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INTERACTION OF PENTAURIDYLIC ACID WITH THE POLY(A)·POLY(U) DOUBLE HELIX

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Abstract. Poly(A)·poly(U) is shown to bind $(pU)_n$ ($n \geqslant 5$) at low temperatures in the presence of Mg^{2+} ions. The binding curve at 1 °C in the Scatchard representation is characteristic of cooperative binding and could be approximated by the following parameters (according to McGhee and von Hippel): $K=790$, $\omega=36$, $n=4.4$. It is proposed that Hoogsten pairs are formed between the poly(A) strand of the poly(A)·poly(U) and oligouridylic acids. Oligouridylic acids ($n \geqslant 5$) could be considered specific ligands for adenine stretches in the RNA double helices.

Key words: poly(A)·poly(U), complexes with ribouridylic acid oligomers, triple helix, binding constant, UV difference spectra, melting temperature.

INTRODUCTION

In recent years a number of low-molecular weight compounds have been found to exhibit different degrees of specificity towards particular nucleotide sequences in double helices. The molecular mechanism underlying the specificity has been elucidated in several cases [1–4]. The three-dimensional structures of the complexes have been established by X-ray investigations [5–7], and some general rules covering the nucleic acid interactions have been drawn.

For single-stranded nucleic acids, sequence-specific ligands can be prepared by making use of the property of the bases to form complementary base pairs, i. e. in order to have a ligand capable of binding to a given sequence of nucleotides one needs nothing but to synthesize the oligonucleotide which is complementary to the polynucleotide strand under investigation [8, 9].

In the case of the double helix the problem is much more complicated. These ligands must meet at least two conditions: (1) There must be a possibility of arranging them into a regular spatial structure to fit the double helix. This condition means that we must deal with polymers. (2) There must be four different kinds of monomers as repeating units in order to bind to four different types of base pairs. Only two different monomeric units are needed when AT/TA base pairs are to be resolved from GC/CG base pairs.

One possibility of attaining the specificity towards the base pairs in DNA follows from the structure of triple helices [10]. Oligouridylic acid is expected to interact with the dA·dT (or A·U) sequences whereas protonated oligocytidylic acid is expected to bind to the dG·dC (or G·C) base pairs from the major groove via the Hoogsten type of pairing. The oligopyrimidines can be regarded as specific ligands capable of recognizing the polypurine sequences in the double helices. The ligands of this kind have been synthesized and shown to interact specifically with the expected nucleotide sequences of DNA [11–13]. It is believed that triple helices could be induced in the homopurine—homopyrimidine tracts of DNA [14–16].

In the present work we have prepared a series of uridylic acid oligomers in order to determine the minimal length of $(pU)_n$ capable of forming a stable complex with ribopolymer poly(A)·poly(U). These oligomers are expected to prefer the A-conformation which is an advantage in the formation of triple helices.

MATERIALS AND METHODS

$(pU)_n$ (n=3...6) was prepared by hydrolysis of poly(U) and was shown to be pure chromatographically. Poly(A) and poly(U) were annealed in 10 mM sodium cacodylate buffer, pH 7.0, containing 30 mM of NaCl at room temperature using poly(A) and poly(U) (SERVA). Poly(dA)·poly(dT) was a product of Boehringer Mannheim GmbH. Experiments were carried out in 1 mM cacodylate buffer, pH 7.0, containing 10 mM NaCl and 10 mM $MgCl_2$. The following extinction coefficients were used for calculations: poly(A) — $\varepsilon_{257}=6000$ $l \cdot M^{-1} \cdot cm^{-1}$; poly(U) — $\varepsilon_{261}=9430$ $l \cdot M^{-1} \cdot cm^{-1}$; $(pU)_5$ — $\varepsilon_{261}=9900$ $l \cdot M^{-1} \cdot cm^{-1}$; poly(A)·poly(U) — $\varepsilon_{260}=6655$ $l \cdot M^{-1} \cdot cm^{-1}$; poly(dA)·poly(dT) — $\varepsilon_{260}=$ $=6000$ $l \cdot M^{-1} \cdot cm^{-1}$; the difference between extinction coefficients of the free and bound oligo(U) under given conditions $\Delta\varepsilon_{260}=5520$ $l \cdot M^{-1} \cdot cm^{-1}$.

