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**Modified polynucleotides. VII. Impaired integrity of a synthetic DNA containing the antiherpetic agent 5-isopropyl-2'-deoxyuridine**

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ABSTRACT

5-Isopropyl-2'-deoxyuridine (ip<sup>5</sup>dU) was recently recognized as a clinically useful antiherpetic (HSV-1) agent. An ip<sup>5</sup>dU-containing polynucleotide, poly(dA-dT,ip<sup>5</sup>dU) was prepared to study how physical and bio-organic properties of the synthetic DNA model poly(dA-dT) would change upon partial substitution of thymidine. Synthesis was carried out with DNA polymerase enzyme and the polymers contained 7-9 % of ip<sup>5</sup>dU. It proved to be less thermostable than poly(dA-dT) and the transition width was highly increased. Although it was a very efficient template for DNA polymerase enzyme, template activity for RNA synthesis was strongly reduced by the presence of ip<sup>5</sup>dU. Diminished stability against enzymic degradation, especially against single-strand-specific Nuclease S<sub>1</sub> was also observed.

INTRODUCTION

A great number of 5-substituted 2'-deoxyuridines is known to possess selective anti-herpesvirus (HSV 1) activity (see reviews 1,2). The selective effect of these thymidine analogs is thought to be accomplished in a similar way: the analog is phosphorylated only in HSV 1-infected cells up to 5'-triphosphate derivative that may become a competitive inhibitor of dTTP incorporation and also a substrate of the viral DNA polymerase enzyme. Effects of incorporated analog on DNA properties are supposed to be mainly responsible for anti-HSV 1 action in most cases(1-3). One of the most effective and the best studied compound is (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU or bv<sup>5</sup>-dU)(2-7). It is incorporated in vitro and in vivo into the DNA of HSV 1(4,5), this DNA becomes labile (4) and HSV 1 polypeptide expression also alters (6,7).

Model studies with poly(dA-dT) analogs, where thymidine was replaced with bv<sup>5</sup>dU also showed changes in physical and

bio-organic properties, compared to poly(dA-dT) (8,9). These changes can partially be related to the alterations observed in vivo.

5-Isopropyl-2'-deoxyuridine (iPDU or ip<sup>5</sup>dU) was also described a few years ago as an anti-HSV 1 compound (1,10). Based on in vitro results it belonged to the group of weak inhibitors of HSV 1 multiplication (1). Surprisingly, in in vivo and later also in clinical trials it proved to be an efficient drug, and this difference is explained presently by the very high degree of stability of N-glycosidic bond against cleavage by mammalian thymidine and uridine phosphorylase enzymes (11).

According to the above theory on possible mode of action of antiherpetic thymidine analogs an ip<sup>5</sup>dU-containing virus DNA should be synthesized in vivo. To model properties of such a DNA we prepared an ip<sup>5</sup>dU-containing poly(dA-dT) type copolymer whose synthesis and some physical and bio-organic properties are presented here.

### EXPERIMENTAL

#### Enzymes and substrates

Klenow fragment enzyme of *E. coli* DNA polymerase I (5700 units/mg), RNA polymerase from *E. coli* (1200 units/mg), pancreatic DNase, snake venom phosphodiesterase and nuclease S<sub>1</sub>, dTTP and dATP were from Boehringer-Mannheim GmbH. [<sup>3</sup>H]dATP (17 Ci/mmol) was purchased from New England Nuclear and [<sup>3</sup>H]ATP (27 Ci/mmol) was from the Radiochemical Center, Amersham.

5-Isopropyl-dUTP was prepared as described earlier (12). [2-<sup>14</sup>C]dTTP (2 mCi/mmol) and [2-<sup>14</sup>C]ip<sup>5</sup>dUTP (27.1 mCi/mmol) were synthesized according to our earlier reports (12,13). Poly-(dA-dT) was from Boehringer-Mannheim GmbH.

#### Methods

Ultraviolet spectra, molar absorbance and thermal transition determinations were carried out with the use of a Specord UV VIS spectrophotometer essentially as described earlier (9). Template activity assays were performed as described in previous publications (9,14), exact conditions are detailed in Figure legends.

