Accelerated Displacement of Duplex DNA Strands by a Synthetic Circular Oligodeoxynucleotide

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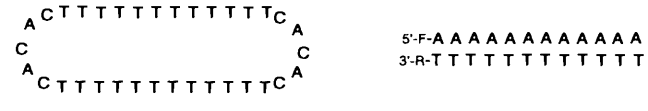
A small circular synthetic DNA oligomer 34 bases in size binds to duplex DNA and actively displaces one of the strands at a rate significantly faster than unassisted dissociation of the duplex.

The sequence-directed targeting of RNA and DNA single strands by oligonucleotides is an increasingly studied field. The presence of secondary structure, such as the short Watson–Crick domains found in folded RNA sequences, can prevent successful hybridization of a probe where bonds are already formed.[1] Spontaneous duplex dissociation is slow, however, and so in natural processes such as transcription, translation and recombination, it is necessary that the disruption of this structure be aided by proteins.[2] We report that a synthetic DNA oligomer can partially mimic this function, significantly increasing the rate at which short DNA duplexes dissociate.

The circular DNA oligomer used in this study has been previously shown to form a strong bimolecular triple helical complex with DNA or RNA oligomers containing the sequence $(A)_{12}$.[3–5] The target sequence is bound between opposite $(T)_{12}$ domains of the circle, and this complex is 9.0 kcal mol^{-1} (1 cal = 4.184 J) more stable than the duplex $d(A)_{12}\cdot d(T)_{12}$ at pH 7.0, 37 °C. Thus, the addition of the complementary circular oligomer to this duplex thermodynamically favours the disruption of duplex structure and the formation of the stable circle complex. DNA binding by displacement by portions of one strand has been observed for other high-affinity DNA-binding groups.[6] However, since DNA is slow to dissociate, this process must occur on a reasonable timescale at physiological temperatures to be useful. Consequently, it is important to determine the rate of this strand displacement in order to determine whether it is feasible to target a strand of DNA or RNA when it is bound in a duplex.



To study the kinetics, we prepared a short DNA duplex with two fluorescent groups: the 5′ end of a $d(A)_{12}$ DNA oligomer was labelled with fluorescein (5′-F) and the 3′ end of its complement, $d(T)_{12}$, was labelled with tetramethylrhodamine (3′R) using published procedures.[7,8]

The duplex formed between the two labelled oligomers shows normal biphasic melting behaviour and displays an affinity similar to that of the unlabelled analogous duplex.† Addition of labelled $d(T)_{12}$ to labelled $d(A)_{12}$ results in decreased fluorescein fluorescence (λ_{max} = 523 nm) and increased tetramethylrhodamine fluorescence (λ_{max} = 590 nm), presumably by energy transfer.[7] By mixing the two strands under pseudo-first-order conditions, and monitoring the rate of the decrease in the fluorescein emission, we obtain an association rate constant of 3.2×10^6 dm^3 mol^{-1} at 10 °C, which agrees well with published rates for association of short DNA oligomers as measured by laser temperature-jump methods.[9,10]

† At a duplex concentration of 0.3 μmol dm^{-3}, with 100 mmol dm^{-3} NaCl, 10 mmol dm^{-3} $MgCl_2$ at pH 7.0, the labelled duplex melts at 39 °C and the unlabelled duplex, 37 °C. A plot of $\ln(C_T/4)$ *vs.* $1/T_M$ for the complex yields $\Delta G°_{10}$ = −19.9 kcal mol^{-1}, $\Delta H°$ = −115 kcal mol^{-1} and $\Delta S°$ = −336 cal mol^{-1} K^{-1} for association (1 cal = 4.184 J).

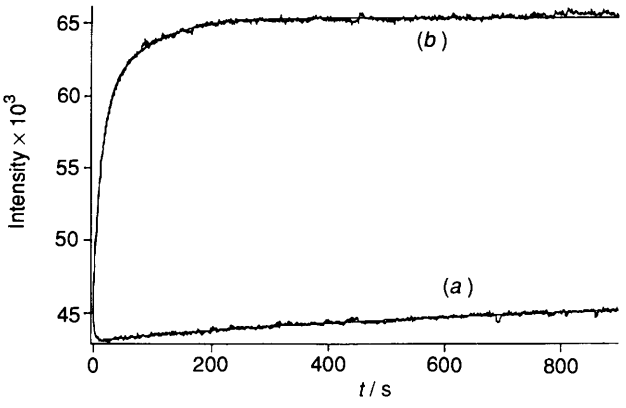


Fig. 1 Fluorescein fluorescence intensity as a function of time following the addition of either (*a*) an unlabelled complementry linear strand of sequence $d(A)_{12}$ or (*b*) a complementary circular oligonucleotide to the fluorescent-labelled DNA duplex, $d(A)_{12}{\cdot}d(T)_{12}$, at 10 °C. Solid lines indicate least-squares fit to the data.

