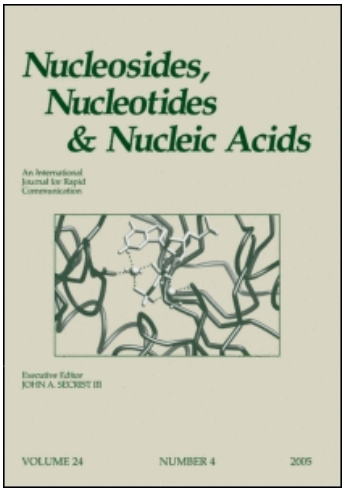

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SYNTHESIS OF SPIN-LABELED RNAS FOR LONG RANGE DISTANCE MEASUREMENTS BY PELDOR

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▫ *Site directed spin labeled RNA duplexes with different interspin distances were synthesized. The radical 2,2,5,5-**t**etramethyl-**p**yrrolin-1-yloxyl-3-**a**cetylene (TPA) was introduced during the solid-phase synthesis through a Sonogashira cross-coupling with 5-iodo-uridine. T_m and CD studies showed that the spin label does not to disturb significantly the A-form of these duplexes. 4-**P**ulse **E**lectron **D**ouble **R**esonance (PELDOR) was then used to measure intramolecular spin-spin distances of 19.3, 33.0 and 40.9 Å, which are in very good agreement with the calculated values of 17.6, 32.1 and 39.1 Å, obtained from **M**olecular **D**ynamics (MD) simulations.*

INTRODUCTION

Site-directed spin labeling (SDSL) in conjunction with EPR spectroscopy has been widely used for proteins[1] but hardly for DNA or RNA. However, this tool allows to study the dynamics of the labeled oligonucleotides,[2] to gather structural information by measuring the distance between two spin labels[3,4] or to study

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TABLE 1 RNA Sequences and Corresponding Data

Name	Sequence	MALDI (m/z)[a]	T_m (°C)[b]	$r_{AB,PELDOR}$ (Å)	$r_{AB,MD}$ (Å)
RNA1	3′CGA C**U**A UAG UCG 5′GCU GAU A**U**C AGC	3958.6/3961.3	60.9/58.8	19.3 ± 1.0	17.6 ± 1.6
RNA2	3′C**U**G ACU AGU CAG 5′GAC UGA UCA G**U**C	5244.3/5248.4	62.3/59.8	33.0 ± 2.0	32.1 ± 1.5
RNA3	3′GC**U**GACUAUAGUCAGC 5′CGACUGAUAUCAG**U**CG	3958.6/3962.1	67.8/67.7	40.9 ± 2.0	39.1 ± 2.6

[a]The first number is the calculated mass, the second one the measured mass.
[b]The first value corresponds to the unmodified RNA, the second one to the labeled RNA.

RNA-ligand interactions.[5] PELDOR, in its 3-[6,7] or 4-pulse[8] version is a pulsed EPR method by which electron-electron coupling can be separated from other spectral contributions. Recently a PELDOR based nanometer distance ruler was developed for DNA,[9] which is here successfully extended to three RNA duplexes.

SYNTHESIS

Three RNAs with the base sequence specified in Table 1 were doubly spin labeled at the positions indicated by a bold **U** using the same conditions as for the DNA (Figure 1). However, three successive cross-couplings with the radical TPA were needed in the case of RNA, whereas two cross-couplings were sufficient for DNA, due to the lower reactivity of 5-iodo-uridine in RNA. The oligonucleotides were cleaved from the controlled pore glass (CPG) with a mixture of ammonia (32%)/MeOH (3/1) over 24 h. The tertbutyldimethylsilyl groups were cleaved with HF in triethylamine over 24 h. After purification of the oligonucleotides via anion exchange chromatography pure samples could be obtained due to an almost