Degradation experiments were carried out in reaction mixtures of 90 μl (15) that contained 0.2 OD<sub>260</sub> units of the copol-

ymers corresponding to 0.33 mM(P) in the case of poly(dA-[<sup>14</sup>C]dT). Reaction was started by addition of the enzyme, and was followed by 10  $\mu$ l samples taken at times indicated in Figures. For DNase I (bovine pancreas) (40 ng/assay) 0.1 M sodium acetate (pH 5) and 5 mM MgCl<sub>2</sub> were applied. Snake venom phosphodiesterase (1.2  $\mu$ g/90  $\mu$ l) reactions were carried out in 0.1 M TRIS.HCl (pH 8.2) and 6 mM MgCl<sub>2</sub>, and Nuclease S<sub>1</sub> from *Aspergillus oryzae* (EC 3.1.30.1) (0.25  $\mu$ g/assay) was tested in 35 mM of sodium acetate (pH 4.5) and 30  $\mu$ M of ZnSO<sub>4</sub>. Radioactivity of acid-insoluble material of the zero minute sample was taken to be 100 % (0 % degradation).

### RESULTS AND DISCUSSION

The 5'-triphosphate of the antiherpetic agent 5-isopropyl-2'-deoxyuridine (ip<sup>5</sup>dUTP) has been tested earlier as a possible substrate (or inhibitor) of several DNA polymerase enzymes. Results with bacterial (13,16), calf thymus alpha- and beta-polymerases (16), HeLa cell alpha-polymerase and HSV 1-coded DNA polymerase (17) and HeLa cell beta-polymerase and HSV 2-induced DNA polymerase (18) were reported. It was a poor substrate of bacterial and cellular polymerases: initial rate of incorporation was under 15 % of that of the dTTP, whereas with HSV-coded enzymes relative rate was described as high as 40-50 % (17,18). Since it is a substrate of bacterial polymerases, the commercially available *E. coli* enzymes were used, as a tool, for the synthesis of polydeoxynucleotides.

De novo polymerization (without exogen template-primer) of dATP and ip<sup>5</sup>dUTP with *E. coli* DNA polymerase I holoenzyme was unsuccessful earlier (13,19), i.e. no high-molecular weight material could be isolated. In a primed system we examined now if ip<sup>5</sup>dUTP can be incorporated into a DNA in a good yield if natural substrate, dTTP, is also present in the reaction mixture. (Without dTTP net synthesis of DNA product was very low.) This system consisted of the synthetic DNA template-primer, poly(dA-dT) and Klenow fragment DNA polymerase enzyme of *E. coli*.

Table 1 shows the results of experiments planned to elucidate conditions for preparation of a polynucleotide with high content of ip<sup>5</sup>dU in good yield. Ratio of dTTP and [<sup>14</sup>C]ip<sup>5</sup>dUTP

Table 1. Analytical experiments designed to elucidate dependence of composition of products on composition of the starting reaction mixture.<sup>x</sup>

Composition of the reaction mixture: dTTP (%) and [ <sup>14</sup> C]ip <sup>5</sup> dUTP (%)	Yield of the new copolymer based on [ <sup>3</sup> H]dAMP incorporation (nmoles) (%)	Incorporation of [ <sup>14</sup> C]ip <sup>5</sup> -dUMP (nmoles)	Ratio of ip <sup>5</sup> dUMP in place of dTMP in newly formed copolymer ( <sup>14</sup> C/ <sup>3</sup> H nmoles x 100) (%)	Ratio of ip <sup>5</sup> dUMP in total polymeric product (%)
100	1.68	0	0	0
75	1.47	0.18	12.2	4.4
50	1.02	0.27	26.5	7.5
25	0.67	0.32	47.8	9.8
0	0.14	0.14	100	5.1

<sup>x</sup>Mixtures of 50  $\mu$ l contained 60 mM of potassium phosphate (pH 7.4), 6 mM of MgCl<sub>2</sub>, 0.15 mM of [<sup>3</sup>H]dATP (5.4 mCi/mmol), 0.1 mM(P) of activated poly(dA-dT) [2.6 nmoles(P) in the 25  $\mu$ l sample taken], 0.4  $\mu$ g of Klenow fragment DNA polymerase enzyme and varying ratio of dTTP and [<sup>14</sup>C]ip<sup>5</sup>dUTP (27 mCi/mmol), as indicated in the Table, in a final concentration of 0.15 mM. 25  $\mu$ l samples were taken after 120 minutes of incubation at 37°C, spotted onto GF/C filters, precipitated in acid, washed, dried and counted.

