OH Radical-Induced Main-Chain Scission of Poly(Ribonucleic Acids) under Anoxic Conditions

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Poly(Ribonucleic Acids), OH Attack, Chain Breakage, Free Radicals, Time-Resolved Light Scattering

Poly(ribonucleic acids), poly A, poly C and poly U, were irradiated in O_2-free dilute aqueous solution at pH 7 to 8 with single pulses (50 to 200 ns) of 16 MeV electrons. With the aid of Rayleigh light scattering measurements main-chain scission, induced by OH radicals, was observed with the three polynucleotides. From the time dependence of the decrease of the light scattering intensity (LSI), the existence of two modes of decrease was inferred, indicating at least two different chemical mechanisms were operative in main-chain degradation. Evidence for the assignment of the slow mode of LSI decrease to the lifetime of a free radical was obtained from quenching experiments with cysteamine. It is noteworthy, that the extent and the lifetime of LSI decrease are not the same for the three polynucleotides. The differences indicate the influence of the chemical nature of the bases on main-chain scission. Consequently, it is concluded that OH attack at carbons in 1′ and/or 2′ position of the ribose moiety contributes essentially to the degradation mechanism.

Introduction

OH radicals attack both the bases and the sugar moieties of polynucleotides [1, 2]. Abstraction from the ribose units in poly(ribonucleic acids), PH, according to

$$\cdot OH + PH \xrightarrow{k_1} H_2O + P\cdot \qquad (1)$$

gives rise to main-chain breaks:

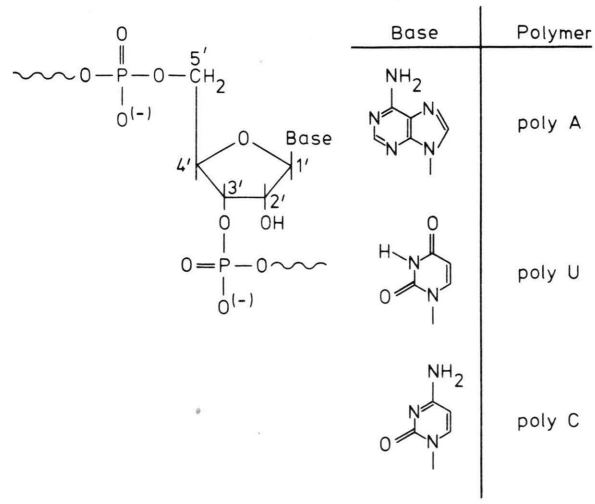
$$P\cdot \xrightarrow{k_2} F_1 + F_2' \qquad (2)$$

where F_1 and F_2' denote fragments of the macroradical P·.

It was the main objective of this work to investigate the kinetics and the mechanisms of main-chain scission processes occurring in the absence of O_2 in single-stranded poly(riboadenylic acid), poly A, poly(ribocytidylic acid), poly C, and poly(ribouridylic acid), poly U.

Impetus to this work came from similar experiments with denatured (single-stranded) calf thymus DNA [3]. In this case, the results were somewhat ambiguous, because of the difficulty in judging the extent and the influence of renaturation. This problem was easily overcome in the present case by working in aqueous solution at pH 7 to 8, where the polynucleotides used here exist as single-stranded molecules and are stable with respect to alkaline hydrolysis under the conditions applied.

The method of pulse radiolysis in conjunction with Rayleigh light scattering was used throughout in order to generate OH radicals in N_2O saturated aqueous solutions and to follow changes of the size of the macromolecules, caused by strand breakage, after irradiation of the solution with single pulses of 16 MeV electrons (duration 50 to 200 ns).



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Materials and Methods

Polynucleotides and preparation of solutions

The polynucleotides used in this work are listed in Table I. Weight average molecular weights $\bar{M}_w$ were determined with a low angle light scattering apparatus (Chromatix KMX-6) in aqueous solutions containing NaCl (10^{-2} mol/l), $(dn/dc) = 0.20$ ml/g. The protein content was determined by the method of Lowry *et al.* [4] (ovalbumin was used as a standard).

For measurements at pH 7 to 8, the polymers were dissolved in sodium phosphate buffer solution. The total ionic strength was adjusted by the addition of $NaClO_4$.

