THE EFFECT OF SATURATED PYRIMIDINE BASES ON RNA CONFORMATION

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1. Introduction

Saturation of the pyrimidine bases in polynucleotides leads to pronounced changes in their biological activity. The template activity of copolymers of 5, 6-dihydrouridine and uridine decreases with increasing 5, 6-dihydrouridine content in an *in vitro* protein synthesizing system [1] and changes its coding properties in an RNA-polymerase system [2]. Photohydration products of uridine, 6-hydroxy-5, 6-dihydrouridine, have been identified as major lethal lesions formed by ultraviolet irradiation in the small coliphage R17 [3]. Saturated derivatives of thymine of the 6-hydroxy(hydroperoxy) 5, 6-dihydrothymine type appear to be major lesions produced by ionizing radiation in bacterial DNA [4, 5]. Besides the importance of the saturated pyrimidines in photo- and radiobiology, 5, 6-dihydrouridine is an ubiquitous minor constituent of transfer RNA. In all transfer RNA species which have been sequenced, the saturated residues were always found in a topographically similar region, termed accordingly as the dihydrouridine loop [6].

The effects of the introduction of saturated residues on the biological activity of polynucleotides may in part be due to changes which occur in the polymer conformation as a result of the modification. It has been observed that the ability of copolymers of 5, 6-dihydrouridine and uridine to form ordered helical complexes with poly A decreased with increasing content of 5, 6-dihydrouridine [7]. This decrease was attributed to two major effects resulting from reduction of the 5, 6-double bond in uridylic acid: 1) a lowering of the hydrogen bonding capability due to an increase in the pK_a of the base; and 2) a loss in stacking forces due to puckering of the heterocyclic portion and shortening of the π-electron system.

In this work, we have attempted to verify experimentally the second part of this interpretation. Copolymers of N^4-acetyl-3, 4, 5, 6-tetrahydrocytidylic acid and CMP were used as models since they allow the investigation of the effects of saturated residues on polymer conformation in the absence of base pairing.

2. Materials and methods

Copolymers of ac^4h_4CMP and CMP were prepared and their composition was determined as described previously [8]. The polymers were further purified by passage through a Sephadex G-200 column and the high molecular weight main peak was used in all experiments. Five copolymers were prepared containing 3.0, 7.9, 10.4, 15.1, and 19.8 percent ac^4h_4CMP and the CD-spectra were recorded at pH 8.5 over a wavelength range from 230 to 360 nm. Maximally about one-third of the residues in poly C can be modified to ac^4h_4CMP by this procedure. Values for percent ac^4h_4CMP obtained by orcinol

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Abbreviations:
ac^4h_4 CMP: N^4-acetyl-3, 4, 5, 6-tetrahydrocytidylic acid; poly (ac^4h_4C, C): random copolymers of ac^4h_4 CMP and CMP; poly A: polyriboadenylic acid; poly C: polyribocytidylic acid.

