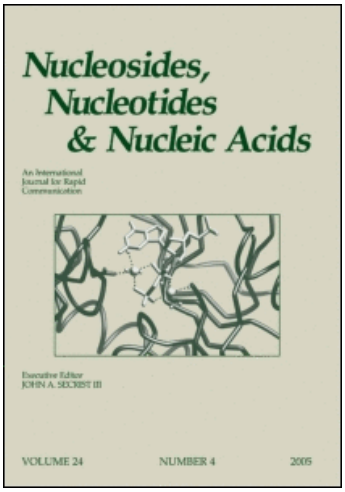

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Instability of the Phosphodiester-Amide Interribonucleotide Bond in Neutral Aqueous Solution

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INSTABILITY OF THE PHOSPHODIESTER-AMIDE INTERRIBONUCLEOTIDE BOND IN NEUTRAL AQUEOUS SOLUTION[1]

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Dedicated to the memory of Professor and Mrs. V. Bruckner

Abstract. $Ap(NH_2)dT$ was synthesized as a model compound for a study of the stability of the phosphodiester-amide interribonucleotide linkage in neutral aqueous solution, by photolyzing $A^{nBn}p(NH_2)dT$ in anhydrous p-dioxane. $Ap(NH_2)dT$ proved to be unstable even under anhydrous conditions and could not be isolated. It was rapidly decomposed in neutral aqueous buffer solution, at 25°C, to give A>p, dT and ApdT. Three unstable intermediates of this decomposition, $A>pNH_2$, $ApNH_2$ and A>pdT were detected by ^{31}P NMR. On the basis of the structure of the products, a mechanism is proposed for the decomposition.

INTRODUCTION

The phosphodiester-amide interribonucleotide bond is an unstable linkage not only in aqueous acidic, but also in aqueous alkaline solutions as a result of the 2'-hydroxyl group in the cis-alpha position[2-4]. For example, ribonucleotidyl nucleoside phosphoramidates (**1**, R_1=H) and their N(P)-alkylated derivatives (**1**, R_1=alkyl), are rapidly decomposed partly with chain cleavage to an equimolar mixture of ribonucleoside 2',3'-cyclic phosphates (**2**) and nucleosides (**3**)[Scheme 1, route A] and partly without chain cleavage to ribonucleotidyl nucleoside isomers (**4**) [Scheme 1, route B], even at moderately alkaline pH[2]. This instability towards both acid and alkali

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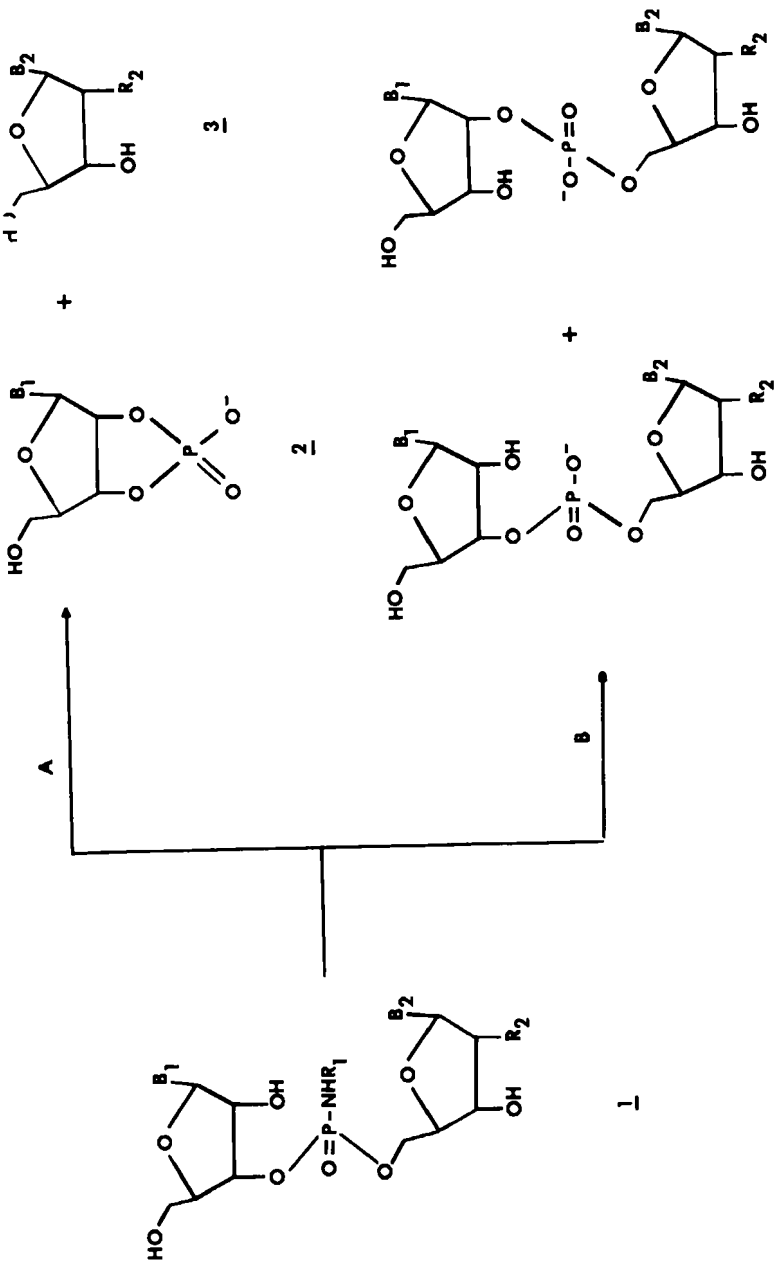


