
Effect of 5-alkyl substitution of uracil on the thermal stability of poly[d(A-r⁵U)] copolymers

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Received 25 April 1979

ABSTRACT

The thermal transition of poly[d(A-r⁵U)] polydeoxynucleotides (where r was a hydrogen atom, or a methyl, ethyl, n-propyl, n-butyl or n-pentyl group) was studied by measuring the derivative melting profiles of the polymers in the range of 0.01-0.36 M K⁺, at pH 6.8. According to the T_m values, polydeoxynucleotide analogues show lower thermal stability than poly[d(A-T)] at any counterion concentration applied. At a given salt concentration, T_m of the alkyl analogues decreased as the number of carbon atoms (n) in the r substituent of poly[d(A-rⁿU)] increased. 1/T_m plotted against 1/n yielded a linear relationship. Cooperativity of the melting of all poly[d(A-U)] analogues decreased with the increase of salt concentration in the solution. This change depended again on 5-substitution of the uracil moiety of poly[d(A-U)]. Smallest decrease was observed in the case of poly[d(A-U)] whereas largest decrease was shown by poly[d(A-pe⁵U)] (pe=pentyl group)

INTRODUCTION

Bacteriophage DNAs which contain natural analogues of thymine (e.g. 5-/4;5'-dihydroxypentyl/uracil¹ or 5-putrescinylyl-thymine²) and DNAs of different organisms in which unnatural base analogues (e.g. 5-ethyluracil³) were incorporated, exhibit unusual physical properties. A very pronounced change was observed in the thermal stability of these DNAs^{1,2,3}. For elucidation of the dependence of structural stability on structural modification of polynucleotides, alkyl-substituted poly[d(A-U)] analogues, the poly[d(A-r⁵U)] copolymers were prepared by E. coli DNA polymerase I enzyme. The alkyl substituents were methyl, ethyl, n-propyl, n-butyl and n-pentyl groups instead of the hydrogen atom in position 5 of uracil in poly[d(A-U)]^{4,5}. In this paper we present results on the dependence of the thermal stability of polydeoxynucleotides on

5-alkyl substitution of the uracil moiety of poly[d(A-U)] and on the cooperativity of the melting of these modified polymers which may be in connection with the primary structure of the polydeoxynucleotides.

METHODS

Poly[d(A-r⁵U)] copolymers were prepared from dATP and r⁵dUTPs by E. coli DNA polymerase I as described in an earlier paper⁴.

Derivative melting profiles were determined by means of a CARY 17 spectrophotometer equipped with a CARY 17909 derivative unit. Temperature was measured by a Cr-Al thermocouple with reference junction at 0°C. Derivative melting curves were recorded by an X-Y recorder /SEFRAM, Paris/ connected with the CARY 17 spectrophotometer and the thermocouple. Rate of temperature increase was 1°C/min. /HAAKE thermostat connected to a synchronous electric motor/. Polynucleotides were dissolved in 10⁻² M phosphate buffer at pH 6.8 and K⁺ concentration was increased by the addition of 2.5 M KF to the starting solution in a 1 cm cell. Polynucleotide concentrations corresponded to 1.0-1.5 O.D.₂₆₀. Thermal transition midpoints were reproducible within ±0.2 to ±0.5°C. Slopes of T_m - (-log[K⁺]), log(ΔT) - (-log[K⁺]) and 1/T_m - 1/n were calculated by the linear regression method of least squares.

RESULTS AND DISCUSSION

Poly[d(A-T)], the duplex alternating copolymer of dAMP and dTMP, is a well defined polynucleotide with high molecular weight^{6,7}. Complete replacement of one of its components by an analogue may greatly affect parameters characteristic of its secondary structure. Since such polymeric analogues can be prepared easily from the preformed substrates by DNA polymerases with or without exogen template-primers^{4,8}, this structure seems to be very advantageous for studying the structure-activity relationship.