Prior to use, the solvents were filtered through membrane filters (Sartorius, pore width 0.2 μm). The polymer solutions were filtered through membrane filters of 0.45 μm pore width.

Irradiation of solutions

Immediately before irradiation, the sample solutions were bubbled with N_2O, purified by Oxisorb (Messer-Griesheim), for 90 min. The flask containing the sample solution was attached to a flow system containing a rectangular irradiation cell made from quartz. The latter was located in front of the exit window of an L-band linear accelerator (Vickers) which produced 16 MeV electrons. The samples were irradiated with pulses of 50, 100, or 200 ns duration. The change of the light scattering intensity after the pulse was recorded as a function of time. Details of the light-scattering detection system have been reported elsewhere [5, 6].

Treatment of light scattering data

It has been shown previously [5, 6] that the extent of the change of the LSI expressed in terms of the relative signal voltage difference $(U_0 - U_\infty)/(U_\infty - U_L)$ can be correlated to the 100 eV-yield of main-chain scission $G(S)$ and to the absorbed dose D_a (in eV/g) according to Eqn. (I)

$$R_\infty^D = \frac{U_0 - U_\infty}{U_\infty - U_L} = \frac{m G(S) D_a}{F N_A \cdot 10^2}. \qquad \text{(I)}$$

The following denotations are used: U: signal voltage (the subscripts 0 and ∞ stand for "before" (0) and a long time "after" (∞) irradiation, L: solvent); m: molecular weight of the repeating unit; N_A: Avogadro's number; $F = 2/(2 + 4 A c P_\vartheta m n_0)$; A: 2nd virial coefficient (in mol · cm³/g²); c: polymer concentration (in g/cm³); P_ϑ: particle scattering factor, n_0: weight average degree of polymerization before irradiation.

The 1st order kinetic treatment for the light scattering intensity decrease due to main-chain scission is based on the relationship [5]:

$$\ln \frac{(U_t - U_L)(U_0 - U_\infty)}{(U_t - U_\infty)(U_0 - U_L)} = k_1 t, \qquad \text{(II)}$$

$$\tau(\mathrm{LSI}) = \frac{\tau_{1/2}(\mathrm{LSI})}{0.69} = k_1^{-1}.$$

Table I. Polynucleotide samples.

Polymer	Source	$\bar{M}_w$*	Protein Content weight %
Poly A-3	Boehringer	5.3×10^5	0.8
Poly A-5	Boehringer	6.8×10^5	0.8
Poly C-1	Sigma	5.9×10^5	<0.1
Poly U-1	Sigma	3.3×10^5	<0.1

* Weight average molecular weight.

Results

Poly(riboadenylic acid)

The polymer undergoes main-chain degradation upon irradiation in N_2O-saturated aqueous solution as is demonstrated in Fig. 1, which shows the decrease of the LSI after the pulse. Obviously, the decrease occurs in two modes as is illustrated quite clearly in Fig. 2, where the change of the signal voltage is plotted *vs.* a logarithmical time scale. The dependence of the total degree of degradation on the absorbed dose per pulse is shown in Fig. 3(a). The difference in the degradation yields observed in Ar and N_2O-saturated solutions is due to the fact that N_2O converts hydrated electrons to OH radicals ($e^-_{aq} + N_2O + H_2O \rightarrow OH^- + \cdot OH + N_2$) and that the reaction of e^-_{aq} with polynucleotides does not induce main-chain degradation. Hydrated electrons and OH radicals are produced in about equal yields. When all OH radicals were scavenged by t-butanol (0.5 mol/l), no change of the LSI was observed (see Fig. 3(a), straight line 3), indicating that OH radical attack is responsible for main-chain scission.

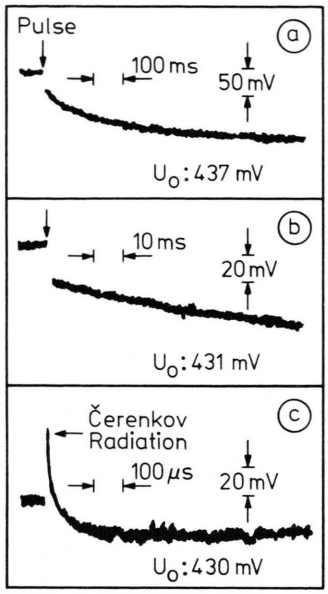


Fig. 1. Main-chain degradation of poly A ($\bar{M}_w = 6.8 \times 10^5$) in N_2O-saturated aqueous solution at pH 7.8 and 23 °C. $[NaClO_4] = 1 \times 10^{-2}$ mol/l; [phosphate buffer] $= 5 \times 10^{-3}$ mol/l; [poly A] = 0.25 g/l. Oscillograms demonstrating the change of LSI after a 100 ns pulse. Absorbed dose per pulse: 22 Gy (2.2 krad).