Scheme 1

indicates that a compound of type 1 could, if at all, be prepared in aqueous solution only via a 2'-blocked derivative, from which the 2'-blocking group can be removed under neutral conditions. We have selected the photolabile o-nitrobenzyl group for 2'-hydroxyl blocking[5] and attempted to synthesize $Ap(NH_2)dT$ [1, $R_1=R_2=H$, B_1=adenin-9-yl, B_2=thymin-1-yl] as a model compound, by photolyzing $A^{nBn}p(NH_2)dT$ under both neutral aqueous and anhydrous conditions. The paper describes these photolysis experiments and demonstrates the instability of $Ap(NH_2)dT$ even in buffered neutral aqueous solution. The synthesis and characterization of $A^{nBn}p(NH_2)dT$ are also presented.

EXPERIMENTAL

Materials

$A^{nBn}pdT$ was prepared from N^6-benzoyl-2'-O-(o-nitrobenzyl)-5'-O-(monomethoxytrityl)-adenosine 3'-phosphate p-chlorophenyl ester[6-8] and 3'-O-acetyl-thymidine[9] with 1-(p-toluenesulfonyl)-4-nitroimidazole[10]. ApdT was synthesized according to a literature procedure[11]. A, dT and A>p as well as 4-nitroimidazole and TPS were purchased from Sigma. DMF was distilled from P_2O_5 and stored over 4 Å molecular sieves (Merck). p-Dioxane was refluxed with sodium for 8 h, distilled and stored over 4 Å molecular sieves. All other chemicals were of reagent grade. For column chromatography DEAE-cellulose (DE-32, Whatman, HCO_3^- form) was used.

Methods

Operations with compounds having an o-nitrobenzyl group, were performed in dim light. Evaporations were carried out using a rotary evaporator (Rotavapor, Büchi) at about 0.2 kPa with a bath temperature of 30°C. Photolysis was performed in 10 cm long quartz tubes with 350 nm UV light (eight concentrically placed "Rayonet" RUL-3500Å lamps, The Southern N. E. Ultraviolet Co., Middleton, Conn.) for 1 h or in 1 cm quartz cuvettes with 366 nm UV light (Desaga

Uvis) for 4 h at 28°C. The distance of lamps was 12 cm from the tubes and 1 cm from the cuvettes.

TLC was performed on cellulose (F_{254}, Merck) in S_1, n-butanol - ethanol - H_2O (16:2:5, v/v); S_2, n-butanol - ethanol - 10^{-1} M TEAB, pH 7.5 (16:2:5); S_3, n-propanol - conc. NH_4OH - H_2O (11:7:2) and S_4, n-butanol - glacial acetic acid (2:1) mixtures, on PEI-cellulose (Polygram Cel 300 PEI/UV_{254}, Macherey-Nagel & Co.) in S_5, 10^{-1} M and S_6, $2x10^{-1}$ M aqueous NaCl solutions as well as on silica gel (60 F_{254}, Merck) chromatosheets in S_7, chloroform - methanol (85:15) mixture. Spots were visualized by UV absorption at 254 nm (Desaga Uvis). R_f values are tabulated in Table 1.

CD spectra were recorded with a JASCO J-40C spectropolarimeter with 10 mm quartz cells, in 10^{-1} M sodium phosphate buffer, pH 7.0, at 24°C. The solutions were $5x10^{-5}$ M in $A^{nBn}p(NH_2)dT$.