was varied. When dTTP was present alone, 40 % of the [ $^3\text{H}$ ]dATP was polymerized during 2 hours of incubation. Yield of the polymeric product decreased with increasing ratio of [ $^{14}\text{C}$ ]ip $^5$ dUTP in the starting mixture. Ratio of ip $^5$ dUMP incorporated was about half of its ratio in the starting mixture. When no dTTP was present newly synthesized copolymer contained only ip $^5$ dUMP and dAMP. However, in the total copolymer, that contains also the starting template-primer, there was only 5 % of ip $^5$ dUMP present since yield was very low (8 % of the dTTP + dATP reaction) and only total copolymer can be isolated. Highest ratio of ip $^5$ dUMP in total copolymer was found to be around 10 % and this could be synthesized from a mixture that contained originally 25 % of dTTP and 75 % of ip $^5$ dUTP.

#### Preparation of poly(dA-dT,ip $^5$ dU)

For a larger scale synthesis of [ $^{14}\text{C}$ ]-labeled polymer the analytical mixture of the above composition was scaled up to 2.5 ml of final volume. For comparison, poly(dA-[ $^{14}\text{C}$ ]dT) was also prepared simultaneously. Highly activated poly(dA-dT) (16) was used as a template-primer [1.75 OD $_{260}$  units/2.5 ml, corresponding to 105  $\mu\text{M}$ (P)] with 0.54 mM of dATP and 0.57 mM of [ $^{14}\text{C}$ ]dTTP (2.0 mCi/mmol), and 0.54 mM of dATP, 0.144 mM of dTTP and 0.426 mM of [ $^{14}\text{C}$ ]ip $^5$ dUTP (27 mCi/mmol), respectively. 10  $\mu\text{g}$  of the Klenow enzyme was applied, and synthesis was followed both by the measurement of radioactivity of acid-insoluble polymeric product with 10  $\mu\text{l}$  samples taken and by ultraviolet hypochromism.

Poly(dA-[ $^{14}\text{C}$ ]dT) synthesis was terminated after 6 hours of incubation at 37°C by addition of NaCl and EDTA (13) and mixture was heated at 70°C for 10 minutes. Polymeric material was precipitated by ethanol, centrifuged, dried and redissolved in diluted buffer. It was then applied to a column (1.5 x 18 cm) containing Bio-Gel A-5m (BIO-RAD Labor., Richmond, exclusion limit for proteins given as 5 x 10 $^6$ ) and eluted with buffer. Ultraviolet absorbing material (at 260 nm) appearing in the void volume was collected, dialyzed and freeze-dried. This high-molecular weight final product was 5.4 OD $_{260}$  units with a specific activity of 0.043 mCi/mmol(P).

Poly(dA-dT[ $^{14}\text{C}$ ]ip $^5$ dU) synthesis was terminated as above

after 25 hours of incubation. High-molecular weight final product was 5.1 OD<sub>260</sub> units (2.9 times netsynthesis) with a specific activity of 2.58 mCi/mmol(P). Based on radioactivity 8.7 % of the total nucleosides of the modified copolymer was [<sup>14</sup>C]ip<sup>5</sup>dU, i.e. 17.4 % of thymidine was replaced by the analog. Thermal stabilities were determined (Table 2), and the radioactive polymers were used for the degradation experiments.