Ultraviolet spectral data showed poly[d(A-r⁵U)] polydeoxy-

nucleotides to be native duplexes with large thermal hyperchromicities (Figure 1). For comparison of the thermal stability of the analogues T_m values were determined in 0.01-0.36 M KF at pH 6.8. T_m values of poly[d(A-U)] and poly[d(A-T)] prepared by the same method as the analogues, were also measured at every K^+ concentration. The derivative melting curves provided T_m values and showed also the cooperativity of the melting by half width values (ΔT), and the underivatized melting curves providing thermal hyperchromicities were simultaneously recorded by the CARY 17 spectrophotometer. Renaturation of all melted polydeoxynucleotides by cooling was quick and complete at any K^+ concentration measured. Therefore the determination of T_m values at different K^+ concentrations could be carried out with the same polydeoxynucleotide solution by adding 10-100 μ l of 2.5 M KF to the starting solution of 0.01 M phosphate buffer. The very high hyperchromicity values did not decrease after 5-6 denaturation-renaturation cycles.

Figure 2 shows the derivative melting profiles of poly-[d(A-pe⁵U)] at six different K^+ concentrations: heat stability

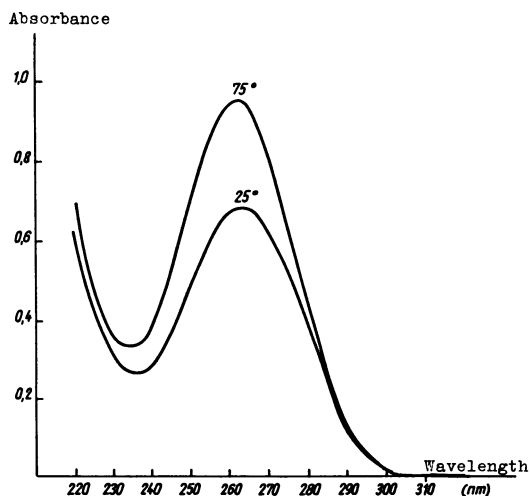


Figure 1. Ultraviolet spectra of poly[d(A-pe⁵U)] in 0.1 M K^+ at pH 7.0

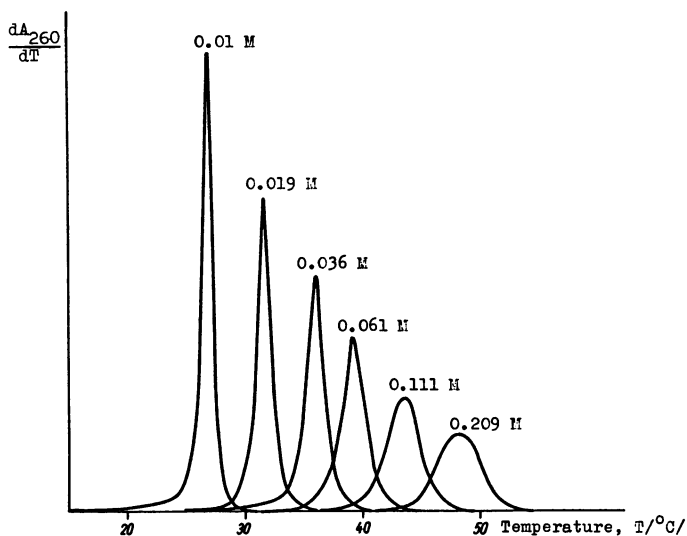


Figure 2. Derivative melting profiles of poly[d(A-pe⁵U)] at six different K⁺ concentrations (pH 6.8)

increased with the increase in salt concentration. The maxima of the curves could be easily determined at lower K⁺ concentrations. With the increase of salt concentration, however, not only T_m values but half width of the curves increased, that is, cooperativity of the heat denaturation decreased, therefore determination of the T_m values above 0.2-0.3 M K⁺ was difficult. Similar characteristics were observed also in the case of the other polydeoxynucleotides (Figure 3 and Table 1). The sharp denaturation profiles at lower K⁺ concentrations and the high hyperchromicity values suggest highly ordered structures for poly[d(A-r⁵U)] copolymers.