^{31}P NMR spectra of $4\text{-}5x10^{-2}$ M solutions in DMSO-d_6 were taken at 36.2 MHz on a JEOL FX 90Q NMR spectrometer with proton decoupling using external H_3PO_4 as reference. Positive chemical shift values are given for signals that occur downfield from H_3PO_4.

Molar quantities of $A^{nBn}pdT$ and $A^{nBn}p(NH_2)dT$ were determined by UV absorption at 260 nm in H_2O, by using ε=25,200. This value was calculated according to the approximate method of Cantor and Tinoco[12] from the equation $\varepsilon=\varepsilon_{dApT} + \varepsilon_{AnBn} - \varepsilon_A$,

Table 1

Compound	R_f values in TLC systems S_1	S_2	S_3	S_4	S_5	S_6	S_7
$A^{nBn}p(NH_2)dT$	0.57	0.40	--	0.65	0.53	0.54	0.19 0.25
$A^{nBn}pdT$	--	--	--	0.47	0.35	--	0.00
dT	0.63	0.63	0.77	--	--	0.87	--
A>p	0.16	0.36	0.68	--	--	0.45	--
ApdT*	0.11	0.26	0.60	--	--	0.59	--
	0.13	0.30	0.61	--	--	0.67	--

*Except system S_6, the faster moving compound is the 3'-5' isomer.

with the values of ε_{dApT}= 22,800[13], ε_{AnB} =17,800[7] and ε_{A}=15,400[13]. This approximation yields ε accurate to about 10%. For calculation of molar quantities of A>p and ApdT, ε_{A} and ε_{dApT} were used.

Synthesis of $A^{nBn}p(NH_2)dT$

A solution of $A^{nBn}pdT$ (145 µmol) in DMF (1.45 ml) containing tri-n-butylamine (69 µl, 290 µmol) was added dropwise to a mechanically stirred solution of TPS (87 mg, 290 µmol) in DMF (1.45 ml), with the exclusion of atmospheric moisture, during a period of 2 min, at 25°C. The homogeneous, pale-yellow solution was stirred for an additional 3 min, then was quickly poured into ice-cold 7.0 N NH_4OH (14.5 ml) under vigorous stirring. The heterogeneous mixture was evaporated to dryness. The residue was triturated with ether (30 ml). The solid was collected by centrifugation, washed with ether (2x30 ml) and dried. It was dissolved in H_2O - p-dioxane (5:1, v/v) mixture (12.0 ml) and percolated through a DEAE-cellulose column (2.0x64.0 cm). The column was washed with H_2O, elution rate: 15.6 ml/20 min/fraction. A large peak with a rear shoulder (fractions 13-30) and two small peaks (fractions 8-9 and 33-35, respectively) emerged. Fractions of the large peak which had absorbancies of A_{260}>10.0 (fractions 16-20), were pooled and evaporated to dryness. The residue was dissolved in H_2O - p-dioxane (5:1) mixture (2.0 ml) and freeze-dried to yield 32.0 mg (31.3%)of the mixture of $A^{nBn}p(NH_2)dT$ diastereoisomers in the form of a white, solid foam. The diastereoisomers were separated by preparative TLC using two successive developments in system S_7 [spotting from and elution by p-dioxane - ethanol (1:1, v/v) mixture, 1-2 mg/sheet].

^{31}P NMR, δ(ppm): 12.82 (R_f=0.25, 51.7% by UV), 12.50 (R_f=0.19, 48.3%). The CD spectra of diastereoisomers between 220 and 380 nm were identical, $[\Phi]x10^{-4}(\lambda_{nm})$: -0.59(325), 1.85(275), -0.36(250), -0.09(240) and -0.59(230). $A^{nBn}p(NH_2)dT$ diastereoisomers were quantitatively converted to $A^{nBn}pdT$ in 1.0 N aqueous HCl during 4 h, at 25°C (TLC in systems S_4 and S_5).

Photolysis of $A^{nBn}p(NH_2)dT$

About $5x10^{-4}M$ solutions of the mixture of $A^{nBn}p(NH_2)dT$ diastereoisomers in p-dioxane or ethanol - 10^{-2} M sodium phosphate buffer, pH 7.0 (1:1, v/v) were photolyzed for 4 h (photolysis mixtures Ia and II). Solutions in p-dioxane were photolyzed for 1 h as well (photolysis mixture Ib). After irradiation the solutions were immediately evaporated to dryness. The residues were dissolved in small amounts of ethanol, and the solutions were evaporated again. The residues thus obtained were analyzed by ^{31}P NMR and TLC, separated by preparative TLC and column chromatography, and subjected to hydrolytic degradations.

