Volume 10 Number 19 1982 Nucleic Acids Research

Modified polynucleotides. VI. Properties of a synthetic DNA containing the anti-herpes agent (E)-5-(2-bromovinyl)-2'-deoxyuridine

J.Sági, A.Czuppon, M.Kajtár*, A.Szabolcs, A.Szemzö and L.Ötvös

Central Research Institute for Chemistry, Hungarian Academy of Sciences, PO Box 17, 1525 Budapest, and *Institute of Organic Chemistry, L.Eötvös University, 1088 Budapest, Muzeum krt. 4/b, Hungary

Received 17 August 1982; Accepted 10 September 1982

ABSTRACT

A new modified polydeoxynucleotide, a copolymer of nucleotides of 2'-deoxyadenosine and the very efficacious anti-herpesvirus agent (E)-5-(2-bromovinyl)-2'-deoxyuridine was synthesized with E. coli DNA polymerase I enzyme. It is characterized by its physical (absorption and circular dichroism spectra, thermal transition, sedimentation analysis) and bioorganic (template activity, stability) properties. Compared to poly[d(A-T)], the modified polydeoxynucleotide had a lower thermal stability but exhibited higher stability against DNases and higher template activity for DNA synthesis. Template activity for RNA synthesis of this template was, however, poor and extent of AMP and UMP incorporation was limited as well.

INTRODUCTION

(E)-5-(2-bromovinyl)-2'-deoxyuridine (bv^5dU) is one of the most effective anti-herpesvirus (type I) nucleoside analogs, in vitro and in vivo [1]. It is phosphorylated in virus-infected cells [2] and the 5'-triphosphate, bv^5dUTP, is able to replace dTTP to different extent in DNA synthesis catalyzed by purified virus--induced, mammalian or bacterial DNA polymerases [3-7]. It was actually incorporated into DNAs of herpes simplex virus type I and host cell in culture, whereas DNA of uninfected cells was not affected [6,7]. Whether there is a correlation between the antiviral activity and the incorporation of bv^5dU into DNA of the virus is not yet known. We wished to model this relation by preparing a poly[d(A-T)] type new modified synthetic DNA containing bv^5dU instead of thymidine. Comparison of its properties with poly[d(A-T)] may provide an insight into the possible changes of properties of a DNA after incorporation of bv^5dU.

© IRL Press Limited, Oxford, England.

0305-1048/82/1019-6051$ 2.00/0

EXPERIMENTAL

Materials

E. coli DNA polymerase I holoenzyme (6670 units/mg) and the Klenow fragment enzyme (5680 units/mg), E. coli RNA polymerase (1170 units/mg), pancreatic DNase, snake venom phosphodiesterase, Staphylococcus aureus micrococcal nuclease and alkaline phosphatase, dATP and dTTP, poly[d(A-T)] and poly(dA)·poly(dT) were the products of Boehringer GmbH, Mannheim. Spleen DNase was from Calbiochem, UTP and poly(U) from Reanal, Hungary. [^{3}H]dATP (17 Ci/mmol) was purchased from New England Nuclear, [^{3}H]ATP (27 Ci/mmol) from the Radiochemical Center, Amersham. [2-^{14}C]dTTP (2 mCi/mmol), bv^{5}dUTP and [2-^{14}C]bv^{5}dUTP (18.2 mCi/mmol) were prepared as described previously [5].

Spectra and thermal transition

Ultraviolet spectra were measured in a Specord UV VIS recording spectrophotometer (Zeiss, GDR) equipped with a modified jacketed cell holder. For the determination of thermal transition increase of temperature (0.35°C/min.) was regulated by a linear temperature programmer. A platinum resistance thermometer (Type 1 Pt 100 Gx 613, Heraeus GmbH, Hanau) was used for the measurement of temperature in the cell. Both temperature and absorbance were digitally displayed (Digital thermometer, DARC and Digital voltmeter, DTV-5, Ganz, Hungary).

Circular dichroism spectra were recorded in a Jobin-Yvon Dichrographe III spectrometer (Roussel-Jouan, France) equipped with a programmable thermostating unit.

Sedimentation analysis

Sedimentation measurements were carried out at 30,000 rev./min. and 25°C in a Beckman model E-HT ultracentrifuge equipped with ultraviolet optics. The polynucleotide sample was sedimented in 10 mM TRIS·HCl (pH 7.4), 1 mM EDTA solution at concentrations of 25, 35 and 50 µg/ml.

