Sequence-Targeted Cleavage of Nucleic Acids by Oligo-α-thymidylate–Phenanthroline Conjugates: Parallel and Antiparallel Double Helices Are Formed with DNA and RNA, Respectively†

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Received December 8, 1987; Revised Manuscript Received February 29, 1988

ABSTRACT: Oligodeoxynucleotides can be synthesized by using the α anomers of nucleoside units. Oligo-α-deoxynucleotides are resistant to nucleases and could be used to regulate gene expression in vivo. Theoretical calculations were carried out to determine the conformational energy of an oligomeric α–β duplex $(dA)_5$·$(dT)_5$ where the adenosine strand contains natural β-deoxyribonucleotides and the thymidine strand contains synthetic α-deoxyribonucleotides. These calculations predict that in the more stable B-like conformation the two strands of the double helix should run parallel to each other whereas in the more stable A-like conformation the two strands should adopt an antiparallel orientation. In order to test these predictions 1,10-phenanthroline was covalently attached to the 5′-end of an α-octathymidylate. In the presence of copper ions and a reducing agent (β-mercaptopropionic acid), the $(phenanthroline)_2$–copper complex generates OH• radicals that cleave phosphodiester bonds in the complementary sequence to which the α-octathymidylate is bound. By use of a 27mer oligo-β-deoxynucleotide containing an octadeoxyadenylate sequence as a target for the phenanthroline-substituted α-$(dT)_8$, cleavage was observed on the 5′-side of the $(dA)_8$ sequence, demonstrating that the α–β DNA–DNA hybrid formed a double helix with *parallel* orientation of the two strands. The same result was obtained when α-$(dT)_8$ was bound to β-$(dA)_n$ with $n = 8$ or 10. When a β-oligoriboadenylate was used as a target, cleavage occurred exclusively on the 3′-side of the $(rA)_8$ or $(rA)_{10}$ sequence, indicating that the α–β DNA–RNA hybrid formed a double helix with an *antiparallel* orientation of the two strands. When a phenanthroline-substituted β-octathymidylate was used instead of the α-octathymidylate, an antiparallel double helix was formed independently of whether the target β sequence was a DNA or an RNA.

During recent years it has been demonstrated that oligonucleotides could be used to selectively block gene expression. In hybrid arrest of translation, a cDNA was used to block the translation of a messenger RNA via cDNA–mRNA hybridization (Paterson et al., 1977; Hastie & Held, 1978). Oligodeoxynucleotides targeted to viral RNAs were shown to inhibit viral development (Zamecnik & Stephenson, 1978; Agris et al., 1986; Zamecnik et al., 1986; Smith et al., 1986; Lemaître et al., 1987; Zérial et al., 1987). Splicing of pre-mRNA (Zamecnik et al., 1986; Smith et al., 1986) could be inhibited as well as translation of mature mRNA (Blake et al., 1985a; Kawazaki, 1985; Cazenave et al., 1986, 1987; Shakin & Liebhaber, 1986; Minshull & Hunt, 1986; Haeuptle et al., 1986; Toulmé et al., 1986; Cornelissen et al., 1986; Walder et al., 1986). Recent studies have shown that living organisms make use of short RNA transcripts to regulate gene expression [see Green et al. (1986) for a review]. This has been the basis for the development of a new strategy for artificial gene regulation using antisense RNAs [see Weintraub et al. (1985) and Green et al. (1986) for reviews]. Antisense RNAs can block translation via hybridization to the complementary mRNAs or prevent transfer of mRNAs from the nucleus to the cytoplasm (Kim & Wold, 1985).

Oligodeoxynucleotides are sensitive to nucleases. They are rapidly hydrolyzed in culture media containing calf serum and inside living cells, e.g., after microinjection into oocytes (Cazenave et al., 1987b). Also, oligodeoxynucleotides do not efficiently cross the membranes of living cells. Both the nuclease sensitivity and the poor penetration limit oligonucleotide use for in vivo studies. Attaching an acridine dye to either (or both) end(s) of an oligodeoxynucleotide increases their penetration into living cells and makes them resistant to exonucleases (those starting from the substituted end) (Asseline et al., 1983, 1984a,b; Cazenave et al., 1987b; Verspieren et al., 1987). The aromatic acridine ring provides an additional binding energy, which stabilizes the complexes formed with a complementary sequence (Asseline et al., 1983, 1984a,b). Nuclear magnetic resonance studies demonstrated that the acridine ring could intercalate between the last two base pairs of the duplex structure (Lancelot et al., 1985; Lancelot & Thuong, 1986). Absorption and fluorescence studies provided evidence for an end-stacked structure in equilibrium with the intercalated structure (Hélène et al., 1985; Asseline et al., 1984a,b). Acridine-substituted oligodeoxynucleotides can block mRNA translation in in vitro systems (Hélène et al., 1985; Toulmé et al., 1986; Cazenave et al., 1987a) or in microinjected oocytes (Cazenave et al., 1987a). They are also able to inhibit viral development (Hélène et al., 1985; Zérial et al., 1987) and to kill trypanosomes in culture (Verspieren et al., 1987). It was recently shown that attachment of an

† This work was supported in part by Rhône-Poulenc-Santé, the Ligue Nationale Française contre le Cancer, and the Fondation pour la Recherche Médicale.