^{31}P NMR

The ^{31}P NMR spectrum of photolysis mixture Ib was recorded after standing in DMSO-d_6 solution 1 h, 2 h, and 4 h, at 25°C. The solution was then diluted with 10% of 1.0 M TEAB, pH 7.5, and the ^{31}P NMR spectrum was repeated after 20 min, 30 min and after 24 h standing in daylight. The ^{31}P NMR spectrum of the photolysis mixture Ia was recorded after 1 h standing, at 25°C. ^{31}P chemical shift values and percentage intensities of the signals are summarized in Table 2. A>p and ApdT were identified by observing the increase of the respective signal intensities on consecutive addition of authentic samples to the solution.

TLC

Photolysis mixtures Ia and II - each from about 10 A_{260} units of $A^{nBn}p(NH_2)dT$ - were analyzed by TLC in systems S_1, S_2 and S_6. Spotting was from DMF. In system S_1, two strong UV absorbing spots, X (R_f=0.25) and Y (R_f=0.21), were shown in mixture Ia. X was more intense than Y. In addition, mixture Ia contained dT and A>p. In mixture II, dT, A>p and ApdT were detected. dT, A>p and ApdT were formed on TLC in systems S_2 and S_6 of both photolysis mixtures, and an additional new spot, Z (R_f=0.21) appeared in system S_2 (only traces in mixture II). Both mixtures also contained

Table 2

^{31}P chemical shift values (ppm) and relative intensities of ^{31}P NMR signals (%) in the 1 h photolysis mixture of $A^{nBn}p(NH_2)dT$ in p-dioxane after standing 1 h (a), 2 h (b), and 4 h (c), at 25°C as well as those in 20 min (d), 30 min (d) and 24 h (e) after the addition of 10% 1.0 M TEAB, pH 7.5. Relative intensities are in parentheses. For details see text.

	$A>pNH_2$	A>pdT	A>p	$Ap(NH_2)dT$ + $A^{nBn}p(NH_2)dT$	$ApNH_2$		ApdT	
a	31.00 30.76 (11.6)			12.72-12.46 four unresolved signals (88.4)				
b	30.96 30.71 (15.4)	18.47 17.70 (8.1)	17.26	12.72, 12.62, 12.47 (76.5)				
c*	30.89 30.66 (28.7)	18.61 17.84 (13.9)	17.30	12.62, 12.55, 12.32 (57.4)				
d			17.20 (7.4)	12.38, 12.25, 12.05 (25.0)	9.09 (29.4)	7.71 (19.1)	0.27 (10.3)	-0.64 (8.8)
e**			17.31 (75.0)				-0.38 (25.0)	-0.53

*The same spectrum was observed for the 4 h photolysis mixture in p-dioxane.

**The spectrum was recorded after 24 h standing on daylight.

unreacted $A^{nBn}p(NH_2)dT$ as detected by systems S_1, S_2 and S_6.

Preparative TLC

The residue of photolysis mixture Ia from 80 A_{260} units of $A^{nBn}p(NH_2)dT$ was dissolved in DMF (80 µl), and the solution was applied onto 8 pieces of cellulose chromatosheets (12.0x20.0 cm). Spotting was 2 cm from the bottom edge of the chromatosheets in the form of 18 cm long, narrow lines. 4 chromatosheets were developed in system S_1, 4 chromatosheets were run in system S_2. The separated UV absorbing zones were cut out, scraped off and eluted with DMF (5.0 ml for each individual zone collected from 4 chromatosheets). A>p and ApdT were only partially resolved in system S_1 and were treated as one, single zone. The cellulose was centrifuged off, and the supernatants were evaporated to dryness. The residues were dissolved in DMF (10.0 µl for each), and the solutions were analyzed by TLC. Solutions of compounds present in zones X and Y, contained, in addition, dT, A>p and ApdT (system S_1). In "the solution of compound/s/ present in zone Z," only A>p was detected (systems S_1 and S_2). The solutions of dT, A>p and ApdT obtained after separation in system S_2 as well as those of dT and A>p + ApdT received after separation in system S_1, were identified by co-chromatography with authentic samples (systems S_2, S_3 and S_6). The solution of ApdT obtained in system S_2 contained traces of A>p, as well. ApdT (1.0 A_{260} unit) was quantitatively hydrolyzed by 0.3 N KOH (10.0 µl) to Ap and dT for 16 h, at 37°C (TLC in systems S_2, S_3 and S_6).

