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Sequence-independent and linear variation of oligonucleotide DNA binding stabilities

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ABSTRACT

The ability to equalize the DNA binding stability of comprehensive sets of oligonucleotides is imperative for the application of sequencing by hybridization technology. By substitution of ribonucleotides into an oligonucleotide composed of deoxyribonucleotides, and vice versa, the duplex stability of the oligonucleotide is changed linearly with the number of serial alternations of sugar configurations within the molecule. Since this effect occurs independently of the actual base sequence, any set of oligonucleotides could be adjusted to a defined level of binding stability.

INTRODUCTION

Sequencing by hybridization (1–4) has huge potential to become the diagnostic tool by which an individual DNA piece can be directly compared to a given standard, such as sequences resulting from the Human Genome Project. Analysis of a given piece of nucleic acid can be carried out by hybridization, at high stringency, to an ordered grid of synthetic oligonucleotides of known sequence. By detection of the binding positions, its sequence can in principle be elucidated. Comprehensive comparison irrespective of a fragment's origin requires the use of a set of sensor oligonucleotides that cover all possible sequence combinations. In order to obtain specific binding to all oligonucleotides under one set of conditions, the duplex stability of the oligonucleotides, which varies significantly with their base composition (5,6), has to be made uniform. The use of tetramethylammonium chloride reduces the difference between G:C and A:T base pairs (7), but the effect is insufficient for short oligonucleotides (6). The proposed variation of the concentration of each particular oligonucleotide on the grid (8) would be technically complex and is incompatible with the existing techniques of grid production (2,3). As a third option, the use of base modifications has been examined extensively (9–11). However, the large differences in the effect they have on oligonucleotide duplex stability, the inherent base dependence of any such effect and also the mere number of substances involved make them unsuitable for the intended use of equalizing oligonucleotide binding stabilities.

As an alternative, an intramolecular mixture of ribo- and deoxyribonucleotides might be expected to alter the conformation of a duplex formed with a DNA molecule and hence its stability, due to variations in the sugar puckering, leaving the base pairing and stacking largely unaffected. Recent theoretical and experimental studies on DNA/RNA hybrid conformations (12,13) suggest such structural characteristics and indicate that the width of the minor groove particularly is affected as compared with pure A- or B-DNA duplexes. In this manuscript results are presented that confirm the applicability of this procedure to the regulation of oligonucleotide duplex stability.

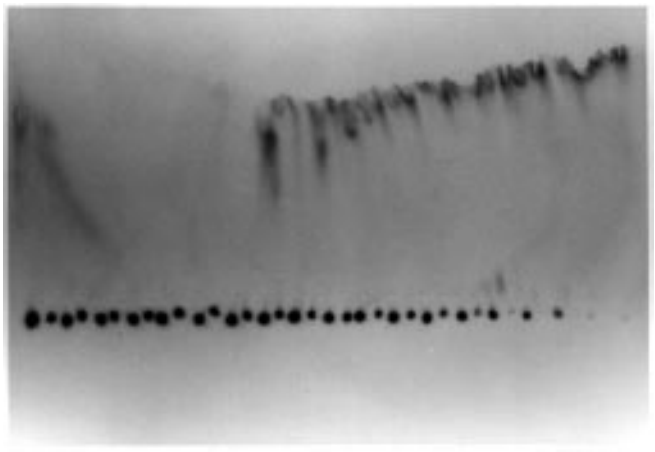

Figure 1. Simultaneous quantitative measurement by TLC of the binding stabilities of oligonucleotides XS-67 (upper row of dots) and XS-100 (lower row). TLC was in 8× SSC buffer perpendicular to a temperature gradient of 3 (left) to 41°C (right).