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0006-2960/88/0427-6039$01.50/0

oligodeoxynucleotide to a polylysine carrier increased the penetration into cells in culture so that biological effects could be detected at low oligonucleotide concentration (less than 1 μM) (Lemaître et al., 1987). In order to overcome the difficulties associated with the nuclease sensitivity of oligonucleotides several strategies have been developed. The phosphodiester linkages of the backbone can be replaced by phosphonates (Blake et al., 1985b; Agris et al., 1986; Smith et al., 1986). Each phosphonate exists under two diastereoisomeric configurations. Therefore, an oligophosphonate with n linkages is a mixture of 2^n compounds that exhibit different affinities toward their complementary sequence. Other modifications of the nucleotide units can be contemplated. For example, it has been shown that replacing β-deoxynucleosides by their α anomers makes oligodeoxynucleotides more resistant to nucleases (Holy, 1973; Sèquin, 1974; Morvan et al., 1987a; Thuong et al., 1987). Oligo-α-deoxynucleotides form stable hybrids with complementary β sequences (Thuong et al., 1987; Morvan et al., 1987; Sun et al., 1987). Model binding studies suggested that α–β hybrids should have their two strands in a parallel 5′→3′ orientation (Sèquin, 1973). Using oligo-α-nucleotides covalently linked to photo-cross-linking reagents, we recently showed that an oligo-α-deoxythymidylate did form a double helix with parallel strands with a complementary oligo-β-deoxyadenylate (Praseuth et al., 1987; Le Doan et al., 1987). Here we present the results of theoretical calculations which predict that the more stable double helical structures of α–β hybrids should have a parallel orientation of the two strands when a B-type conformation is adopted but, in contrast, an antiparallel orientation when an A-type conformation is favored. We have used an oligo-α-thymidylate covalently linked to 1,10-phenanthroline to test these predictions. An oligo-β-adenylate sequence was chosen as a target either in a DNA or in an RNA fragment with the expectation that α–β hybrids will adopt a B-like conformation when the β sequence is a DNA and an A-like conformation when the β sequence is an RNA.

The 2:1 phenanthroline–copper complex was previously shown to produce OH• radicals in the presence of a reducing agent (Sigman, 1986). Covalent attachment of phenanthroline to an oligonucleotide brings the OH•-radical-producing complex in close proximity to the deoxyriboses or riboses of the oligonucleotide complementary sequence (Chen & Sigman, 1986; François et al., 1988). The OH• radicals attack the sugar moieties and induce strand-cleavage reactions in the target sequence at well-defined sites. The location of the cleavage sites allows us to deduce the relative orientation of the phenanthroline-containing oligonucleotide with respect to its target sequence. Here we show that α-T_8–β-A_8 hybrids have parallel orientation of the two strands when the β sequence is a DNA and an antiparallel orientation when the β sequence is an RNA. An oligo-α-thymidylate was chosen to carry out these investigations because its inherent symmetric sequence allows it to bind in either orientation with respect to its complementary oligo-β-adenylate sequence.

Materials and Methods

Oligonucleotides. The oligo-β-deoxynucleotide 27 nucleotides in length whose sequence is shown in Figure 4 was synthesized on a Pharmacia automatic synthesizer by phosphoramidite chemistry. It was purified by HPLC on Pharmacia HR 5/5 columns and then by gel electrophoresis on denaturing 20% polyacrylamide gels. Oligoriboadenylates $(rA)_8$ and $(rA)_{10}$ were purchased from PL-Biochemicals and used without further purification. α-Thymidine was purchased from Sigma, and its 5′-dimethoxytrityl and 3′-p-chlorophenyl derivatives were synthesized as previously described for β-nucleosides (Thuong et al., 1981). The phosphotriester method in solution was used to synthesize both β- and α-octathymidylates. A thiophosphate group was attached to the 3′-end of β-T_8 and to the 5′-end of α-T_8 as previously described (Praseuth et al., 1987; Thuong & Chassignol, 1987). The terminal thiophosphate was then reacted with 5-(iodoacetamido)-1,10-phenanthroline, a gift from Dr. David Sigman, UCLA. The phenanthroline-substituted octathymidylates were purified by reverse-phase chromatography on Pharmacia HR 5/5 columns. The resulting compounds will be referred to as (OP)-α-T_8 and β-T_8-(OP).

