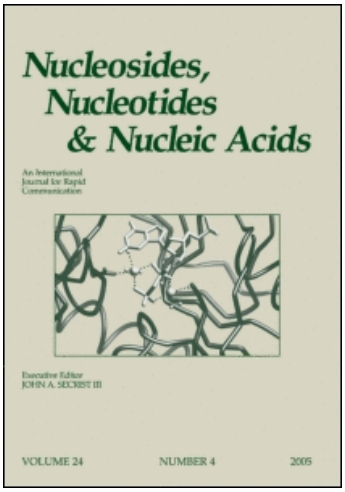

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POSSIBILITIES OF TRIPLEX-FORMATION BY 21-MER OLIGODEOXYRIBONUCLEOTIDES AS ANALOGS OF *TETRAHYMENA* PRE-rRNA FRAGMENTS

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ABSTRACT: A potential DNA triple helix of 21-mer oligodeoxyribonucleotides was synthesized and characterized. The strands were chosen to study the interaction of internal guide and intervening sequences analogs as well as adjacent 3′and 5′exon parts around the splicing site of *Tetrahymena* pre-rRNA. Further in parallel works a series of different RNA and DNA strands was synthesized and combined yielding a suitable order of stability. Here we want to show an isolated examination of a DNA-strand triple helix with defined sequences containing a central mismatched base arrangement and T-A-T bases at the ends.

INTRODUCTION

Highly organized and cooperative tertiary biopolymeric structures are not only prerequisites for performing complex molecular functions, but seem, moreover, indicative of molecular hystereses, basal operation modes for oscillations and rhythm generations, memory imprints and information processings in biomesogenic regulations. In poly(deoxy)ribonucleotides these phenomena appear to be intimately connected with triplex arrangements [1-10].

While in self-splicing RNAs internal guide (IGS) and intervening (IVS) sequences, displaying mutual exon-intron recognition facilities, have long been discussed in terms of appropriate

positioning of reactive parts [11-19], and an informative base triple had been proposed years ago [16], the base patterns around the splicing site of *Tetrahymena* pre-rRNA (Fig. 1) might be suggestive of building up even more extended transient triplex organizations.

A selected 7-mer sequence of this splicing site arrangements forming a modified Cech [16] (Fig. 2) analog GGT base triple with groups of T and A bases (7 base triples) at the 5′ and 3′ ends were synthesized. This formation was used preferring a switching of the all purine leading pattern via the base-triple between two triplex parts.

UV-absorption thermal denaturation studies, CD-spectroscopy and molecular modelling were used to study the possibities of triplex formations of the synthesized 21-mer DNA oligodeoxyribonucleotides.

MATERIALS AND METHODS

The 21-mers of the oligodeoxyribonucleotides (Fig. 3) were synthesized using standard procedures via phosphoramidite chemistry (starting materials were purchased from Roth, Karlsruhe). The deprotected oligomers were purified on a 20 % denaturating polyacrylamide gel (PAGE). Subsequently HPLC analyses were carried out on a BioRad Model 2700 System (software series 800 HPLC System, Version 2.30.1a) equipped with column oven and with an AS-100T HPLC automatic sampling system. A BioRad UV-1806 UV/VIS detector was used (Gen-Pak 60 °C). The average coupling efficiencies were about 98.9 %. All solutions were prepared using a salt concentration of 10 mM sodium chloride. Oligodeoxyribonucleotide concentrations were determined spectrophotometrically by measuring the absorbance at 260 nm using the following single strand extinction coefficients (S1: $1.88 \cdot 10^5$ l/(cm·mol), S2: $2.16 \cdot 10^5$ l/(cm·mol)and S3: $2.13 \cdot 10^5$ l/(cm·mol)) at 20 °C, as calculated by the nearest-neighbor approximation [20].

The thermal denaturations of the three component single strand 21-mers (S1, S2 and S3), the 21-mer duplex (S2·S3, „·“ for Watson-Crick arrangement) and the 21-mer triplex (S1xS2·S3, „x“ for (reversed) Hoogsteen- and Zhurkin- arrangement) [21-26] (Fig. 4) were carried out with a Perkin-Elmer Lambda2 UV/VIS spectrometer, using 1 cm optical path length quartz cuvettes. The cuvette was heated and reheated by a PTP-1 Perkin-Elmer at a rate of 1 °C/min while the temperature and the absorbance of the sample at 260 nm were recordered every 12 sec. The samples for thermal denaturation analysis were prepared by mixing equimolar amounts of denaturated (80 °C, 5 min) strands S2 and S3. After hybridization at 4°C for 5 days

