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MODIFIED POLYNUCLEOTIDES. V. SLOW-DOWN OF NUCLEASE ACTION BY 5-ALKYLURACIL-CONTAINING DNAs

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Summary

Poly d/(^{3}H]A-r^{5}U/ type of synthetic models of bacteriophage DNAs containing thymine analogues were prepared by DNA polymerase and tested for stability against nucleases /r was a n-alkyl group from methyl to pentyl/. The 5-pentyluracil-containing copolymer was found to be most stable: 50 % degradation with pancreatic DNase, spleen DNase, snake venom phosphodiesterase or micrococcal nuclease required 3-15 times as much time as that of poly d/A-T/.

Six modified bases substituting thymine or cytosine in bacteriophage DNAs have been isolated: uracil /1/, 5-hydroxymethyluracil /2/, 5-/4',5'-dihydroxypentyl/uracil /3/, 5-/4-aminobutylaminomethyl/uracil /4/, 5-methylcytosine /5/ and 5-hydroxymethylcytosine /6/. Beyond the unusual physical properties of these DNAs /e.g. in thermal stability/ no exact biological function was attributed to the modified bases.

dTTP analogues, like 5-alkyl-dUTPs can be incorporated into synthetic or natural DNAs by bacterial or mammalian DNA polymerases /7-9/. With synthetic poly d/A-r^{5}U/ copolymers /7/ we recently modeled the change in thermal stability /10/ and studied the effect of base modification on template activity of the copolymers for enzymatic DNA and RNA synthesis /11/. Here we pre-

Abbrevations: two-letter symbols were used for 5-alkyl substitution of uracil in poly d/A-r^{5}U/, where r was a h, me, et, pr, bu or pe group which stand for hydrogen atom, methyl, ethyl, n-propyl, n-butyl and n-pentyl group, respectively.

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sent data on enzyme catalyzed hydrolytic reactions of the high molecular weight double helical [^{3}H]dAMP-labelled poly d/A-r^{5}U/ copolymers /r was a hydrogen atom, or a methyl, ethyl, n-propyl, n-butyl or n-pentyl group/.

Materials and Methods

Snake venom phosphodiesterase and micrococcal nuclease were the products of Boehringer-Mannheim GmbH. Pancreatic DNase was from Miles-Serayac, spleen DNase from Calbiochem.

Synthesis of [^{3}H]dAMP-labelled poly d/A-r^{5}U/ copolymers was carried out as described earlier /7/. Specific activities were 6.61 ± 0.4 x 10^5 dpm/1 A_{260} unit of the polymer. Molar extinction coefficients of the modified polynucleotides were determined by measuring hyperchromic changes upon the degradation by pancreatic DNase and snake venom phosphodiesterase. Digestion up to nucleotides was checked with DE 81 /Whatman/ paper chromatography. ε/P/ values were 6660, 6640, 6680, 6740, 6750 and 6810 $M^{-1}cm^{-1}$ for the r = h, me, et, pr, bu and pe copolymers, respectively.

Nuclease reactions were carried out in a final volume of 90 µl which contained 0.136 A_{260} units of the copolymer. Degradations were followed with 10 µl samples taken at different time intervals up to 120 min., spotted onto GF/C filters /Whatman/, precipitated in acid, washed, dried and counted. Radioactivity of the acid-insoluble material of the 0 min. sample /around 10000 dpm/ was taken to be 100 % /0 % degradation/. Reproducibilities were ± 0.5 - ± 5 %. Acid-solubility of the degradation products of different polymers was similar based on DE 81 paper chromatography.

The reaction mixture for pancreatic DNase /EC 3.1.4.5./ contained 0.1 M TRIS.HCl /pH 7.0/, 6 mM $MgCl_2$ and 72 ng of the enzyme in 90 µl. The mixture for spleen DNase /EC 3.1.4.6./ contained 0.1 M sodium acetate buffer /pH 4.6/, 0.75 mM $MgCl_2$ and 4.2 µg of the enzyme.

The reaction mixture for snake venom phosphodiesterase /EC 3.1.4.1./ contained 0.1 M TRIS.HCl /pH 8.0/, 6 mM $MgCl_2$ and 2 µg of the enzyme. Staphylococcus aureus micrococcal nuclease was assayed in 0.02 M TRIS.HCl /pH 8.8/, 0.01 M $CaCl_2$ and 2 ng of nuclease /EC 3.1.4.7./ was applied per reaction.

