The mixture was reprecipitated by the same technique and the precipitate was treated with 15 *N* methanolic ammonia. The unprotected nucleotides (16%) were subjected to DEAE-cellulose column chromatography as shown in Figure 1. Aliquots of materials in peak II, III, and IV were treated with bacterial alkaline phosphatase. Dephosphorylated oligonucleotides were digested with spleen phosphodiesterase. The ratio of thymidine to Tp were: peak II, 1.00:0.93; peak III, 1.00:1.94; peak IV, 1.00:2.98. A main portion of the reaction mixture (84%) was subjected to chromatography on a column (1.7 × 65 cm) of DEAE-cellulose (bicarbonate form) using a linear gradient of triethylammonium bicarbonate (0–0.45 *M*). The total volume was 3 l. The tetranucleotide was eluted in two peaks, 871 A_{260} and 409 A_{260}. Materials from these two peaks were identical, and the combined material was rechromatographed to give a symmetrical single peak using an identical condition.

Analysis of a Specific Photoreaction in Oligo- and Polydeoxyadenylic Acids

Dietmar Pörschke

Contribution from the Max-Planck-Institut für Biophysikalische Chemie, Göttingen, Germany. *Received July 31, 1973*

Abstract: In contrast to oligo- and polyriboadenylic acids, which are relatively stable against uv irradiation, oligo- and polydeoxyadenylic acids were found to be rather sensitive to uv irradiation at neutral pH. A specific photoreaction was detected in deoxyadenylic acid chains with chain lengths $n \geq 2$. The photoproduct formation was recorded both by changes in the uv spectrum and in the CD spectrum. Solutions flushed with nitrogen showed the same reaction as solutions that had not been deaerated. The major photoproduct resulting from irradiation of $d(pA)_2$ was isolated by DEAE chromatography. The uv absorption of the $d(pA)_2$ photoproduct, denoted $d(pA)_2$*, shows a maximum extinction coefficient of 10.0×10^3 per mole of phosphorus (λ_{max} 264 nm), which is only slightly lower than that of $d(pA)_2$, indicating the existence of an aromatic system in the photoproduct. $d(pA)_2$* shows a characteristic CD spectrum, which remains almost unchanged in 100% ethanol solution, indicating a rather stable asymmetric structure, which is possibly maintained by a covalent bridging bond. $d(pA)_2$* is characterized by a p*K* of 7.0. One phosphate group of $d(pA)_2$* can be removed by the action of bacterial alkaline phosphatase. Phosphodiesterase from snake venom apparently does not have any effect on $d(pA)_2$*. The quantum efficiency of the $d(pA)_2$ degradation by irradiation at 248 nm is around $\phi = 10^{-3}$. The quantum efficiency increases with increasing deoxyadenylic acid chain length *n*: $\phi(n = 3) = 1.7 \times 10^{-3}$, $\phi(n = 4) = 2.1 \times 10^{-3}$, $\phi(n = \infty) = 2.5 \times 10^{-3}$. The quantum yield of the $d(pA)_2$ degradation shows a relatively small dependence upon the wavelength of the irradiated light in the range of 240–280 nm. Irradiation experiments performed under different solvent conditions demonstrate that the photoreaction is solvent dependent. The base-pairing ability of irradiated deoxyadenylic acid chains to the complementary poly(U) is inhibited.

The radiation sensitivity of living organisms has been attributed mainly to radiation induced damages in the carriers of the genetic information, *i.e.*, the nucleic acids.[1–5] In the attempt to understand the radiation damages, a lot of activity has been devoted to the radiation biology and chemistry of the nucleic acids. Most of the investigations have been concerned with photoreactions of the pyrimidine bases (uracil, thymine, and cytidine) since these bases have proved to be rather sensitive to radiation. In contrast to the pyrimidine bases the purine bases have been considered to be stable against radiation, since rather high radiation doses are required to bring about any chemical change in adenine or guanine.

In a recent investigation[6] it has been demonstrated that the usually high photoresistance of adenine bases may be reduced considerably, when the adenine residues are incorporated into a specific polymer structure. Whereas the adenine bases are rather photoresistant in poly(A), a specific photoproduct is formed in poly(dA) with quantum yields of 2.5×10^{-3} mol/einstein. This result demonstrated that photoreactions in purine bases cannot be neglected in the interpretation of photolesions in living cells. The present investigation is concerned with a characterization of the photoreaction in deoxyadenylic acid chains by using oligonucleotides of defined chain length.

Experimental Section

Materials and Methods. Poly(dA) was purchased from LP Biochemicals, Inc., Milwaukee, Wis. 53205, and was used without further purification ($s_{20,w} = 7.5$, ϵ_{260} 10.1×10^3 M^{-1} cm^{-1}). Oligodeoxyriboadenylic acids were prepared and purified as described by Ralph and Khorana.[7] Poly(A) was obtained from Miles Laboratories (Lot No. 74) and ApA was from Zellstoffabrik Waldhof, Mannheim. Concentrations were determined according to the following extinction coefficients (all values in 10^3 M^{-1} cm^{-1} at 20°): $d(pA)_2$, ϵ_{260} 12.8; $d(pA)_3$, ϵ_{260} 11.9; $d(pA)_4$, ϵ_{260} 11.3; poly(A), ϵ_{257} 10.0; ApA, ϵ_{259} 13.7. The extinction coefficient of the photoproduct was calculated from a phosphorus determination according to Eibl and Lands.[8] Alkaline phosphatase (EC 3.1.3.1) and phosphodiesterase (EC 3.1.4.1) were purchased from Boehringer, Mannheim.