Column chromatography

Photolysis mixture II of 80 A_{260} units of $A^{nBn}p(NH_2)dT$ was evaporated to about one-fourth of its original volume, then was applied onto a DEAE-cellulose column (1.4x30.0 cm). Elution was performed with H_2O (100-120 ml), then with a linear gradient of TEAB, pH 7.5 (0→ 0.3 M, 1000 ml, elution rate 12.8 ml/fraction/20 min). The peaks emerged in the following order: dT + unidentified photoproduct/s/

(in fractions 5-6), $A^{nBn}p(NH_2)dT$ (in fractions 9-14), ApdT (double peak in fractions 24-27, 0.80 μmol) and A>p (in fractions 28-30, 1.81 μmol). The compounds were identified as above.

Hydrolytic degradations

The residue upon evaporation of photolysis mixture Ia from 50 A_{260} units of $An^{Bn}p(NH_2)dT$ was dissolved in an ethanol - p-dioxane (1:1, v/v) mixture (100 μl). 10 μl aliquots of this solution were diluted with equal volumes of 10^{-1} M aqueous sodium phosphate buffer, pH 7.0 (a) or 10^{-1} M TEAB, pH 7.5 (b). Mixtures a and b were analyzed by TLC in systems S_1 and S_2, after standing 0.5, 1 and 24 h, at 25°C. Compounds present in spots X and Y were quantitatively decomposed in both mixtures within 0.5 h. The compound present in spot Z was found after 1 h, but quantitatively degraded during a period of 24 h.

RESULTS

Synthesis and characterization of $A^{nBn}p(NH_2)dT$

$A^{nBn}p(NH_2)dT$ was prepared from $A^{nBn}pdT$ according to the same procedure used for the synthesis of $dTp(NH_2)dT$ recently[14] (Scheme 2). The product was an approximately 1:1 mixture of diastereoisomers, which were separated by adsorption chromatography.

The structure of the diastereoisomers was verified by CD and ^{31}P NMR spectroscopies as well as by acid hydrolysis. The CD spectra of diastereoisomers between 220 and 380 nm, were identical. They closely resembled the CD spectrum of A^{nBn} 7. The observed ^{31}P NMR signals were characteristic of a phosphodiester-amide structure[14-15]. The diastereoisomers

$$A^{nBn}pdT \xrightarrow[2.\ 7.0\ N\ NH_4OH,\ 1\ min,\ 0^{\circ}C]{1.\ TPS,\ Bu_3N,\ DMF,\ 5\ min,\ 25^{\circ}C} A^{nBn}p(NH_2)dT$$

Scheme 2

were quantitatively reconverted to $A^{nBn}pdT$ upon treatment with acid.

Photolysis of $A^{nBn}p(NH_2)dT$

The photolysis was performed either in anhydrous p-dioxane or in 50% ethanolic aqueous neutral buffer solutions. The photolysis product, $Ap(NH_2)dT$, could be detected only in anhydrous p-dioxane. $Ap(NH_2)dT$ was, however, unstable even in this solvent and decomposed to a mixture of dT, $A>pNH_2$ and A>pdT. $A>pNH_2$ and A>pdT were formed in an approximate molar ratio of 75:25. The decomposition was more than 50% in 4 h, at 25°C. As a result, the isolation of $Ap(NH_2)dT$ in pure form, could not be accomplished. $A>pNH_2$ and A>pdT were further degraded to A>p and ApdT by the action of aqueous neutral buffer solution, at 25°C. The decomposition of $A>pNH_2$ proceeded via $ApNH_2$ and was completed within 24 h. The degradation of A>pdT was finished within 30 min.

In 50% ethanolic aqueous neutral buffer solution, only dT, A>p and ApdT could be detected. An approximate molar ratio of A>p : ApdT - 70 : 30, was observed.

Structural verification of $Ap(NH_2)dT$ and its decomposition products

Structural assignments for $Ap(NH_2)dT$ and its decomposition products were based on the following arguments.

$ApNH_2$, ^{31}P NMR, δ(ppm): 9.09 and 7.71; spot Z in TLC system S_2.

1/ These two signals which appeared after the addition of TEAB, pH 7.5, in the ^{31}P NMR spectrum of $A^{nBn}p(NH_2)dT$ photolysis mixture in p-dioxane, are characteristic of a nucleoside phosphoramidate structure [16,17] and may result from nucleoside 2' - and 3' - phosphoramidate isomers.

2/ The compound was quantitatively converted by the action of TEAB, pH 7.5, to A>p, within 24 h, at 25°C (^{31}P NMR and TLC).

$A>pNH_2$, ^{31}P NMR, δ(ppm): 31.00 and 30.76.