Preparative syntheses were repeated with non-labeled nucleotides in final volumes of 25 ml. 8.9 OD<sub>260</sub> units of activated poly(dA-dT) were used as template-primer in each of the two mixtures. Progress of synthesis was followed by hypochromicity measurements at 260 nm. Workup of mixtures was carried out as above. 122 OD<sub>260</sub> units of pure high-molecular weight poly(dA-dT) was obtained (71 % yield, 12.7 times net synthesis). Amount of poly(dA-dT,ip<sup>5</sup>dU) was 86 OD<sub>260</sub> units corresponding to 48 % yield and 8.7 times net synthesis. Base compositions were determined from enzymic (DNase I, venom phosphodiesterase and alkaline phosphatase) hydrolysate on Silica gel 60 F<sub>254</sub> plate with ethyl-acetate-methanol (8:2) mixture as described earlier (9). R<sub>f</sub> values for dA, dT and ip<sup>5</sup>dU were 0.28, 0.54 and 0.69, respectively. Based on quantitative determination of the nucleosides (Shimadzu TLC Scanner, CS-920) 7.1 % of the total nucleosides was ip<sup>5</sup>dU in poly(dA-dT,ip<sup>5</sup>dU), i.e. 14.2 % of thymidine was replaced. Spectra, molar absorbances and thermal stabilities were determined and these polymers were used for template activity measurements.

### Spectral properties

Ultraviolet spectra of poly(dA-dT,ip<sup>5</sup>dU) (not shown) was found to be very similar to that of the poly(dA-dT), presumably as a consequence of low percent of substitution and similar molar absorbances of dTMP and ip<sup>5</sup>dUMP (13), values in bracket are that of the poly(dA-dT):  $\lambda_{\max} = 262 \text{ nm}$  (262),  $\lambda_{\min} = 234 \text{ nm}$  (235),  $A_{\max/\min} = 2.74$  (2.89),  $A_{280/260} = 0.575$  (0.570), measured in 0.1 M phosphate buffer (pH 7) at 25°C. At 70°C  $\lambda_{\max}$  was shifted to 260 nm (260),  $\lambda_{\min}$  to 233 nm (233) with thermal hyperchromic values of 42 % (Table 2). The only difference observed was in the molar absorbance. It was determined from spectral data measured at 260 nm before and after degradation of the polynucleotides to nucleotides by DNase I and phosphodiesterase (9). To-

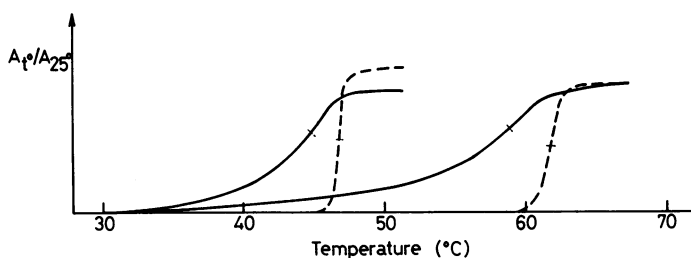


Figure 1. Melting curves in buffers of 0.024 M and 0.1 M  $\text{Na}^+$  concentrations of poly(dA-dT), (---), and poly(dA-dT, ip<sup>5</sup>dU), (—).

tal hyperchromic change was 76.8 % (85.2 %). Calculation was based on  $\epsilon_{260}$  values of 15300, 9300 and 9600 for dAMP, dTMP and ip<sup>5</sup>dUMP, respectively, taking into account of an 8 % substitution. In this way  $\epsilon_{260}$  was  $6950 \text{ M}^{-1}\text{cm}^{-1}$  (6650).

#### Thermal stability

Figure 1 shows heat-denaturation curves and Table 2 contains data of thermal transitions determined at two sodium ion concentrations of both the modified and unmodified polymers.

$T_m$ -value of poly(dA-dT) was decreased only by 2-3°C when 14-17 % of thymidine was replaced by 5-isopropyl-2'-deoxyuridine. Decrease in stability of poly(dA-dT) upon substitution of the methyl group of thymidine is known, e.g. with n-alkyl groups (ethyl-pentyl), and on this complete substitution desta-