A β-octathymidylate was synthesized with 2-methoxy-6-chloro-9-aminoacridine attached to the 3′-phosphate via a pentamethylene linker as previously described (Asseline et al., 1986). Phenanthroline was covalently linked to its 5′-end as described above. This compound will be referred to as (OP)-β-T_8-Acr.

Lucifer yellow (Aldrich) was covalently linked to the (terminal) 3′-ribose of oligo$(rA)_{10}$ after opening of this ribose by treatment with sodium periodate followed by reduction with sodium borohydride. This compound was a gift from Dr. J. F. Hau (C.B.M., Orléans).

Cleavage Reactions. The 27mer, $(rA)_8$, and $(rA)_{10}$ were 5′-end-labeled by using polynucleotide kinase and [γ-^{32}P]ATP (Amersham). The labeled oligonucleotide was mixed with the (OP)-substituted oligothymidylate, copper sulfate added, and the cleavage reaction started by adding β-mercaptopropionic acid (2 mM). In most cases, free 1,10-phenanthroline was added to the incubation mixtures before adding β-mercaptopropionic acid. Concentrations will be indicated in the text or in the figure legends. The mixtures were incubated for 3 h at 4 °C. The reaction was stopped by adding an excess of bathocuproine (400 μM). Salt was removed by ethanol precipitation. The samples were electrophoresed on denaturing 20% polyacrylamide gels, which were then autoradiographed with Fuji films at −70 °C using a screen amplifier.

Theoretical Calculations. Theoretical calculations of the conformational energy of DNA oligomeric duplexes were carried out with the recently developed JUMNA (junction minimization of nucleic acids) algorithm (Lavery, 1987). This technique allows rapid energy optimization of nucleic acid segments to be performed by using as variables the helical parameters describing the position in space of each nucleotide in addition to the glycosidic angle, the torsion and valence angles describing the sugar puckering, and the phosphodiester torsions of the backbone. Closure of the DNA backbone between neighboring nucleotides is achieved by using a constraint approach (Lavery et al., 1986a; Lavery, 1987). A careful definition of the helical parameters yields a commuting set of variables and consequently allows analytic first derivatives of the energy to be employed in a conjugate gradient minimization algorithm.

The conformational energies (E) of the DNA segments studied are calculated by using the pairwise additive formula shown (Lavery, 1987; Lavery et al., 1986a,b):

$$E = \sum Q_i Q_j / \epsilon(R) R_{ij} + \sum(-A_{ij}/R_{ij}^6 + B_{ij}/R_{ij}^{12}) + \sum[(\cos\theta)(-A_{ij}^{HB}/R_{ij}^6 + B_{ij}^{HB}/R_{ij}^{12}) + (1 - \cos\theta)(-A_{ij}/R_{ij}^6 + B_{ij}/R_{ij}^{12})] + \sum Vs/2(1 \pm \cos N_{sts}) + \sum va(\sigma a - \sigma a^\circ)^2$$

The first term of this formula is the electrostatic energy, calculated as the sum of interactions between atomic monopoles Q_i (obtained from reparameterized Huckel–Del Re calculations) (Lavery et al., 1984) and damped by a dielectric

Table I: B-like Energy-Optimized Conformation of $(dA)_5 \cdot (dT)_5$

duplex[a]	Xsh[b] (Å)	Zsh (Å)	Tlt[c] (deg)	Pro (deg)	Wdg (deg)	Gly[d] (deg)	energy (kcal/mol)
β–β (AP)	1.3	3.1	2.7	19.7	38.2	79.5/66.3	−12.9
β–α (AP)	2.0	3.0	6.6	28.1	37.9	68.9/131.9	39.7
β–α (P)	1.6	3.1	8.5	21.3	37.8	79.5/−45.4	9.8

[a] AP, antiparallel strands; P, parallel strands. [b] The helical parameters shown correspond to our previously defined set. Xsh, displacement of base pairs toward the minor groove; Zsh, separation of base pairs along the helical axis; Tlt, rotation of the base pairs around the local dyad axis; Pro, contrarotation of the base pairs around their long axis; Wdg, rotation of successive base pairs around the helical axis. Tlt and Pro have the conventionally accepted signs. [c] Tilt values given in Tables I and II are averaged values for the two strands. In fact, AT pairs are found to exhibit considerable heteronomy leading to a "buckling" of the base pairs, which can be seen in the illustration of the β–α duplexes. [d] Glycosidic angles for the adenosine and thymidine strands, respectively.