With all polydeoxynucleotides studied the linear dependence of T_m on $-\log[K^+]$ was observed. The slopes were similar therefore the T_m values of the modified polydeoxynucleotides could be compared at any salt concentration applied. Table 2 shows this comparison at 0.1 M K⁺: maximal stability was observed in the case of poly[d(A-T)]. By replacement of the 5-methyl group of all thymine residues by a hydrogen atom or

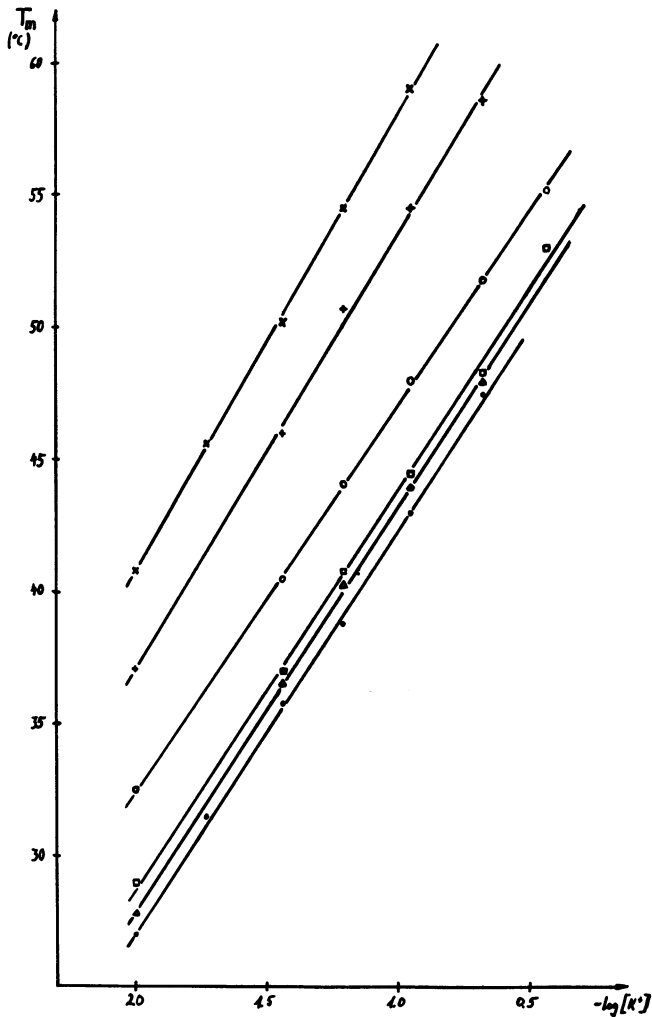


Figure 3. Dependence of T_m values of poly[d(A-r⁵U)] copolymers on salt concentration of the solution: x-x, poly[d(A-T)]; +-+, poly[d(A-U)]; o-o, poly[d(A-et⁵U)]; □-□, poly[d(A-pr⁵U)]; ▲-▲, poly[d(A-bu⁵U)]; and •-•, poly[d(A-pe⁵U)];

longer alkyl chains in the polymer, thermal stability decreased.

The lower stability of poly[d(A-U)] compared to poly[d(A-T)] is well known⁹. Example for the change of T_m on comp-

Table 1. Data of the thermal transitions of poly[d(A-r⁵U)] copolymers in buffered KF at pH 6.8

K ⁺ conc. (M)	poly[d(A-r ⁵ U)]											
	r = h		r = me		r = et		r = pr		r = bu		r = pe	
	T _m (°C)	ΔT (°C)	T _m (°C)	ΔT (°C)	T _m (°C)	ΔT (°C)	T _m (°C)	ΔT (°C)	T _m (°C)	ΔT (°C)	T _m (°C)	ΔT (°C)
0.010	37.1	1.75	40.8	1.25	32.5	1.75	29.0	1.25	27.8	1.5	27.0	0.9
0.019	-	-	45.6	1.25	-	-	-	-	-	-	31.5	1.25
0.036	46.0	2.0	50.2	1.5	40.5	2.0	37.0	1.5	36.6	2.0	35.8	1.75
0.061	50.7	2.3	54.5	1.75	44.1	2.5	40.8	1.75	40.3	2.5	38.8	2.25
0.111	54.5	2.5	59.0	2.0	48.0	3.0	44.5	2.25	44.0	3.0	43.0	3.25
0.209	58.6	2.75	-	-	51.8	3.25	48.3	2.75	48.0	4.0	47.5	4.5
0.360	-	-	-	-	55.2	4.0	53.0	3.0	-	-	-	-
Hyperchromicity at 0.01 M K ⁺ (%)	50.0		50.0		51.0		49.3		51.0		46.6	
d(T _m)/d(-log[K ⁺])	16.5		17.3		14.7		15.2		15.4		15.3	

lete substitution of thymine residues by any 5-alkyluracil in model polynucleotides is known in the case of the ribohomo-polynucleotide triplex poly(A).2poly(et⁵U) whose thermal stability was about 77 % of that observed for poly(A).2poly(T) in 0.1 M Na⁺ 10. Partial replacement of thymine by 5-ethyluracil in the DNA of different organisms caused smaller decrease in T_m 3.