Determination of molar absorbance and base composition

Molar extinction coefficient (ϵ/P/) of the polymer was determined by measuring hyperchromic change upon degradation to nucleosides with nucleases. 80 µg of polynucleotide in 2.5 ml of 10 mM phosphate buffer (pH 7.0) and 5 mM $MgCl_2$ was digested with 20 µg of pancreatic DNase, 10 µg of snake venom phosphodiesterase and fi-

nally with 20 μg alkaline phosphatase. For base composition analysis of the hydrolysate proteins were precipitated with ethanol, centrifuged, and the solution was freeze-dried. Redissolved material was spotted onto Kieselgel 60 F_{254} DC-Alufolien (Merck) plate and developed with ethylacetate-methanol (8:2) mixture. R_f value for dA was 0.22, for bv^5dU 0.73. Quantitative determination of nucleosides was carried out in a Shimadzu High Speed TLC Scanner (CS-920) at 260 nm.

Triple strand formation

Complex formation with poly(U) was studied by mixing 1.2 ml of 100 μM(P) poly[d(A-T)], poly(dA)·poly(dT) or poly[d(A-bv^5U)] with 1.2 ml of 50 μM(P) poly(U) in a 1 cm light path cell at 25°C. Spectra were recorded before and after mixing and after 5 hours of incubation at 37°C.

Measurement of template activity for DNA synthesis

Template activity was determined essentially as described previously [8]. 250 μM of [^{3}H]dATP (18.6 dpm/pmol) and 250 μM of [^{14}C]-dTTP (3.5 dpm/pmol) were used. For the time-course experiments mixtures of 82 μl were used that contained 0.4 μg of the Klenow enzyme and 100 μM(P) of the template-primer, and 10 μl samples were taken for acid-insolubility test. When measuring dependence of replication rate on template concentration, template-primers of 25-350 μM(P) concentration were applied with 0.2 μg of enzyme in 40 μl volume. 25 μl samples were taken and spotted onto GF/C filters at 20 minutes of incubation at 37°C. Limited synthesis was carried out in the presence of [^{3}H]dATP alone in 40 μl with 200 μM(P) polynucleotide. 25 μl samples were taken at 60 minutes.

Template activity for RNA synthesis

Measurements were carried out as described in a previous paper [8]. [^{3}H]ATP (12.5 dpm/pmol) and UTP were used in 200-200 μM concentrations. For the determination of time-course of RNA synthesis 12.4 μg of RNA polymerase and 200 μM(P) of template were applied in 82 μl volume, and 10 μl samples were taken. For the concentration-dependence experiments 6.2 μg of enzyme was used in 40 μl volume, and 25 μl samples were taken at 10 minutes of incubation at 37°C for acid-insolubility test. To determine inhibition of RNA synthesis on poly[d(A-T)] template of 100 μM(P) by poly-

[d(A-bv^{5}U)] of different concentrations, 40 μl volume reactions were used.

Enzymatic hydrolysis

Nuclease reactions were carried out in 90 μl of reaction mixtures that contained 175 μM(P) of double-labelled polydeoxynucleotide, as it was described in a previous publication [9]. Samples of 10 μl were taken at times indicated in Figure 9. Radioactivity of the acid-insoluble material of the 0 min. sample was taken to be 100 % (0 % degradation). Reaction mixture for pancreatic DNase (EC 3.1.21.1.) contained 0.1 M sodium acetate (pH 5), 5 mM $MgCl_2$ and 25 ng of enzyme. For spleen DNase (EC 3.1.4.6) 0.1 M acetate buffer (pH 4.6), 0.75 mM $MgCl_2$ and 4 μg of enzyme were used. In micrococcal nuclease (EC 3.1.4.7.) reactions 0.02 M TRIS·HCl (pH 8.8) was applied with 0.01 M $CaCl_2$ and 3 ng of enzyme.

RESULTS

Preparation of the modified copolymer

Synthesis of copolymers from dATP and bv^{5}dUTP, [^{3}H]dATP and [^{14}C]-bv^{5}dUTP, or [^{3}H]dATP and [^{14}C]dTTP was carried out de novo with E. coli DNA polymerase I enzyme similarly as it was described in a previous paper for poly[d(A-T)] and some of its analogs [10], based on the original report of Schachman et al [11]. Polymerization was followed by a measurement of hypochromic change at 260 nm or by an incorporation of labelled precursors into an acid--insoluble product. Only high molecular weight polydeoxynucleotides excluded by gel filtration on a Sephadex G-200 column were analyzed and used for experiments. Yield of poly[d(A-bv^{5}U)] was 27 % based on molar absorbancies. For double-labeled copolymer prepared from [^{3}H]dATP (18.6 dpm/pmol) and [^{14}C]bv^{5}dUTP (41.7 dpm/pmol) yield was 28 % based on radioactivity or absorbancies. Specific activity was 9.1 dpm/pmol for ^{3}H-label and 20.6 dpm/pmol for ^{14}C-label. Yield of the double-labeled poly[d(A-T)] prepared from the above [^{3}H]dATP and [^{14}C]dTTP (3.5 dpm/pmol) was 52 % based on radioactivity or absorbancies. Specific activity was 9.0 dpm/pmol for ^{3}H-label and 1.7 dpm/pmol for ^{14}C-label. T_m value in buffered 0.01 M K^+ was 41.1^{o}C, with a hyperchromic effect of 48.1 %.