(1) D. Shugar in "The Nucleic Acids," Vol. III, J. N. Davidson and E. Chargaff, Ed., Academic Press, New York, N. Y., 1960, pp 39–104.
(2) A. D. McLaren and D. Shugar in "Photochemistry of Proteins and Nucleic Acids," Pergamon Press, Elmsford, N. Y., 1964, pp 163–278.
(3) S. Okada, "Radiation Biochemistry," Academic Press, New York, N. Y., 1970.
(4) A. J. Lomant and J. R. Fresco, *Progr. Nucl. Acid Res., Mol. Biol.*, **12**, 1 (1972).
(5) R. B. Setlow and J. K. Setlow, *Annu. Rev. Biophys. Bioeng.*, **1**, 293 (1972).
(6) D. Pörschke, *Proc. Nat. Acad. Sci. U. S.*, **70**, 2683 (1973).
(7) R. N. Ralph and H. G. Khorana, *J. Amer. Chem. Soc.*, **83**, 2926 (1961).
(8) H. Eibl and W. E. M. Lands, *Anal. Biochem.*, **30**, 51 (1969).

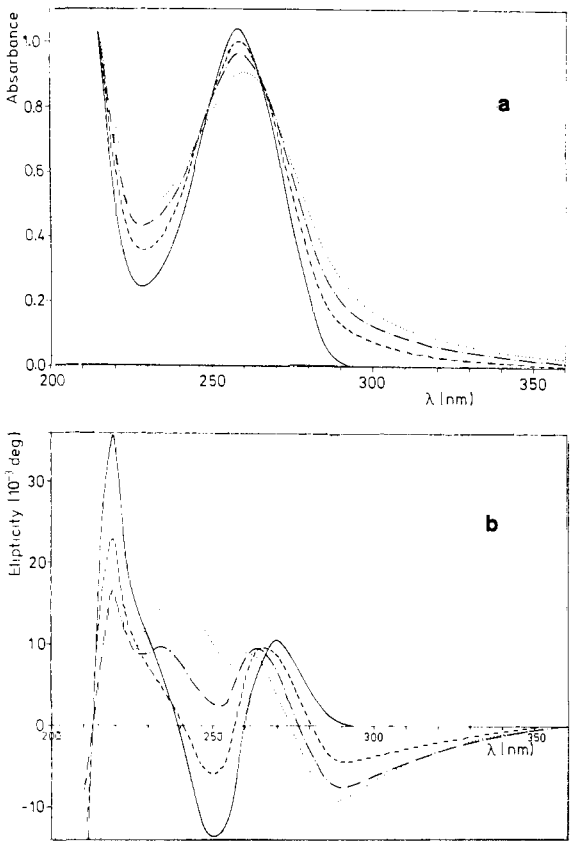

Figure 1. Uv absorbance (a) and CD spectrum (b) of $d(pA)_2$ in 0.1 *M* NaCl–0.1 *M* sodium cacodylate (pH 6.9), 20° at various degrees of uv irradiation at 248 nm: 0 min (——), 30 min (– – –), 60 min (-·-·-), 105 min (· · ·).

Irradiations, actinometer determinations, and uv and CD measurements were performed as described previously.[6] Due to some aging process of the light source, the irradiation light flux into the cuvette decreased from initially 2.2×10^{-6} einstein/min to 1.8×10^{-6} einstein/min at later stages of the experiments.

Electrophoresis experiments were performed on cellulose thin layers, purchased from E. Merck, Darmstadt, using a Camag thin-layer electrophoresis chamber.

Results

(a) **Photoreactions in $d(pA)_n$ Oligomers.** 5′-Deoxyadenylic acid is rather photoresistant.[6] Very high uv irradiation doses are required to induce changes of its optical properties. This photoresistance is reduced considerably when two deoxyadenylic acid monomers are connected by a 3′–5′ phosphodiester bond. A specific photoreaction in $d(pA)_2$ is indicated by characteristic changes both in the uv absorption and the CD spectrum (Figures 1a and b). These changes are similar to those observed previously in poly(dA),[6] although the uv irradiation doses, required to induce these changes in $d(pA)_2$, are higher than for poly(dA). Corresponding results are obtained for higher oligomers. Both $d(pA)_3$ and $d(pA)_4$ exhibit the characteristic changes of the uv absorbance and CD spectra upon irradiation at 248 nm. With increasing chain length the uv dose required for the induction of the photochemical conversion decreases slightly. These irradiation experiments, indicating a photosensitivity starting at the dimer level and increasing slightly with chain length strongly suggest a photoreaction between adjacent deoxyadenylic acid residues.

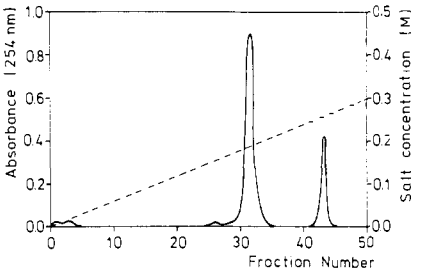

Figure 2. Column chromatography of an irradiated sample of $d(pA)_2$ on DEAE cellulose using a linear gradient of triethylammonium hydrogen carbonate (pH 7) (– – –, left scale).

