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Single strand conformation of adenylate chains analysed by a specific photoreaction. Determination of structure by 5' residue

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ABSTRACT

The photoreactivity was analysed in various oligo- and polyadenylates: 1) The quantum yields of the specific photoreaction in poly(dA) and dApdA decrease with increasing temperature, whereas the quantum yield of the photodegradation in poly(rA) increases. 2) The photoreactivities of poly(2'MeA) and poly(2'EtA) closely correspond to that of poly(rA). 3) The photodegradation of rApdA is very similar to that of rAprA, whereas dAprA shows the same specific photoreaction as observed for dApdA.

These data support the view, that the specific photoreaction observed in oligo(dA) and poly(dA) is dependent upon a specific conformation, which is not accessible to oligo(rA), poly(rA), poly(2'MeA) and poly(2'EtA). The specific conformation is determined by the nucleotide, which carries the internucleotide bond in the 3'-position.

INTRODUCTION

It is well known that the conformation of single stranded oligo- and polynucleotides depends upon the nature of the sugar component. The exchange of a ribose against a deoxyribose in an adenylate chain induces a change in the conformation reflected by an alteration in e.g. CD parameters.[1] This difference in single strand conformation also seems to be the reason for the great difference in photochemical reactivity observed in deoxyriboadenylates versus riboadenylates.[2] Oligo(dA) and poly(dA) shows a specific UV photoreaction with a relatively high quantum yield, whereas oligo(rA) and poly(rA) exhibit an unspecific photodegradation with a low quantum yield.[2,3] Apparently the geometry of the adenine stacks is quite different in deoxyribo-

versus riboadenylate chains.

In the present investigation the dependence of photoreactivity upon single strand conformation is analysed in further detail and is used to characterize the conformations of some oligo- and polynucleotides.

MATERIALS AND METHODS

rAprA, poly(rA), $d(pA)_2$ and poly(dA) were the same samples as described previously (cf. ref. 3). dApdA was prepared from $d(pA)_2$ by the action of alkaline phosphatase. Adenylyl(2'-5') adenosine was obtained from Pharma Waldhof, Düsseldorf. 2'Deoxyadenylyl(3'-5')adenosine [dAprA] and adenylyl(3'-5')2'deoxyadenosine [rApdA] were kindly provided by F. Eckstein. Poly (2'methyladenylate) [poly(2'MeA)] and poly(2'ethyladenylate) [poly(2'EtA)] were the generous gift of J. L. Alderfer.

Irradiations, actinometer determinations, UV and CD measurements in general were performed as described previously (cf. ref. 2). The temperature of solutions during irradiations was maintained at constant values by a thermostated cuvette holder. Irradiations were performed at a given temperature and then the reaction characterized by CD measurements at 20°C. Quantum yields were determined as described in ref. 3. All the photoreactions described in the present communication were induced by irradiation at 248 nm.

RESULTS

The conformations of single stranded polynucleotides are known to depend upon the temperature. The single strand helical conformations found at low temperatures are mainly converted to random coil forms at high temperatures.[1,4] This dependence may be used for an analysis of the photoreactivity in oligo- and polyadenylates. Hence the photoreactions of poly(rA), poly(dA) and dApdA were characterized at a series of different temperatures. As shown in Fig. 1 the quantum yields

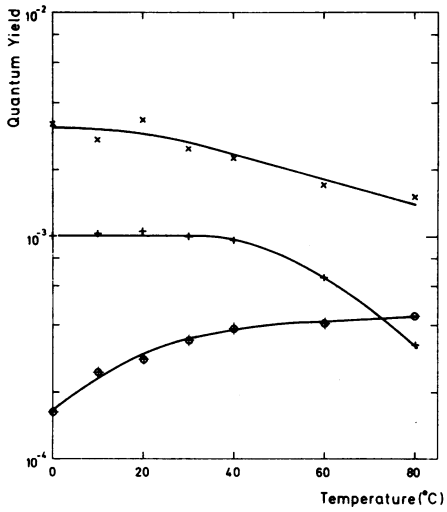

Fig. 1 Quantum yield in moles/einstein at 248 nm for poly(dA) (x), dApdA (+) and poly(rA) (o) as a function of the temperature in 0.1 M Na-cacodylate, pH 6.9.

determined for the specific photoreaction in poly(dA) and dApdA decrease with increasing temperature. In contrast the quantum yields of the unspecific photoreaction in poly(rA) increase with increasing temperature. These results are consistent with the known temperature dependences of the single strand conformations. The specific photoreaction apparently is dependent upon a specific conformation formed in poly(dA) and dApdA at low temperatures. Thus dissociation of this conformation at high temperature leads to a reduction in the quantum yield. In the opposite way the photoreaction of poly(rA) is inhibited by the formation of its single strand helical conformation at low temperatures. At increasing temperatures the dissociation of the helical conformation in poly(rA) is reflected by an increase in the quantum yield.

It has often been claimed that the difference in the conformations of poly(rA) and poly(dA) is due to hydrogen bonding of the 2'OH group in poly(rA).[5,6] In recent years evidence has been collected, which disfavors this hypothesis. Thus the properties of poly(2'MeA) and poly(2'EtA) were shown to be similar

to that of poly(rA), although the hydrogen donor group is substituted in these polymers.[7-9] The analysis of photoreactivity may now be used as a method to characterize the conformation of the modified adenylate polymers. The quantum yields in poly(2'MeA) and poly(2'EtA) were determined in 0.1 M $Na_2B_4O_7$ pH 9.2, in order to prevent any double helix formation.[7-9] (Test experiments did not show any pH dependence of the photoreactivity in poly(rA) and poly(dA) in the pH range 6.9 to 9.2, within experimental accuracy.) There is no indication of any specific photoreaction in the substituted polymers. The CD bands of the "native" polymers simply disappear without appearance of any new CD bands. Thus there seems to be no similarity of the poly(2'MeA) and poly(2'EtA) conformations to that of poly(dA). The close correspondence of the quantum yields in poly(rA), poly(2'MeA) and poly(2'EtA) (cf. Table I) suggests, that their conformations are quite similar. This result supplies additional evidence against a determination of single strand conformation by hydrogen bonding of the 2'OH in poly(rA).