Table II: A-like Energy-Optimized Conformations of $(dA)_5 \cdot (dT)_5$[a]

duplex	Xsh (Å)	Zsh (Å)	Tlt (deg)	Pro (deg)	Wdg (deg)	Gly (deg)	energy (kcal/mol)
β–β (AP)	3.1	3.0	14.6	16.2	33.8	37.1/37.1	−9.7
β–α (AP)	9.6	2.1	23.9	11.1	24.0	24.4/133.4	29.4
β–α (P)	−0.4	3.5	−1.0	44.7	39.4	65.4/−40.6	96.0

[a] See Table I for the definitions of the parameters.

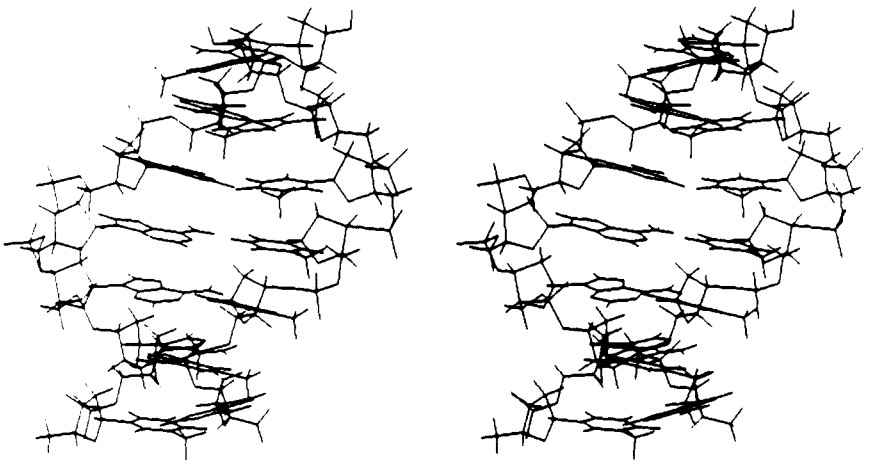


FIGURE 1: Stereoviews of the energy-minimized double helix with *parallel* strands formed by β-$(dA)_5$ with α-$(dT)_5$ in a B-like conformation.

function $\epsilon(R)$ similar to that proposed by Hingerty et al. (1985). The next three terms represent the dispersion–repulsion energy calculated with a 6–12 dependence and using in part the parameter set developed by the group of Poltiev (Zhurkin et al., 1980). Hydrogen bonds are dealt by the latter two of these terms, which account for angular dependence by mixing together two sets of *A,B* parameters using a cosine function of the angle formed by the vectors X–H and H–Y for a bond X–H···Y. All of these terms are summed only over pairs of atoms separated by at least three chemical bonds in order to avoid calculating contributions that cannot change within our model. It should consequently be remarked that the absolute values of the energies presented do not correspond to chemical formation energies and only differences in energy between different conformations have a sense. The final two terms take into account the distortion energy associated with torsion angles τs (including anomeric effects) and valence angles σa.

RESULTS

(*1*) *Conformational Energies of α–β Duplexes in the B-like and A-like Conformations.* Calculations have been performed for oligomeric DNA segments $(dA)_5 \cdot (dT)_5$, where the adenosine strand contains a normal β-deoxyribose and the thymidine strand contains the modified α-deoxyribose. Energy-optimized conformations have been obtained for both B-like and A-like allomorphs maintaining mononucleotides repeat symmetry in the helical parameters but allowing for heteronomy between the two strands of the duplexes (see Materials and Methods).

The conformational parameters and energies obtained for the B-like β–α duplexes are given in Table I. Of the tow possibilities investigated, it is found that the duplex with parallel strands is by far the more stable. This result is principally associated with a destabilization of the antiparallel duplex due to phosphate–phosphate repulsion between the two backbones of this duplex, which are abnormally close to one another. The parallel duplex has helical parameters close to those of a normal β–β B-DNA (also shown in Table I for comparison) with the exception of a larger propeller twist (Figure 1). Both the α-thymidine and the β-adenosine residues are in the anti conformation. The sugar conformations found for this duplex are C2′-endo and C4′-endo for the A and T strands, respectively. In contrast, the antiparallel duplex has sugars in C2′-endo and C1′-endo conformations for the A and T strands, respectively.