As may be expected, the photoreaction is not influenced by the presence of the 5′-phosphate group. d(ApA) shows the same dose effect curves as $d(pA)_2$ within experimental error.

All the experiments described above were performed with solutions that were not deaerated prior to irradiation. In order to test for any oxygen dependence a control experiment was conducted using a $d(pA)_2$ solution flushed with nitrogen for 20 min in comparison to the same solution used without nitrogen treatment. After an irradiation period of 90 min both solutions showed almost the same changes in the uv absorption and in the CD spectrum. Differences that were detected were close to the experimental error. Some slow changes of the optical properties following the irradiation were observed in both solutions. These changes occurred within days after the irradiation process, the apparent half-time being around 15 hr. In both cases the uv absorbance and the CD effects partly went back to those of the unirradiated solutions. This back-amplitude was around 10–15% of the changes produced in the irradiation process.

(b) Isolation of the Major Photoproduct from $d(pA)_2$ and Some of Its Properties. The photoproduct resulting from irradiation of $d(pA)_2$ may be readily separated by DEAE column chromatography. Application of a linear salt gradient (triethylammonium bicarbonate (pH 7.5)) results in the separation of two major peaks (Figure 2), the first one being unreacted $d(pA)_2$. The material obtained in the second peak is homogeneous as judged by thin-layer electrophoresis in the pH range from 2.6 to 9.2. The uv absorbance and CD spectra of $d(pA)_2$ and the photoproduct are shown in Figures 3a and b. Compared to the $d(pA)_2$ absorbance spectrum the maximum of the absorbance in the photoproduct around 260 nm is reduced by 22%. The maximum of the absorbance in the photoproduct is shifted to longer wavelengths and the absorbance extends to far longer wavelengths than observed in $d(pA)_2$. The CD spectrum of the photoproduct is completely different from that of $d(pA)_2$. Two major Cotton effects are observed in the wavelength range down to 220 nm. The difference in sign compared to the Cotton effects of $d(pA)_2$ may indicate a different sense of asymmetry. In the following sections the photoproduct of $d(pA)_2$ is denoted $d(pA)_2$*.

Both uv absorbance and CD spectra of $d(pA)_2$* are pH dependent within the neutral pH range (see Figure 4). Since there are no changes of the optical properties of $d(pA)_2$ within the same pH range, the chemical function responsible for these changes obviously is pro-

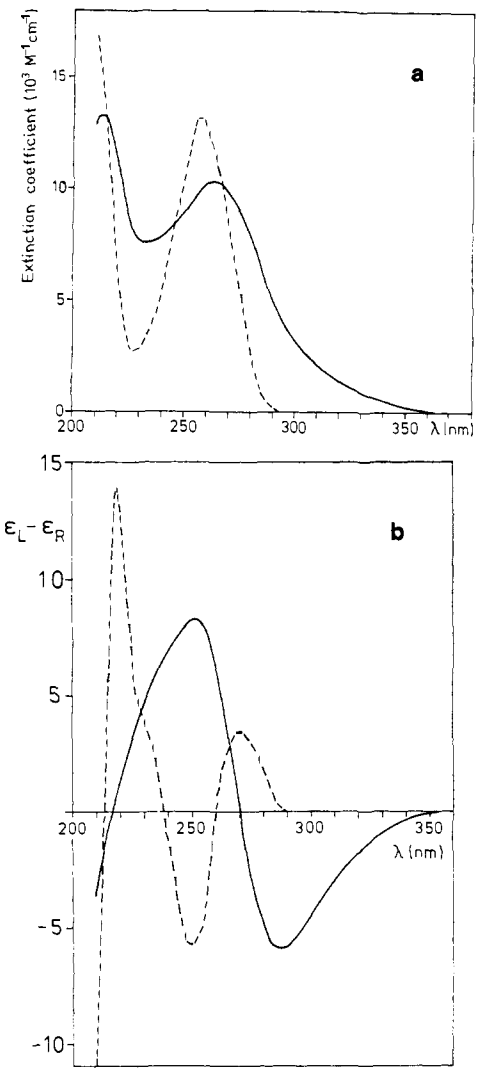

Figure 3. Uv absorbance (a) and CD spectrum (b) of $d(pA)_2$ (- - -) and its photoproduct, $d(pA)_2$* (——), in 0.1 *M* NaCl–0.1 *M* sodium cacodylate (pH 6.9), 20°.

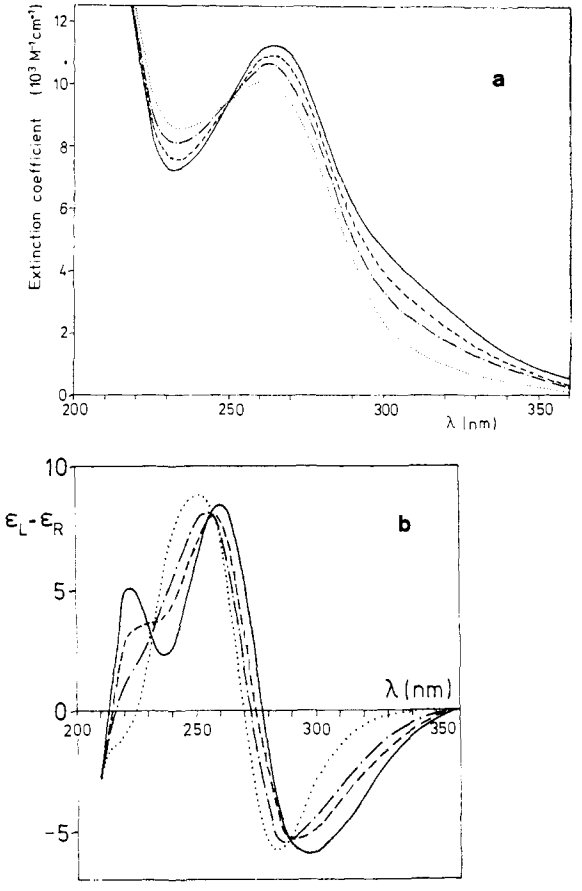

Figure 4. Uv absorbance (a) and CD spectrum (b) of $d(pA)_2$* in H_2O at different pH values (20°): pH 10.4 (——), pH 8.0 (- - -), pH 7.4 (-·-·-), pH 6.15 (···).