1/ These two signals appearing in the ^{31}P NMR spectrum of $A^{nBn}p(NH_2)dT$ photolysis mixture in p-dioxane, are in

good agreement with the structure of A>pNH_2 diastereoisomer. A nitrogen-for-oxygen displacement at tetracoordinate phosphorus atom causes a downfield shift of 11-13 ppm[18,19], and $\delta_{A>p}$ was 17.20-17.30 ppm.

2/ The compound was quantitatively converted by the action of TEAB, pH 7.5, to ApNH_2, within 30 min, at 25 °C (^{31}P NMR).

A>pdT, ^{31}P NMR, δ(ppm): 18.61 and 17.84.

1/ These two signals appearing in the ^{31}P NMR spectrum of $A^{nBn}p(NH_2)dT$ photolysis mixture in p-dioxane after standing at 25°C, correspond with the structure of A>pdT diastereoisomers. The ^{31}P chemical shift values of nucleoside 2', 3'-cyclic phosphates and their O(P)-alkyl derivatives differ insignificantly [20-22]. On this basis, it is to be expected that A>p and A>pdT will have very close δ values.

2/ The compound was quantitatively converted by the action of TEAB, pH 7.5, to ApdT within 30 min, at 25 °C (^{31}P NMR).

Ap(NH_2)dT, ^{31}P NMR, δ(ppm): two signals between 12.46 and 12.72.

1/ The four signals between 12.46 and 12.72 ppm in the ^{31}P NMR spectrum of $A^{nBn}p(NH_2)dT$ photolysis mixture in p-dioxane, may be caused by Ap(NH_2)dT and unreacted $A^{nBn}p$-(NH_2)dT diastereoisomers. ^{31}P chemical shift values of these two compounds expectedly differ insignificantly, and $A^{nBn}p(NH_2)dT$ diastereoisomers have δ values of 12.82 and 12.50 ppm.

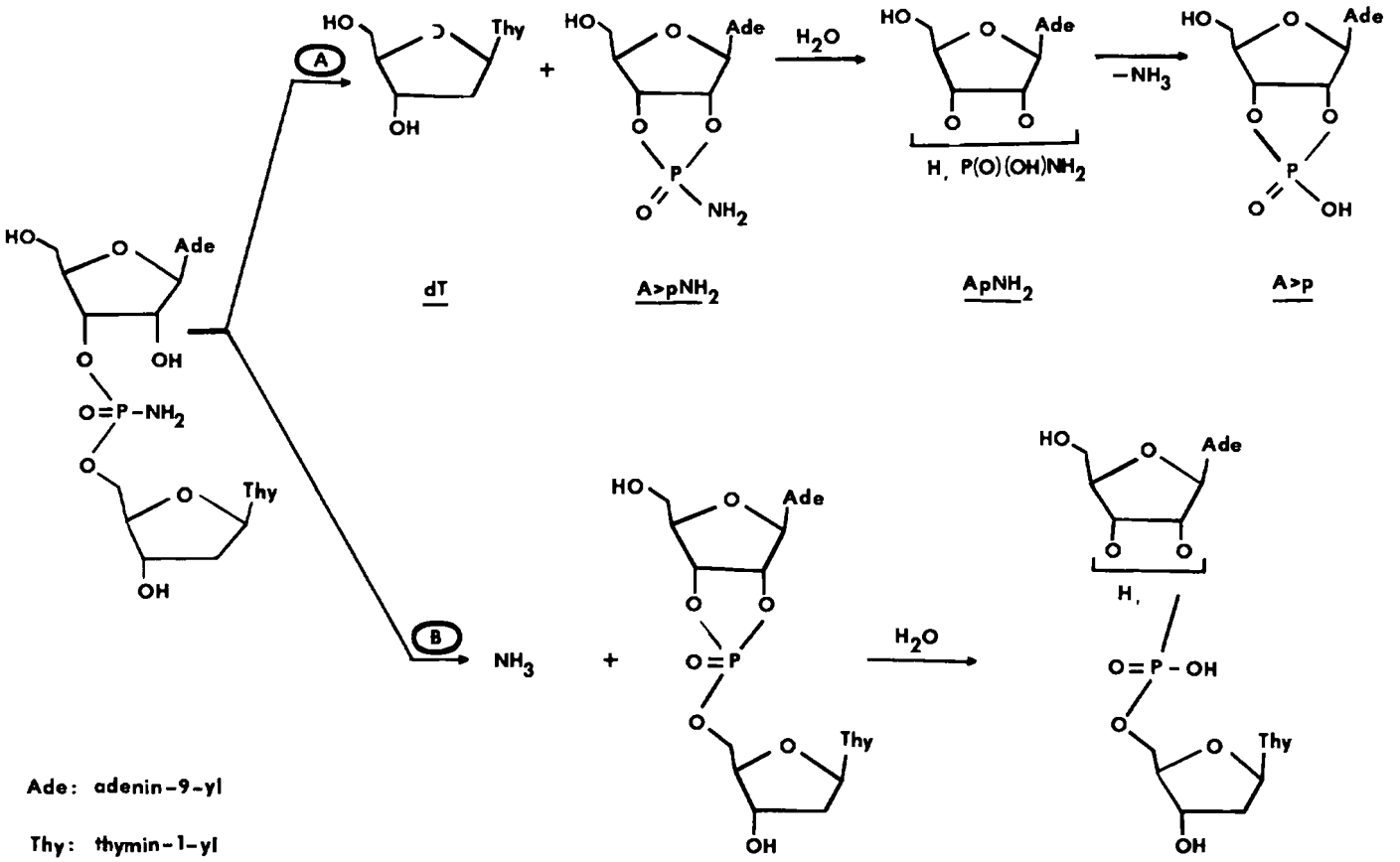
2/ On standing in anhydrous solvent, the compound was decomposed to a mixture of A>pNH_2 and A>pdT (^{31}P NMR).