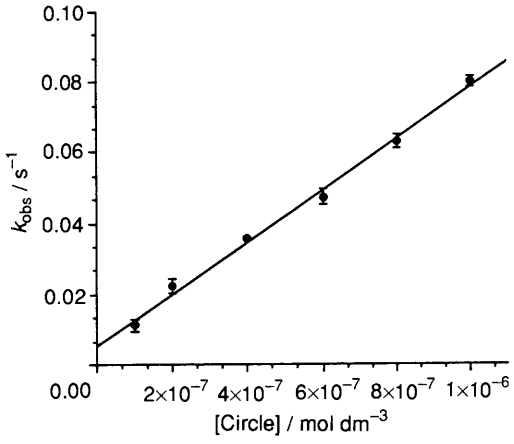
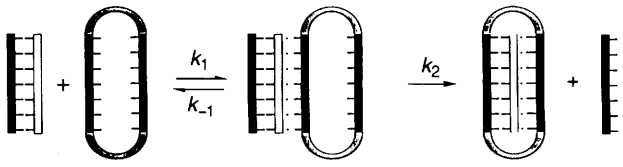


Fig. 2 Plot of observed pseudo-first-order rate constant, k_{obs}, for the reaction as a function of circle concentration

As a measure of the rate at which an added third strand can exchange with strands in a duplex, we added a large excess of an unlabelled single strand [$d(A)_{12}$ or $d(T)_{12}$], and followed the rate of increase in the fluorescein emission as the duplex strands separate. Fig. 1(*a*) shows a typical kinetic run for the reaction of the preformed labelled 1:1 duplex (0.01 μmol dm^{-3}) with unlabelled $d(A)_{12}$ strand (1.0 μmol dm^{-3}) at 10 °C. The observed first-order rate constant is similar for the addition of either unlabelled $d(A)_{12}$ or $d(T)_{12}$ single strands and is independent of unlabelled strand concentration (0.1–0.4 μmol dm^{-3}). Under the reaction conditions, the exchange is a slow process, with a half-life of 58 min at 10 °C, which is 30 °C below the melting temperature for the duplex. This reflects the slow rates at which even short duplexes dissociate.[10,11]

In contrast to the above behaviour, when the complementary circle is instead added to the duplex, the rate of increase in the fluorescein emission is considerably faster [Fig. 1(*b*)]. The experimental first-order-rate constant for the reaction of duplex with added circle (100-fold excess) at 10 °C is $2.3 \times 10^{-2}\ s^{-1}$, a half-life of only 30 s. The rate of this reaction is dependent on the concentration of added circle (Fig. 2); a plot of [circle] *vs.* k_{obs} is linear with a slope of $7.3 \times 10^4\ dm^3\ mol^{-1}\ s^{-1}$.

Based on this kinetic behaviour, we propose the following mechanism for the observed acceleration of duplex DNA strand dissociation (Scheme 1). The circle preassociates with



Scheme 1

the duplex by forming Hoogsteen-type hydrogen bonds between one $d(T)_{12}$ domain in the circle and the adenines in the major groove of the duplex. This circle·duplex preassociation leaves the second $d(T)_{12}$ domain in the circle unbound and in high local concentration to trap the $d(A)_{12}$ strand when the duplex partially opens, either through a fraying or slipping mechanism. The importance of this secondary binding domain for the acceleration of duplex dissociation is demonstrated by the observation that a single $d(T)_{12}$ strand, which can also form a triple helix, undergoes only slow exchange. In support of a preassociation mechanism, the prior addition of a $d(T)_{12}$ strand completely inhibits subsequent duplex dissociation by the circle.

The second-order rate constant, $7.3 \times 10^4\ dm^3\ mol^{-1}\ s^{-1}$, for duplex dissociation by circle is similar to literature values for triple-helix formation.[12] This suggests that the rate-limiting step in the proposed mechanism is initial complexation, which is also supported by the fact that we have not observed saturation kinetics. The rate constant for dissociation, k_2, is greater than $0.08\ s^{-1}$, the largest observed rate constant.

Our studies demonstrate that it is possible to design a synthetic DNA molecule which can bind to duplex DNA by active displacement of the secondary structure. It has three important features: the ability to preassociate, which results in a high local concentration; a second binding domain, which allows it to replace one strand of the duplex; and a high affinity, which drives the reaction to completion. Further experiments will be necessary to determine the generality and the mechanism of this acceleration. Although the substrate duplex in this reaction is a simple model for a portion of a larger duplex DNA or for a folded RNA segment, the results indicate that it may be possible on a practical level to target single strands of nucleic acids even when they are folded within secondary structure.

We thank the National Institutes of Health (GM46625), the National Science Foundation (CHE-9007673 and CHE-9057092) and the Office of Naval Research (N00014-92-J-1740) for support.

Received, 10th September 1992; Com. 2/04872H

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