Table I Quantum yields in mol/einstein at 248 nm (0.1 M NaCl-0.1 M Na-cacodylate pH 6.9, except for poly(2'MeA) and poly(2'EtA) in 0.1 M $Na_2B_4O_7$ pH 9.2, cf. text).

poly(dA)	2.5×10^{-3}
poly(rA)	3.4×10^{-4}
poly(2'MeA)	3.2×10^{-4}
poly(2'EtA)	3.7×10^{-4}
3'-5'-(dApdA)	1.0×10^{-3}
3'-5'-(dAprA)	1.0×10^{-3}
3'-5'-(rApdA)	6.3×10^{-5}
3'-5'-(rAprA)	6.2×10^{-5}
2'-5'-(rAprA)	2.6×10^{-4}

According to the data described above the conformation of oligo and poly(dA) seems to be unique. The nature of this specific conformation may be characterized further by analysis of various sequence isomers: dApdA, dAprA, rApdA, rAprA. The results

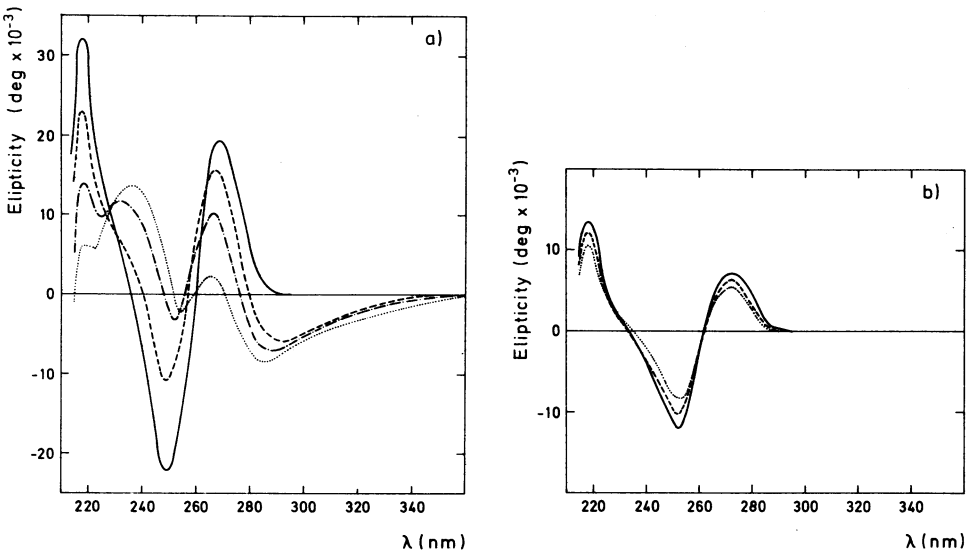

Fig. 2 CD-spectrum of dAprA (a) and rApdA (b) in 0.1 M NaCl-0.1 M Na-cacodylate (pH 6.9) at various times during UV irradiation at 248 nm.
a) 0 min (-); 40 min (---); 90 min (-·-·); 150 min (···)
b) 0 min (-); 421 min (---); 1124 min (···).

of this analysis are given in Fig. 2 and Table I. dAprA clearly shows the same specific photoreaction as dApdA, whereas rApdA exhibits the same unspecific photodegradation as rAprA. Thus the conformation leading to the specific photoreaction is determined by the nucleoside, which carries the internucleotide bond in the 3'-position and it does not matter, whether the other residue is a ribo- or a deoxyriboadenylate.

The photoreactivity of one further isomer, 2'-5'-rAprA, has also been characterized. It shows a higher quantum yield of photodegradation than 3'-5'-rAprA. However, there is no indication of a specific photoreaction in 2'-5'-rAprA.

DISCUSSION

The method applied in the present investigation is quite unusual in the characterization of polynucleotide conformations. The ana-

lysis of photoreactivity obviously does not allow to obtain information about absolute conformations. However, the method provides a very simple way to compare the conformations of various oligo- and polyadenylates. As a result the conformations of various oligomers and polymers may be classified and finally the factors may be analysed, which influence the different conformations.

The results of the present investigations clearly suggest, that the conformation of single stranded oligo and poly(dA) is different from that of single stranded oligo and poly(rA), poly (2'MeA) and poly(2'EtA). Similar results were obtained by other methods.[1,7-9] Alderfer et al.[9] found a decrease of base stacking in the series $(dA)_n$ > $(rA)_n$ > $(r2'\text{-}MeA)_n$ > $(r2'\text{-}EtA)_n$. They explained their results by steric hindrance due to an increasing size of the C-2'-substituents. Their interpretation may also be used to explain the photochemical data: steric hindrance may prevent the stacking geometry necessary for the specific photoreaction.

The photochemical data obtained from the various adenylate dimers are consistent with the division of these dimers into two groups performed by Maurizot et al..[10] The data are also consistent with the assigment of a stacking order from hypochromicity and PMR dimerisation shifts given by Kondo et al.[11], yet do not fit as well to the stacking order derived from CD amplitudes by the same authors. The photochemical data clearly support the idea, that the single strand conformation is determined by the 5' nucleoside. Whether this is due to steric hindrance, the influence of the sugar conformation or some not yet identified molecular interaction, remains for further investigation.

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