UV spectra and temperature dependencies were recorded using a Pye-Unicam SP8-150 spectrophotometer equipped with a SPX 876 Series 2 Temperature Programme Controller. The absorbance measurements were performed in 0.1 cm cuvettes; both beams of the spectrophotometer contained two cuvettes. To have the positive peak in the difference spectrum, poly(A)·poly(U) and oligouridylic acid were placed in the sample beam in separate cuvettes one after the other, whereas the cuvettes containing the buffer alone and the complex were in the zero beam. $(pU)_n$ was always added to pre-existing double helical poly(A)·poly(U).

CD spectra were recorded using a Jobin-Yvon Model 3 spectrometer.

Absorbance spectra were measured at 1 °C and 25 °C; thereafter, the absorbance difference at 260 nm was found and used to calculate the fractions of the free and bound ligand. The molar difference spectrum, which corresponds to 100%-bound ligand, was found from experiments with a high oligomer/polymer ratio.

Binding curves were calculated for 1.0 °C and the respective parameters found according to the McGhee—von Hippel formula for cooperative binding [17]. The binding of a single oligomer molecule to the double helix is characterized by the binding constant K; molecules, which bind to sites adjacent to this (already occupied) site, have a ω times bigger binding constant (i.e. $\omega \cdot K$); n stands for the length of the site (the number of base pairs which could be covered by a single ligand molecule). The best fit was found by least squares.

RESULTS

Upon mixing $(pU)_n$ with pre-existing double helical poly(A)·poly(U) and cooling the mixtures to 1 °C in the cuvette compartment of the spectrometer, hypochromicities were recorded as a function of time. After the initial rapid development of hypochromicity, an additional decrease of absorption during the following four hours was observed at large oligomer/polymer ratios.

Reliable changes in hypochromicity were found for oligomers $n \geqslant 5$ (Fig. 1). The melting of the complexes depended upon the concentration of the salt added (Fig. 2); when the concentration of $MgCl_2$ was 10 mM or more, a plateau region was observed in the vicinity of 0 °C. The UV difference spectrum (Fig. 3) as well as the CD spectrum of the transition (data not shown) were quite similar to those observed in the case of poly(A)·2poly(U).

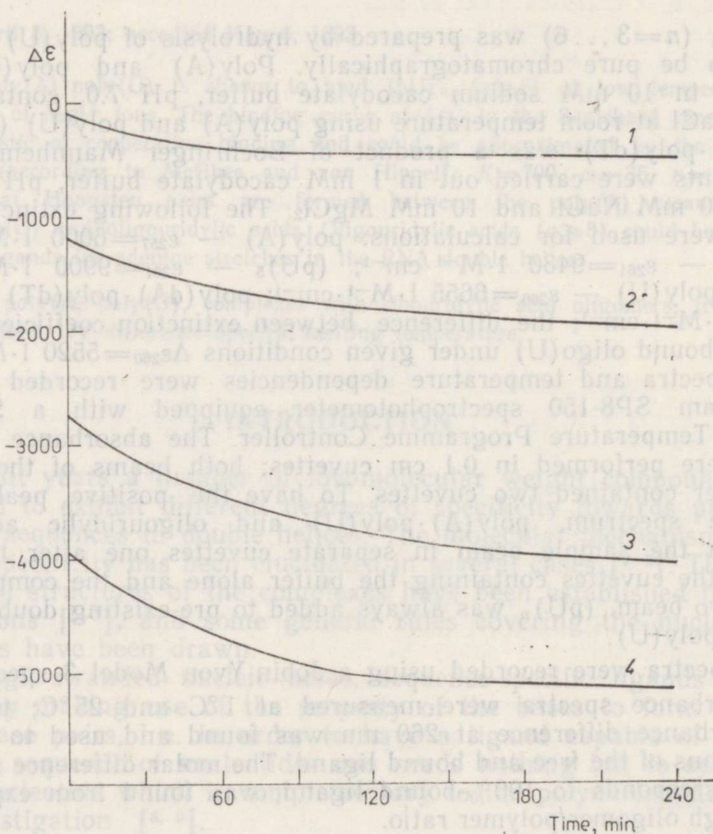

Fig. 1. Kinetics of the reaction of poly(A)·poly(U) with $(pU)_5$ as observed at 260 nm. $(pU)_5$ was added to double helical poly(A)·poly(U) at room temperature and cooled down in the cuvette compartment of the spectrophotometer. The count of time began from the moment the temperature 1 °C was reached. Ordinate axis: change in absorbance per base pair of poly(A)·poly(U) for the following ratios of molar concentrations [U]/[A·U]: *1* — 0.46, *2* — 0.78, *3* — 1.35, *4* — 1.79.