The results for the A-like conformations are given in Table II. Note that these conformations were obtained with a reduced charge on the phosphate groups (−0.5e), which has been found to be more appropriate for modeling A conformations in β–β duplexes. Consequently, the energies of Table II should not be directly compared with those in Table I. For

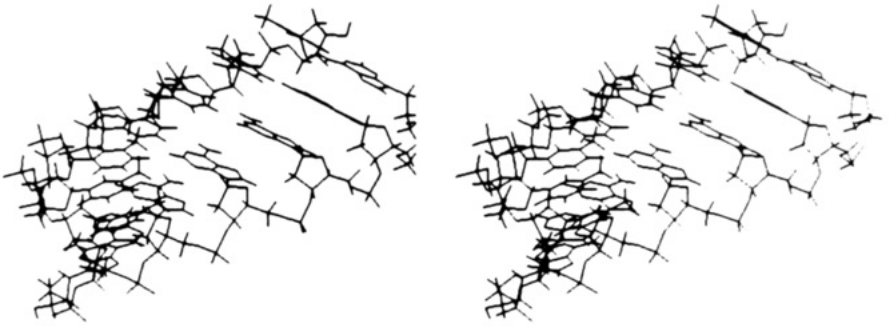


FIGURE 2: Stereoviews of the energy-minimized double helix with *antiparallel* strands formed by β-$(dA)_5$ with α-$(dT)_5$ in an A-like conformation.

these conformations it is the antiparallel duplex that is found to be more stable. However, as can be seen from the helical parameters in Table II and the illustration in Figure 2, there are considerable differences from the standard A-DNA structure. The most notable change is a large increase of the displacement of the base pairs toward the minor groove (Xsh). This change is associated with a smaller displacement between successive base pairs, a decreased twist angle, and a positive roll of the base pairs (13°). In addition, the thymidine nucleosides are found to be in the syn conformation. Overall, this leads to a "ribbonlike" conformation of the double helix. The parallel duplex in the A-like conformation (which in contrast to the antiparallel duplex has a very small base pair displacement and a very large propeller twist) is much less stable due to the proximity of its two phosphodiester backbones. In both β–α A-like duplexes the adenosine strand is found to have C3′-endo sugars while the thymidine sugars are in the C1′-endo conformation.

The calculations described above include the dielectric damping effects of aqueous solution, but not the specific hydrogen-bonding effects of first-shell waters. For the A form of DNA, counterions were modeled by the reduction of phosphate net charges to –0.5. This level of modeling has been found to be sufficient for producing good structures of the various allomorphs of DNA and for studying sequence-dependent effects (Lavery, 1987). Between very different allomorphic conformations there is certainly a hydration term to be taken into account, but it is undoubtedly small compared to the important energy differences established in this study and leading to, among other things, the prediction of antiparallel strands in the A conformation of the β–α duplex.

For both allomorphs studied it can be noted that the mixed β–α duplexes are found to be less stable than the corresponding β–β duplexes (by roughly 20 kcal/mol for the "B" conformation and by roughly 40 kcal/mol for the "A" conformation). It should also be noted, however, that other β–α duplexes with guanine, adenine, or cytosine in the α strand show similar stabilities to the A–T conformations presented. These studies will be the subject of a future publication.

(2) Cleavage of Single-Stranded DNA by Oligo-α- and Oligo-β-thymidylates Covalently Linked to 1,10-Phenanthroline. In order to correlate the energy minimization calculations with experimental results, 1,10-phenanthroline [abbreviated as (OP)] was attached to the 5′-end of an oligo-α-thymidylate [(OP)-α-T_8] and, for comparison purposes, to the 3′-end of an oligo-β-thymidylate [β-T_8-(OP)] or to the 5′-end of an oligo-β-thymidylate bearing an acridine derivative (Acr) covalently attached to the 3′-phosphate via a pentamethylene linker [(OP)-β-T_8-Acr]. An oligodeoxyadenylate

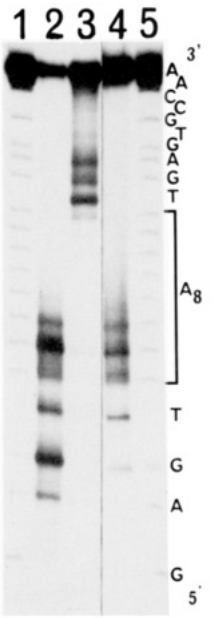


FIGURE 3: Cleavage of the 27mer DNA fragment by phenanthroline-substituted oligothymidylates. Part of the sequence is shown on the right of the figure with the $(dA)_8$ sequence indicated by a bracket. Incubation was carried out for 3 h at 4 °C in a pH 7.5 buffer containing 10 mM sodium phosphate and 1 M NaCl in the presence of 1 μM $CuSO_4$, 1 μM 1,10-phenanthroline, and 2 mM β-mercaptopropionic acid. The concentration of the 5′-end-labeled 27mer DNA fragment was 10 nM. Concentrations of oligothymidylates were 5 μM for β-T_8-(OP) (lane 2), 5 μM for (OP)-β-T_8-Acr (lane 3), and 20 μM for (OP)-α-T_8 (lane 4). Lanes 1 and 5 are the (G + A) sequence of the 27mer fragment.