MATERIAL AND METHODS

Measurement of dissociation temperatures

The mixed ribo/deoxyribo oligonucleotides were purchased from a commercial source (MWG-Biotech). 2′-*O*-Fpmp protection groups were removed by incubation in acidic solution as recommended by the manufacturer. Oligonucleotides were radioactively labelled at the 5′-end and purified by acrylamide gel electrophoresis. The dissociation temperatures were determined as described in detail earlier (9). Single-stranded target DNA was prepared by either *Hin*dIII or *Eco*RI cleavage of plasmid pTZ18R (14), followed by an exonuclease III digestion. Oligonucleotide probe (3.0 fmol) was annealed to plasmid DNA (0.95 pmol) and spotted on TLC plates (Polygram CEL300, DEAE/HR-1/15). The plates were developed in 8× SSC (1.2 M NaCl, 0.12 M sodium citrate, pH 7.5) perpendicular to a linear temperature gradient. Bound oligonucleotide was retained at the origin by the plasmid DNA, while free oligonucleotide moved with the buffer. Very many pairwise combinations of oligonucleotides were examined simul-

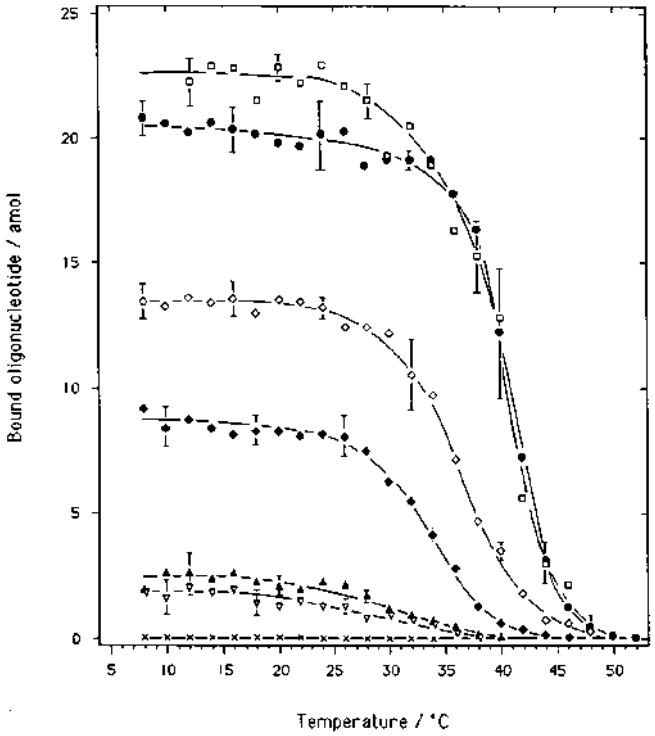

Figure 2. Dissociation curves of the SX series oligonucleotides. Each spot represents the average of 10–18 individual measurements. Hybridizations were with oligonucleotides SX-0 (●), SX-17 (♦), SX-42 (▲), SX-58 (▽), SX-83 (◇) and SX-100 (□), as well as SX-0 to its own sequence as a target (×). For each fourth point the standard deviation is indicated.

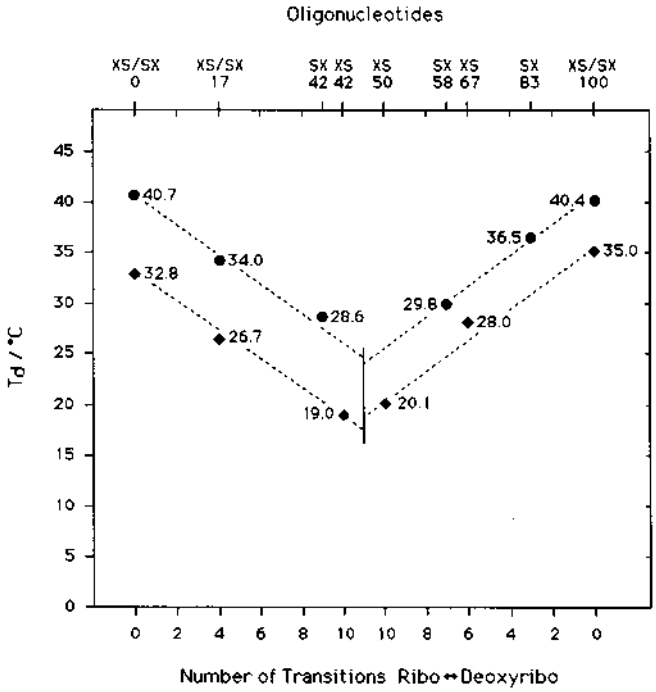

Figure 3. Variation of the dissociation temperature of the SX (●) and XS oligonucleotides (♦) is dependent on the number of serial alternations from deoxyribo- to ribonucleotides and vice versa.

taneously on a single TLC plate to control for variations between individual experiments. After autoradiography of the plates, in the presence of spotted probe dilutions of known concentration as standards, the autoradiograms were scanned and the amount of oligonucleotide at the origin was determined using the NIH Image software package (version 1.56).