TLC results suggest that A>pNH_2, A>pdT and Ap(NH_2)dT appear only partially resolved in system S_1, as spots X and Y.

dT, A>p and ApdT were identified by TLC and ^{31}P NMR comparison (A>p and ApdT) with authentic samples. The structure of ApdT was further verified by alkaline hydrolysis.

DISCUSSION

The results clearly indicate the instability of Ap(NH_2)dT in anhydrous p-dioxane and DMSO. Neutral aqueous buffer



Scheme 3

solutions markedly accelerate the decomposition. The failure of the isolation of $Ap(NH_2)dT$ by partition chromatography in n-butanol - ethanol - H_2O mixture and of its detection after ion-exchange TLC in aqueous sodium chloride solution or after partition TLC in n-butanol - ethanol - 10^{-1} M TEAB, pH 7.5, mixture, can thus be understood.

On the basis of the structure of degradation products, the mechanism of decomposition of $Ap(NH_2)dT$ may be formulated as follows (Scheme 3). The decomposition occurs by the action of the 2'-hydroxyl group and proceeds according to two parallel routes. According to route A, $Ap(NH_2)dT$ is degraded to dT and $A>pNH_2$. $A>pNH_2$ then hydrolyzes to $ApNH_2$, which cyclizes with the loss of NH_3 to A>p. According to route B, $Ap(NH_2)dT$ is transformed with the loss of NH_3 into A>pdT. A>pdT then hydrolyzes to ApdT[23]. In accordance with this scheme, $A>pNH_2$ and A>pdT were detected as first degradation products under anhydrous conditions, while $ApNH_2$ was formed by the action of neutral aqueous buffer solution.

The synthesis of $Up(NH_2)U$[24] and 2'-5' $Ap(NH_2)Ap(NH_2)A$[25] under anhydrous conditions, has been reported recently. $Up(NH_2)U$ was characterized by a ^{31}P chemical shift value of -0.50 ppm, which is more characteristic of a phosphodiester than for a phosphodiester-amide structure[4,14-15]. 2'-5' $Ap(NH_2)Ap(NH_2)A$ was not characterized at all. $Up(NH_2)U$ was used, among others, for enzymic studies[24]. 2'-5' $Ap(NH_2)Ap(NH_2)A$ was tested as protein synthesis inhibitor[25]. The stability of the compounds in aqueous solution was not reported. We believe that the observed instability of $Ap(NH_2)dT$ even in neutral aqueous buffer solution, is a general feature of oligoribonucleotide derivatives having phosphodiester-amide interribonucleotide bond/s/. On this basis, we may conclude that the application of these derivatives for biochemical studies is rather doubtful since, instead of them, their degradation products will probably be tested.