sequence included in a 27mer single-stranded DNA fragment was used as a target for the octathymidylates. Previous works have shown that, in the presence of copper ions and a reducing agent, phenanthroline–copper complexes generate OH• radicals that attack the sugar residues located in the immediate vicinity of phenanthroline (Sigman, 1986). This reaction leads to a cleavage of the phosphodiester backbone. In Figure 3 it can be seen that the 27mer DNA is cleaved at well-defined locations when it is incubated in the presence of (OP)-α-T_8, β-T_8-(OP), and (OP)-β-T_8-Acr and the reaction started by adding mercaptopropionic acid as a reducing agent . The location of the cleavage sites with respect to the $(dA)_8$ sequence allowed us to determine the relative orientation of the oligothymidylate with respect to the target β sequence (Figure 4). As expected, the β–β DNA hybrids involved two antiparallel strands, as observed in natural DNA. In contrast, the α–β DNA hybrid was characterized by a parallel orientation of the two strands. A similar result was previously obtained with

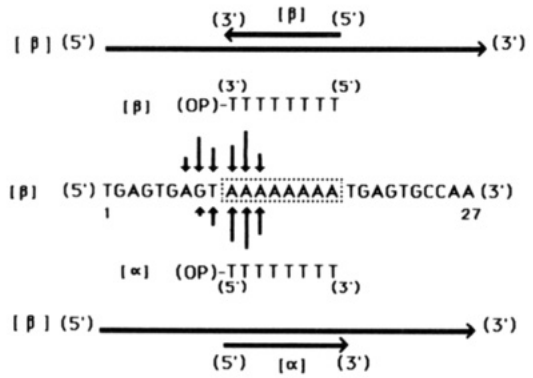


FIGURE 4: Sequence of the 27mer DNA fragment. The arrows indicate the sites of cleavage by β-T_8-(OP) above the sequence and (OP)-α-T_8 below the sequence. The relative orientation of the chains is shown above for β-T_8 and below for α-T_8.

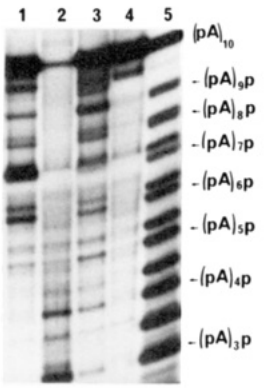
photo-cross-linking reagents (*p*-azidophenacyl or azidoproflavine derivatives) to probe the orientation of the oligothymidylate with respect to its target sequence (Praseuth et al., 1987; Le Doan et al., 1977).

In order to determine whether the flanking sequences of the $(dA)_8$ segment were playing any role in the cleavage reaction, the experiments described above were repeated with two oligo-β-deoxyadenylates oligo$(dA)_8$ and oligo$(dA)_{10}$ as targets. In both cases, the results were identical with those obtained with the $(dA)_8$ segment included in the 27mer DNA fragment (results not shown): β–β duplexes exhibited the expected antiparallel orientation of the two strands, whereas α–β duplexes were characterized by a parallel orientation of the two strands.

(*3*) *Cleavage of Single-Stranded RNA by Oligo-α- and Oligo-β-thymidylates Covalently Linked to 1,10-Phenanthroline.* In Figure 5 are shown the results obtained when oligothymidylate–phenanthroline conjugates were incubated in the presence of $(rA)_{10}$. As expected, β-T_8-(OP), which bears (OP) linked to the 3′-end, cleaved on the 5′-side of the $(rA)_{10}$ sequence, indicating that the β-DNA–β-RNA hybrid involved an antiparallel orientation of the two strands. (OP)-β-T_8-Acr, which bears the acridine derivative at the 3′-end and phenanthroline at the 5′-end, induced cleavage reactions at the 3′-end of the $(rA)_{10}$ sequence in agreement with an antiparallel orientation of the two strands. When (OP)-α-T_8 [with (OP) attached to the 5′-end] was used, the cleavage sites were located on the 3′-side of the $(rA)_{10}$ sequence. The main band appeared at the location of $(pA)_7p$, indicating that the main reaction induced by the phenanthroline–copper complex occurred at the eighth adenine base (Figure 5). This result indicated that the α-DNA–β-RNA hybrid also had an antiparallel orientation of the two strands. This orientation is the reverse of that observed when the target sequence of (OP)-α-T_8 was a DNA fragment rather than an RNA fragment (Figure 4). This result is, however, in agreement with the energy minimization calculations presented above. The same conclusions were reached when an oligo$(rA)_8$ was used as a target instead of oligo$(rA)_{10}$ (results not shown).

Triple helix formation (le Doan et al., 1987; Praseuth et al., 1988) involving one oligo$(rA)_{10}$ and two α-T_8 might have been responsible for the observed cleavage if one α-T_8 strand was parallel and the other antiparallel to the adenine-containing strand. However, absorption (Thuong et al., 1987) and circular dichroism studies (Durand et al., 1988) have provided evidence for the formation of only 1:1 complexes between α-T_8 and poly(rA) or oligo$(rA)_8$.