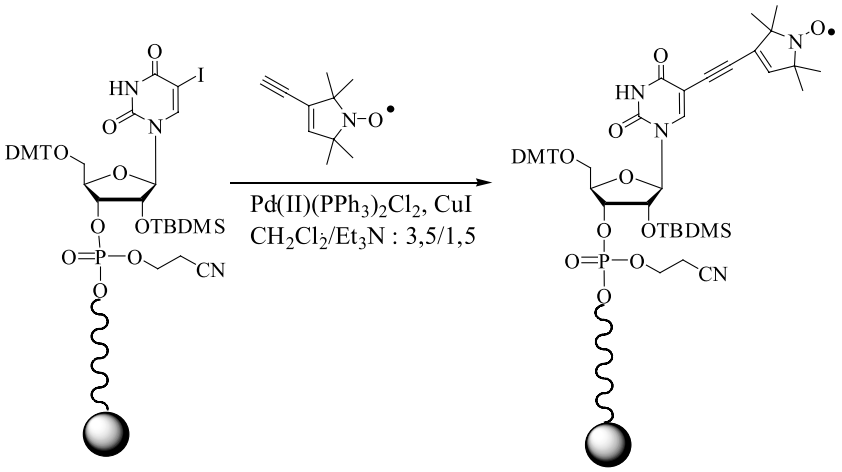

FIGURE 1 Spin labeling during the solid-phase synthesis.

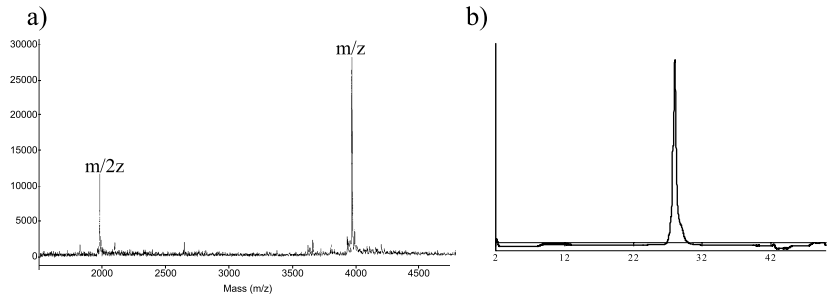

FIGURE 2 a) MALDI-Tof spectrum (calc: 3958.6/meas.: 3961.3) and b) analytical anion exchange HPLC of RNA 1, gradient: 1M LiCl/DEPC water: 0/100 to 50/50% in 50 min.

quantitative yield of the palladium cross-coupling (Figure 2). In order to investigate the thermodynamic effects of the nitroxide on the RNA, UV-vis detected thermal denaturation experiments were performed. These experiments showed a slight destabilisation of 0.1 to 2.5°C of the labeled RNAs in comparison to the unmodified RNAs. However, CD measurements confirmed that the A-form of the helix is conserved in the labeled RNAs although they reveal a lower ellipticity (Figure 3).

PELDOR

The dead-time free 4-Pulse ELDOR (Figure 4) measurements were done on the doubly labeled oligonucleotides 1–3, dissolved in a 10 mM phosphate buffer containing NaCl (140 mM, pH 7) and 20% ethylene glycol. The obtained PELDOR time traces (Figure 5a) were Fourier transformed and the frequency ν_{AB} was read

