we wash the lab benches in strong base to denature the RNase enzymes, and we treat our solutions with reagents like RNasin or diethylpyrocarbonate (DEPC). We keep weak bases out of the solutions, so that RNA is not hydrolyzed on its own. With DNA, we worry less. Enzymes that degrade DNA are less likely to be lurking in our glassware or on our benchtops, and DNA is stable on its own over a wide pH range. All of these differences are, of course, a result of the 2′-OH group, which makes the hydrolysis of RNA much more facile [2].

The biological roles of DNA and RNA exploit beautifully the difference in hydrolytic lability. The integrity of the genome is safely protected in the hydrolytically more inert DNA, whereas multiple copies of RNA are sent out into the cytoplasm to transfer the information and then strategically degraded to avoid overexpression. Translational regulation is realized through secondary structure elements that confer RNA stability [3]. Most dramatically, RNA can catalyze its own hydrolysis to produce self-splicing [4].

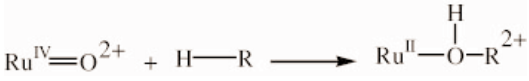
So although our conception of RNA as a fleeting carrier of the genetic message is well founded chemically and biologically, the stability of DNA does not necessarily reach to all kinds of reactions. Here, I present evidence from a number of chemical cleavage and model experiments showing that RNA sugars are less reactive oxidatively than those in DNA [5–7]. Although these observations counter the intuitive notions discussed above, the higher reactivity of DNA towards oxidation is readily understood based on simple concepts from physical organic chemistry [8]. So, like Jack in Wilde's play, RNA represents itself differently to different reactants: labile to those interested in hydrolysis, stable to those interested in oxidation. Consideration of the chemical origins of this dichotomy reveals that, like Jack, RNA is rightfully entitled to this dual role.

Evidence for greater oxidative stability of RNA

Chemical nucleases have been used for a number of years to study the structures of nucleic acids and nucleoprotein complexes [9–13]. Originally, oxidizing reagents were shown to cleave the DNA backbone, generally by C–H activation on the deoxyribose sugar [9]. Binding of a protein at the oxidized site prevents such cleavage reactions, which can therefore be used to generate very high-resolution footprints of DNA-binding proteins [9]. Later, similar reagents were shown to cleave RNA by related reactions [14], and early indications were for a higher selectivity for RNA cleavage. For example, Sigman and coworkers [14] showed that single-stranded regions of RNA were selectively oxidized by copper-phenanthroline over double-stranded regions. Celander and Cech [15] used Fe–EDTA to selectively cleave the 'outside' of a complex RNA, revealing its secondary structure. Chow and Barton [12] found high selectivities for sugar cleavage by rhodium(III) photooxidants. Finally, Hecht [7] examined the action of iron bleomycin (FeBLM) on RNA and found far fewer cleavage sites in a number of diverse RNAs than had been observed in DNA.

These observations prompted parallel experiments on chemical cleavage of DNA and RNA analogues with similar sequences and structures. Holmes and Hecht [16] examined the action of FeBLM on the DNA analogue of a tRNA, termed a 'tDNA'. Although the tDNA exhibited a number of intense cleavage sites, the tRNA was cleaved at only a single site, even though tRNA bound FeBLM more strongly than did tDNA. Similar experiments were carried out for reactions of enediynes and rhodium(III) photooxidants [12,17]. Our group [18–20] examined the reactions of oxoruthenium(IV) complexes with tRNA and the stem–loop structures found in the TAR RNA and the iron responsive element (IRE) RNA. In each of these cases, generally more cleavage sites were observed for DNA than for the analogous RNA.

The collective evidence suggests that C–H bond activation might be more demanding chemically in RNA than in DNA, and that this difference might contribute, along with differences in structure, to the greater selectivity observed for oxidative RNA cleavage. Figure 1 shows the pathways of deoxyribose oxidation that have been established [21]. The oxoruthenium(IV) reagents oxidize DNA sugars through activation of the 1′ C–H bond along the pathway shown in Figure 1 [22]. Isotopic labeling shows that the oxo ligand of the metal complex is transferred to the DNA and winds up in the furanone product [20]. The collective evidence on the chemistry of oxoruthenium(IV) with small molecules and with DNA suggests an inner-sphere reaction pathway, in which the initial oxidation step is insertion of the oxo ligand into the 1′ C–H bond [23–26]:



(1)

