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STRUCTURAL FEATURES ASSOCIATED WITH α- ANOMERIE IN OLIGO-α-THYMIDYLATES

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Comparison between gel electrophoresis migrations of oligo-α-thymidylates and oligo-ß-thymidylates indicates that the migration of α-oligonucleotides under native conditions is different from the migration of ß-oligonucleotides when the number of thymines, part of the sequence, is higher than 5. Such difference disappears when the gels are run under denaturing conditions. This, together with UV spectra, indicates that the structure of α-oligonucleotides is more organized than the structure of ß-oligonucleotides and that such an organisation appears for a length higher than 5 monomeric units.

Among the compounds investigated so far to control gene expression, various nuclease resistant antisense oligonucleotide analogues have been synthesized (1-9). These unnatural oligomers which differ from the natural oligomers only by exhibiting the α-configuration at the anomeric carbon atom (C'1), have been shown to be more resistant to nucleases than their natural analogues (2,8). Furthermore they are able to form duplex with complementary ß- or α- strands in which the Watson-Crick base pairing specificity is retained (9). The heteroduplex obtained from hybridization between complementary α- and ß- strands belong to the B-DNA family and they show parallel polarity (4,5,7). Thermodynamic parameters corresponding to the binding of α-oligonucleotides to their complementary sequences have been estimated (10,11) leading to the conclusion that the nature of the α- sequence could interfere with the energy of formation of these duplex when compared with natural ß- sequences. It appears that one of the factors responsible for such sequence dependant energy of formation could

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be the structural organization of the single stranded α-oligonucleotide. We report here experiments, based upon gel electrophoresis study and UV spectra, leading to the conclusion that such structure does exist when studying model α-oligonucleotidic sequences: α-$(Tp)_{n-1}T$.

MATERIALS AND METHODS

UV determinations were carried out with a UVIKON 810 thermostated spectrophotometer. The temperature of the cells was adjusted with a circulating water bath.

The α-oligothymidylate, α-$(Tp)_{11}T$ was synthesized by J.L. Imbach (Montpellier), using the phosphotriester method as previously described (6). Extinction coefficients of α-oligonucleotides were determined after digestion by 3'-exonuclease from *Crotalus Durissus* (Boehringer). Total degradation was checked by 5' end labelling of the oligonucleotide and migration on 20% polyacrylamide sequencing gels.

Strand labelling: 10 µM of oligonucleotides were incubated for 1 hr at 37°C in 20 mM TRIS.HCl pH 7.5, 10 mM $MgCl_2$, 100 mM KCl and 1mM DTTin the presence of 1µl of [γ^{32}p]ATP and 1 µl of T_4 polynucleotide kinase: 10 u/µl (Biolab).

Oligonucleotide degradation: labelled oligonucleotides (5mM) were incubated at 37°C with *Crotalus Durissus* phosphodiesterase (Boehringer): 1µl of enzyme at 200 µg/ml for α- anomers or 1µl of enzyme at 20 µg/ml for ß- anomers, in 50 mM TRIS-HCl pH 9, 5 mM $MgCl_2$. The reaction was terminated by adding a stop buffer: 90 mM TRIS-Borate pH 8, 3 mM EDTA, 50% Glycerol, 0.25% xylene cyanol and 0.25% bromophenol blue.

Gel electrophoresis: Oligonucleotidic fragments were separated on gel electrophoresis under two different conditions

denaturing gels were performed on 20% acrylamide, 7M urea for 2 hours at 1800V. The temperature of migration was kept constant at 65°C.

non denaturing gels were performed on 20% acrylamide for 15 hours at 800 V in the cold room. The temperature of migration was 8°C.

After migration and autoradiography, the migration was normalized relatively to the xylene cyanol migration.