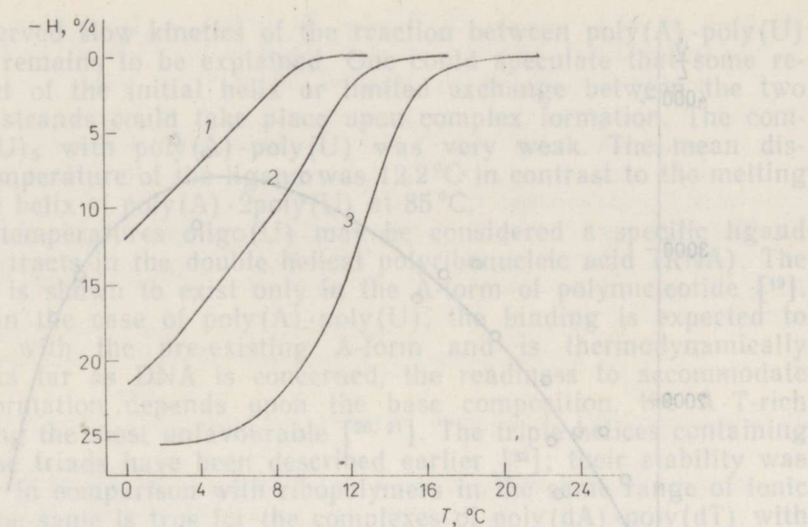

Fig. 2. Melting curves of the complexes poly(A)·poly(U)+$(pU)_5$ in 1 m*M* cacodylate buffer, pH 7.0, containing 10 m*M* NaCl and *1* — 4 m*M*, *2* — 6 m*M* or *3* — 10 m*M* $MgCl_2$. Before the measurements the specimens were held four hours at 1.0 °C in the cuvette compartment of the spectrophotometer in order to achieve equilibrium. The hypochromicity of the specimens is given with respect to [P⁻] of the ligand molecule.