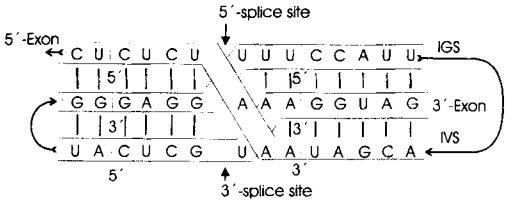

FIG. 1: Schematically cut of the *Tetrahymena* pre-rRNA intron - modified by visualization of hypothetical triplex formation facilities of internal guide sequence yielding a GGU base triple mismatch. In black lines the picted 7-mer core region is marked (Figs. 2-4)

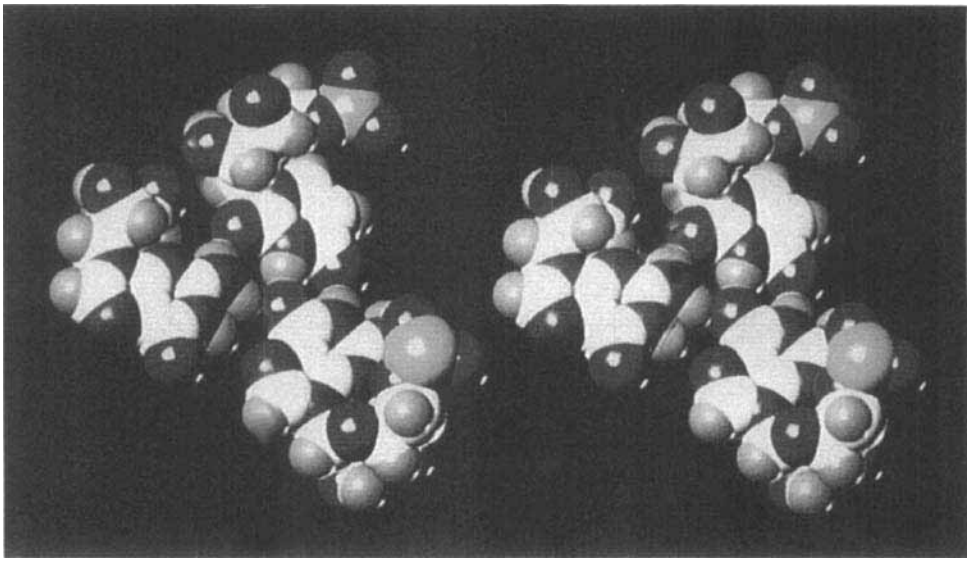

FIG. 2: Stereo CPK-presentation of a modified Cech GGU mismatched triple [16]

the sample was dissolved in 10 mM sodium chloride, an equimolar amount of the third denaturated (80 °C, 5 min) single strand S3 was added and the solution incubated at 4 °C for 7 days. The representative thermal denaturation curves of the S2·S3 duplex and the triple formation S1xS2·S3 are shown in Fig. 4. CD-spectra (Fig. 6) were obtained on a spectropolarimeter JASCO J-720 in 1xPBS at 20 and 80 °C.

The original RNA triplex 7-mer core-region (Fig. 5) was generated by SYBYL 6.2 programs. For optimization Gasteiger-Marsili algorithms were used.

S1 [5′-d(TTT-TTT-TCT-C**T**A-AAT-TTT-TTT)-3′]
S2 [3′-d(AAA-AAA-AGA-G**G**T-TTT-TTT-TTT)-5′]
S3 [5′-d(TTT-TTT-TCT-C**G**T-AAA-AAA-AAA)-3′]

