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# A FLUORESCENT STUDY OF TRYPTOPHAN DERIVATIVES OF OLIGONUCLEOTIDES AND THEIR HELICAL COMPLEXES WITH POLYURIDYLIC ACID

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## 1. Introduction

To investigate the nature of protein–nucleic acid interaction, model compounds in which protein fragments (amino acids) and those of nucleic acids (nucleotides) are covalently bound, have been studied [1,2]. The present paper deals with a new model system, a complementary complex formed between oligoadenylates covalently bound to tryptophan, and polyuridylic acid. TrpOMe-dp($A_3$) · 2 poly(U)* complex makes it possible to study the interaction of an aromatic amino acid residue with structures resembling helices of nucleic acids. Elucidation of the structure of such a complex is of interest, one of the reasons its use of chemically joining oligonucleotides on a polynucleotide template [3].

The data obtained by fluorescence indicate that in the amidates of mono- and oligonucleotides the tryptophan residue interacts with the nucleic acid basis. In the complex TrpOMe-dp($A_3$) · 2 poly (U) intercalation of in the indol ring of Trp between the bases of the complex is, however, hardly possible.

## 2. Materials and methods

Tryptophan derivatives of mononucleotides and phosphoric acid methyl ester were prepared by the carbodiimide method [4] and were in agreement with the specifications in the literature [1]. TrpOMe-dp($A_2$) and TrpOMe-dp($A_3$) were prepared by a modified carbodiimide method [5]. Methyl ester of DL-tryptophan was used in the synthesis. The characteristics of these compounds corresponded to those of analogous phenylalanine derivatives [6]. Poly(U) was from Calbiochem.

Fluorescence was measured by an Aminco-Bowman spectrophotofluorometer in thermostated 1 cm quartz cuvettes at a 90° angle to the exciting light beam. Quantum yields ($\varphi$) were estimated relative to that of fluorescence of Trp in water for which the value 100 was adopted, the optical density per tryptophan being equal [7]. The errors in determining $\varphi$ were about ± 20%, those of $\lambda_{max}$ of the fluorescence were ± 3nm. The correction for the screening effect of nucleotides was calculated as in [8].

Absorption spectra and absorbance temperature dependence were measured in a Cary 15 spectrophotometer.

## 3. Results and discussion

3.1. *Properties of mono- and oligonucleotide amidates*

As shown in table 1, tryptophan derivatives of mono- and oligonucleotides (compounds I-VII) have anomalously low fluorescence quantum yields $\varphi$. The values were corrected for the weak screening effect of nucleotides (see Materials and methods). Fluorescence quenching in these compounds is not a consequence of tryptophan phosphorylation, as no fluorescence quenching in Trp-pOMe (X) and TrpOMe-pOMe (XI) in comparison with Trp (VIII) and TrpOMe (IX), respectively, was detected. The fluorescence quantum yield of nucleotide amidates depends on the nature of

* Abbreviations: TrpOMe-pU, uridylyl-(5'→N)-tryptophan, methyl ester; TrpOME-dp($A_2$), deoxyadenylyl-(5' 3')-deoxyadenylyl-(5'→N)-tryptophan, methyl ester; TrpOME-pOMe *N*(methylphospho)-tryptophan, methyl ester; poly(U), polyuridylic acid.

Table 1
Relative fluorescence quantum yields (φ) and emission spectra maxima ($\lambda_{max}$) of tryptophan derivatives (0.02 M phosphate buffer, pH 7.5, 22°C, concentration per tryptophan - $1.9 \times 10^{-5}$ M, excitation λ 285 nm)

| Compounds | | φ | λ max (nm) |
|---|---|---|---|
| TrpOMe-pA | (I) | 14 | 364 |
| TrpOMe-dpA | (II) | 14 | 364 |
| TrpOMe-dp($A_2$) | (III) | 14 | 364 |
| TrpOMe-dp($A_3$) | (IV) | 14 | 364 |
| Trp-pU | (V) | 29 | 367 |
| TrpOMe-pU | (VI) | 10 | 364 |
| TrpOMe-pG | (VII) | 17 | 364 |
| Trp | (VIII) | 100 | 362 |
| TrpOMe | (IX) | 70 | 360 |
| Trp-pOMe | (X) | 250 | 367 |
| TrpOMe-pOMe | (XI) | 73 | 364 |
| TrpOMe-dp($A_3$)* | | 25 | |
| TrpOMe-pOMe* | | 130 | |
| TrpOMe-dp($A_3$)·2 poly(U)* | | 32 | |