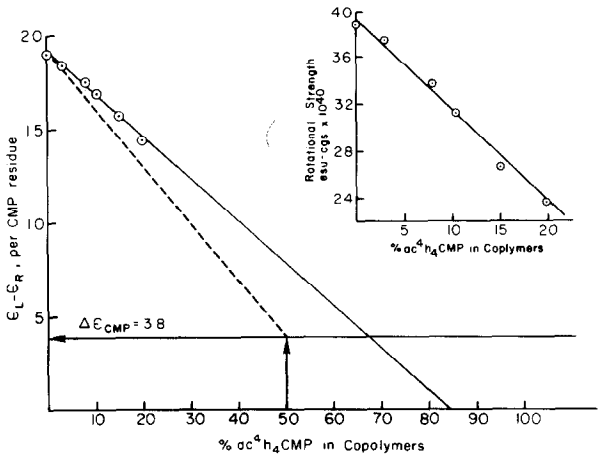

Fig. 1. Decrease of the differential molar absorptivity of the 276 nm band in the CD-spectrum with increasing ac^4h_4 CMP content. Circular dichroism spectra were recorded over a wavelength range of 230 to 360 nm, using a Cary 60 recording spectropolarimeter equipped with a 6001 dichroism accessory. The cell used for all measurements had a 1 cm pathlength. Samples were thermostated at 27 ± 1°. Circular dichroism results are reported as the molar dichroism absorptivity ($\epsilon_L - \epsilon_R$). Solutions used for circular dichroism measurements were of the same dilution from stock as those used for composition analyses to avoid the errors involved in handling small volumes of concentrated solutions. Concentrations were in the range of $1-3 \times 10^{-4}$ moles of nucleotide residue per liter. All spectra were recorded in 0.02 M Tris buffer, 0.01 M in NaCl, pH 8.5. The dotted theoretical curve is for the case where long range effects are excluded and the saturated residues are assumed to be distributed non-randomly in the polymer so that clustering of the modified residues is completely avoided.

Inset: Expanded representation of experimental data. Rotational strength (R) was calculated by numerical integration of the function $(\epsilon_L - \epsilon_R)/\lambda$ vs λ, with $(\epsilon_L - \epsilon_R)$ based on PO_4-residue concentration,

$$R = 22.95 \times 10^{-40} \int_0^\infty [(\epsilon_L - \epsilon_R)/\lambda]\, d\lambda \dagger$$

† I. Tinoco, Jr. and C.R. Cantor, "Application of Optical Rotatory Dispersion and Circular Dichroism to the Study of Biopolymers" in Methods of Biochemical Analysis, Vol. 18, p. 81. Interscience, New York, 1970.

assay and by difference from percent CMP determined by UV absorption were comparable for each sample. The polymer phosphate concentration was an independent criterion for the validity of the composition determinations.

3. Results and discussion

Qualitatively all polymers showed the non-conservative CD-spectrum which is characteristic of the single-stranded structure of poly C at neutral or slightly alkaline pH [9, 10]. Upon introduction of ac^4h_4 CMP, there was no shift in the maximum of the long wavelength peak from the 276 nm observed for poly C, and the point of crossover was the same for all samples (approximately 245 nm). However, as shown in the inset of fig. 1, the rotational strength of the 276 nm peak decreased in a linear fashion as the content of ac^4h_4 CMP in the polymer increased. There were also small changes in the depth of the trough at 235 nm, but the shoulder at 255 nm displayed almost no change in intensity.

Our results show that the dissymmetric structure of the single-stranded polymers which mostly reflects base stacking interaction decreases linearly with increasing ac^4h_4 CMP content. An important question asked in these studies was whether the saturated residues exert a long range distortion effect on the ordered conformation of the polymer or whether their effect is localized. Our results favor the latter interpretation. This conclusion is based on the following considerations. It is likely that saturation of a residue leads to a complete loss of the contribution to the CD-spectrum originating from its stacking interactions with neighboring bases. Maximal loss of the dissymmetric structure would be achieved at a given level of modification if the saturated residues are always separated by unmodified residues, i.e., if product clustering is completely excluded. At 50% ac^4h_4 CMP content, an alternating copolymer would be obtained with a CD-spectrum which should resemble that of monomeric CMP ($\Delta\epsilon = 3.8$). The dotted curve in fig. 1 gives the expected decrease in the differential molar absorptivity for this case. A steeper slope of the curve would be expected if long range distortion effects would exist additionally. As is evident from fig. 1, the slope of the experimental curve is smaller, rather than larger, than that of the theoretical curve. If it can be assumed that the distribution of the saturated residues in our polymers is strictly random, our results indicate that the effect of the saturated residues is mostly localized. Some product clustering is expected in a random copolymer and may explain the observed difference between the theoretical and experimental curves. Since

long range effects appear to be unimportant, it may be appropriate to compare the saturated residues to electronic insulators. It has to be kept in mind, of course, that exclusively those properties of the polymer have been studied which are reflected in the CD-spectrum and that the saturated residues may additionally introduce changes in the polymer conformation which cannot be detected by this technique.

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