Our results show direct dependence of T_m value of the 5-alkyluracil-containing polydeoxynucleotides on the size of the 5-substituent in the case of complete replacement of thymine by 5-alkyluracils in poly[d(A-T)]. The T_m value of poly[d(A-r⁵U)]s decreased as the number of carbon atoms (n) in r substituent increased (Table 2). The decrease of T_m values was found to be, however, a nonlinear function of n. Largest decrease in T_m (-11°C) was observed between the T_m values of poly[d(A-T)] and poly[d(A-et⁵U)]; only some slight differences (-0.7 and -0.9°C) could be observed between T_m values of poly[d(A-pr⁵U)], poly[d(A-bu⁵U)] and poly[d(A-pe⁵U)], respectively. On the other hand, 1/T_m plotted against 1/n yielded a linear relationship (Figure 4). In this way in 0.1 M K⁺, at

Table 2. T_m values of poly[d(A-r⁵U)] copolymers at 0.1 M K⁺

substituent in poly[d(A-r ⁵ U)]	\underline{n}	T_m (°C)	ΔT_m (°C)
h	0	53.6	-4.5
me	1	58.1	0
et	2	47.1	-11.0
pr	3	44.0	-14.1
bu	4	43.3	-14.8
pe	5	42.4	-15.7

pH 6.8, $T_m = 1/(-0.007958/n + 0.0252)$ as determined in the case of $\underline{n} = 1-5$ for poly[d(A-r⁵U)]s.

No definite explanation of these results has been given as yet. Presumably the change in the hydrophobic character of

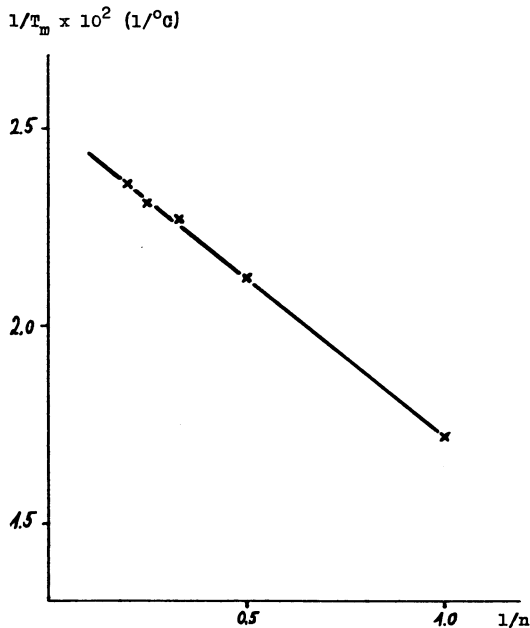


Figure 4. Dependence of T_m values of poly[d(A-r⁵U)] copolymers on the number \underline{n} of carbon atoms (n) in the \underline{r} substituents.

modified polydeoxynucleotides is mainly responsible for the decrease in stability, due to the change in the ion-solvating capacity of phosphate anions.

The broadening of melting curves at higher salt concentrations was observed earlier only for poly[d(A-T)] and poly-[d(A-U)] copolymers which have strictly alternating sequences⁷. (In the case of natural DNAs half width decreases and with the homopolymer duplex poly[d(A)].poly[d(T)] half width does not change with the increase in salt concentration of the solution from 10^{-3} to 1.0 M Na^+ ⁷.) Quick and completely reversible renaturation after thermal denaturation was observed also only in the case of strictly alternating copolymers in 10^{-1} - 10^{-3} M Na^+ ⁷. In this way the decrease in the cooperativity of melting of all our poly[d(A-r⁵U)]s with the increase of K^+ concentration and the quick and complete renaturation of the melted polydeoxynucleotides at any K^+ concentration used may point to a similar primary structure of the copolymers.