* 0.004 M phosphate buffer, pH 7.5, 0.2 M NaCl, 0.075 M $MgCl_2$, 2°C, concentration $8 \times 10^{-5}$ M per mononucleotide.

the heterocycle (table 1) and its ionization. For example, the fluorescence of Trp-pU(V) in the uracil ionization increases more than two-fold (fig.1). The fluorescence is not affected by the structure of the sugar, and the existence of the quenching effect

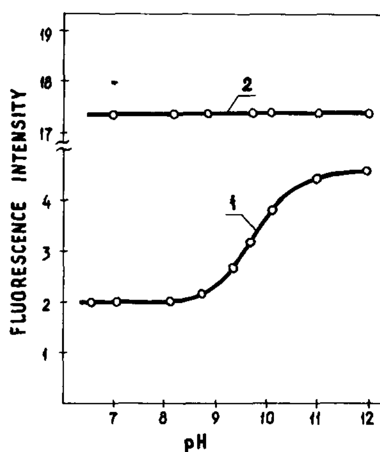


Fig.1. pH dependence of fluorescence intensity: 1, Trp-pU (V); 2, Trp-pOMe (X). Concentration $1.9 \times 10^{-5}$ M, 22°C, 0.02 M phosphate buffer, pH 6.5–9, and 0.02 M glycine buffer, pH 9–12; excitation λ 285 nm, fluorescence λ 365 nm.

does not depend on whether the carboxy group is free or methylated (table 1).

These data allow one to conclude that the fluorescence quenching of tryptophan derivatives of mono- or oligonucleotides should be ascribed to intramolecular non-covalent interaction of the tryptophan residue and the heterocyclic bases. This interaction, as was previously shown [1,2] gives rise to a specific parallel conformation between the planes of indole and the base. The fluorescence quantum yields of Trp-OMe-dpA (II), TrpOMe-dp($A_2$) (III) and TrpOMe-dp($A_3$) (IV) being equal suggests that the tryptophan residue interacts with the nearest oligonucleotide base in the same fashion as with the base in TrpOMe-dpA.

### 3.2. *Properties of TrpOMe-dp($A_3$) · 2 poly(U) complex*

We have shown by absorption spectroscopy that in 0.004 M phosphate buffer, pH 7.5, containing 0.2 M NaCl and 0.075 M $MgCl_2$, TrpOMe-dp($A_3$), like all other trideoxyadenylate amidates [6], forms with poly(U) a triple helical TrpOMe-dp($A_3$) · 2 poly(U) complex. The mixing curves (see also [6]) gave precise 2:1 stoichiometry which excludes the possibility that the tryptophan takes the place of an adenine base in the oligo(dA) stack. The presence in this complex of a covalently bound tryptophan residue results in its destabilization by 8°C as compared to the dp($A_3$) · 2 poly(U) complex.

As is seen in fig.2,A, dissociation of the TrpOMe-dp($A_3$) · 2 poly(U) complex induces a cooperative decrease in the fluorescence intensity. It shows that Trp does not gradually leave the complex but only when the oligo(dA) dissociates (see for comparison curves a and b in the insert of fig.2,A). The fluorescence quantum yield of the complex is 25% higher than that of TrpOMe-dp($A_3$) (at 2°C), but much lower than that of TrpOMe-pOMe (table 1), which indicates that the trytophan residue is not free in the complex, but the interaction is not so strongly expressed as in TrOMe-dp($A_3$).