RESULTS AND DISCUSSION

UV spectra corresponding to α-$(Tp)_{11}T$ and ß-$(Tp)_{11}T$ before and after exonuclease degradation are shown on figure 1. It appears that the wavelength corresponding to the maximum of the spectrum for each compound is different. The maximum of absorbency for the ß- anomer is found at 264 nm when the maximum for the α- anomer is found at 270 nm. This difference may be explained by taking into account a slight difference in the conformation of these oligonucleotides. Durand et al (22) have attributed differences between CD spectra of α- and ß oligonucleotides to changes in the 3'OH and 4'CH_2OH groups which are not the same for

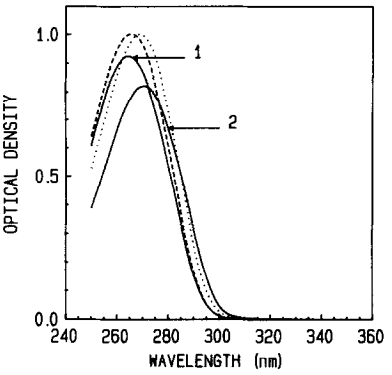

Figure 1: UV absorption spectra of $(Tp)_{11}T$ oligonucleotides before (1: ß-$(Tp)_{11}T$, 2: α-$(Tp)_{11}T$) and after (--- : ß-$(Tp)_{11}T$, ... : α-$(Tp)_{11}T$) degradation with *Crotalus Durissus* exonuclease. Spectra were normalized to 1.

both compounds and to a possible difference in the structure of both the sugar conformation and the sugar-base torsion angle.

Upon degradation of the oligothymidylates, we observe an increase in the optical density together with a slight displacement of the maximum of the absorption toward the higher wavelengths for ß-anomer (from 264 nm to 266 nm) and toward the lower wavelengths for α- anomers (from 270 nm to 268 nm). At the same time, the hyperchromic change is higher for α-$(Tp)_{11}T$ than that observed for ß-$(Tp)_{11}T$: 23.1 % and 8.8 % respectively. Such difference in the hyperchromicity induced by nuclease degradation which converts oligonucleotides to monomeric units, suggests that the structure of α-$(Tp)_{11}T$ is different from the one corresponding to ß-$(Tp)_{11}T$ and that this difference could reflect a difference between the stacking of the α-monomeric units forming the α- oligonucleotide and the stacking of the ß- monomeric units in the ß- oligonucleotide. If such close association (for example stacking) actually takes place in the α- anomer and to a lesser extent in the ß- anomer, we should expect the overall compaction of the α- oligonucleotide to be different from the one associated to the natural ß- anomer.

By digesting the oligonucleotides for different incubation times, we were able to generate an oligomeric population whose size ranges from 1 to 12 nucleotides. Gel migration of these oligonucleotides was followed in native and denaturing conditions for α- oligonucleotides and ß- oligonucleotides. The pattern corresponding to the migration in these conditions is shown on figure 2. We observe that under denaturing conditions both type of

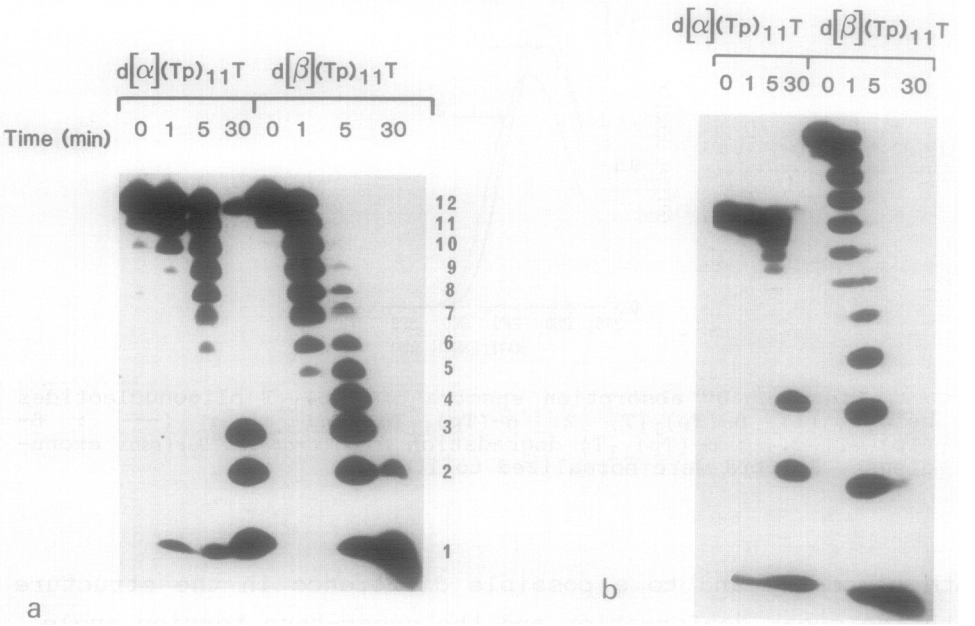

Figure 2: Gel electrophoresis of digested $(Tp)_{11}T$ oligonucleotides. Digestion was performed for the indicated amounts of time leading to oligonucleotides whose size ranges from 1 to 12. Panel a: denaturing gel, panel b: non denaturing gel.