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Abbreviations: Ap(NH_2)dT, adenylyl-(3'-5')-thymidine (P→N)amide; $A^{nBn}p(NH_2)dT$, 2'-O-(o-nitrobenzyl)-adenylyl-(3'-5')-thymidine (P→N)amide; $A^{nBn}pdT$, 2'-O-(o-nitrobenzyl)--adenylyl-(3'-5')-thymidine; ApdT, adenylyl[2'(3')-5']-thymidine; A, adenosine; dT, thymidine; A>p, adenosine 2',3'-cyclic phosphate; TPS, 2,4,6-triisopropylbenzenesulfonyl chloride; DMF, N,N-dimethylformamide; TLC, thin-layer chromatography; TEAB, aqueous triethylammonium bicarbonate solution; dApT, 2'-deoxyadenylyl-(3'-5')-thymidine; A^{nBn}, 2'-O-(o-nitrobenzyl)-adenosine; Ap, adenosine 2'(3')-phosphate; dTp(NH_2)dT, thymidylyl-(3'-5')-thymidine (P→N)amide; A>pNH_2, adenosine 2',3'-cyclic phosphoramidate; A>pdT, adenylyl-(2',3'-5')-thymidine; ApNH_2, adenosine 2'(3')-phosphoramidate; DMSO, dimethylsulfoxide; Up(NH_2)U, uridylyl-(3'-5')-uridine (P→N)amide; 2'-5' Ap(NH_2)Ap(NH_2)A, adenylyl-(2'-5')-adenylyl-(2'-5')--adenosine (P→N)bis-amide.

REFERENCES AND NOTES

1. Presented in part at the International Conference on "Synthetic Oligonucleotides in Molecular Biology," Uppsala, Sweden, August 16-20, **1982**, Abstracts p. 51.
2. Shabarova, Z. A. Progr. Nucl. Acids Res. Mol. Biol, **1970**, 10, 145 and references.
3. Juodka, B. A.; Smrt, J. Collect. Czech. Chem. Commun. **1974**, 39, 963.
4. Tomasz, J.; Simoncsits, A. Tetrahedron Lett. **1981**, 22, 3905.
5. This blocking group has been introduced into the synthetic oligonucleotide chemistry by Ohtsuka, E.; Tanaka, S.; Ikehara, M. Nucleic Acids Res. **1974**, 1, 1351.

6. Ohtsuka, E.; Tanaka, S.; Ikehara, M. Chem. Pharm. Bull. **1977**, 25, 949.
7. Ohtsuka, E.; Wakabayashi, T.; Tanaka, S.; Tanaka, T.; Oshie, K.; Hasegawa, A.; Ikehara, M. Chem. Pharm. Bull. **1981**, 29, 318.
8. Ikehara, M.; Oshie, K.; Hasegawa, A.; Ohtsuka, E. Nucleic Acids Res. **1981**, 9, 2003.
9. Verheyden, J. P.; Moffatt, J. G. Synthetic Procedures in Nucleic Acid Chemistry, (Zorbach, W. W. and Tipson, R. S. eds.) **1968**, Vol. I, 383.
10. Gough, G. R.; Singleton, C. K.; Weith, H. L.; Gilham P. T. Nucleic Acids Res. **1979**, 6, 1557.
11. Michelson, A. M. J. Chem. Soc. **1959**, 3655.
12. Cantor, C. R; Tinoco, I., Jr. J. Mol. Biol. **1965**, 13, 65.
13. Cantor, C. R.; Warshaw, M. M.; Shapiro, H. Biopolymers **1970**, 9, 1059.
14. Tomasz, J. Nucleosides and Nucleotides **1983**, 2, 51.
15. Lebedev. A. V.; Rezvukhin, A. I. Izv. Sib. Otd. Akad. Nauk SSR, ser. khim. nauk, **1975**, 2, 149.
16. Tomasz, J. J. Carbohydrates-Nucleosides-Nucleotides **1981**, 8, 551.
17. Tomasz, J. Nucleosides and Nucleotides **1983**, 2, 63.
18. van Wazer, J. R.; Callis, C. F.; Shoolery, J. N.; Jones, R. C. J. Am. Chem. Soc. **1956**, 78, 5715.
19. Nielsen, M. L.; Pustinger, J. V., Jr. J. Phys. Chem. **1964**, 68, 152.
20. Blackburn, G. M.; Cohen, J. S.; Lord Todd Tetrahedron Lett. **1964**, 2873.
21. Drutsa, V. F.; Zarytova, V. F.; Knorre, D. G.; Lebedev, A. V.; Sokolova, N. I.; Shabarova, Z. A. Nucleic Acids Res. **1978**, 5, 185.
22. Zarytova, V. F.; Ryte, V. C.; Chernikova, T. S. Bioorg. Khim. **1977**, 3, 1626.
23. Scheme 3 is basically similar to that proposed by Shabarova for the alkaline degradation of N(P) alkylated diribonucleoside phosphoramidates[2].

24. Nemer, M. J.; Ogilvie, K. K. Tetrahedron Lett. **1980**, 21, 4149.
25. Jurovcik, M.; Smrt, J. FEBS Lett. **1981**, 133, 178.

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