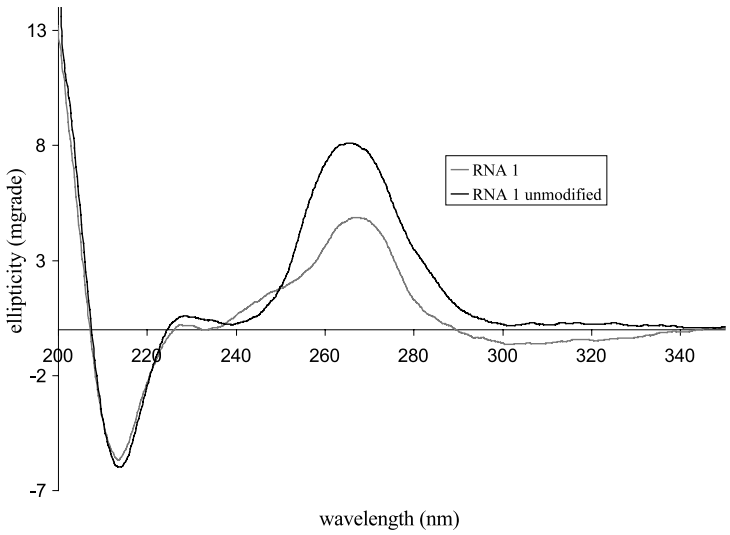

FIGURE 3 Comparison of CD spectra of RNA1 and the corresponding unmodified RNA.

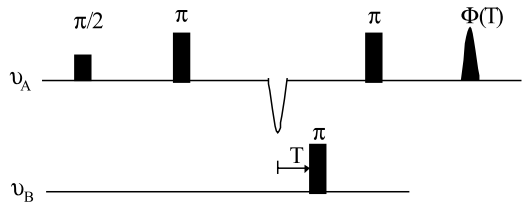

FIGURE 4 Pulse sequence for a 4-pulse ELDOR experiment.

off from the singularity at $\theta = 90°$ (Figure 5b). The distance between the two spin labels was then calculated parameter free with the following equations:

$$\nu_{AB} = \nu_{Dip} \cdot (1 - 3\cos^2\theta)$$

$$\nu_{Dip} = \frac{\mu_B^2 \cdot g_A \cdot g_B \cdot \mu_0}{4 \cdot \pi \cdot h} \cdot \frac{1}{r_{AB}^3}$$

θ: angle between the magnetic field and the dipole vector
μ_B: Bohr magneton
μ_0: magnetic field constant
g_A and g_B: g values of spin A and spin B, respectively
h: Planck constant

MD SIMULATIONS

All MD simulations were performed using the GROMACS program package,[10] the AMBER98 all-atom force field,[11] explicit TIP3P water solvent,[12] and a density functional theory parametrization of the spin label molecules.[9] Following equilibration, the MD distances were obtained from 10 ns NTV runs. The results for all the three RNAs are gathered in Table 1. The distances obtained from the PELDOR measurements are in very good agreement with the distances found in the MD simulations.

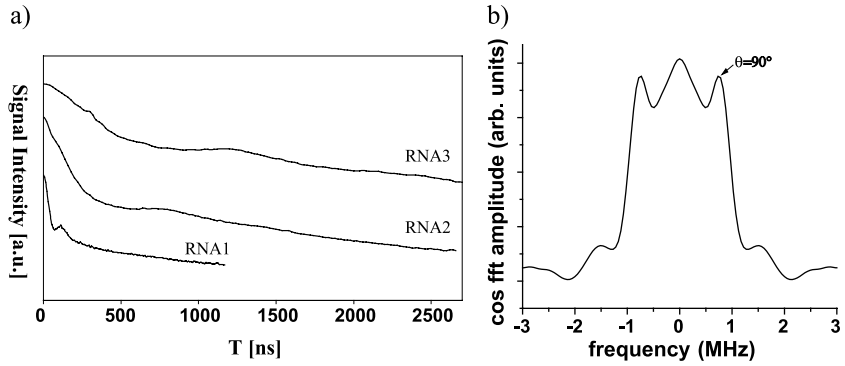

FIGURE 5 a) PELDOR time traces of RNAs 1–3; b) Fourier transformed time traces of RNA 3 as an example.

OUTLOOK

In order to investigate more complex biological systems, we synthesized 5-iodo-cytidine, 5-iodo-tubercidine,[13,14] and 2-iodo-adenosine[15] as fully protected phosphoramidites which allow the derivatization in the major and minor groove respectively. Further experiments are underway and will be reported in due course.

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