anomers, α- or ß-, migrate at the same distance which depends only upon the size of the oligomers. On the contrary, under native conditions, it is obvious that the α-$(Tp)_{11}T$ oligonucleotide migrates much faster than the ß-$(Tp)_{11}T$ oligonucleotide. This behaviour of α-$(Tp)_{11}T$ is correlated to native conditions since it disappears under denaturation and we can assume that a difference in the structure of the oligonucleotides provides an explanation. Figure 3 represents the relation between the number of nucleotides and the distance of migration. Under denaturing conditions,

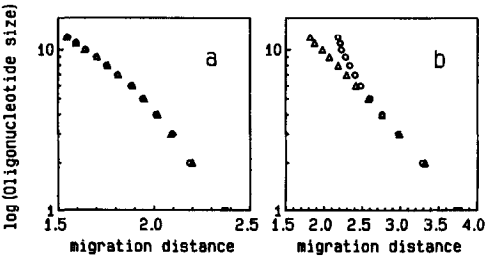

Figure 3: Representation of the logarithm of the oligonucleotide size as a function of the distance of migration. Panel a: denaturing gel, panel b: non denaturing gel: -Δ- : ß-(Tp)n-1T, -○- α-(Tp)n-1T.

the distance migrated by α- or ß- oligonucleotides is the same whatever the length of the oligonucleotide (figure 3a). When migration is performed under native conditions (figure 3b), a difference between the migrations of α- anomers and ß-anomers becomes apparent for a chain length greater than 5 nucleotides and is more and more pronounced when the length of the oligonucleotide increases at least up to 12 nucleotides. Such difference between the migrations of the α- and ß-oligonucleotides has to be compared to the difference observed in the spectra of these compounds. The higher hyperchromicity associated to the degradation of oligonucleotides is assumed to correspond to a more stacked structure of the bases. Such stacking arising on the single strand from base to base, leads to a diminution in the average molar absorption of one isolated residue in the oligomer (13). The variation of the hypochromicity, normalized to 1 for the monomer, as a function of 1/n is linear. On figure 4, such linearity is actually observed by plotting $H_n = f(1/n)$ for ß-$(Tp)_{n-1}T$ oligonucleotide. This linearity indicates self association of the thymines. For α-$(Tp)_{n-1}T$, the hypochromicity is roughly linear up to 5 or 6 residues, but for higher lengths the plot is no more linear. It can be concluded, from this result, that a self association model is no more valid. In this case, it seems that the hypochromicity could result from some kind of base to base interaction leading to the formation of a more complex structure. This conclusion is sustained by the gel electrophoresis experiments. A highly organised structure of the α-oligonucleotide, appearing for a number of nucleotides higher than 6 would account

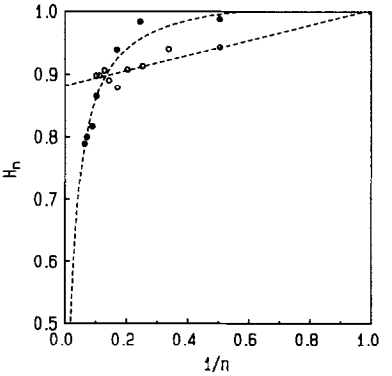

Figure 4: Plot of the hyperchromicity H_n (ϵ_n/ϵ_1) as a function of the reverse of the nucleotide number in the oligonucleotide (1/n). ϵ_n and ϵ_1 are the molar absorbance of the oligonucleotide and the mononucleotide respectively.
-○- ß-$(Tp)_{n-1}T$, -●- α-$(Tp)_{n-1}T$.