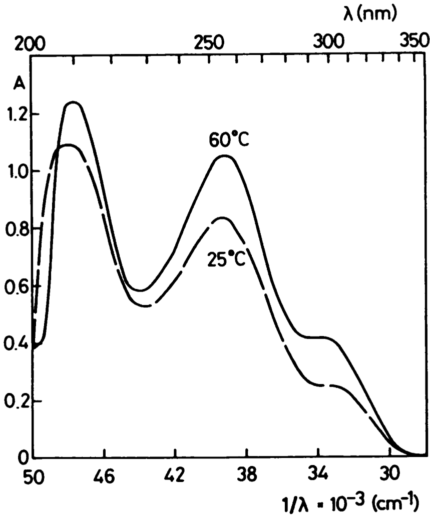

Figure 1. Ultraviolet absorption spectra of poly[d(A-bv^5U)] in buffered 0.1 M KCl, pH 7.

Ultraviolet and circular dichroism spectra

Figure 1 shows the UV spectra of poly [d(A-bv^5U)] at 25 and 60°C (coil form) in 0.1 M buffered KCl. (The nucleoside bv^5dU has a two-maximum spectrum [12].) Spectral data are summarized in Table 1.

Figure 2 shows the CD spectra of poly[d(A-bv^5U)] at 25 and

Table 1. Spectral data of poly [d(A-bv^5U)]

	25°C	60°C
λ_{min} (nm)	228	227
$A_{228(7)}/A_{260}$	0.68	0.57
λ_{max} (nm)	254	255
$A_{254(5)}/A_{260}$	1.08	1.04
$\lambda_{plateau}$ (nm)	293-302	288-300
$A_{300(294)}/A_{260}$	0.32	0.41
ϵ_{260} ($M^{-1}cm^{-1}$)	7730	10100

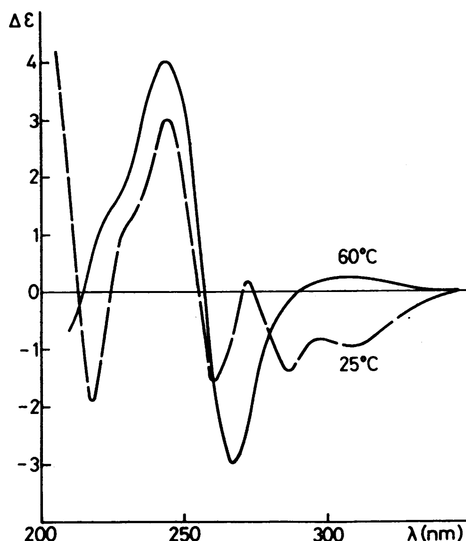

Figure 2. Circular dichroism spectra of poly[d(A-bv^{5}U)] in buffered 0.1 M KCl, pH 7.

60°C in 0.1 M K^+, pH 7. The unusual spectra may be explained by the absorption properties.

Sedimentation in ultracentrifuge

Sedimentation velocity was measured in dilute neutral solution, $s^{o}_{20,w}$ = 13.8 Svedberg units was found for the modified copolymer.

Determination of base composition and molar extinction coefficient

Previous incorporation studies with radioactive precursors showed that dATP and bv^{5}dUTP could be completely incorporated into a polymeric product in the presence of the alternating sequence poly-[d(A-T)] or activated natural DNA. With poly(dA)·poly(dT) only a limited incorporation of the substrates was observed. The synthetic product with poly[d(A-T)] contained dAMP and bv^{5}dUMP in a ratio of 1:1 [5]. The same ratio was found during the present preparative scale synthesis of the double-labelled poly[d(A-bv^{5}U)], and also with the isolated and purified polymer precipitated in acid onto GF/C filter (for specific activities, see Experimental). In addition to these results nucleoside ratio was determined from hydrolyzate of the copolymer as well. Product of enzymatic degradation to nucleosides (see below) was chromatographied and quantitative-

ly analyzed as described in Experimental section. Result of the analysis yielded also a ratio of 1:1 for dA and bv^5dU.