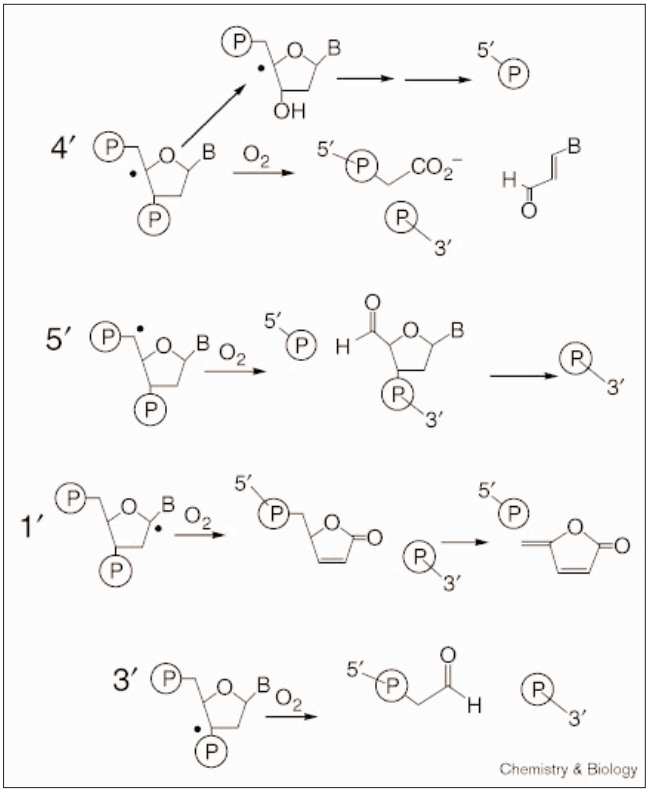
The coordinated alcohol then undergoes further oxidation eventually to produce ketone products, such as the furanone derived from the DNA reaction.

A simple prediction from the reaction pathway shown in equation 1 is a significant accumulation of positive charge on the carbon of the activated C–H bond. An advantage of the oxoruthenium(IV) system is that the reduction of oxoruthenium(IV) can be monitored in real time by stopped-flow spectrophotometry and these results can be correlated with cleavage yields on sequencing gels [22]. We have measured the rates of reduction of RuO^{2+} by the mononucleotides of DNA and RNA. In general, we observe about a fivefold decrease in the oxidation rate for 2′-OH mononucleotides over the corresponding 2′-H deoxymononucleotides [24]. This result was interpreted as evidence of a polar effect of the 2′-OH on the activation at the 1′ position, which implies significant positive charge accumulation in the transition state for the reaction. This notion provides a physical basis for the greater oxidative stability of RNA than that of DNA.

Crich and Mo [6] have also addressed this 'DNA/RNA paradox' in oxidation of sugars that are models of RNA and DNA. In this study, sugars were synthesized that produced a cation radical at the 3′ position, which was less stable when there was a 2′-OMe substituent than in the 2′-deoxy case. The reaction performed by Crich and Mo is shown in Figure 2.

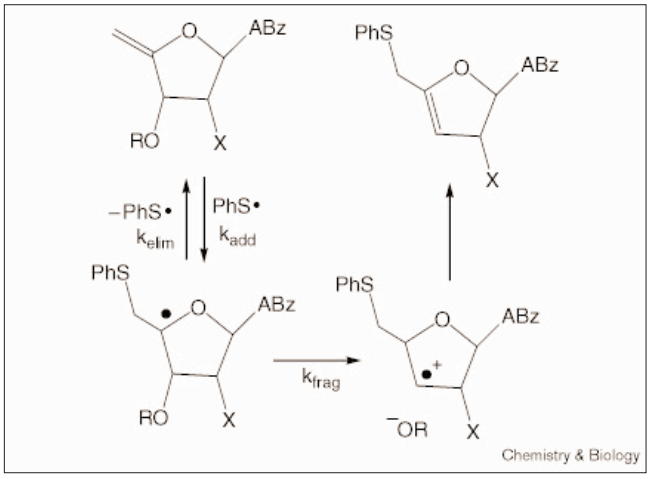
We have now applied this idea to the study of DNA oligonucleotides with novel 2′ substituents that are commercially available as phosphoramidites [5]. Reaction of RuO^{2+} with oligonucleotides where a single nucleotide contains either a 2′-H, NH_2, OMe or F substituent produces decreasing extents of cleavage at the derivatized site according to the polar Hammett parameter of the substituent, that is, H > NH_2 > OMe > F. The extent of reaction at the unsubstituted nucleotides does not change as a function of the substituent. Analysis of the extent of cleavage at the substituted site suggests a Hammett ρ value of –1.4 (Figure 3), which is typical of reactions with large accumulations of positive charge on the oxidized carbon [27]. The ratio of rate constants for 2′-OH and 2′-H mononucleotides [24] falls on the line defined by the electrophoresis data (square, Figure 3).