RESULTS

To test for the effect on duplex stability of the introduction into deoxyribo oligonucleotides of an increasing number of ribonucleotide residues dodecamer sequences which had been used in earlier experiments (10) were chosen in order to allow direct comparisons (Table 1). Quantitative measurement was by TLC (e.g. Fig. 1), which accurately represents the conditions of standard format hybridization experiments (9). There was no non-specific binding of the oligonucleotides to DNA or the matrix of the TLC plate (Fig. 2). As observed earlier (10), the two purely DNA-containing dodecamers XS-0 and SX-0 showed a large difference in their dissociation behaviour (Fig. 3), although from nearest neighbour analysis data (5), which are widely used for predicting duplex stabilities, they should be of identical stability. With each substitution that caused an additional serial RNA to DNA or DNA to RNA transition within a molecule, indicated in the sequences listed in Table 1 by a change of case nN or Nn, the dissociation temperature (T_d) decreased by 1.40°C, with no apparent effect from the base of the substituted nucleotide or the position of the substitution within the oligonucleotide (Figs 2 and 3). By extrapolation, the minimal T_d occurs at the maximum possible number of 11 changes. Beyond this point, further insertions, and hence a reduction in the number of intramolecular transitions, increased the T_d linearly by 1.55°C each. As observed with oligonucleotides containing base modifications, concomitant with a change in T_d there was a shift in the total amount of oligonucleotide bound at low temperature (Fig. 2).

Table 1. Oligonucleotide sequences

Oligonucleotide	Sequence[a]	Oligonucleotide	Sequence[a]
XS-0	TCTAGAGTCGAC	SX-0	GTCGACTCTAGA
XS-17	TCTaGAGTcGAC	SX-17	GTcGACTCTaGA
XS-42	TcTaGAgTcGaC	SX-42	gTcGaCTcTaGA
XS-50	TcTagAgTcGaC	SX-58	gTcgacTcTagA
XS-67	TcTagagTcgaC	SX-83	gTcgactctagA
XS-100	tctagagtcgac	SX-100	gtcgactctaga

[a]Capital letters designate deoxyribonucleotides, small letters ribonucleotides.

The mixed oligonucleotides were found to be substrates for both bovine pancreas RNase A and, as a duplex with DNA, *Escherichia coli* RNase H when ribonucleotides were located at the appropriate positions (not shown). However, although no special precautions were taken during the annealing process with plasmid DNA (generally isolated by alkaline lysis but also by polymerase chain reaction) and the subsequent measurement by TLC, no degradation of the oligonucleotide probe could be observed by acrylamide gel electrophoresis under such conditions, even over extended periods (data not presented). From these results, RNase degradation

is not expected to be a problem for practical application of the mixed oligonucleotides.

DISCUSSION

After the establishment of techniques to synthesize large arrays of oligonucleotides (2,3), the ability to vary the duplex stabilities of oligonucleotides with no major effect on their sequence specificity overcomes the second principal obstacle which prevents a practical application of comprehensive oligonucleotide arrays for analyses. The extent of the observed variation in duplex stabilities is such that a complete set of oligonucleotides could be synthesized that bind at a defined (low) level of stability. The third major problem of oligonucleotide hybridization technology, the short read length caused by the repeated occurrence of identical oligonucleotide sequences within a DNA fragment, exists only for *de novo* sequence determination. It disappears when at least part of the sequence of the analysed fragment is known (15). For this reason, and also because of the proven efficiency of the established gel-based methods, the future of the sequencing by hybridization technique will most likely not be in sequencing large DNA fragments of unknown sequence, but in application of the arrays as a diagnostic tool comparing individual sequences to a reference sequence, which for man, for instance, will soon be available as a result of the Human Genome Project.

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