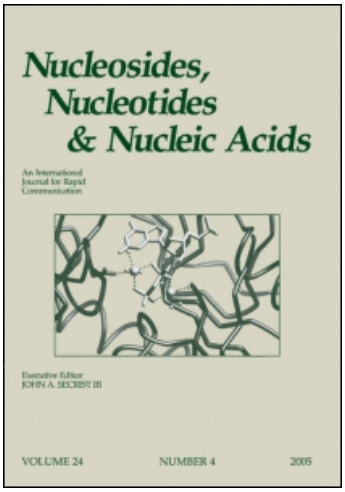

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Analysis of DNA Sequencing Reaction Products Made with 7-Halo-7-deaza-2'-deoxyguanosine-5'-triphosphate

Mark G. McDougall[a]; Louis P. Hosta[a]; Shiv Kumar[a]; Carl W. Fuller[a]
[a] Amersham Pharmacia Biotech, Cleveland, OH, USA

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ANALYSIS OF DNA SEQUENCING REACTION PRODUCTS MADE WITH 7-HALO-7-DEAZA-2'-DEOXYGUANOSINE-5'-TRIPHOSPHATE

Mark G. McDougall, Louis P Hosta, Shiv Kumar*, and Carl W. Fuller

Amersham Pharmacia Biotech, 26111 miles Rd., Cleveland, OH, USA 44128

ABSTRACT : 7-Chloro- and 7- Iodo-7-Deaza-2'-Deoxyguanosine-5'-Triphosphates were employed as substrates replacing dGTP, dITP, or 7-Deaza-2'-Deoxyguanosine-5'-Triphosphate in sequencing reactions with Thermo Sequenase™. Analysis of the reaction products by denaturing gel electrophoresis indicates DNA containing these halogenated analogs can form strong secondary structures.

We report a series of experiments in which 7-Chloro- and 7- Iodo-7-Deaza-2'-Deoxyguanosine-5'-Triphosphate[1], dITP, and 7-Deaza-2'-Deoxyguanosine-5'-Triphosphate (dZTP) were used as substrates replacing dGTP in dye primer cycle sequencing reactions with Thermo Sequenase™ on double stranded pGEM 3Zf(+), starting at the -28 reverse primer. The ratio of the two halogenated analogues to ddGTP (300 : 1) in the sequencing reaction mixtures were the same as typically used for dGTP or dZTP.[2] Both 7-Cl-dZTP and 7-I-dZTP were observed as good substrates in the sequencing reactions with no significant difference in band uniformity when compared to dGTP. Bands were sharp and well-resolved and the sequences could be read well past 400 bases. The pGEM template contains a number of sites where 2 or 3 consecutive G's occur in the newly synthesized strand. These did not result in any apparent difficulties in incorporation of the 7-Iodo substrate, even though such repeats in the same strand is destabilizing and favors A-DNA like structures.[3]

The common term for irregularities of band spacing found in sequencing gels is "compression". These are actually the result of faster than normal migration of some DNA fragments. Such behavior is caused by the formation of thermodynamically stable secondary structures[4] in the single-stranded DNA fragments, despite the denaturing environment of 7-8 M urea and elevated temperatures (40° to 50°C) used for electrophoresis. In the 460 base sequence of which comprise pGEM 3Zf(+) studied, we have found that sequence products made using dGTP, 7-Cl-dZTP, and 7-I-dZTP have six strong compression artifacts which prohibit correct sequence interpretation. Sequences made with dZTP resolved all but two of these compressions, and dITP gave a completely correct sequence.