Figure 1



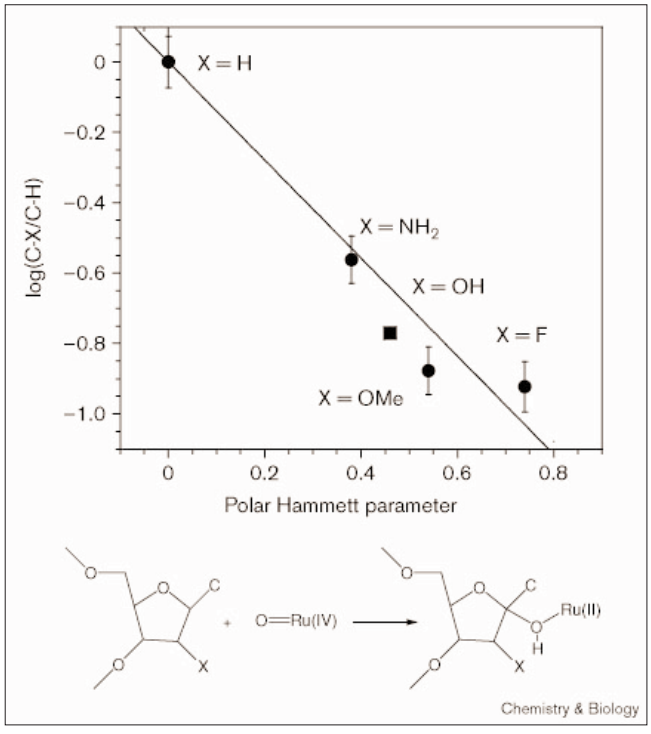
Elucidated pathways of strand scission following activation of C–H bonds in DNA. These pathways are reviewed in detail in [21].

Figure 2



Reaction studied by Crich and Mo [6] as a model for nucleotide oxidation. When X = H, the reaction proceeds much faster than when X = OH, which is attributed to a difference in k_{frag}. ABz = 6-N-benzoyladenine, R = P(O)(OEt)$_2$.

Figure 3



Results on the modulation of DNA cleavage by oxoruthenium(IV) complexes in intact oligonucleotides containing a substituted cytidine nucleotide. The graph shows relative rates obtained from gel electrophoresis for inner-sphere C–H bond activation by the likely mechanism shown below.

Potential biological benefits of differences in oxidative reactivity

Given the accumulated evidence on cleavage studies of related DNAs and RNAs and the more focused studies of Crich and our group, we now have a firm theoretical basis for the greater oxidative stability of RNA. This situation raises the question of whether there is a biological advantage of this RNA stability. *In vivo*, DNA is sensitive to oxidative damage of the sugar, either through direct attack of oxidants on the C–H bonds of the sugar or through transfer of oxidative damage from the nucleobases to the backbone [28,29]. Base complementarity, the double-stranded structure and DNA repair enzymes combine to help combat this oxidative damage and preserve the integrity of the genome. The nuclear membrane and histones presumably provide greater protection from oxidants.

In contrast, RNA does not have such highly developed protective mechanisms. RNA generally does not have an extra complementary strand to keep a backup of the sequence information [30], and the single-stranded structure provides greater solvent accessibility of the bases and backbone to small oxidants. Nonetheless, RNA ventures out into the cytoplasm where oxidative stress is high [3]. Of course, RNA is not required to maintain the genetic information indefinitely, but resistance to oxidative damage might be an advantage in getting the right sequence out of the nucleus to a ribosome where it can be translated. The oxidative resistance of RNA could therefore provide an advantage as a messenger of genetic information to the rest of the cell.

Because the modern flow of genetic information from DNA to RNA to protein clearly predates the accumulation of oxygen in the atmosphere [31], it is difficult to speculate that the oxidative stability of RNA was part of its selection as the cytoplasmic messenger. The 'RNA world' hypothesis has centered on the hydrolytic properties of RNA and its ability to form diverse structures [32], and it is doubtful that these new findings on the oxidative reactivity will add to that discussion. However, the stability of RNA may have provided part of the means by which life was able to survive the accumulation of oxygen in the biosphere [31] and the subsequent onslaught of reactive oxygen species [29]. Biological machinery based on signaling by oxygen radicals, such as systemic acquired resistance in plants, activation of NF-κB and apoptosis, could have been enabled by RNA stability, as could the nitric oxygen signaling pathways [33].

Summary

There is considerable evidence now that although RNA is hydrolytically much more labile than DNA, it is oxidatively more stable [5,6]. This property has a simple origin in physical organic chemistry, as polar substituents like –OH destabilize the cationic transition states necessitated by oxidation of biomolecules [8]. This property and the 'DNA/RNA paradox' [6] could simply be coincidences brought about by the need for DNA to be hydrolytically

stable and for RNA to perform hydrolytic chemistry and form diverse structures. Furthermore, the oxidative stability of RNA may not have enabled the accommodation of oxygen by cells or the development of signaling pathways based on reactive oxygen species. There are plenty of other reasons for RNA and DNA to have their own identities. On the other hand, we may learn soon that the dual life of RNA as a hydrolytic flibbertijibbet and an oxidative stoic is more than a physical organic relic left over from the need for control of stability or the simple differentiation of DNA and RNA backbones.

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