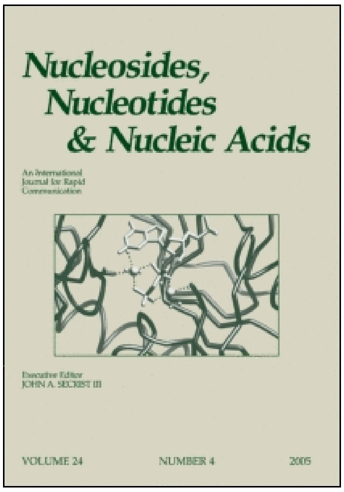

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Selective Recognition of Triplex and Duplex Melting in Triple-Helical Nucleic Acids by Circular Dichroism Spectroscopy

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SELECTIVE RECOGNITION OF TRIPLEX AND DUPLEX MELTING IN TRIPLE-HELICAL NUCLEIC ACIDS BY CIRCULAR DICHROISM SPECTROSCOPY

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ABSTRACT: Here we present a method based on CD spectroscopy to evaluate duplex and triplex melting independent from each other in cases where UV melting curves show only one transition.

In recent years, studies of sequence-specific triplex formation of short oligonucleotides are of prime interest because this approach is applicable to biological and biochemical studies as site specific cleavage of DNA and inhibition of gene expression[1].

The most common motif is the pyrimidine×purine•pyrimidine motif in which the third strand is located in the major groove of the duplex in a parallel orientation due to the purine strand. Thymines and protonated cytosines in the third strand form hydrogen bonds with adenines and guanines, respectively, in the Hoogsteen type[1].

Here we present a pH dependent investigation of thermal stability of triple helical nucleic acids by UV- and CD-spectroscopy. The investigated triple helices are:

5'-rUUUUCUUCUUCUUUU	5'-dTTTTC TTCTT CTTTT
5'-dCCCAAAAGAAGAAGAAAACCC	5'-dCCCAAAAGAAGAAGAAAACCC
3'-dGGGTTTT C TTCTT C TTTTGGG	3'-dGGGTTTT C TTCTT C TTTTGGG

The results indicate that a more stable triplex is formed in the case that the third strand is RNA instead of DNA (see Table 1).

The duplex melting temperature is between 63 and 64 °C, independent of the pH.

Normally UV melting experiments of triple-helices result in biphasic strand dissociation according to the transitions triplex → duplex + single strand → single strands. In some cases, like here with RNA as third strand at pH 5.0, it is only possible to recognize

TABLE 1: Comparison of the melting temperatures (T_m) of triplexes with RNA or DNA as third strand obtained by UV melting experiments (Errors ± 0.5 °C)

pH	Third strand RNA	Third strand DNA
5.0	*	49 °C
6.0	45 °C	34 °C
7.0	32 °C	21 °C
7.4	26 °C	16 °C

* In this case there is only one transition for duplex and triplex melting at 60 °C.

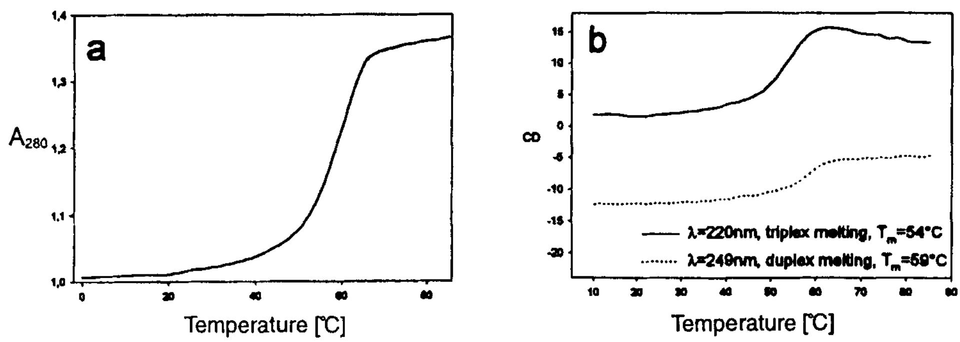

FIG. 1: [a] UV melting curve with a RNA third strand at pH 5.0, [b] CD melting curves at 220 nm (triplex melting) and 249 nm (duplex melting) with a RNA third strand at pH 5.0

one transition, so that it remains unclarified if a stable triple helix which melts together with the duplex has been formed or not. If this happens it is feasible to detect the transition triplex → duplex + single strand independent of the duplex melting by using a CD melting experiment at a selected wavelength. The most suitable wavelength can be chosen from a wavelength dependent CD spectrum. Triplex melting can be shown at a wavelength between 210 and 220 nm, duplex melting around 250 nm.

There are only a few examples for the use of CD melting experiments of triple helical nucleic acids in the literature[2], in all these cases the third strand is DNA. A CD melting experiment at a selected wavelength with RNA as the third strand is reported here for the first time.

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