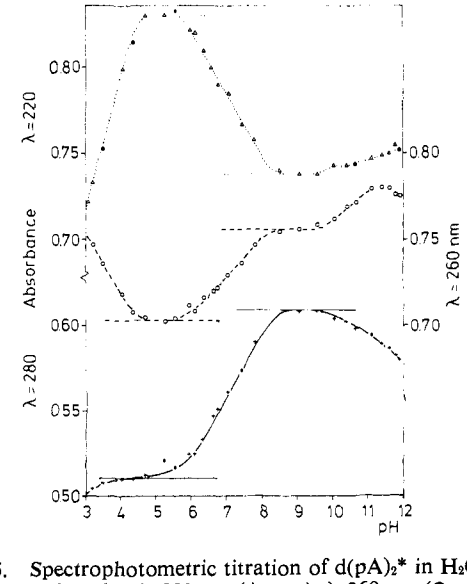

Figure 5. Spectrophotometric titration of $d(pA)_2$* in H_2O (20°) at different wavelengths: λ 220 nm (Δ, ···), λ 260 nm (○, - - -), and λ 280 nm (+, ——).

duced in the course of the photoreaction. The large changes of the optical properties of $d(pA)_2$* within the titration suggest that the chemical function is directly connected to the aromatic system. The apparent p*K* of this function in the photoproduct is 7.0 (*cf.* Figure 5). From the elution position in DEAE chromatography and from electrophoretic mobilities (Figure 6), it can be concluded that the chemical function carries a negative charge on the alkaline side of the titration. The electrophoretic mobilities indicate the dissociation of two protons in the range from pH 5.5 to pH 8.5. The more acidic one apparently is due to a primary phosphate group at the 5′-end and the other one due to the yet unidentified chemical function in the aromatic system of $d(pA)_2$*. Further titratable groups are observed in the pH ranges around 3 and 11.5 (*cf.* Figure 5).

Some evidence for the chemical structure of $d(pA)_2$* may be taken from enzyme degradation experiments. The primary phosphate group present in $d(pA)_2$ is also present in $d(pA)_2$*, since treatment with bacterial alkaline phosphatase results in the complete formation of a homogeneous material (as judged by electrophoresis), which exhibits an electrophoretic mobility lower than that of $d(pA)_2$* (1.9 cm/hr for d(ApA)* compared to 6.6 cm/hr for $d(pA)_2$*, both at pH 6.8).

Venom phosphodiesterase apparently does not have any effect on $d(pA)_2$*. Under conditions leading to the complete degradation of $d(pA)_2$ to the mononucleotides $d(pA)_2$* remained unreacted. This result suggests that the photoreaction leads to the formation of a

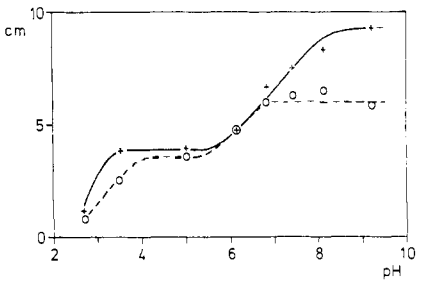

Figure 6. Electrophoretic mobility (cm/hr at 1200 V) of $d(pA)_2$* (+, ——) and $d(pA)_2$ (O, – – –) as a function of the pH value (cellulose thin-layer plates at 2°).

bond between the two nucleotide moieties. It should be noted, however, that some modified dinucleoside phosphates without a covalent bond between the nucleoside moieties have been described, which were not degraded by phosphodiesterase.[9]

Further evidence for a chemical linkage, probably between modified adenine bases, is obtained from CD measurements of the photoproduct. The CD spectrum of the photoproduct dissolved in ethanol is almost the same as that obtained from aqueous solutions. This is in contrast to the behavior of dinucleotides like $d(pA)_2$, which show a strong reduction of the CD amplitudes observed in aqueous solution, when they are dissolved in organic solvents like ethanol.[10] The reduction of the CD amplitudes of dinucleotides like $d(pA)_2$ in solvents of decreasing polarity is explained by the reduction of the stacking interaction between the bases resulting in the dissociation of the asymmetric stacked structure. Since the CD spectrum of $d(pA)_2$* is essentially the same in aqueous and in ethanol solution, the asymmetric structure is probably not maintained by any stacking interaction but by a covalent linkage.

For the evaluation of irradiation dose effect functions it is important to know about the radiation sensitivity of $d(pA)_2$*. $d(pA)_2$* is degraded by uv irradiation at similar rates as $d(pA)_2$. The quantum yield of this photodegradation is estimated from the rate of the decrease in the CD amplitudes to be around 10^{-3}. From this result it is clear that $d(pA)_2$* cannot be produced by irradiation of $d(pA)_2$ in quantitative yield. Any $d(pA)_2$* that is produced by $d(pA)_2$ irradiation is subjected to photodegradation itself.