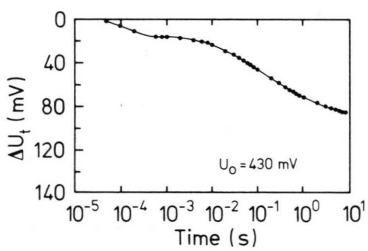


Fig. 2. Main-chain degradation of poly A in N_2O saturated aqueous solution (for other conditions refer to caption to Fig. 1). Change of the signal voltage corresponding to the LSI as a function of time after the pulse. ($\Delta U = U_0 - U_t$).

1st order kinetics hold for both modes of LSI decrease. A typical 1st order plot according to Eqn. (II) is shown in Fig. 4. As can be seen from Fig. 3 (b) the halflife of the slow mode is independent of the absorbed dose in the dose range investigated whereas the halflife of the fast mode decreases slightly with increasing absorbed dose. From the temperature dependence of the 1st order rate constants of the LSI decrease depicted in Fig. 5, the activation energies were determined as 14.6 kJ/mol (fast mode) and 24.7 kJ/mol (slow mode). In the case of the fast

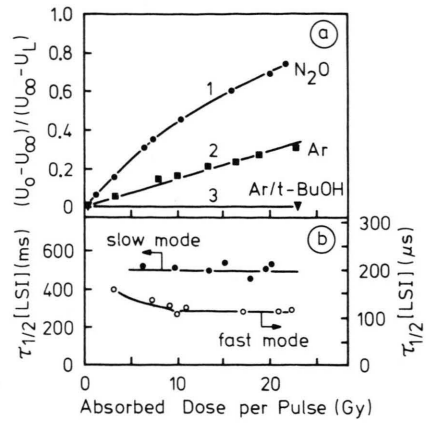


Fig. 3. Main-chain degradation of poly A in aqueous solution (for other conditions refer to caption to Fig. 1). (a) Extent of the change of LSI as a function of the absorbed dose. In the case of curve 3 the solution contained *tert.*-butanol (0.5 mol/l). (b) Halflife of the fast and the slow mode of LSI decrease vs. the absorbed dose (1 Gray (Gy) = 100 rad).

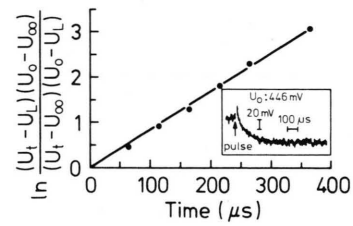


Fig. 4. Main-chain degradation of poly A in N_2O-saturated aqueous solution. Absorbed dose per pulse: 33 Gy. For other conditions refer to caption to Fig. 1. 1st order plot of fast mode of LSI decrease according to Eqn. (II).

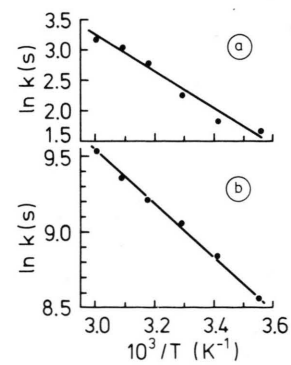


Fig. 5. Main-chain degradation of poly A ($\bar{M}_w = 5.3 \times 10^5$) in aqueous solution. Arrhenius plots of the rate constants of LSI decrease, (a): slow mode, (b): fast mode. Absorbed dose per pulse: 10.5 Gy. For other conditions refer to caption to Fig. 1.