Determination of molar extinction coefficient [$\epsilon(P)$] for poly-[d(A-bv^5U)] was based on the measurement of hyperchromic change upon enzymatic degradation. From five determinations total hyperchromicity was 173.4 % at 260 nm. Based on molar absorbancies of dA and bv^5dU at 260 nm (15000 and 11800 $M^{-1}cm^{-1}$, resp.) and on the 1:1 ratio of the nucleosides in the polymer, $\epsilon(P)$ for poly-[d(A-bv^5U)] was calculated 7730 $\pm$ 50 $M^{-1}cm^{-1}$.

Thermal transition

Data of thermal transition of poly[d(A-bv^5U)] and poly[d(A-T)] were determined by plotting A_t/A_{25^o} values (measured at 260 nm) against corresponding temperature values (t), both obtained from digital readouts (Figure 3). Table 2 shows the data determined at four different K^+ concentrations.

T_m values of the bv^5dU-containing copolymer were lower, on an avarage, by 7°C than that of the poly[d(A-T)]. Plotting of T_m values against -log[K^+] yields straight lines with both polynucleotides. Slopes were similar. Thermal hyperchromicity of the modified copolymer was also smaller at 260 nm than that of the thymidine-containing one, but was high at 295 nm. ΔT values were higher, showing less cooperative transitions for the modified pol-

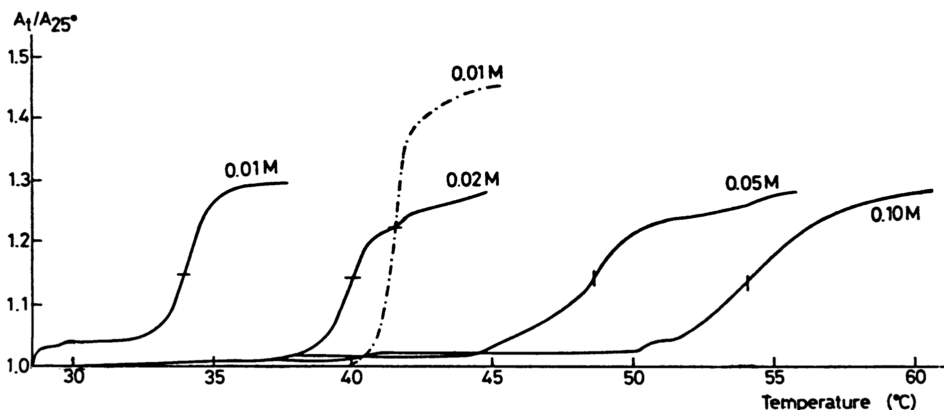

Figure 3. Thermal transition of poly[d(A-bv^5U)] (——) and, in comparison, poly[d(A-T)] (–·–) in buffered KCl solutions (pH 7). K^+ concentrations are indicated on the curves.

Table 2. Data of thermal transitions

K^+ conc. (M)	poly[d(A-T)] T_m (°C)	ΔT^x (°C)	h_{260}^{xx} (%)	poly[d(A-bv^{5}U)] T_m (°C)	$-\Delta T_m$ (°C)	ΔT (°C)	h_{260} (%)	h_{295} (%)
0.01	41.3	0.6	45.0	34.0	7.3	1.5	29.8	64.9
0.02	47.6	0.8	44.3	40.2	7.4	1.7	26.7	66.9
0.o5	54.7	1.2	44.4	48.7	6.0	3.1	27.1	65.5
0.10	61.0	1.5	47.1	54.1	6.9	3.4	27.4	67.5
$\frac{d(T_m)}{d(-\log[K^+])}$	19.7			20.4				

^{x} ΔT is width of the transition between 25 and 75 % of the thermal hyperchromicity

^{xx} h_{260} is thermal hyperchromicity measured at 260 nm

ymer. ΔT values of both copolymers increased with the growth in salt concentration. This property was shown only by copolymers of strictly alternating sequence [13]. Quick and reversible renaturation after thermal denaturation was also found with both polymers at all K^+ concentrations examined.

Complex formation

Complexing with poly(U), i.e. formation of a triple helix is a characteristic feature of the homopolymer duplex structure, like that of the poly(dA)·poly(dT), whereas such complexing could not be observed with copolymers of alternating sequence [14]. Our mixing experiments were carried out with poly(dA)·poly(dT), poly-[d(A-T)] and poly[d(A-bv^{5}U)]. Of the three polymers examined, hypochromic change upon mixing with poly(U), as described in Experimental section, was observed only with poly(dA)·poly(dT). It was 13.6 % at 260 nm.