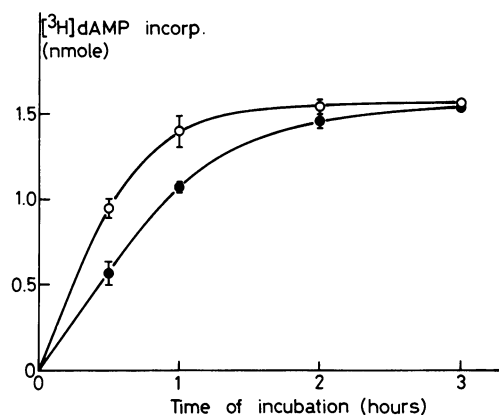
Table 2. Data of thermal transitions

$\text{Na}^+$ conc. (M)	poly(dA-dT)			poly(dA-dT, ip <sup>5</sup> dU)				
	$T_m$ (°C)	$\Delta T^a$ (°C)	$H_{262}^b$ (%)	% of ip <sup>5</sup> dU	$T_m$ (°C)	$\Delta T_m$ (°C)	$\Delta T^a$ (°C)	$H_{262}^b$ (%)
0.024	46.6	0.7	46.4	7.1	44.5	-2.1	4.8	38.5
0.10	61.7	1.5	41.7	7.1	58.8	-2.9	7.4	42.2
0.10 <sup>c</sup>	60.2	1.4	46.1	8.7	58.5	-1.7	6.6	43.2

<sup>a</sup> $\Delta T$  is the width of transition between 25 and 75 % of total thermal hyperchromicity

<sup>b</sup> $H_{262}$  is the total thermal hyperchromicity at 262 nm

<sup>c</sup>Data of the radioactive copolymer

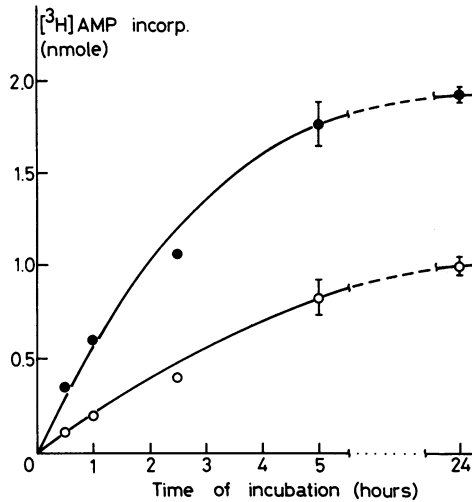


**Figure 2.** Time-course of DNA synthesis. Polymerase reactions were carried out in 60 mM of phosphate buffer (pH 7.4) and 6 mM of  $MgCl_2$ , using 0.1 mM(P) of polynucleotides, 0.2 mM of [ $^3H$ ]-dATP (5.4 mCi/mmo), 0.2 mM of dTTP and 0.4  $\mu g$  of Klenow DNA polymerase enzyme in final volumes of 100  $\mu l$ . 20  $\mu l$  samples were taken and analysed for radioactivity of acid-insoluble material. Reactions were carried out in triplicate. Poly(dA-dT), ●-●, and poly(dA-dT, ip<sup>5</sup>dU), o-o.

bilization was high, 11-16°C (20). Complete replacement of methyl group by 2-bromovinyl group could also be accomplished and decrease in  $T_m$ -value was 7°C (9). Destabilizations were explained by the theory of hydrophobic destabilization (20) that can also be applied for the present case. However, a further effect can be seen here: the highly increased transition width.

The most striking feature of the curves is this decrease in cooperativity of melting of poly(dA-dT, ip<sup>5</sup>dU) ( $\Delta T$ -value). (Renaturation on cooling was complete in all cases, curves not shown.) The increased  $\Delta T$ -values may point to the steric effect of the bulky isopropyl side chain, i.e. insertion of a 5-isopropyluracil base between two adenine bases in a polynucleotide chain can not be without any effect on the secondary structure (21). It leads to the distortion of the helix, which in turn, may lead to the formation of loops and/or unpaired ends. This may be reflected in the increased width of transition and is further supported by the increased sensitivity to Nuclease  $S_1$  enzyme (see later). Similar findings were observed with poly-