In order to test for the presence of any radical function in the photoproduct esr measurements were performed. However, these measurements did not yield any evidence for the presence of unpaired electrons.

For comparison, a $d(pA)_3$ sample was irradiated at 248 nm and the products were analyzed by DEAE column chromatography using a linear gradient of triethylammonium hydrogen carbonate. Three major peaks were eluted: the first one appeared at about 0.25 *M* salt and was unreacted $d(pA)_3$; at 0.32 and 0.34 *M* salt, two adjacent but clearly separated peaks were observed. Analysis of their uv absorbance and CD spectra showed a close similarity to the spectra obtained for the $d(pA)_2$ photoproduct (*cf.* Figure 7).

(9) K. H. Scheit and A. Holy, *Biochim. Biophys. Acta*, **149**, 344 (1967).

(10) A. J. Adler, L. Grossman, and G. D. Fasman, *Biochemistry*, **8**, 3846 (1969).

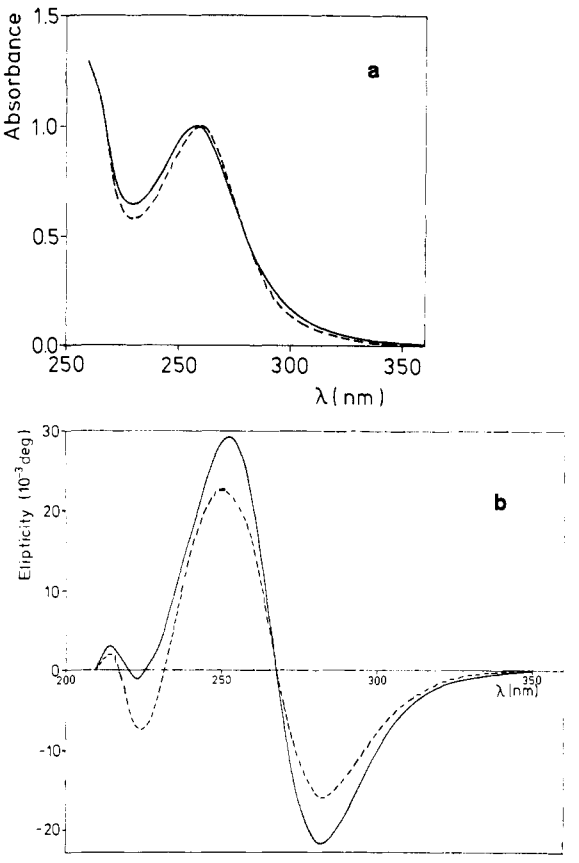

Figure 7. Uv absorbance (a) and CD spectrum (b) of the $d(pA)_3$ photoproducts isolated by DEAE chromatography at 0.32 *M* salt (——) and at 0.34 *M* salt (– –). Uv and CD spectra are normalized to concentrations resulting in absorbance 1.00 at the maximum around 260 nm (20°, aqueous solution, pH 6.3).

This result suggests that there are two isomeric photoproducts in $d(pA)_3$, probably d(pApApA) and d(pApApA).

(c) Quantum Yield as Function of the Chain Length and the Wavelength. The isolation of the photoproduct of $d(pA)_2$ and the characterization of its properties provide a basis for a quantitative analysis of the photoreaction. The CD spectra given in Figure 3 indicate a rather convenient possibility for such analysis: the highest amplitude within the CD spectrum of $d(pA)_2$ is observed at a wavelength, where the CD spectrum of the $d(pA)_2$ photoproduct is characterized by a zero amplitude. Thus the degradation of $d(pA)_2$ can be recorded specifically. Furthermore the trough of the $d(pA)_2$* CD spectrum appears at a wavelength where hardly any CD amplitude of $d(pA)_2$ is observed. Using these specific CD properties the concentrations of both $d(pA)_2$ and $d(pA)_2$* were evaluated at different times of a $d(pA)_2$ irradiation experiment (see Figure 8). In the initial phase of the irradiation the rate of $d(pA)_2$ degradation is close to the rate of $d(pA)_2$* production. At later stages of the irradiation the photodegradation of $d(pA)_2$* is observed to be the major photoprocess. According to the results given in Figure 7 the quantum yield of $d(pA)_2$* degradation should be less than that of $d(pA)_2$ degradation. Thus the quantum yield obtained from separate irradiation of $d(pA)_2$* is somewhat higher than that deduced from the analysis of the $d(pA)_2$ irradiation scheme. Further detailed studies

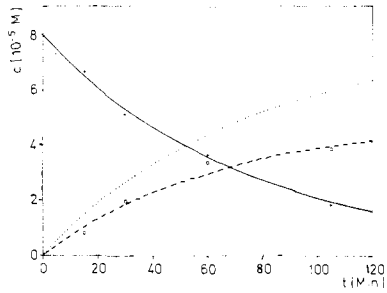

Figure 8. Concentration of $d(pA)_2$ (+, ——) and of $d(pA)_2^*$ (O, – – –) at different times of irradiation; (· · · ·) the concentration of $d(pA)_2^*$ that would be expected if $d(pA)_2$ would be converted to $d(pA)_2^*$ quantitatively.