FIG. 3: Single strand oligodeoxyribonucleotides sequences

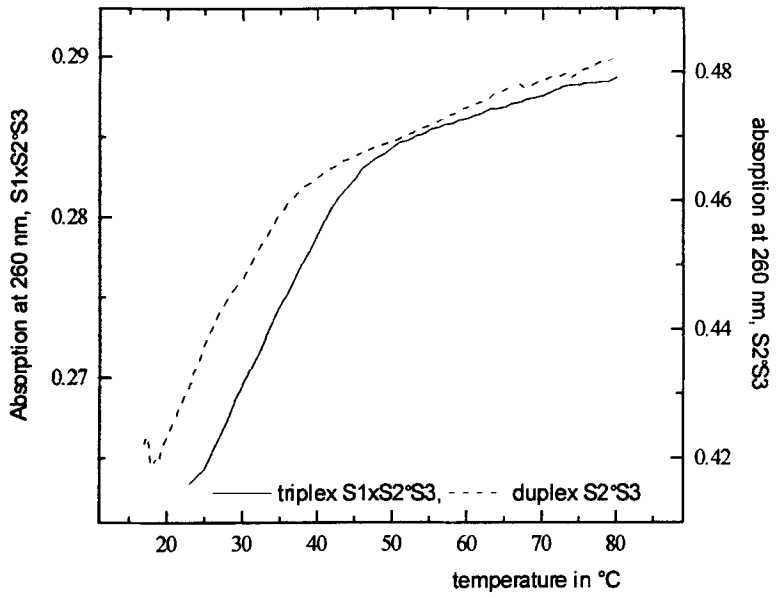

FIG. 4: Thermal denaturation profiles of triplex and duplex formation

RESULTS AND DISCUSSION

The thermal denaturation studies of the single strands S1, S2 and S3 demonstrated that the 21-mers at 20 °C possess considerable order with experimental found T_m-values of 21 °C (S1), 30 °C (S2) and 24 °C (S3). On the other hand more than one T_m-transitions regions were observed according to possible intra- or intermolecular self complementary arrangements.

This feature must be recognized for a selective hybridization to higher ordered DNA duplex and triplex structures from intermolecular associations of their single strands. Therefore all single strands were denaturated at 80 °C for 5 min before mixing. For first studies the partially mismatched 7-mer triplex (GGT base triple mismatch), see also Fig. 5, was modified by oligo(dA) and/or oligo(dT) at the 5′- and 3′-DNA strands for better Watson-Crick and

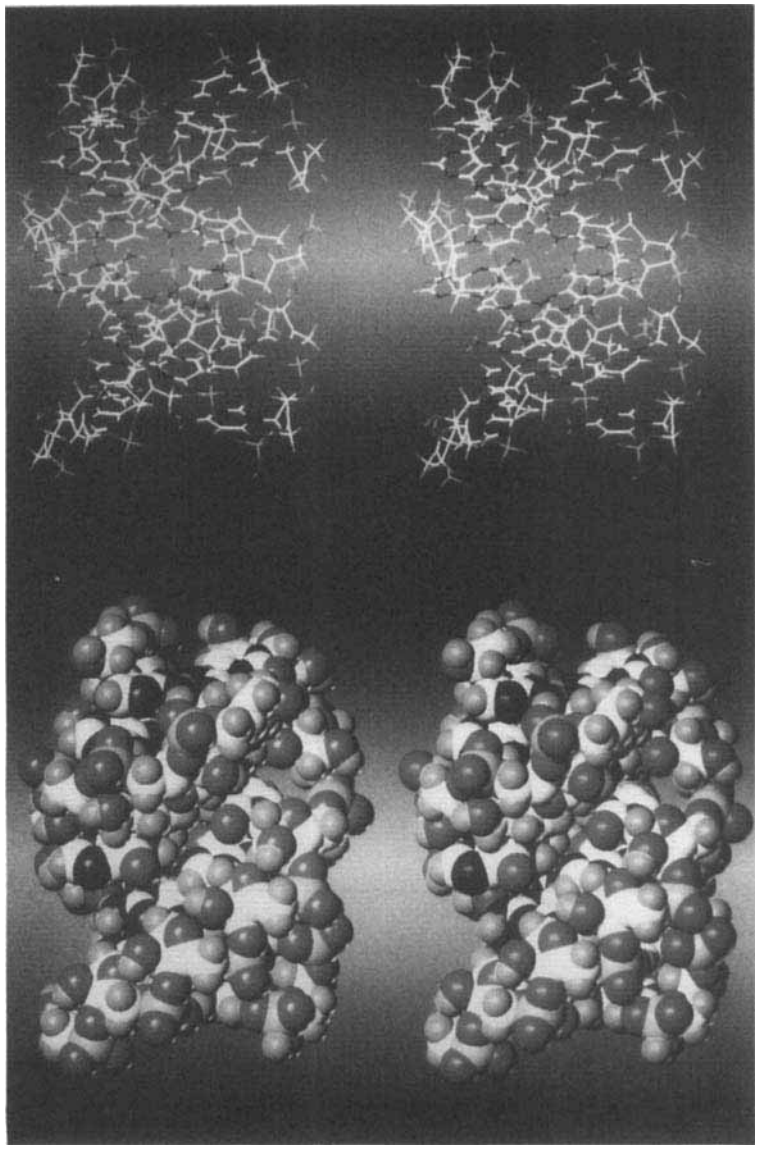

FIG. 5: Original RNA 7-mer-core visualization around the modified Cech-triple [16] by SYBYL programs