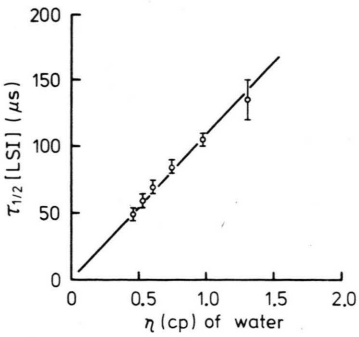


Fig. 6. Main-chain degradation of poly A (0.25 g/l, $\bar{M}_w$: 5.3×10^5) in N_2O-saturated aqueous solution at pH 7.5 and temperatures between 9 and 62 °C. [$NaClO_4$] = 1×10^{-2} mol/l; [phosphate buffer] = 5×10^{-3} mol/l. Absorbed dose per pulse: 10 Gy. The halflife $\tau_{1/2}$ of the fast mode of the LSI decrease vs. the viscosity of water η. (η values were adopted from ref. [10].)

mode the temperature dependence of the halflife corresponds to the temperature dependence of the viscosity of the solvent, water (see Fig. 6). This behavior is expected if the rate of the decrease of the LSI is determined by the rate of diffusion of the fragments generated by a rapid chemical reaction involving main-chain scission. In this case the chemical reaction occurs much faster than the diffusion of the fragments. Such rapid main-chain scission reactions have been observed, for example, in the flash photolysis of poly-1-phenyl-2-propene-1-one [6, 7] or in the pulse radiolysis of polyisobutene [8] in solution.

In order to obtain information about the nature of the slow mode of LSI decrease, experiments in the presence of cysteamine hydrochloride were carried out. Cysteamine (RSH) is known to react with free carbon radicals P· according to the reaction

$$P\cdot + RSH \xrightarrow{k_3} PH + RS\cdot . \qquad (3)$$

Provided reaction (3) competes with reaction (2), the lifetime of the radicals P· should diminish by cysteamine being present in the solution. Thus, halflifes $\tau_{1/2}$(LSI) were expected to decrease by increasing the concentration [RSH], provided the rate of LSI decrease corresponds to the rate of the decay of free radicals. As shown in Fig. 7 (a) and reported briefly already [9], $\tau_{1/2}$(LSI) decreased upon the addition of cysteamine. This is evidence for the slow mode of LSI decrease corresponding to the lifetime of a relatively long-lived free radical at the sugar moiety. The evaluation of the data of Fig. 7 (a) with respect to the competition of reaction (2) with reaction (3) yields, with [RSH] = const. and

$$[P\cdot]_t = [P\cdot]_0 \exp - (k_2 + k_3 [RSH])\, t \qquad (III)$$

$k_2 = 1.7 \pm 0.1\ s^{-1}$ and $k_3 = 3.4 \times 10^6$ l/mol s.

In the experiments concerning the data in Fig. 7 (a) the highest cysteamine concentration was 1×10^{-5} mol/l. At this concentration about 10% of the OH radicals are scavenged by RSH (on the basis of $k_{OH+RSH} = 8.5 \times 10^9$ l/mol s [13] and $k_{OH+PH} =$

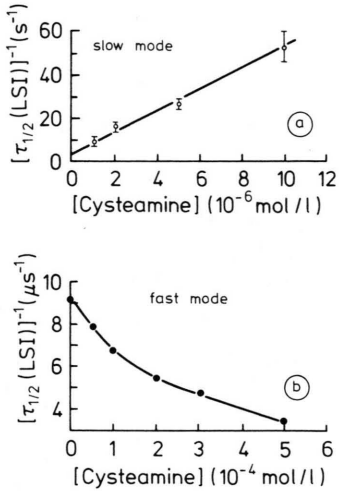


Fig. 7. Influence of cysteamine on the rate of LSI decrease in N_2O-saturated aqueous solution of poly A (0.3 g/l, $\bar{M}_w = 6.8 \times 10^5$) at pH 7.8 and 23 °C. Reciprocal halflife of the slow mode (a) and of the fast mode (b) of the LSI decrease vs. the concentration of cysteamine hydrochloride. [phosphate buffer] = 5×10^{-3} mol/l; [$NaClO_4$] = 1×10^{-2} mol/l; absorbed dose per pulse: 10.5 Gy (a) and 37 Gy (b).