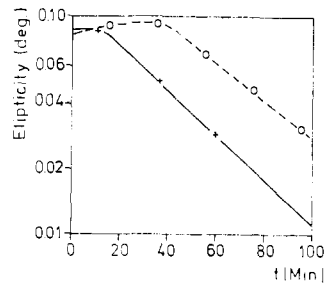

Figure 9. Logarithmic plot of the CD amplitude *vs.* irradiation time: poly(A) (λ 262 nm, O, – – –) and poly(dA) (λ 261 nm, +, ——) in 0.01 *M* sodium formate (pH 3.6).

will be necessary to elucidate the exact quantum yield of $d(pA)_2^*$ degradation.

In spite of this uncertainty of the $d(pA)_2^*$ photoyield the overall photoreaction scheme

$$d(pA)_2 \xrightarrow{h\nu} d(pA)_2^* \xrightarrow{h\nu} \text{products}$$

allows a satisfactory description of the experimental data. The quantum yield of $d(pA)_2$ degradation (calculated on the basis of the absorbed part of the irradiated light) is given in Table I together with the quantum yields of d(pA), $d(pA)_3$, $d(pA)_4$, and poly(dA). The evaluation of the quantum yields for $d(pA)_n$ with $n > 2$ was performed according to the same principle as described for $d(pA)_2$.

Table I. Quantum Yield of $d(pA)_n$ Degradation[a]

	n				
	1	2	3	4	∞
Quantum yield (10^{-3} mol/einstein)	<0.1	1.0	1.7	2.1	2.5

[a] Irradiation at room temperature in 0.1 *M* NaCl–0.1 *M* sodium cacodylate (pH 6.9) at 248 nm. Quantum yields for $n = 1$ and $n = \infty$ taken from ref 6.

Some irradiation experiments using $d(pA)_2$ were also performed at a series of different wavelengths. Although the changes of the spectral properties resulting from the irradiation, *e.g.*, at 280 nm were similar to those found after irradiation at 248 nm, some differences in the spectral changes were observed. These may be partly due to a difference in the action spectrum of $d(pA)_2$ and $d(pA)_2^*$ and partly due to a different intermediate, which seems to be induced by irradiation at 280 nm, indicated by a small negative CD effect centered around 320 nm and a small positive CD effect centered around 300 nm. These CD effects are observed in the initial phase of irradiations at 280 nm. At later stages of the irradiation the changes of the CD spectrum essentially correspond to those observed in irradiation experiments performed at 248 nm. Quantum yields at the different irradiation wavelengths were determined again from the decrease of the $d(pA)_2$ CD amplitude at 218 nm. The values given in Table II (calculated on the basis of the absorbed part of the irradiated light) demonstrate a rather small decrease of the quantum yield with increasing wavelength.

(d) Conformation and Solvent Dependence of the Photosensitivity. The difference of the photosensitivity in poly(A) and poly(dA) at neutral pH apparently is due to a difference of the single-strand conformation.[6] Since both poly(A) and poly(dA) undergo conformational changes, when titrated to acidic pH, these polymers represent convenient models for a further study of the conformation dependence of the photoreactivity. Poly(A) is known to form a double-stranded helical structure[11–13] at pH values below 6. Although in poly(dA) the titration behavior is different from that found in poly(A), evidence has been presented that poly(dA) forms an aggregated helical structure similar to that of poly(A) at low pH values.[10]

Table II. Quantum Yield of $d(pA)_2$ Degradation at Different Wavelengths[a]

	Wavelength (nm)				
	240	248	260	270	280
Quantum yield (10^{-3} mol/einstein)	1.1	1.0	0.8	0.5	0.6

[a] Irradiation at room temperature in 0.1 *M* NaCl–0.1 *M* sodium cacodylate (pH 6.9).

Both polymers were investigated in a 0.01 *M* sodium formate solution at pH 3.6. Under these conditions poly(A) and poly(dA) exhibit closely similar CD spectra. Upon irradiation the CD amplitudes decrease after some induction period, which is longer in poly(A) than in poly(dA) (*cf.* Figure 9). In both cases uv irradiation causes a simple reduction of the existent CD band without appearance of new CD effects. Thus the specific photoreaction observed in poly(dA) at neutral pH is inhibited at acidic pH. The photochemical damage induced in the polymers is also reflected in the uv spectra. In both polymers the photoprocess is indicated by the appearance of a new absorption band centered around 300 nm, while the main absorption band around 260 nm is strongly reduced (Figure 10). The induction period of irradiation observed in the CD spectra is also found in the uv spectra in both poly(A) and poly(dA).

In further experiments ApA and $d(pA)_2$ were irradiated in 0.01 *M* sodium formate (pH 3.6) solution. These oligomers do not form any double helical complex at the low concentrations (10^{-4} *M*) used in the irradiation experiment. Due to the protonation of the adenine bases, which leads to a reduced stability of stacked structures, the CD amplitudes in ApA and $d(pA)_2$ at pH 3.6 are rather small. Irradiation led to a

(11) A. Rich, D. R. Davies, F. H. C. Crick, and J. D. Watson, *J. Mol. Biol.*, 3, 71 (1961).
(12) V. Luzzati, A. Mathis, F. Masson, and J. Witz, *J. Mol. Biol.*, 10, 28 (1964).
(13) D. N. Holcomb and I. Tinoco, *Biopolymers*, 3, 121 (1965).

further decrease of these amplitudes. The absorbance spectrum of ApA and $d(pA)_2$ showed similar spectral changes as those observed in poly(A) and poly(dA) at pH 3.6. The uv doses required to induce these changes were also quite similar in oligomers and polymers. Thus the photoreaction observed at pH 3.6 seems to be due to the state of protonation more than due to the state of helix aggregation.