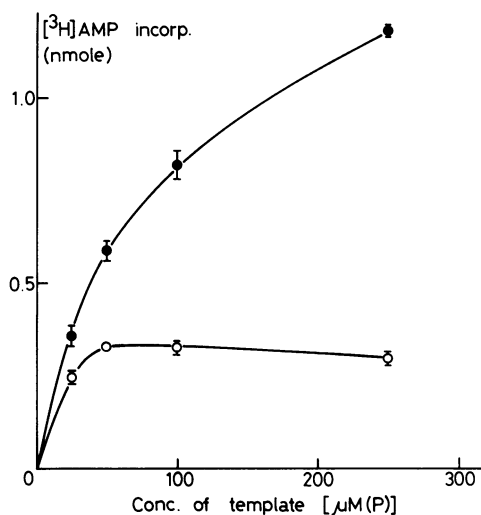
**Figure 3.** Time-course of RNA synthesis on poly(dA-dT), ●-●, and poly(dA-dT, ip<sup>5</sup>dU), ○-○, templates. Reactions were carried out in 40 mM TRIS.HCl (pH 7.9) and 10 mM MgCl<sub>2</sub> as described earlier (9,14) in 100 μl volume using 2.4 μg of *E. coli* RNA polymerase enzyme with 0.2 mM of [<sup>3</sup>H]ATP (5.9 mCi/mmol), 0.2 mM UTP and 0.2 mM (P) of the templates. 10 μl samples were analysed for radio-activity of the acid-insoluble polymeric product.

(dA-bv<sup>5</sup>dU), where bv stands for 2-bromovinyl group, but the effect ( $\Delta T$ ) was smaller (9).

Alteration in the secondary structure of DNA implies a series of consequences. From these, replication and transcription template activities and rate of enzymatic hydrolysis were examined, the former two with bacterial enzymes. Therefore, results are not intended to be extrapolated directly to virus-infected in vivo systems. However, since a comparison with unmodified poly(dA-dT) was always used the differences observed may suggest the nature of changes in an analog-containing viral DNA, as well.

#### Template activity for DNA synthesis

Time-course of replication ([<sup>3</sup>H]dATP + dTTP copolymerization) on poly(dA-dT, ip<sup>5</sup>dU), in comparison with poly(dA-dT), catalyzed by the Klenow fragment enzyme of *E. coli* DNA polymerase I is shown on Figure 2. Initial rates were different: the modified copolymer exhibited higher activity. Measuring the dependence



**Figure 4.** Dependence of transcription rate on concentration of poly(dA-dT), ●-●, and poly(dA-dT,ip<sup>5</sup>dU), o-o. 1.2  $\mu\text{g}$  of *E. coli* RNA polymerase was applied in a final volume of 50  $\mu\text{l}$ . Samples of 25  $\mu\text{l}$  were taken at 30 minutes of incubation at 37°C.

of initial incorporation rate on the concentration of polynucleotides, again, ip<sup>5</sup>dU-containing poly(dA-dT) is a more active template than poly(dA-dT) (curves not shown).

In a similar way, increased template activity for replication was also observed with a series of poly(dA-r<sup>5</sup>U) copolymers, where r stands for n-alkyl, (14) and also with poly(dA-bv<sup>5</sup>dU) (9). Accordingly, these substituents in position 5 of the uracil base do not produce any steric hindrance to the DNA polymerase enzyme and the structural and conformational changes caused by the presence of the analogs seem to be favourable, as well.

#### Template activity for RNA synthesis

Figure 3 shows time-course of transcription ([ $^3\text{H}$ ]ATP + UTP polymerization) by *E. coli* RNA polymerase enzyme on poly(dA-dT,ip<sup>5</sup>dU) in comparison with poly(dA-dT) template. Contrary to the observations with DNA polymerase enzyme, the ip<sup>5</sup>dU-containing poly(dA-dT) proved to be less efficient template than poly(dA-dT). Relative rate was 32 % at 30 minutes of incubation and did not exceed 52 % at 24 hours. A similar feature can be seen on

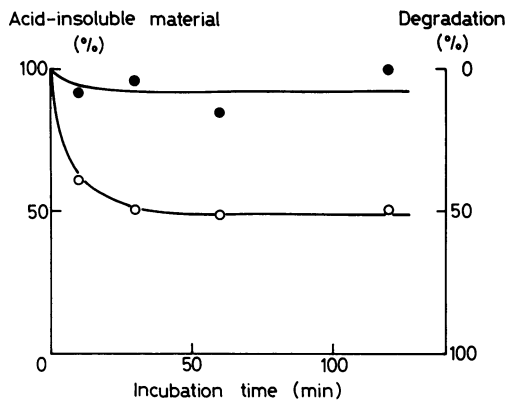


Figure 5. Hydrolysis of poly(dA-[<sup>14</sup>C]dT), ●-●, and poly(dA-dT, [<sup>14</sup>C]ip<sup>5</sup>dU), ○-○, by single-strand-specific S<sub>1</sub> nuclease.