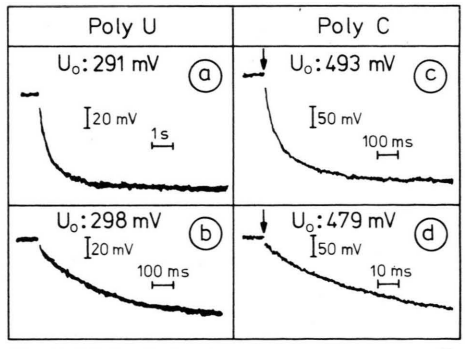


Fig. 8. Main-chain degradation of poly U ($\bar{M}_w = 3.3 \times 10^5$) and poly C ($\bar{M}_w = 5.9 \times 10^5$) in N_2O-saturated aqueous solution at pH 7.8 and 23 °C. [poly U] = 0.25 g/l; [poly C] = 0.25 g/l; [$NaClO_4$] = 1×10^{-2} mol/l; [phosphate buffer] = 5×10^{-3} mol/l; absorbed dose per pulse: 25 Gy. Oscillograms demonstrating the decrease of the LSI after the pulse at various time scales.

Table II. Comparison of data obtained with poly A, poly C and poly U in aqueous solution (pH 7.8) containing phosphate buffer (5×10^{-3} mol/l) and $NaClO_4$ (1×10^{-2} mol/l).

Polymer	$\bar{M}_{w,o}$[a]	$\tau_{1/2}(LSI)_{slow}$[b] [ms]	R_∞^D (at 10 Gy)	E_a[c] slow mode [kJ/mol]	E_a[c] fast mode [kJ/mol]	f_{fast}[d] (%)
Poly C	5.9×10^5	150	0.75	34		ca. 3
Poly U	3.3×10^5	510	0.33	38		8
Poly A	6.8×10^5	500	0.38	24.7	14.6	20

[a] Initial weight average molecular weight.
[b] At 23° C.
[c] Activation energy of 1st order rate constant.
[d] Fraction of fast mode of LSI decrease, R_∞^D (fast)/R_∞^D (total), determined at 10 Gy.

9.4×10^8 l/base-mol s). Therefore, the fast mode should not be affected significantly at rather low cysteamine concentrations. At higher concentrations, however, the fast mode is strongly affected as is shown in Fig. 7(b). The halflife increases, *i.e.* $[\tau_{1/2}(LSI)]^{-1}$ decreases, in the concentration range up to 5×10^{-4} mol/l, which was investigated. This results confirms the conclusion arrived at earlier, that the fast mode reflects a diffusion process. Namely, the scavenging of OH radicals by RSH causes less attack on the polymer, with the consequence that the average number of scissions per initial macromolecule becomes smaller and thus the average fragment size larger. Longer fragments diffuse more slowly than smaller ones. Therefore, the diffusion rate decreases with increasing cysteamine concentration at constant absorbed dose.

Poly(ribocytidylic acid) and poly(ribouridylic acid)

With poly C and poly U similar results were obtained as with poly A. From the oscilloscope traces shown in Fig. 8 it can be seen that there are also two modes of LSI decrease. The fraction of the fast mode, however, is significantly smaller than in the case of poly A, as can be seen from Table II. The dependence of the extent of LSI decrease R_∞^D and of the halflife of the slow mode of LSI decrease on the absorbed dose is illustrated in Fig. 9. R_∞^D increases and $\tau_{1/2}(LSI)_{slow}$ remains constant with increasing dose per pulse. As is shown in Table II, R_∞^D (at a given dose) is significantly higher and $\tau_{1/2}(LSI)_{slow}$ is more than three times lower for poly C than for the other two polynucleotides.

Preliminary experiments with cysteamine demonstrated that the halflife of the slow mode of LSI decrease diminished with increasing cysteamine concentration as in the case of poly A [11]. Therefore, it seems to be established that, in all three cases, the slow mode of LSI decrease reflects the existence of rather long-lived free radicals.

Discussion

The three polynucleotides exhibit a similar behavior as far as the facts are concerned that OH radical