Results and Discussion

Figure 1-4 show the degradation patterns of poly d/A-T/ analogues with the different nucleases. For better comparison of the hydrolysis rates, incubation time required for 50 % degradation of the polymers was determined from the curves /see Table 1/. Comparisons were based on poly d/A-T/ containing the bases found in natural unmodified DNAs.

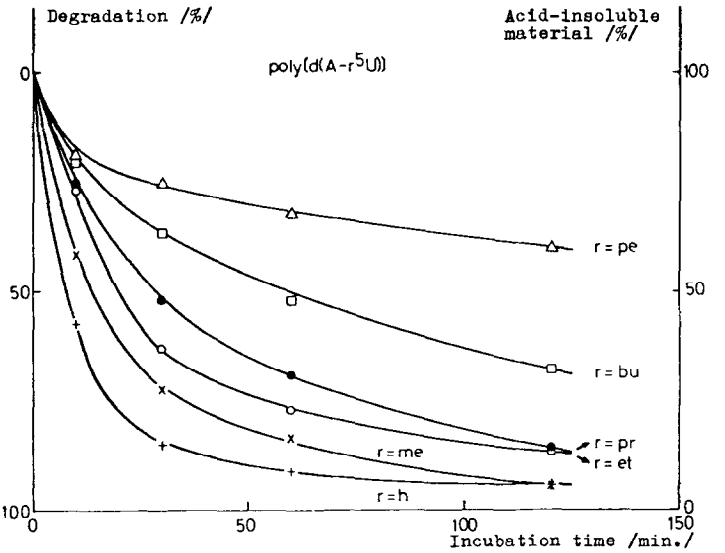

Figure 1. Hydrolysis of the cop lymers with pancreatic DNase

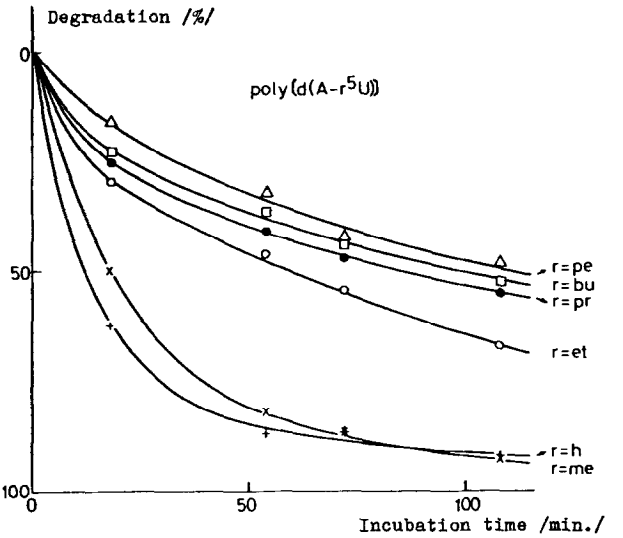

Figure 2. Hydrolysis of the copolymers with spleen DNase

Upon replacement of thymine in poly d/A-T/ by uracil or 5-alkyluracils a change in susceptibility of the polymers to nucleases could be observed. Poly d/A-U/ showed highest rate of hydrolysis with each enzyme of the copolymers studied. As the number of carbon atoms in the r substituent of poly d/A-r^5U/