Figure 4. At any template concentration initial rate of transcription was lower on the modified copolymer. Since molecular weights were in the range of millions diminished and limited transcription may come from either a direct effect of the bulky isopropyl group or, more probably, from the changes of the secondary structure. A very similar behavior was observed earlier also with poly(dA-bv<sup>5</sup>dU) (9). The limited transcription observed with isolated enzyme may be relevant to the alterations of HSV 1 polypeptide expression in vivo caused by (E)-5-(2-bromovinyl)-2'-deoxyuridine (6,7).

#### Enzymatic hydrolysis

Relative stability of poly(dA-dT, [<sup>14</sup>C]ip<sup>5</sup>dU) against enzymatic degradation was examined in three different enzyme systems: pancreatic DNase, snake venom phosphodiesterase and single-strand-specific Nuclease S<sub>1</sub> (Figure 5). Data obtained with the first two enzymes are summarized in Table 3.

Poly(dA-dT, ip<sup>5</sup>dU) turned out to be less stable than poly(dA-dT) against degradation enzymes studied. The observations with single-strand-specific enzyme Nuclease S<sub>1</sub> were especially interesting. More than 90 % of poly(dA-dT) remained acid-insoluble after treating with this enzyme for 120 minutes, whereas around 50 % of poly(dA-dT, ip<sup>5</sup>dU) was degraded to acid-soluble product during the same period of time. Doubling the concentration of Nuclease S<sub>1</sub> gave the same results with poly(dA-dT),

Table 3. Enzymatic hydrolysis of the copolymers

Copolymer	Pancreatic DNase		Snake venom phosphodiesterase	
	50 % digestion time (min.)	rel. stability (%)	50 % digestion time (min.)	rel. stability (%)
poly(dA-[ <sup>14</sup> C]dT)	38	100	24	100
poly(dA-dT, [ <sup>14</sup> C]ip <sup>5</sup> dU)	17	45	7	29

while degradation of poly(dA-dT,ip<sup>5</sup>dU) leveled off at 70 % during the same period of incubation time. This is in agreement with the conclusion drawn from the decrease in cooperativity of melting, i.e. there are much more single stranded region in the modified copolymer than in poly(dA-dT).

On the other hand, poly(dA-r<sup>5</sup>dU) copolymers, where r = n-alkyl from ethyl to pentyl, proved to be more resistant to enzymatic degradation than poly(dA-dT) (15). The only exception found was with the phosphodiesterase enzyme in the case of r = ethyl and n-propyl. Poly(dA-bv<sup>5</sup>dU) proved to be more stable than poly(dA-dT) against pancreatic and spleen DNases and micrococcal nuclease (9) whereas it was less stable against venom phosphodiesterase and behaved in a very similar manner against Nuclease S<sub>1</sub> as poly(dA-dT,ip<sup>5</sup>dU) (unpublished observation). This may be in connection with the in vivo observed instability of HSV 1 DNA containing bv<sup>5</sup>dU (2,4). In this way ip<sup>5</sup>dU exerts the same effects as bv<sup>5</sup>dU at molecular level (on T<sub>m</sub>, ΔT, inhibition of transcription and decreased stability against enzymic degradation) but at much lower degree of incorporation.

To summarize, a new modified DNA analog containing the anti-herpetic thymidine analog 5-isopropyl-2'-deoxyuridine was prepared with DNA polymerase enzyme. The poly(dA-dT,ip<sup>5</sup>dU)-s contained 7-9 % of 5-isopropyl-2'-deoxyuridine. Compared to poly(dA-dT), its template activity for DNA synthesis was not ruined at all. Data of thermal transition, however, hinted at the presence of high ratio of single stranded region in the duplex,

and this was confirmed by a highly decreased resistance against single-strand-specific enzyme, Nuclease S<sub>1</sub>. It proved to be less stable also against other enzymes of nucleic acids' degradation. Furthermore, template activity for RNA synthesis of poly(dA-dT,ip<sup>5</sup>dU) was significantly decreased probably as a consequence of the distorted secondary structure of the modified copolymer, compared to poly(dA-dT).

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