Avoidance of sulfur loss during ammonia treatment of oligonucleotide phosphorothioates

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ABSTRACT

Sulfur loss during the unblocking of phosphorothioate analogues of oligonucleotides with concentrated aqueous ammonia can be completely suppressed by the addition of 2-mercaptopoethanol.

Most synthetic oligodeoxyribonucleotide phosphorothioates (Scheme 1, 1a) that have been described so far have been prepared by solid phase synthesis (1,2). Phosphoramidite monomers are generally used in solid phase oligonucleotide phosphorothioate synthesis, and a sulfur transfer step is then required in each synthetic cycle. The assembled oligonucleotide sequences are removed from the solid support and unblocked in a two-step process which involves heating with concentrated aqueous ammonia at 50–55°C, followed by treatment with dilute acid.

Analysis of the completely unblocked material by 31P NMR spectroscopy (3) usually reveals a small percentage (perhaps ~1%) of phosphodiester (as in Scheme 1, 1b) amongst a developed reagents (3,4), sulfur transfer may well not be quantitative in each synthetic cycle. The assembled oligonucleotide sequences are removed from the solid support and unblocked in a two-step process which involves heating with concentrated aqueous ammonia at 50–55°C, followed by treatment with dilute acid.

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Figure 1. $^{31}$P NMR Spectra ($D_2O$) of $d\{Gp(s)A\}$, unblocked at $50^\circ C$ for 15 h with (a) conc. aq. NH$_3$–HSCH$_2$CH$_2$OH (9:1 v/v), and (b) conc. aq. NH$_3$ only. The insets are corresponding reverse phase HPLC profiles. Signals (peaks) relating to sulfur-free components are indicated by arrows.

(Fig. 1b), the presence of $d\{GpA\}$ was detected both by $^{31}$P NMR spectroscopy ($\delta_P$ ~0; estimate ~1.3%) and HPLC ($R_t$ 10.91 min; estimate ~1.8%). It is further clear from Figure 2a and b that $d\{Cp(s)Tp(s)Gp(s)A\}$ was obtained without any detectable sulfur loss when 2-mercaptoethanol was added to the unblocking medium, but that ~1% loss of sulfur occurred when it was not added. It is similarly clear from Figure 2c and d that 2-mercaptoethanol prevented an ~0.8% loss of sulfur when it was added to the aqueous ammonia solution used to unblock $d\{Tp(s)Tp(s)Gp(s)Gp(s)Gp(s)Tp(s)T\}$. It was independently confirmed that the four main base residues, adenine, cytosine, guanine and thymine are not affected by the addition of 2-mercaptoethanol to the concentrated aqueous ammonia.

In the solid phase synthesis of oligonucleotide phosphoro-thioates, it is impossible to estimate how much sulfur loss is due to incomplete sulfur transfer and how much occurs during unblocking in ammonia solution. However, it seems reasonable to conclude that contamination with material containing phosphodiester internucleotide linkages would be diminished significantly if 2-mercaptoethanol were added to the ammonia unblocking solution.

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REFERENCES