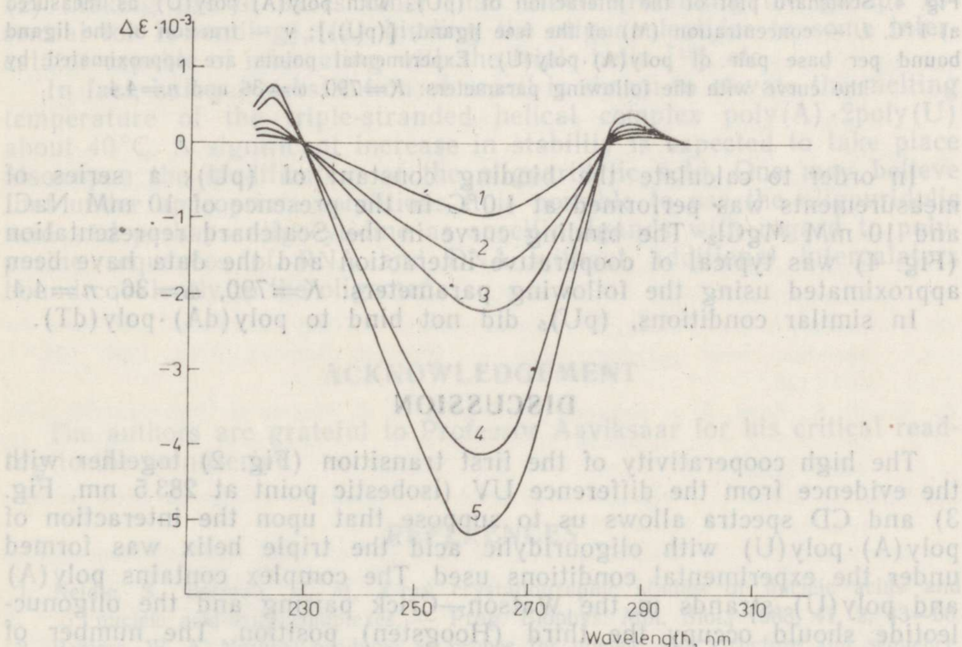

Fig. 3. Difference spectra of the interaction of $(pU)_5$ with poly(A)·poly(U) at 1 °C with respect to room temperature for the following ratios of molar concentrations [U]/[U·A]: *1* — 0.46, *2* — 0.56, *3* — 0.78, *4* — 1.26, *5* — 1.79. The interaction is accompanied with hypochromic change in the uracil absorption band. The isobestic point is at 283.5 nm as expected for the triple helix [24]. The concentration of poly(A)·poly(U) was $4.0 \cdot 10^{-4}$ *M*. The absorbance difference at 260 nm was used to calculate the fraction of bound ligand.

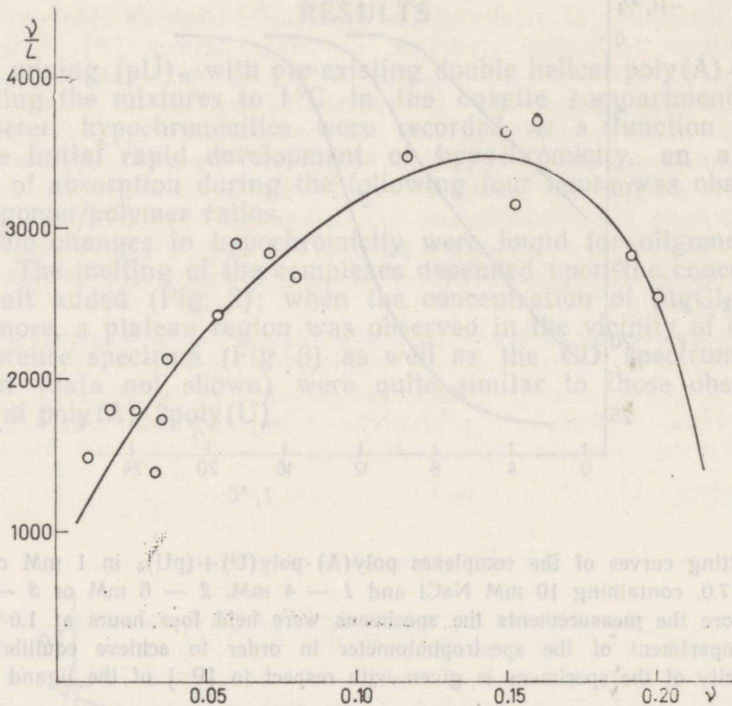

Fig. 4. Scatchard plot of the interaction of $(pU)_5$ with poly(A)·poly(U) as measured at 1 °C. L — concentration (M) of the free ligand, $[(pU)_5]$; ν — fraction of the ligand bound per base pair of poly(A)·poly(U). Experimental points are approximated by the curve with the following parameters: $K=790$, $\omega=36$ and $n=4.4$.

In order to calculate the binding constant of $(pU)_5$ a series of measurements was performed at 1.0 °C in the presence of 10 mM NaCl and 10 mM $MgCl_2$. The binding curve in the Scatchard representation (Fig. 4) was typical of cooperative interaction and the data have been approximated using the following parameters: $K=790$, $\omega=36$, $n=4.4$. In similar conditions, $(pU)_5$ did not bind to poly(dA)·poly(dT).

DISCUSSION

The high cooperativity of the first transition (Fig. 2) together with the evidence from the difference UV (isobestic point at 283.5 nm, Fig. 3) and CD spectra allows us to suppose that upon the interaction of poly(A)·poly(U) with oligouridylic acid the triple helix was formed under the experimental conditions used. The complex contains poly(A) and poly(U) strands in the Watson—Crick pairing and the oligonucleotide should occupy the third (Hoogsten) position. The number of base pairs covered by a ligand as found from the Scatchard plot was close to the expected value. In the case of 1 : 1 stoichiometry of poly(A) and oligo(U), the formation of stable double helices has been observed beginning with $n=7$ (50 mM cacodylate containing 100 mM NaCl and 10 mM $MgCl_2$) [18].