Template activity for DNA synthesis

Figure 4. shows time-course of replication of poly[d(A-bv^{5}U)] in comparison with poly[d(A-T)] at 100-100 μM(P) concentrations. Substrates were [^{3}H]dATP and [^{14}C]dTTP and the Klenow fragment enzyme of E. coli DNA polymerase I was used. Equimolar incorporation of the nucleotides was observed. After 23 hours of incubation at 37°C, complete utilization of the triphosphates was ob-

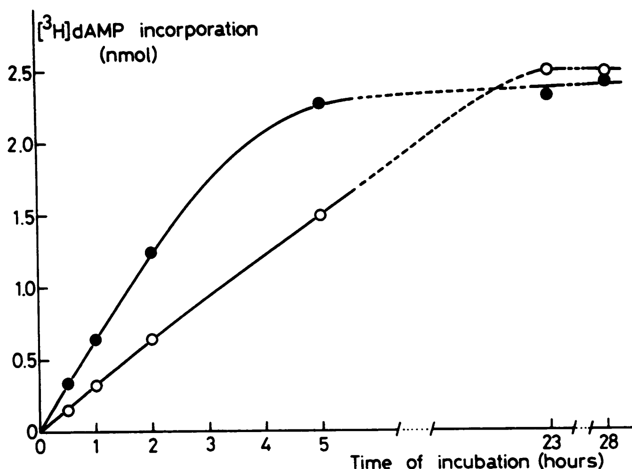

Figure 4. Time-course of replication catalyzed by E. coli DNA polymerase I Klenow fragment enzyme in the presence of poly[d(A-bv5U)], ●-●; and poly[d(A-T)], o-o.

served in both systems. Initial replication rates were, however, different. Template activity of the modified polynucleotide was 2.2, 1.8, 1.9 and 1.5-fold that of the poly[d(A-T)] at 30, 60, 120 and 300 minutes, respectively.

Figure 5 shows the dependence on polynucleotide concentration of the template activity. Higher activity of the modified copolymer was observed throughout the concentration interval examined. At 350 μM(P) the ratio was 1.5:1.

Limited synthesis (replication in the presence of one triphosphate only) is also a characteristic of the sequence of polynucleotide [15]. Results summarized in Table 3 show that the modified copolymer exhibits incorporation patterns in the one-substrate reactions similar to that of the poly[d(A-T)] and are in contrast with poly(dA)·poly(dT).

Template activity for RNA synthesis

Figure 6 shows time-course of transcription. Contrary to the replication by E. coli DNA polymerase, for transcription with E.coli RNA polymerase poly[d(A-bv^{5}U)] was a poor template. Relative incorporation rate of [^{3}H]AMP at 10 min. was 22 % with this template compared to poly[d(A-T)]. At 5 or 22 hours of incubation relative amount of incorporated AMP did not exceed 35 %. This means that

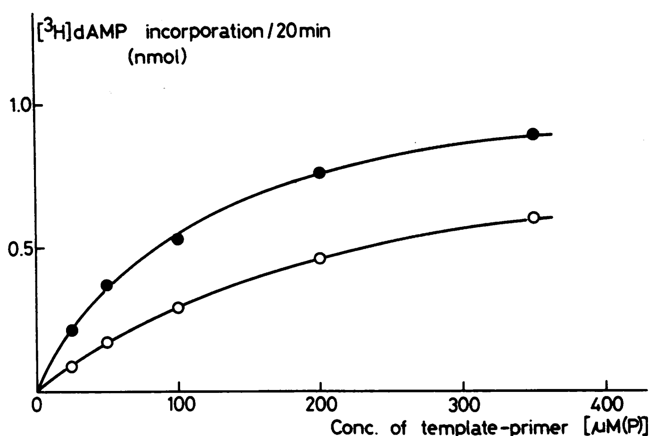

Figure 5. Replication by Klenow fragment enzyme of poly[d(A-bv^{5}U)], ●-●; and poly[d(A-T)] o-o.

incorporation is limited as well. Similar low relative incorporation rate of AMP was observed, on an avarage 23 %, with templates of 25-350 μM(P) concentrations in the 10 minute reactions (Figure 7). Figure 8 indicates that in the presence of the modified copolymer, rate of transcription of poly[d(A-T)] decreases as well. Re-

Table 3. Limited synthesis with Klenow fragment DNA polymerase

Substrates	Incorporation of labelled substrates (nmol/60 min/25 μl)		
	poly[d(A-T)]	poly[d(A-bv^{5}U)]	poly(dA)·poly(dT)
[^{3}H]dATP	<0.01	<0.01	0.33
[^{14}C]dTTP	<0.01	<0.01	0.45
[^{3}H]dATP + [^{14}C]dTTP	1.31 1.35	2.52 2.58	2.21 2.14