The homopolymeric nature of oligothymidylates and oligoadenylates allows for sliding of one oligonucleotide with



1. (OP)-[α]-T_8
2. [β]-T_8-(OP)
3. (OP)-[β]-T_8-Acr
4. [α]-T_8-(OP)
5. $(pA)_{10}$ · piperidine

FIGURE 5: Cleavage of 5′-end-labeled oligo$(rA)_{10}$ by phenanthroline-substituted octathymidylates. Incubation was carried out for 3 h at 4 °C in a pH 7.5 buffer containing 50 mM sodium phosphate and 0.2 M NaCl in the presence of the following: (lane 1) 5 μM (OP)-α-T_8, 1 μM $CuSO_4$, and 5 μM 1,10-phenanthroline; (lane 2) 20 μM β-T_8-(OP), 5 μM $CuSO_4$, and 20 μM 1,10-phenanthroline; (lane 3) 20 μM (OP)-β-T_8-Acr, 5 μM $CuSO_4$, and 20 μM 1,10-phenanthroline; (lane 4) 20 μM α-T_8, 5 μM $CuSO_4$, and 40 μM 1,10-phenanthroline. Lane 5 is oligo$(rA)_{10}$ treated with 1 M piperidine at 90 °C for 5 min. Cleavage generates doublets corresponding to 2′,3′-cyclic phosphate and 3′(2′)-phosphate. The bands corresponding to phosphate termini are indicated by arrows on the right part of the figure.

respect to the other. This could induce the formation of multimeric structures from such staggered duplexes. If such structures existed, then it would be obviously impossible to deduce the relative orientation of the T and A strands from the location of the cleavage sites induced by phenanthroline–copper complexes attached to one end of the oligothymidylates. It should be noted that this was not the case for oligo-β-thymidylates bound to either oligo-β-riboadenylates or oligo-β-deoxyriboadenylates since the sites of the cleavage reactions were in agreement with the expected antiparallel orientation of the two strands. Also, as described above, α-$(dT)_8$ was bound in a parallel orientation with respect to a β-$(dA)_8$ sequence independently of whether this sequence was included in a longer DNA fragment (where no sliding could occur) or used without surrounding bases (in which case sliding could occur). However, one could not exclude that the α-$(dT)_8$–β-$(rA)_n$ complex (n = 8 or 10) was a very special case where sliding and aggregation took place. In order to prevent sliding, a bulky negatively charged dye, lucifer yellow, was covalently attached to the 3′-end of $(rA)_{10}$. When this substituted $(rA)_{10}$ was used as a target for (OP)-α-T_8, cleavage sites were observed on the 3′-side of the $(rA)_{10}$ sequence at the same location as those observed with the unsubstituted $(rA)_{10}$ (results not shown). Therefore, these experiments seem to exclude sliding as an artifact leading to an apparent antiparallel orientation of the two strands and lead to the conclusion that the α-T_8–β-$(rA)_8$ duplex is indeed characterized by an antiparallel orientation of its two strands.

Discussion

The energy minimization calculations and the experiments describing the site-specific cleavage of a target sequence were aimed at understanding how an oligo-α-deoxynucleotide binds to its complementary natural β sequences. We used an oligo-α-thymidylate because of its inherent sequence symmetry, which allowed it to bind in either orientation with respect to

its complementary β sequence. The experimentally observed complex therefore represents the more stable structure.

Energy minimization studies predicted that an oligo-α-$(dT)_n$ should form a stable double helix with an oligo-β-$(dA)_n$ provided the two strands have a parallel 5′→3′ orientation. This is what was experimentally observed as deduced from the cleavage of the complementary sequence by an oligo-α-$(dT)_n$–phenanthroline conjugate.

In contrast, energy minimization predicted that the more stable A-type α–β double helix should have antiparallel strands. This antiparallel orientation was experimentally observed by using the same strategy of site-targeted cleavage reactions induced by an oligo-α-$(dT)_n$–phenanthroline conjugate bound to a complementary oligo-β-$(rA)_n$.

When an oligo-β-$(dT)_n$ was used to target the phenanthroline–copper reaction to a complementary oligo-β-$(dA)_n$ or oligo-β-$(rA)_n$, the cleavage sites occurred in both cases at locations that indicated an antiparallel orientation of the two strands, as expected from the long-established structure of natural DNA–DNA and DNA–RNA double helices.