This also shows that intercalation of Trp indole ring between the bases of the complex is hardly probable, because it should cause complete fluorescence quenching [9]. The absence of intercalation is also supported by the fact that the tryptophan residues in the complex are accessible to quencher molecules like potassium iodide. As is shown in fig.2,B, KI quenches the fluorescence of Trp in the completely

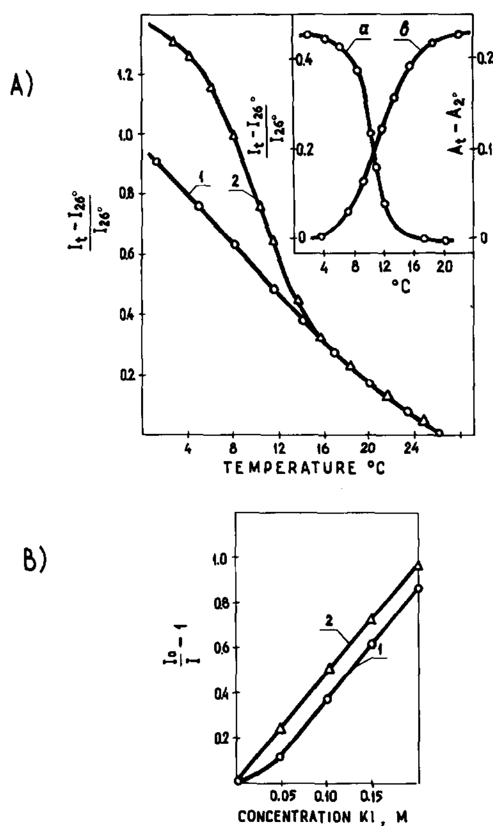


Fig.2,A) Temperature dependence of the relative fluorescence intensity increment: 1, TrpOMe-dp($A_3$); 2, TrpOMe-dp($A_3$) · 2 poly(U). Concentration 8 × $10^{-5}$ M per mononucleotide, 0.004 M phosphate buffer, pH 7.5, 0.2 M NaCl and 0.075 M $MgCl_2$ ; excitation λ 285 nm, fluorescence λ 365 nm. Insert: a, a difference curve for curves 1 and 2; b, the absorbance temperature profile of the complex (at 260 nm), the same conditions. B) Effect of potassium iodide on the fluorescence of TrpOMe-dp($A_3$) · 2 poly(U): 1, at 2°C; 2, at 25°C. Concentration 8 × $10^{-5}$ M per mononucleotide, 0.004 M phosphate buffer, pH 7.5, 0.075 M $MgCl_2$, 0.2 M (NaCl + KI), concentration of KI is given in figure. $I_0$ and I, fluorescence intensity at zero and given concentration of KI. Excitation λ 285 nm, fluorescence λ 365 nm.

formed complex (curve 1) in a similar manner as in the dissociated complex (mixture of poly(U) and TrpOMe-dp($A_3$) at 25°C, curve 2). Quenching decreases upon complex formation, but remains sufficiently pronounced. In the case of the complexes between dyes and DNA, intercalation renders the dyes inaccessible to KI [10].

From the data presented here and our previous work [6] several possibilities for the arrangement of the Trp residue in the triple stranded complex between TrpOMe-dp($A_3$) and poly(U) can be ruled out: a) The absence of complete quenching and accessibility of Trp to KI exclude intercalation. b) The precise 2:1 stoichiometry of all oligonucleotide amidate complexes shows that all uracil residues are regularly bound to adenine residues. This excludes the possibility that Trp may replace adenine in the oligo(dA) stack.

There are only limited possibilities to account for the cooperative dissociation and the low quantum yield of the fluorescence of Trp in the complex. Model building of an A · U · U helix leaves little space for interaction of the nucleic acid bases with Trp. Since the Trp residue must be in the small groove of the Watson-Crick part of the triple helix only interaction with the $N_3$ part of adenine is reasonably steric. The interaction between the tryptophan residue and accessible parts of the nucleic acid bases is less in the complex than in TrpOMe-dp($A_3$), which renders the denaturated state preferable. This may explain why the TrpOMe-dp($A_3$) · 2 poly(U) complex has a lower thermal stability than the dp($A_3$) · 2 poly(U) complex.

The question whether all TrpOMe-dp($A_3$) have the same polarity is presently being studied in our laboratory.

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