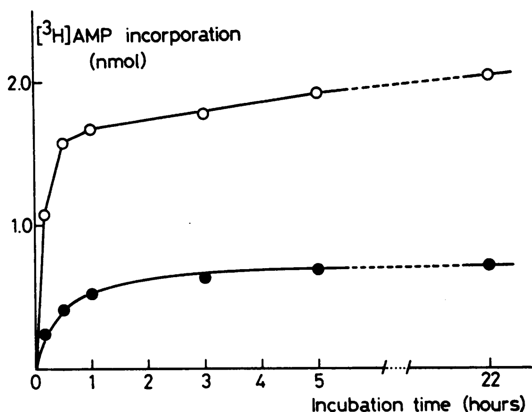

Figure 6. Transcription by E. coli RNA polymerase of poly-[d(A-bv^{5}U)], ●-●; and poly[d(A-T)], o-o.

duction of the rate was around 50 % with a two-fold molar excess of poly[d(A-bv^{5}U)].

Enzymatic hydrolysis

Based on similar solubility in trichloroacetic acid of the degradation products of poly[d(A-T)] and poly[d(A-bv^{5}U)] (proved with DE 81 paper chromatography [9]) we examined the relative stability of the modified copolymer against some nucleases. Figure 9 presents reactions with pancreatic DNase, as an example, of the two double-labelled copolymers as a function of incubation time. Table 4 displays data of the degradation reactions: incubation time

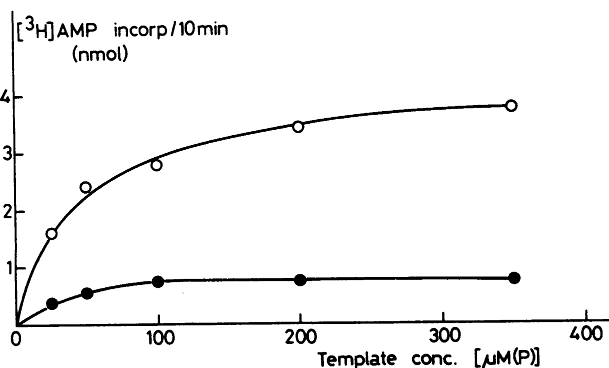

Figure 7. Transcription rate as a function of concentration of poly[d(A-bv^{5}U)], ●-●; and poly[d(A-T)], o-o.

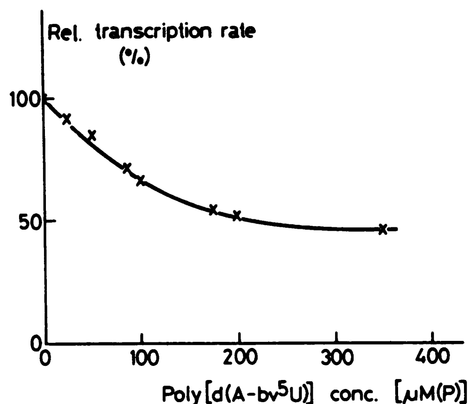

Figure 8. Inhibition of transcription on poly[d(A-T)] of 100 μM(P) by the modified copolymer.

for 50 % degradation of the polynucleotide and relative stability value based on the former data. The modified copolymer proved to be more stable than the natural one against pancreatic and spleen

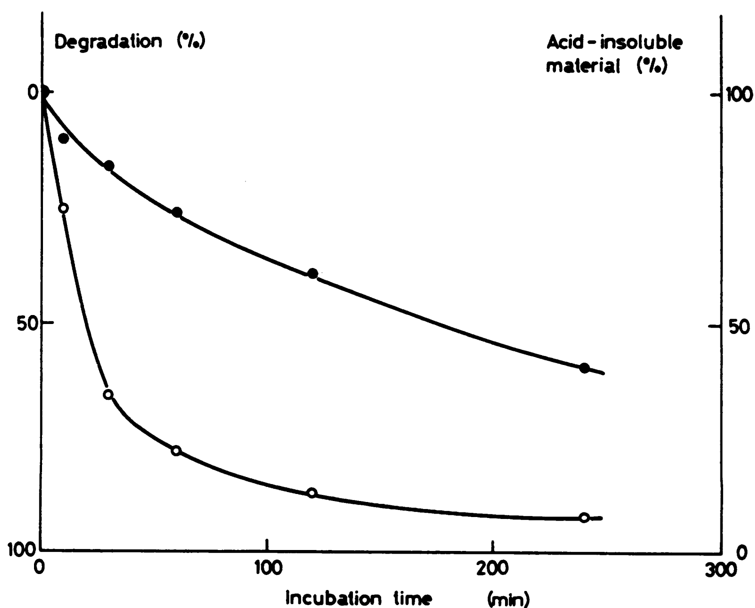

Figure 9. Time-course of degradation by pancreatic DNase of poly-[d(A-bv5U)], ●-●, and poly[d(A-T)], o-o.