Cleavage of DNA phosphodiester backbone by phenanthroline–copper chelates involves a 2:1 complex (Sigman, 1986). We observed cleavage reactions in the absence of added free phenanthroline even though the oligothymidylates carried only one phenanthroline ring. However, it should be kept in mind that the oligonucleotide–(OP) conjugate was always used in large excess as compared to the target sequence. Therefore the two phenanthrolines of the 2:1 $(OP)_2Cu$ complex could be provided by two different oligonucleotides, one of them being hydrogen-bonded to the target sequence. A similar behavior was previously reported by Chen and Sigman (1986). Addition of free phenanthroline at low oligonucleotide–(OP) concentration stimulated the cleavage reaction as expected if the added phenanthroline provided the second ligand in the $(OP)_2Cu$ complex (results not shown).

The energy calculations predict that α–β hybrids should be less stable than their β–β counterparts in both the B-like and A-like conformations. This is in disagreement with experimental observations (Thuong et al., 1987), which showed that an α-$(dT)_8$–$\beta(rA)_8$ complex was more stable than the β-$(dT)_8$–β-$(rA)_8$ complex. There is no clear explanation for this discrepancy. However, it should be noted that A and T can form Hoogsteen as well as Watson–Crick base pairs. Energy minimization calculations were carried out by assuming the formation of Watson–Crick base pairs. Further studies should indicate the base pair configuration that is experimentally adopted in the α–β hybrids. It should also be noted that energy minimization was carried out on an A-type conformation of a double-stranded DNA structure and not on that of an RNA–DNA hybrid.

The B-like parallel conformation of an α–β duplex has helical parameters close to those of natural B-DNA (Figure 1, Table I). Recent nuclear magnetic resonance studies have shown that an α-DNA–β-DNA complex forms a right-handed B-type double helix with parallel orientation of the two strands, nucleosides in the anti conformation and sugars in the C-3′-exo conformation (Lancelot et al., 1987; Morvan et al., 1987b). In contrast, the A-like antiparallel conformation is quite different from that of a standard A-DNA structure (Figure 2, Table II). The cleavage reactions induced by phenanthroline–copper complexes have quite different efficiencies in the α–β DNA–DNA and DNA–RNA duplexes (compare Figures 3 and 5). Previous studies showed that DNA was cleaved more efficiently than RNA or DNA–RNA hybrids (Sigman, 1986). Phenanthroline–copper complexes induce strand breaks from the minor groove of DNA. When phenanthroline is tethered to an oligonucleotide, the location of the phenanthroline ring with respect to the duplex grooves and the position of the sites of attack on the sugar (C-4′ and C-1′) are expected to be quite different in an α–β duplex as compared to a β–β duplex. Further studies on the conformational properties of such duplexes should help in understanding their different behaviors with respect to strand-cleavage reactions.

Oligo-α-deoxynucleotides are much more resistant to nucleases than their β analogues (Thuong et al., 1987; Morvan et al., 1987a). They could be used to selectively block gene expression if they are targeted, e.g., to messenger or viral RNAs. They can be covalently linked to intercalating agents in order to stabilize the complexes formed with their complementary sequence. They could also be targeted to DNA in order to inhibit transcription or replication processes (Hélène et al., 1985). In order to synthesize the appropriate sequence of an oligo-α-deoxynucleotide it is of course necessary to know in which orientation it binds to its target sequence. The parallel orientation of the two strands in α-DNA–β-DNA hybrids is a general property that does not appear to depend on the base sequence (Praseuth et al., 1987; Sun et al., 1987; Morvan et al., 1987b; Lancelot et al., 1987). The experimental results described above for α-DNA–β-RNA duplexes deal with an α-$(dT)_8$ sequence. It remains to be determined whether the antiparallel orientation observed for the complex of α-$(dT)_8$ with β-$(rA)_8$ is a special property due to the formation of adjacent AT base pairs involving, e.g., Hoogsteen rather than Watson–Crick hydrogen-bonding interactions.

Oligo-β-deoxynucleotides are efficient inhibitors of mRNA translation (see the introduction). It has been recently shown that this efficiency is due—at least partly—to an induced degradation of the mRNA by RNase H that recognizes the oligodeoxynucleotide–mRNA hybrid and cleaves the mRNA strand (Minshull & Hunt, 1986; Haeuptle et al., 1986; Cazenave et al., 1987a). Hybrids formed by oligo-α-deoxynucleotides and mRNAs are poor substrates for RNase H (unpublished results). This would limit the efficiency of oligo-α-deoxynucleotides in inhibiting mRNA translation. The results reported in the present study show that it is possible to attach active reagents to oligo-α-deoxynucleotides and to make them efficient in cleaving their target mRNA. Such "artificial nucleases" should be helpful in investigating gene expression and might provide a starting point for the development of new therapeutic strategies.

ACKNOWLEDGMENTS

We thank Dr. D. S. Sigman (UCLA) for a gift of 5-(iodoacetamido)-1,10-phenanthroline and Dr. J. F. Hau (C.B.M., Orléans) for the synthesis of $(rA)_{10}$ covalently linked to lucifer yellow.

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