The increase in the half width of derivative melting curves of poly[d(A-r⁵U)]s was not the same for the different analogues. Best comparison of the half width increase of the analogues could be obtained from the double logarithmic plot of ΔT and $[\text{K}^+]$ (ΔT plotted versus $[\text{K}^+]$ showed saturation type curves). $\log(\Delta T)$ changes linearly with $-\log[\text{K}^+]$ and the slopes depend on the size of the substituent of poly[d(A-r⁵U)] (Figure 5 and Table 3). Upon the increase of salt concentration, smallest decrease in the cooperativity of melting was observed in the case of poly[d(A-U)]l, whereas largest decrease was

Table 3. Dependence of the cooperativity of melting of poly[d(A-r⁵U)] copolymers on the structure of r substituent

substituent in poly[d(A-r ⁵ U)]	$d(\log \Delta T)/d(-\log [\text{K}^+])$
h	0.153
me	0.211
et	0.237
pr	0.264
bu	0.321
pe	0.530

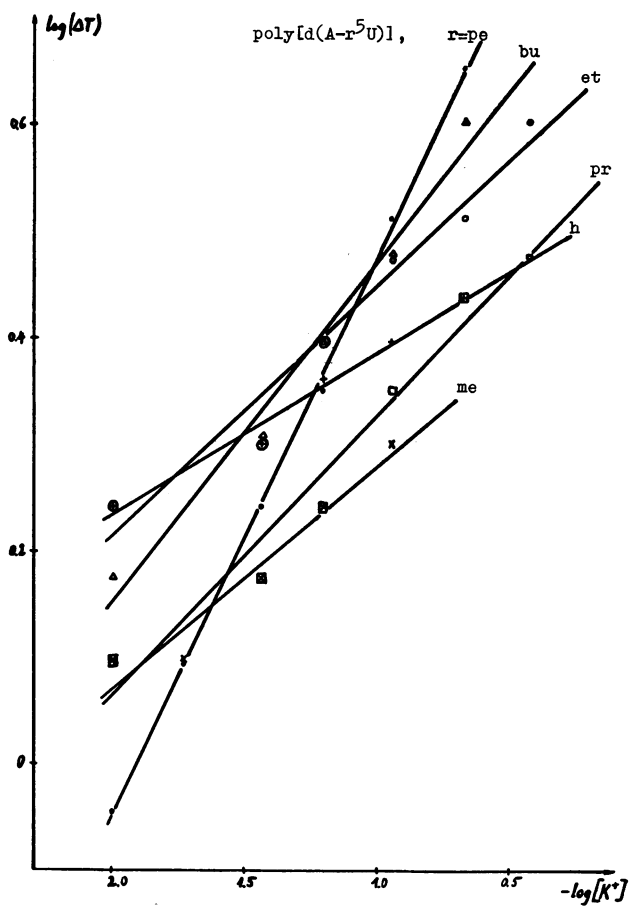


Figure 5. Dependence of half width values of the thermal transition of poly[d(A-r⁵U)] copolymers on salt concentration of the solution: x-x, poly[d(A-T)]₅; +--+ , poly[d(A-U)]₅; s-o, poly[d(A-et⁵U)]₅; □-□, poly[d(A-pr⁵U)]₅; Δ-Δ, poly[d(A-bu⁵U)]₅; •-•, poly[d(A-pe⁵U)]₅;

observed in the case of poly[d(A-pe⁵U)]₅. Poly[d(A-pe⁵U)]₅ melting was most cooperative at 0.01 M K⁺ and the least cooperative at 0.2 M K⁺ among the poly[d(A-r⁵U)]₅s studied.

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5. Abbreviations: two-letter symbols were used for 5-alkyl substitutions of uracil in poly[d(A-r⁵U)]_n, where r stands for alkyl group; et, pr, bu and pe stand for ethyl, n-propyl, n-butyl and n-pentyl, respectively. ΔT is the half width of the derivative melting curve; n is the number of carbon atoms in r substituent of poly[d(A-r⁵U)]_n.
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