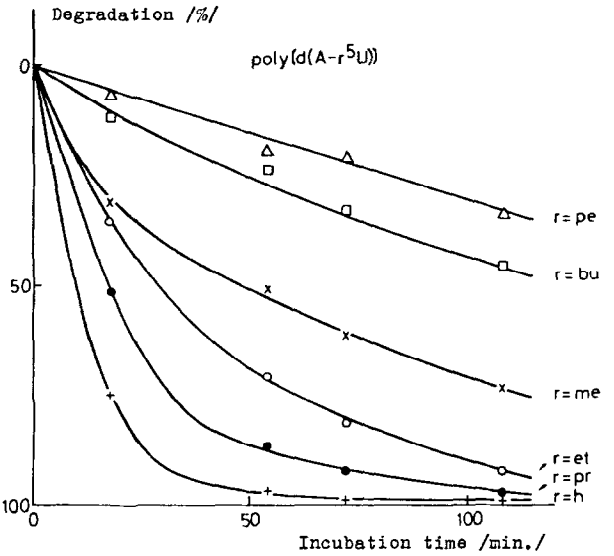

Figure 3. Hydrolysis of the copolymers with snake venom phosphodiesterase

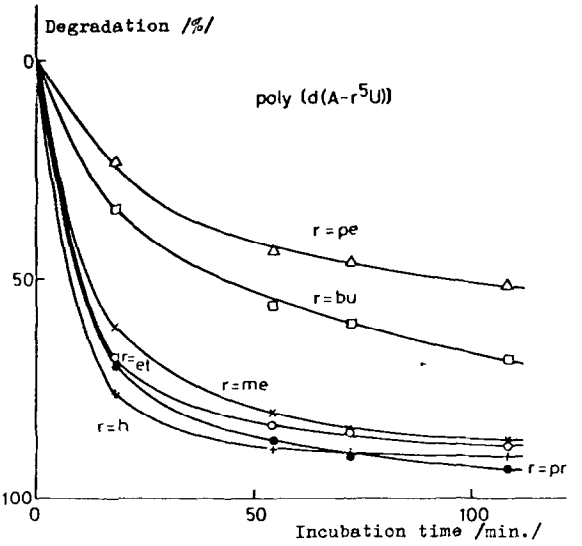

Figure 4. Hydrolysis of the copolymers with micrococcal nuclease

increased, the rate of degradation by the 2'-deoxypentose-specific endonuclease DNases decreased. Relative stability of the 5-pentyluracil-containing poly d/A-pe^{5}U/ was 6-15 times that of the poly d/A-T/.

Table 1. Data of the enzymatic hydrolysis of poly d/A-r^{5}U/ copolymers

r in poly d/A-r^{5}U/	pancreatic DNase		spleen DNase		snake venom phosphodiesterase		micrococcal nuclease	
	50 % digestion time /min./	rel. stability	50 % digestion time /min./	rel. stability	50 % digestion time /min./	rel. stability	50 % digestion time /min./	rel. stability
h	8	0.6	12	0.7	9.5	0.2	8	0.7
me	13.5	1.0	18	1.0	47	1.0	12	1.0
et	20	1.5	59.5	3.3	28.5	0.6	10	0.8
pr	28.5	2.1	84.5	4.7	17.5	0.4	9.5	0.8
bu	58.5	4.3	98	5.4	120.5	2.6	43	3.6
pe	197	14.6	111	6.2	163	3.5	92	7.7

Non-pentose-specific snake venom phosphodiesterase and micrococcal nuclease hydrolysed poly d/A-et^{5}U/ and poly d/A-pr^{5}U/ at a slightly higher rate than observed for poly d/A-T/. Poly d/A-bu^{5}U/ and poly d/A-pe^{5}U/ were degraded, however, with much smaller rate than poly d/A-T/.

Poly d/A-pe^{5}U/ proved to be the less susceptible to nucleases of the modified polydeoxynucleotides studied. Despite the increased resistance, larger amounts of enzymes are capable of its complete degradation to acid-soluble products. 2.5 μg of pancreatic DNase and 1 μg of phosphodiesterase completely hydrolysed 20 nmol/P/ of poly d/A-pe^{5}U/ in 90 μl to acid-solubles in 10 minutes.

The alkyl substituents, protruding into the large groove of the double helix, change the normal hydration shell around phosphate esters due to their increased hydrophobic character. This changes the rate of enzyme-catalyzed hydrolysis. The effect of the substituent on the rate was, however, different for the enzymes studied. This may reflect the different mode of action of these nucleases.

Modification of the structure of nucleic acid components changes macromolecular properties as well. In our system, modeling possible properties of some modified phage DNAs, complete replacement of thymine in poly d/A-T/ by 5-alkyluracils of increasing chain length resulted in decrease of thermal stability /10/, in increase of molar extinction coefficient of the polymers. An increase was also observed in the template activity for DNA synthesis, and a change of activity for RNA synthesis of poly d/A-r^{5}U/s /11/. Resistance to nucleases has also changed: the long alkyl chain-containing poly d/A-bu^{5}U/ and poly d/A-pe^{5}U/ were found to be 3-15 times more stable than poly d/A-T/. We can assume that the main roles of long chain thymine analogues present in some phage DNAs, as e.g. 5-/4';5'-dihydroxypentyl/uracil in SP-15 DNA /3/, are the stabilization of DNA against degradation by cellular nucleases, and the increase of template activity for replication.

Several 5-alkyl analogues of thymidine, as 5-ethyl-, 5-n-propyl- and also 5-n-butyl-2'-deoxyuridines, are known to selectively inhibit herpes virus replication /12,13/. A number of possible targets were proposed for their antiviral action. Impairment of virus DNA functions after incorporation of 5-alkyl-dUMP into DNA seems to be improbable, based on the non-mutagenic nature of 5-alkyluracils /14/, on our present and former results /11/. However, reduction of replication rate in the presence of 5-alkyl-dUTPs may contribute to the antiviral action of these thymidine analogues /9/.

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