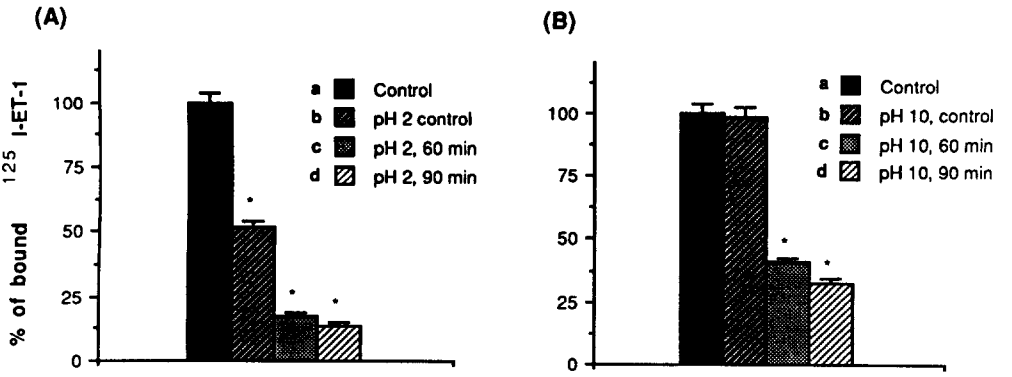

Figure 4 Dissociation of bound [^{125}I]-ET-1 by pH 2 (A) or 10 (B) from solubilized receptors. a: solubilized membrane proteins were incubated with 8.5×10^{-11}M [^{125}I]-ET-1 at 37°C for 60 min; b: solubilized membrane proteins were preincubated at pH 2(A) or 10(B) at 4°C for 60 min and then the pH was restored to 7.4 by the addition of 0.1 N HCl or 0.1N NaOH followed by incubation with [^{125}I]-ET-1 at 37°C for 60 min; c and d: solubilized membrane proteins were preincubated with [^{125}I]-ET-1 at pH 7.4 at 37°C for 60 min, and then the pH was adjusted to 2(A) or 10(B), followed by incubation at 4°C for 60(c) or 90(d) min. Each value represents mean ± SEM of 3 separate experiments. Duplicate estimates were used for each experiment.

results show that the [^{125}I]-ET-1 is able to bind to the solubilized receptors and the bound [^{125}I]-ET-1 cannot be dissociated by 2×10^{-7}M unlabelled ET-1. Therefore, it does

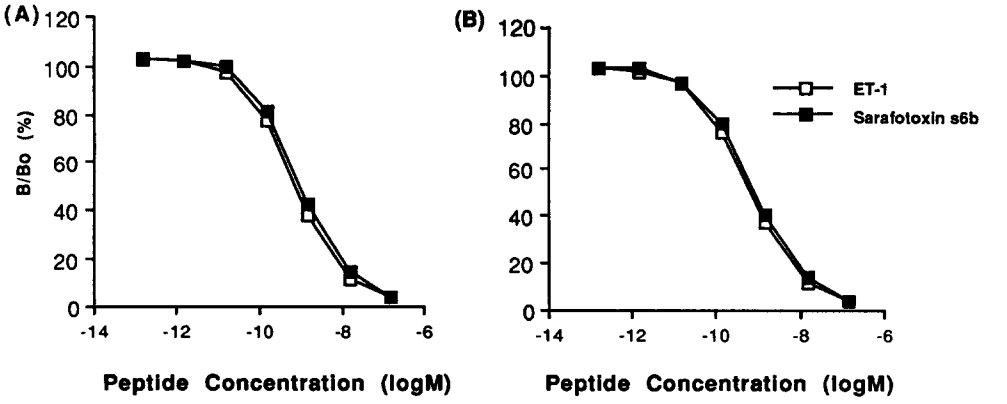

Figure 5 Competition by unlabelled ET-1(endothelin-1)(□) and sarafotoxin S6b (■) for the specific binding of [^{125}I]ET-1 to cardiac membranes (A) and solubilized membrane receptors (B). B/Bo (%) refers to percent of [^{125}I]-ET-1 bound relative to amount bound in the absence of either unlabelled ET-1 or sarafotoxin S6b. Similar estimates were obtained from three separate experiments. Duplicate estimates were used for each experiment.