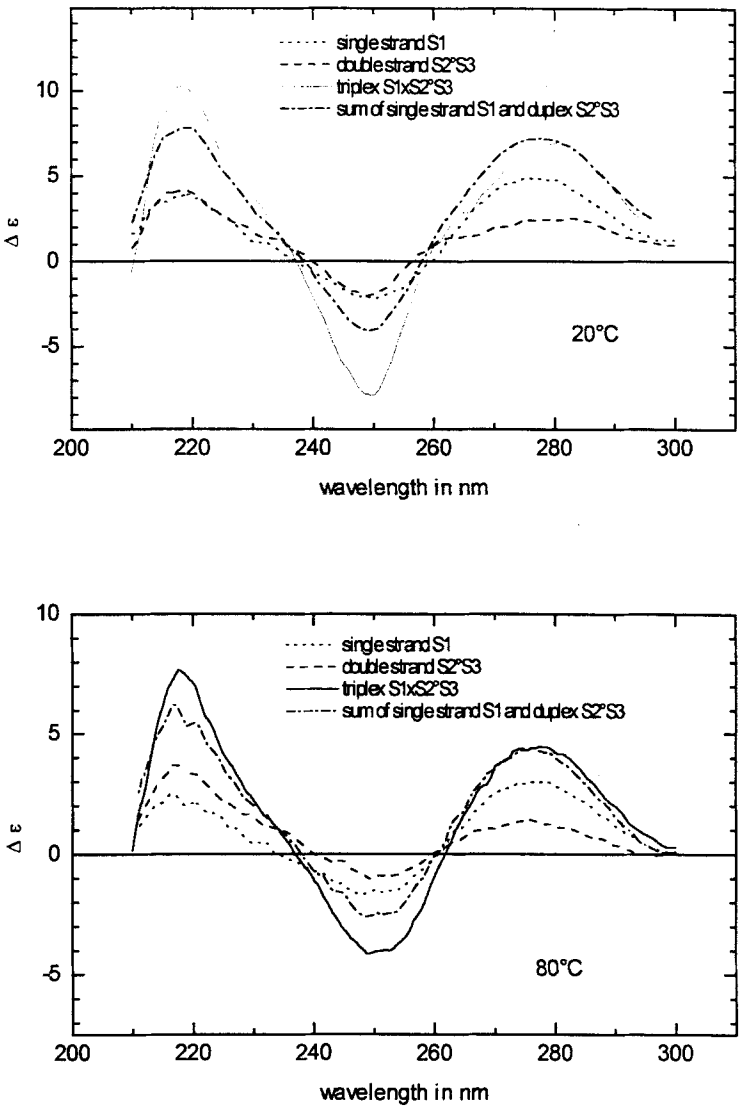

FIG. 6: CD-spectra of 21-mer at 20 and 80 °C

Hoogsteen base pairing. The Watson-Crick association of DNA strand S2 and S3 shows at 260 nm a sharp cooperative transition with a T_m of 24 °C (Fig. 4). After annealing of S1 to the duplex S2·S3 the melting profile shows a more stable behavior than in case of duplex.

CD-spectra (Fig. 6) additionally show clearly that at room temperature the triple arrangement of the 21-mer (7-mer with additional T-A-T parts) occur. Further at 80°C the single strand of S1 (see Fig. 3) was separated from the double helices S2·S3.

Molecular modelling studies from different start geometries could be correlated in a certain degree to the experimental found results. For triplex formation a stabilizing effect in H-bond energies were found as around 50% more favourable in comparison to duplex S2·S3- or S1·S2- formation. Details of this study will be published elsewere [27].

Starting from a possible structure of *Tetrahymena* splicing site (Fig. 1-3 and 5) published by Cech et. al. [16] we tried to form a DNA-triplex arrangement supported by T-A-T coils (Fig. 3). The formation of this triplex could assumpted by interpretation of CD-spectra (Fig. 6) and partially thermal denaturation curves (Fig. 4) at temperature ≤ 20 °C. Above 40 °C only duplex formation of the S2 and S3 strand occur. Comparison of the triplex, duplex and single strands CD-measurements show at wavelengths above 270 nm a characteristic shape, which depends on the nature of interaction. At 20 °C the maximum in case of triplex formation was observed at 281 nm. In case of duplex or by addition of duplex and single strand S1 measurements we found 278 and 277 nm. At 80 °C the maxima of all peaks are nearly the same between 276 and 277 nm. So an interaction between the duplex strand and the S1 strand at 20 °C could be proposed, while at 80 °C the three strands show the comparable characteristic, neither dependent on the presence of the other strands, nor being interpretable in terms of special formations.

OUTLOOK

While splicing-models of *Tetrahymena* pre-rRNA so far emphasized the mutual binding of the guide and intervening sequences to parts of the 5'- and 3'-exons, the formerly presented hypothesis had been intended to offer possibilities for a dynamically aligned core region of the splicing complex, that could provide cooperative contacts between all partners involved in splicing and exon ligating reactions.

Independently of its native relevance, the investigations seem interesting in that they might offer a first example of a hypothetical triplex, consisting of both Arnott- and Zhurkin domains, switching the all-purine leading pattern via a base triple mismatch between the two triplex parts.

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