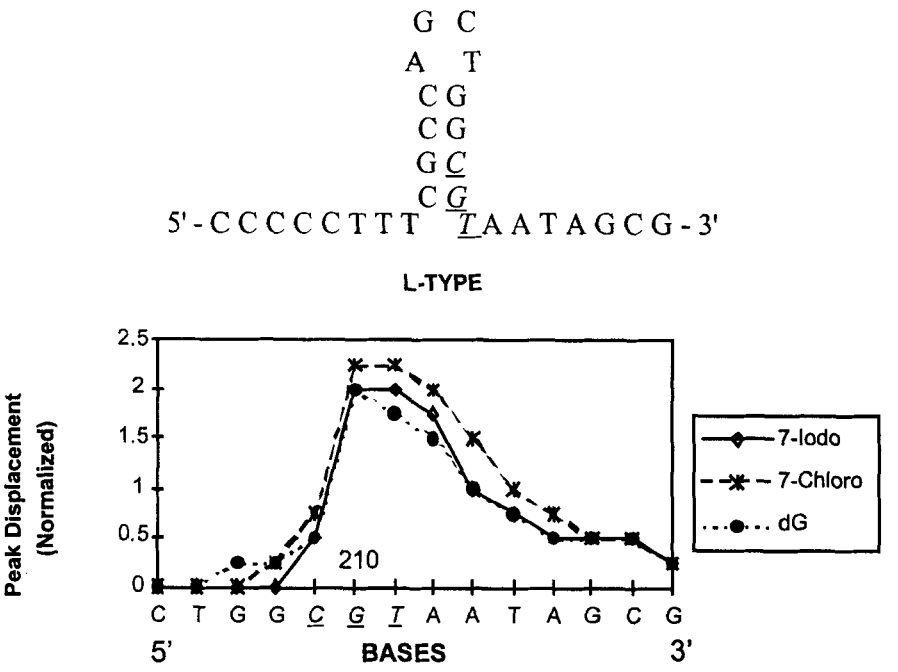

FIGURE 1. Example of compression analysis of a L-type hairpin centered at 210 bases. The underline bases are those fragments which migrated to about the same position in the gel. The x-axis represent the relative strength of the hairpin for each DNA fragment (y-axis).

These strong compressions all have at least one DNA fragment band which migrates faster than the band for the fragment one base smaller (i.e. its predecessor in the sequence). This shift in band position makes sequence interpretation inaccurate. Since the sequence of this DNA is known, and band spacing in these gels is quite regular, it is possible to accurately estimate the magnitude of the shift in band position, normalized for the average space between bands. By measuring the shifts in band positions observed in the compressed regions of the sequences, we have found that the relative "strength" of the secondary structures (hairpins) causing the compression can be determined. Thus, the secondary structure which causes to be displaced by three normal band spacings from its normal position is assumed to be more stable than a secondary structure which causes a displacement of only two spacings. It is interesting to note that displacement of subsequent bands in the sequence decreases in magnitude until the bands apparently migrate at their "normal" positions and displacement is zero. The number of bases required to reach this condition also seems to vary with the apparent strength of the causative secondary structure. This is readily seen in histograms of displacement magnitude plotted against position in the sequence.

Five compressions were found to be of the L-type (those stabilized by long G:C stems) as defined by Yamakawa Nakajima and Ohara[4]. Here fragments containing the 7-chloro analogue had intrinsically stronger structures than those containing 7-Iodo,

regardless if the stem contained alternating G:C and C:G, or G:C and G:C pairs. This is surprising due to the observations by Seela and Ramzaeva,[3] which indicate that duplexes with alternating G:C structures containing 7-Iodo are more stabilized than ones containing 7-bromo, while homooligonucleotide duplexes show the opposite effect. Perhaps hydrogen bonding strength contributes more to these hairpin structures than base stacking effects. In some cases both halo-analogues were bound stronger than dG and other cases dG showed greater displacement. This is an indication that even in L-type motifs the base content within the loop is important. In one example of an M-type motif[4] which is proposed to be stabilized by G(2-NH_2):A(7-N) and G(3-N):A(6-NH_2) hydrogen bonding in the hairpin loop[5], an exactly similar strength was observed for dG, 7-Cl-dZ, and 7-I-dZ containing fragments. Residues containing dZ, both L-type and M-type were weaker than those containing dG or halogenated analogues.

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