Finally some irradiation experiments were performed using $d(pA)_2$ in ethanol solution. In this solvent the stacked structure observed in aqueous $d(pA)_2$ solution cannot be formed as is indicated by the almost complete absence of CD amplitudes (*cf.* ref 10). Thus the irradiation process could be recorded by changes in the absorbance spectrum only. After some induction period, similar to that observed in the irradiations performed at pH 3.6, the absorbance spectrum exhibited changes corresponding to those shown in Figure 10. The induction periods observed for irradiations at pH 3.6 (*cf.* Figure 9) and in ethanol indicate that the corresponding photoreactions cannot be described by a single hit process (*cf.* ref 14).

These examples of irradiations may be sufficient to demonstrate that the photoreactions in adenylic acids are solvent dependent. The specific photoreaction observed in oligo- and poly(dA) at neutral pH was not observed in acidic nor in ethanol solution. The difference of the photoreactivity in ribo- *vs.* deoxyriboadenylic acid chains observed at neutral pH was not found at any similar extent in acidic solutions. Because of the far greater importance of the photoreactions in aqueous solutions of neutral pH, the photoreactions described in the present section were not analyzed further.

(e) Influence of the Photoreaction upon the Base-Pairing Reaction. It has been demonstrated in the previous investigation[6] that the photoreaction in poly(dA) inhibits its base-pairing capacity to the complementary polynucleotide poly(T). In the present investigation it was found that the photoproduct in oligo- and poly(dA) is characterized by a pK around 7.0 leading to additional negative charges on oligo- and poly(dA) nucleotides around neutral pH. These additional negative charges on the adenylic acid chains may be responsible for the reduction of the base-pairing stability observed in the experiments reported previously.

In order to test for the influence of these charges, base-pairing experiments using irradiated oligodeoxyadenylic acids have been performed at three different pH values. The base-paired helical complex used in these experiments was the triple-stranded structure formed from $d(pA)_4$ and poly(U).[15] In all cases, at pH 4.6, 6.9, and 8.75 (1 M NaCl and 0.066 M phosphate, 0.05M sodium cacodylate, and 0.05 M sodium borate, respectively), both the melting temperature and the degree of hypochromicity decreased with increasing degree of irradiation of $d(pA)_4$. These results demonstrate that the inhibition of the base-pairing reaction is not primarily due to a charge density effect.

Discussion

The present investigations allow some further insight into the mechanism of the specific deoxyadenylic acid photoreaction. Rather strong evidence has been collected that suggests a photoinduced reaction between adjacent deoxyadenylic acid residues leading to a chemical linkage between modified adenine bases. A final proof of this mechanism could be supplied by an elucidation of the chemical structure of $d(pA)_2$*. At present the proposal of this mechanism is based upon the chain length dependence of the photosensitivity and the properties of the $d(pA)_2$ photoproduct, such as the CD spectrum of $d(pA)_2$* in ethanol and the resistance of $d(pA)_2$* against phosphodiesterase.

The photoreaction in deoxyadenylic acid chains bears some similarity to the photodimerization of pyrimidine bases observed in oligo- and polynucleotides containing adjacent pyrimidine nucleotide residues. The formation of the pyrimidine dimers, the structure of the various species, and their properties have been investigated extensively.[1-5] The general mechanism of the photoreaction may be similar in deoxyadenylic acid chains. With respect to the properties of the photoproduct, however, an important difference is observed. Whereas the aromatic system of the pyrimidine bases is decomposed completely within the photoproduct, some aromatic system is maintained within the photoproduct obtained in deoxyadenylic acid chains. This difference may be easily explained by the existence of two aromatic systems within the adenine base. If the photoreaction between the adjacent ade-

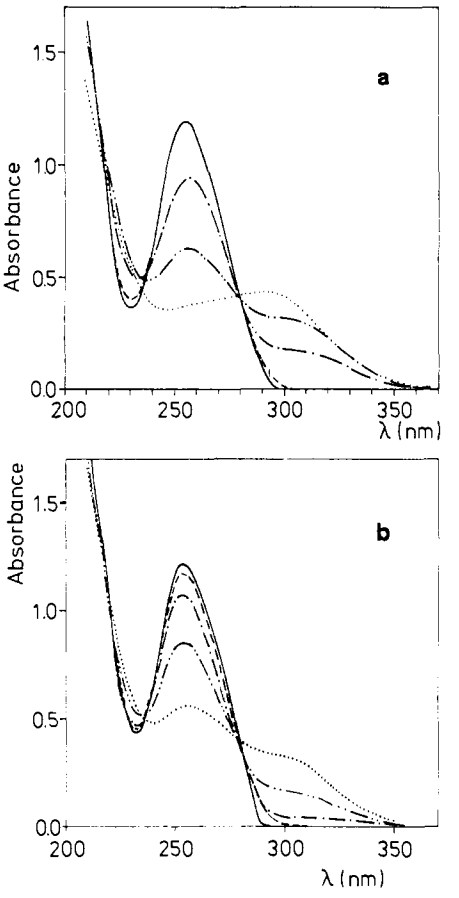

Figure 10. (a) Uv absorbance spectrum of poly(dA) in 0.01 M sodium formate (pH 3.6), 20° at various degrees of uv irradiation at 248 nm: 0 min (——), 10 min (– – –), 36 min (-·-·-), 60 min (-··-··-), 120 min (····). (b) Corresponding data obtained for poly(A): 0 min (——), 15 min (– – –), 35 min (-·-·-), 55 min (-··-··-), 95 min (···).