Table 4. Data of enzymatic hydrolysis of the copolymers

Copolymers	pancreatic DNase 50% digestion time /min./	pancreatic DNase rel. stability	spleen DNase 50% digestion time /min./	spleen DNase rel. stability	micrococcal nuclease 50% digestion time /min./	micrococcal nuclease rel. stability
poly[d([^{3}H]A-[^{14}C]T)]	20	1	15	1	19	1
poly[d(^{3}H]A-[^{14}C]bv^{5}U)]	176	8.8	58	3.9	82	4.3

DNases and micrococcal nuclease.

DISCUSSION

Synthesis by DNA polymerase enzyme and study of the properties of a copolymer containing nucleotides of 2′-deoxyadenosine and (E)-5-(2-bromovinyl)-2′-deoxyuridine are described in this paper. This is the presentation of a new modified polydeoxynucleotide, and the results may contribute to the knowledge of the mode of action of the very effective anti-herpesvirus (type I) agent (E)-5-(2-bromovinyl)-2′-deoxyuridine (bv^{5}dU). Since its incorporation into DNA of the virus was established [6,7], we searched for possible alterations in properties of a modified DNA of this kind with a synthetic model of DNA.

For this purpose it was advantegous to have a DNA that contains the modified base in high percentage, can easily be prepared and structure can be well determined. These requirements are best accomplished by a copolymer containing only two nucleotides and prepared by E. coli DNA polymerase I enzyme. Numerous copolymers were de novo synthesized by this enzyme from dATP and 5-substituted dUTPs, and all had a strictly alternating sequence [11, 16-19]. The copolymer poly[d(A-T)] and the homopolymer duplex poly(dA)·poly(dT) are very well characterized in the literature [13-15, 20-23]. Comparison of characteristic data of a new modified polynucleotide with that of the former polymers may help in structure determination.

In the case of the polydeoxynucleotide containing dA and bv^{5}dU in a ratio of 1:1, increase of melting width upon the increase of salt concentration of the solution (Table 2), results of the lim-

ited DNA synthesis experiments (Table 3) and the absence of complex formation with poly(U) prove a poly[d(A-T)] type copolymer structure for the high molecular weight polynucleotide ($S^{o}_{20,w}$ = 13.8), designated as poly[d(A-bv^5U)]. It is double helical, the duplex is less stable than that of the poly[d(A-T)], and exhibits smaller thermal hyperchromicity (Table 2). Total hyperchromic effect, obtained by enzymatic degradation to nucleosides, was, however, high: 73.4 % compared to 78.6 % of poly[d(A-T)] at 260 nm.

In many of its physical and bioorganic properties poly-[d(A-bv^5U)] resembles to the 5-alkyluracil-containing poly-[d(A-r^5U)] copolymers, where r was an ethyl or a n-propyl group. Such properties were e.g. the reduced thermal stability [24] (compared to poly[d(A-T)] at 0.1 M K^+ ΔT_m values were -6.9, -11.0 and -14.1^oC for the bv^5dU-, et^5dU- and pr^5dU-containing copolymers), the higher template activity for DNA synthesis [8] (initial rates at 100 µM(P) concentration were 1.8, 1.7 and 3.1-fold of that of the poly[d(A-T)]) and the higher stability against some DNases [9] (relative stabilities against pancreatic DNase were 8.8, 1.5 and 2.1, against spleen DNase 3.9, 3.3 and 4.7).

In comparison with poly[d(A-T)], the decrease in thermal stability of poly[d(A-bv^5U)] was not so high as that of the 5-alkyluracil-containing copolymers. In the latter case decrease of T_m could be correlated with the increase of hydrophobic character of the substituent [24]. Since bromovinyl group is even more hydrophobic than ethyl group [25], higher T_m value of poly[d(A-bv^5U)] may be the result of the effect of the bromovinyl side chain on stabilizing forces, like stacking. The smaller thermal and the high total hyperchromicity point also to a high stacking in the coil form.

Higher template activity with DNA polymerase enzyme (Figures 4 and 5) may be in connection with the lower thermal stability [8]. (Copolymers cited are double helical at 37^oC under conditions of the experiments.)

Enhanced stability against nucleases of poly[d(A-bv^5U)] (Table 4) fits well the properties observed with other modified polynucleotides [9]. It is established against a wide variety of enzymes also for natural DNAs [26].

Template activity of poly[d(A-bv^5U)] for RNA synthesis with

E. coli RNA polymerase proved to be strongly reduced compared to both poly[d(A-T)] (Figures 6 and 7) and the 5-alkyluracil-containing copolymer analogs mentioned above [8]. Not only initial rate of incorporation of AMP and UMP was low but the amount of substrates polymerized on this template was limited as well. Saturating value was 35 % that of observed with poly[d(A-T)].

