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Stability of oligodeoxynucleoside phosphorodithioates and phosphorothioates in aqueous ammonia

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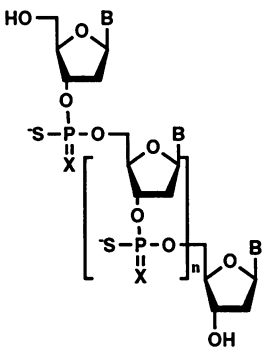
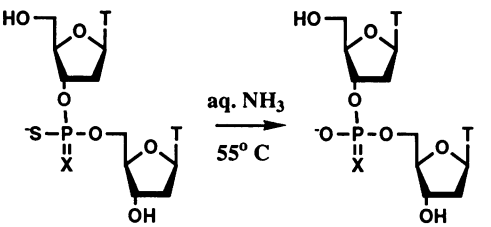
Oligonucleotide analogues like oligodeoxynucleoside phosphorothioates and phosphorodithioates (Fig. 1) are of interest for antisense applications (1). Efficient methods exist for the preparation of both analogues, but the products often contain less sulfur than intended, as shown by the presence of variable amounts of phosphate linkages in oligonucleoside phosphorothioates, and phosphorothioate linkages in oligonucleoside phosphorodithioates. Incomplete oxidation by the sulfurization reagents used in phosphoramidite or thiophosphoramidite methods could explain the appearance of these impurities, but cannot explain why products prepared by triester methods are also often contaminated. We have recently described a dithiophosphortriester method that gives oligodeoxynucleoside phosphorodithioates free of phosphorothioate impurities (2). However, when the oligonucleoside phosphorodithioates were kept in prolonged contact with aqueous ammonia during deblocking, small amounts of phosphorothioates sometimes appeared. The amounts did not vary in any systematic way with contact time, dilution of the aqueous ammonia, or temperature. Here we describe our experiments to determine the factors responsible for this loss of sulfur atoms in aqueous ammonia, based on data from the treatment of the TT dimers TpsT or Tps_2T (Fig. 2) with aqueous ammonia under different conditions.



phosphorothioate, X = O
phosphorodithioate, X = S

Figure 1. Structure of oligodeoxynucleoside phosphorothioates and phosphorodithioates.



TpsT, X = O
Tps_2T, X = S

Figure 2. Sulfur removal by hydrolysis of the TT dimers TpsT and Tps_2T in aqueous ammonia.

In a typical experiment, ~0.1 mmol TpsT or Tps_2T was dissolved in 1 ml of aqueous ammonia (32%, Merck 5426, undiluted or diluted four times with glass-distilled water) in a 5 mm NMR tube which was sealed and kept at 55°C in an oven. The tubes were removed at selected intervals and the reactions monitored by ^{31}P NMR (JEOL FX90Q at 36.24 MHz or Varian Unit 400 at 161.91 MHz). Since the concentration of ammonia was ≥ 3.7 M pseudo first order conditions were assumed in the calculation of k' and $t_{1/2}$. Selected results are shown in Table 1.

The results show that loss of sulfur is catalyzed by metal ions like Fe^{2+}, Ni^{2+} and Cr^{3+}, and that the effect can be reduced by addition of EDTA. The above metal ions are likely contaminants of ammonia, particularly when solutions are transfered using syringes with metal needles. We have observed 2–3% of phosphorothioate contaminations in previously pure oligodeoxynucleoside phosphorodithioates after a few days in 4 M aqueous ammonia when the solutions had been in contact with metal needles. A recent observation of the appearance of phosphate impurities in oligodeoxynucleoside phosphorothioates during treatment with conc. aqueous ammonia (3) may be explained similarly. The addition of small amounts of EDTA to ammonia deblocking solutions is therefore strongly recommended in order to preserve the integrity of phosphorothioate and phosphorodithioate oligonucleotides. Tps_2T, and presumably oligodeoxynucleoside phosphorodithioates, in a conc. aqueous ammonia solution with 0.01 M EDTA will form only 0.012% of monothioate after 10 h at 55°C, which is clearly acceptable.

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Table 1. Pseudo first order rate constants k′ and halftimes $t_{1/2}$ for sulfur removal by hydrolysis of phosphorodithioate (Tps_2T) and phosphorothioate (TpsT) dimers, at 55°C in aqueous ammonia with or without additives

TT dimer	CNH_3 (M)	Additive	k′ (h^{-1})	$t_{1/2}$ (days)
Tps_2T [a]	~16.7[b]	none	9.8×10^{-5}	300[c]
	~16.7b	0.01 M EDTA	1.2×10^{-5}	2500
	4.18	0.01 M EDTA	1.1×10^{-5}	2700
	3.80	$<10^{-4}$ M $FeSO_4$[d]	1.8×10^{-4}	160
	3.73	10^{-4} M $NiCl_2$	2.9×10^{-4}	100
	3.76	$<10^{-4}$ M $Cr_2(SO_4)_3$[d]	7.9×10^{-4}	35
TpsT [a]	~16.7[b]	none	2.0×10^{-5}	1400[c]
	4.18	0.01 M EDTA	7.0×10^{-6}	4100
	3.80	$<10^{-4}$ M $Cr_2(SO_4)_3$[d]	4.7×10^{-5}	610

[a]Tps_2T was prepared as the *t*-butylammonium salt by a triester method analogous to that of ref. 2. TpsT was obtained as the *t*-butylammonium salt by a standard phosphoramidite coupling followed by oxidation with sulfur. Both dimers were purified by column chromatography on silica before the protecting groups were removed. Tps_2T contained 1.0% of the phosphorothioate, and TpsT 0.6% of the phosphate; these values were subtracted from the measured amounts before calculations of k′ and $t_{1/2}$.

[b]32% aqueous ammonia (Merck 5426).

[c]Metal needle used.

[d]Some precipitation occurred.

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