(14) H. Dertinger and H. Jung, "Molecular Radiation Biology," Springer-Verlag, West Berlin, 1970.
(15) M. N. Lipsett, L. A. Heppel, and D. F. Bradley, *J. Biol. Chem.*, 236, 857 (1961).

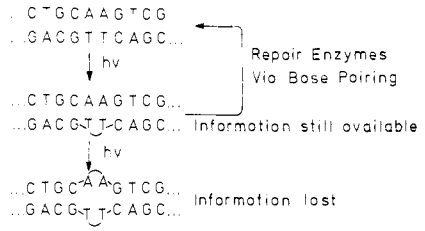

Figure 11. Schematic representation of DNA photolesions in complementary sites.

nine bases proceeds *via* the opening of one aromatic system of each base, the second aromatic system of each base may remain unaffected. Due to the presence of an aromatic system within the photoproduct, which is correspondingly characterized by a strong uv absorbance, photoreactions in deoxyadenylic acid chains cannot be detected by uv absorbance measurements as easily as, *e.g.*, the photodimerization of pyrimidine bases. Some types of photoreactions may even be rather difficult to detect by uv absorbance measurements. In such cases measurements of the circular dichroism would be expected to detect photodefects with much higher sensitivity.

Strong evidence for the proposed mechanism of the photoreaction in deoxyadenylic acid chains is obtained from the chain length dependence of the photoreactivity. A simple model based upon the reaction probability of an excited adenine with an adjacent adenine residue would predict a chain length dependence of photoreactivity according to the following scheme

$$P_1 = 0, P_2 = 1, P_3 = 1.33, P_4 = 1.5, P_\infty = 2$$

where P_n are photoreactivities in nucleic acid chains of chain lengths n. Although this scheme is too simple to describe the observed chain length dependence of the quantum yields correctly, it may serve to illustrate a possible major reason for the existence of this dependence. A chain length dependence of the nucleotide conformation may also give rise to some variation of the photoreactivity. Furthermore, transfer of excitation energy between adjacent nucleotide residues may be a process, that has to be considered for a more quantitative understanding of the photoreaction.

Finally the measuring procedure applied in the present investigation may give rise to some simulated chain length dependence in the measured quantum yields. The Cotton effects of single-stranded polynucleotides such as poly(dA) are due to the secondary structure induced by the stacking interactions between adjacent nucleotide residues. Thus a photoreaction between adjacent nucleotides will not only affect the stacking interaction between the reacting residues but at the same time influence the stacking interaction to their neighbors. Accordingly a single photoreaction in the deoxyadenylic acid chain may lead to the dissociation of three stacks accompanied by a corresponding reduction of the CD amplitude. This neighbor effect may lead to the simulation of a higher quantum yield than the "intrinsic" value. However, quantum yields obtained from CD measurements will accurately reflect the photosensitivity with respect to changes in the secondary structure.

The photoreaction analyzed in the present investigation merits particular attention from different points of view. One interesting aspect of this reaction is its high specificity which has been discussed already in a previous publication. It is very likely that the high specificity of the photoreaction is due to a specific difference of the conformations in single-stranded oligomers and polymers of deoxy- *vs.* riboadenylic acids.[6]

Another probably more important aspect of the photoreactivity in deoxyadenylic acid chains is the now evident possibility of a new type of photolesions in DNA (*cf.* Figure 11). Previous discussions of DNA photolesions have been based on the assumption that purine bases are almost completely photoresistant. Any photolesions induced in pyrimidine bases will damage one of two complementary sites only. This type of photolesions may be repaired by repair enzyme systems.[5,16] If, however, both complementary sites are damaged, the genetic information is lost and thus a repair becomes impossible (*cf.* Figure 11). The probability for this type of photolesion may be rather low, since two photoreactions have to be induced at the same site. On the other hand, the biological effect of this type of photolesion may be considerable, resulting in mutation or even inactivation (*cf.* ref 17).

Acknowledgment. Thanks are due to Drs. W. McClure, F. Eckstein, and K. H. Scheit for critical comments on the manuscript, to Mrs. A. Augustin for phosphate determinations, to Mr. H. Möhwald for esr measurements, and to Mr. J. Ronnenberg for technical assistance.

(16) J. Berndt, *Angew. Chem.*, **85**, 289 (1973).
(17) E. Freese, *Angew. Chem.*, **81**, 1 (1969).

Communications to the Editor

Specific Cation Participation in Alkali–Carbanion Initiated Epoxide Cleavage Reactions

Sir:

In a recent investigation on the cation solvating power of a number of small-ring ethers we found the rate of cleavage of ethylene oxide (EO) and similar epoxides by carbanions to be highly dependent on alkali cation. This was of particular interest since we were able to determine the extent and structure of ion pairing in the systems investigated making possible a correlation between ion pair structure and reactivity.

The fluoradenyl carbanion[1,2] (FD^-) was used in this

(1) H. Rapoport and G. Smolinski, *J. Amer. Chem. Soc.*, **82**, 934 (1960).
(2) T. E. Hogen-Esch, *ibid.*, **95**, 639 (1973).