It would be of interest to examine whether (E)-5-(2-bromovinyl)-2'-deoxyuridine will have a similar effect on transcription after its incorporation into virus DNA in vivo.

ACKNOWLEDGEMENT

Authors are grateful to Mrs. I. Fritzsche for her technical assistance, and to dr. L. Koresz for his help in ultracentrifugation experiments.

REFERENCES

1. De Clercq, E., Descamps, J., De Somer, P., Barr, P.J., Jones, A.S. and Walker, R.T. (1979) Proc. Natl. Acad. Sci. USA 76, 2947-2951; and Maudgal, P.C., De Clercq, E., Descamps, J., Missotten, L., De Somer, P., Busson, R., Vanderhaeghe, H., Verhelst, G., Walker, R.T. and Jones, A.S. (1980) Antimicrob. Agents Chemother. 17, 8-12.
2. Cheng, Y.-C., Dutschman, G., De Clercq, E., Jones, A.S., Rahim, R.G., Verhelst, G. and Walker, R.T. (1981) Mol. Pharmacol. 20, 230-233.
3. Allaudeen, H.S., Kozarich, J.W., Bertino, J.R. and De Clercq, E. (1981) Proc. Natl. Acad. Sci. USA 78, 2698-2702.
4. Ruth, J.L. and Cheng, Y.-C. (1981) Mol. Pharmacol. 20, 415-422.
5. Sági, J., Szabolcs, A., Szemző, A. and Ötvös, L. (1981) Nucleic Acids Res. 9, 6985-6994.
6. Allaudeen, H.S., Chen, M.S., Lee, J.J., De Clercq, E. and Prusoff, W.H. (1982) J. Biol. Chem. 257, 603-606.
7. Larsson, A. and Öberg, B. (1981) Antimicrob. Agents Chemother. 19, 927-929.
8. Sági, J. and Ötvös, L.(1979) Nucleic Acids Res. 7, 1593-1601.
9. Sági, J. and Ötvös, L. (1980) Biochem. Biophys. Res. Commun. 95, 156-162.
10. Sági, J., Szabolcs, A., Szemző, A. and Ötvös, L. (1977) Nucleic Acids Res. 4, 2767-2777.
11. Schachman, H.K., Adler, J., Radding, C.M., Lehman, I.R. and Kornberg, A. (1960) J. Biol. Chem. 235, 3242-3249.
12. Jones, A.S., Verhelst, G. and Walker, R.T. (1979) Tetrahedron Letters 45, 4415-4418; and Barr, P.J., Jones, A.S., Verhelst, G. and Walker, R.T. (1981) J.Chem. Soc. Perkin I, 1665-1670.
13. Wells, R.D., Larson, J.E., Grant, R.C., Shortle, B.E. and Cantor, C.R. (1970) J. Mol. Biol. 54, 465-497.
14. Morgan, A.R. and Wells, R.D. (1968) J. Mol. Biol. 37, 63-80.
15. Chamberlin, J.M. (1965) Fed. Proc. 24, 1446-1457.
16. Inman, R.B. and Baldwin, R.L. (1964) J. Mol. Biol. 8, 452-469.

17. Cassady, P., Kahan, F. and Alegria, A. (1965) Fed.Proc. 24, 226.
18. Dale, R.M.K., Livingstone, D.C. and Ward, D.C. (1973) Proc. Natl. Acad. Sci. USA 70, 2238-2242.
19. Dale, R.M.K. and Ward, D.C. (1975) Biochem. 14, 2458-2469.
20. Greve, J., Maestre, M.F. and Levin, A. (1977) Biopolymers 16, 1489-1504.
21. Pilet, J., Blicharski, J. and Brahms, J. (1975) Biochem. 14, 1869-1876.
22. Lieberman, M.W., Harvan, D.J., Amacher, D.E. and Patterson, J.B. (1976) Biochim. Biophys. Acta 425, 265-277.
23. Szybalski, W. (1968) in Methods in Enzymology, Grossman, L. and Moldave, K. eds., Vol. XII/B, pp. 330-360, Academic Press, New-York and London.
24. Sági, J., Brahms, S., Brahms, J. and Ötvös, L. (1979) Nucleic Acids Res. 6, 2839-2848.
25. Hansch, C., Leo, A., Unger, S.H., Kim, K.H., Nikaitani, D. and Lien, E.J. (1973) J. Med. Chem. 16, 1207-1216.
26. Huang, L.-H., Farnet, C.M., Ehrlich, K.C. and Ehrlich, M. (1982) Nucleic Acids Res. 10, 1579-1591.