After dissociation of the ligand, the rearrangement of poly(A)·poly(U) to the triple helix followed at 64.5 °C (in the presence of 10 mM $MgCl_2$) accompanied by the increase in light scattering. In the case of the triple helical poly(A)·2poly(U), the melting of the complex followed at 85 °C.

The observed slow kinetics of the reaction between poly(A)·poly(U) and $(pU)_5$ remains to be explained. One could speculate that some rearrangement of the initial helix or limited exchange between the two pyrimidine strands could take place upon complex formation. The complex of $(pU)_5$ with poly(A)·poly(U) was very weak. The mean dissociation temperature of the ligand was 12.2 °C in contrast to the melting of the triple helix of poly(A)·2poly(U) at 85 °C.

At low temperatures oligo(U) may be considered a specific ligand to the A·U tracts in the double helical polyribonucleic acid (RNA). The triple helix is shown to exist only in the A-form of polynucleotide [19]. Therefore, in the case of poly(A)·poly(U), the binding is expected to take place with the pre-existing A-form and is thermodynamically favoured. As far as DNA is concerned, the readiness to accommodate the A-conformation depends upon the base composition, the A·T-rich regions being the most unfavourable [20, 21]. The triple helices containing dA·2dT base triads have been described earlier [22]; their stability was much lower in comparison with ribopolymers in the same range of ionic strength. The same is true for the complexes of poly(dA)·poly(dT) with poly(U) as the third strand [22]. This seems to be the reason why poly(dA)·poly(dT) does not bind short oligomers of the oligouridylic acid.

The interaction of the oligouridylic acid with double-stranded poly(A)·poly(U) was cooperative. One may expect the value of the binding constant to increase when the longer oligonucleotides are used.

There are additional possibilities of increasing the binding constant, for instance, (1) introducing methyl or halogen groups into uracil [23], (2) making use of bases other than uracil (retaining the groups essential for H-bonding), (3) binding the oligonucleotides to some intercalator capable of interacting with the triple helix [13], etc.

In fact, halogen substitution of uracil is shown to elevate the melting temperature of the triple-stranded helical complex poly(A)·2poly(U) about 40 °C. A significant increase in stability is expected to take place also upon the modification of the oligouridylic acid. One may believe that under appropriate conditions it is possible to use the oligouridylic acid and perhaps oligocytidine as specific ligands with regard to polypurine sequences of RNA and DNA without additional intercalators bound covalently to the oligomer.

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PENTAURIDÜÜLHAPPE KOMPLEKSID KAKSIKHELIKAALSE POLY(A)·POLY(U)-GA

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On näidatud, et uridiini oligomeerid üldvalemiga $(pU)_n$ ($n \geqslant 5$) seostuvad madalal temperatuuril magneesiumioonide manulusel ribopolümeeriga poly(A)·poly(U). Seostumiskõver Scatchardi koordinaadistikus on iseloomulik kooperatiivsele seostumisele ja lähendatav järgmiste parameetritega: $K=790$, $\omega=36$, $n=4{,}4$. Oligouridiin seostub kaksikhelikaalse ribopolümeeriga Hoogsteni skeemi kohaselt kolmanda ahelana. Seega võib oligouridiini vaadelda kaksikheeliksi koostises olevate poly(A)-de järjestuse suhtes spetsiifilise ligandina.

ВЗАИМОДЕЙСТВИЕ ПЕНТАУРИДИЛОВОЙ КИСЛОТЫ С ДВОЙНОЙ СПИРАЛЬЮ ПОЛИ(A)·ПОЛИ(U)

Эрго РАУКАС, Кай КООЛИ, Виталий ЯМКОВОЙ

Показано, что при низкой температуре и в присутствии ионов магния поли(A)·поли(U) связывает олигоуридилаты $(pU)_n$ при условии $n \geqslant 5$. В представлении Скэчарда, кривая связывания имеет при 1°C кооперативный характер и может быть описана константами $K=790$, $\omega=36$, $n=4{,}4$ (согласно формуле МакГи—фон Хиппеля). Высказано предположение, что олигоуридилаты связываются с цепочкой поли(A) в составе двойной спирали поли(A)·поли(U) посредством пар типа Гугстена. Таким образом, олигоуридилаты можно рассматривать как лиганды, специфические к полиадениновым последовательностям в составе двойной спирали РНК.