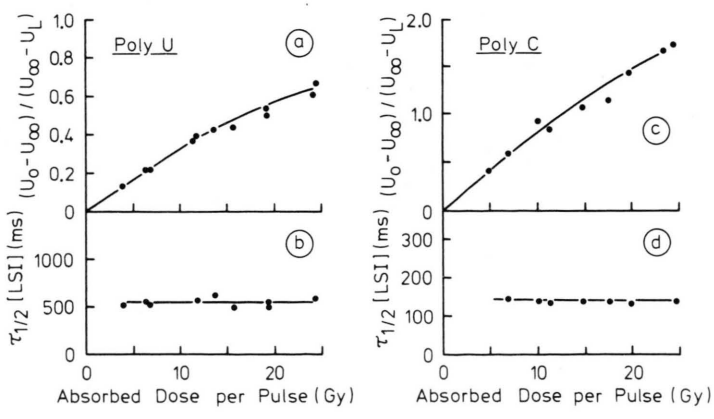
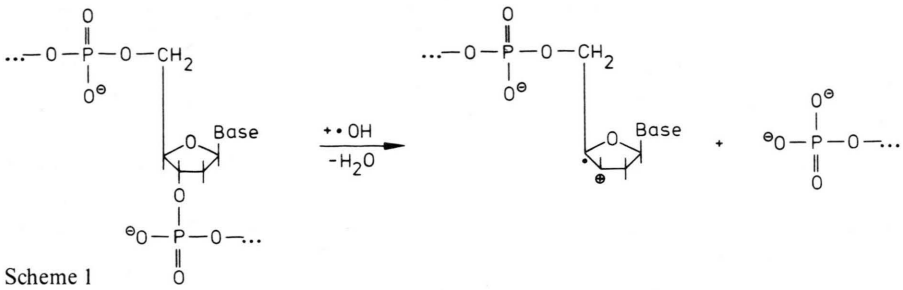


Fig. 9. Main-chain degradation of poly U (a) and (b), and of poly C (c) and (d) in N_2O-saturated aqueous solution. For other conditions refer to caption to Fig. 8. Extent of LSI decrease ((a) and (c)) and halflife of the slow mode of LSI decrease ((b) and (d)) as a function of the absorbed dose per pulse



Scheme 1

attack induces main-chain scission and that, on the basis of the time dependence of the LSI decrease, main-chain scission comprises two different chemical processes.

The first process occurs so rapidly, that the rate of LSI decrease cannot reflect the rate of a chemical reaction but the rate of the diffusion of the fragments generated by main-chain rupture. This is concluded from the low value of the activation energy of the rate constant for LSI decrease and from the dependence of the rate constant on the viscosity (see Fig. 6). The chemical reaction responsible for the rapid mode of LSI decrease occurs at times definitely shorter than 50 μs.

The second process, *i.e.*, the slow mode of the LSI decrease comprises the major portion of the total LSI decrease in all three cases. Since it was proven that the slow mode corresponds to a chemical reaction, probably involving free radicals, it might be concluded that the same is true also for denatured DNA. From this point of view, assumptions assigning the slow mode of LSI decrease, observed in the case of single-stranded DNA [3], to segment detachment in renatured portions of the macromolecules can be discarded.

In accordance with this work are results obtained by pulse radiolysis of poly(ribouridylic acid) in conjunction with electrical conductivity measurements [12]. These investigations led to the conclusion that main-chain scission involves macroradicals with a halflife of approx. 1.3 s (at pH 7.8) which is in the same order as the halflife of the slow mode of the light scattering intensity decrease observed with poly U (0.51 s, see Table II).

Direct information concerning the chemical nature of the free radicals leading to main-chain rupture cannot be obtained from the present light scattering measurements. In a former publication [9] it was suggested that the rapid process is initiated by an attack of OH at the C-atom in 4′-position. According to von Sonntag and Schulte-Frohlinde [2] the radical thus formed induces main-chain scission in the case of DNA as shown in Scheme 1.

Actually, the H atoms in the positions 1′, 2′, 3′ and 5′ can also be abstracted and the question arises as to whether the ensuing free radicals can give rise to main-chain scission*. Provided a sequence of reactions is involved in such a process, all possible carbon radicals could initiate main-chain scission. In this connection, it is noteworthy that the base exerts a significant influence on both the halflife and on the fraction of the fast mode of LSI decrease, as can be seen from Table II. Therefore, it is probable that free radicals generated by H abstraction in 1′ or 2′-position are responsible for the slowly occurring main-chain ruptures. The importance of the bases for main-chain rupture processes is emphasized as a result of this work. It appears to be necessary to employ various additional experimental techniques in future work in order to elucidate the mechanism in more detail.

Acknowledgements

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* Polyribonucleotides have – in the 2′-position – only one hydrogen which is more likely to be abstracted by OH than the secondary hydrogens in 2′-position in the case of DNA.

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