SYNTHESIS AND PROPERTIES OF POLY-2,4-DITHIOURIDYLIC ACID, A NEW ANALOG OF POLY URIDYLIC ACID

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1. Introduction

Recently the enzymatic synthesis and properties of poly s^4U were reported [1]. Although poly s^4U was found to form a double helical complex with poly A, evidence emerged from spectroscopic [1] and X-ray diffraction studies [2] that helix formation does not involve hydrogen bonding between the 4-thioketo group and the N^6-adenine protons. It seemed therefore promising to attempt the synthesis of poly s^2s^4U**, where both uracil keto groups are substituted by thioketo groups.

2. Materials and methods

Poly A was purchased from Miles Labroratories, Elkart, USA. The synthesis of s^2s^4UDP will be described elsewhere [3]. The polymerization of s^2s^4UDP by polynucleotide phosphorylase from *E. coli* was carried out as already described [1]. Ultraviolet absorption spectra were recorded on a Cary 14 Spectrophotometer. Ultraviolet absorption temperature profiles were measured in a Zeiss PMQII using thermostated cuvettes. A thermistor served as temperature indicator. Spectrophotometric titrations and sedimentation velocity analysis were performed as described recently [1].

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** *Abbreviations*:
poly-2,4-dithiouridylic acid, poly s^2s^4U;
2,4-dithouridine diphosphate, s^2s^4UDP;
2,4-dithiouridine phosphate, s^2s^4UMP.

3. Results and discussion

Chemically synthesized s^2s^4UDP [3] was shown to be a substrate for polynucleotide phosphorylase from *E. coli*. s^2s^4UDP showed exchange of its β-phosphate residue in the presence of polynucleotide phosphorylase and was polymerized to poly s^2s^4U (K_m (s^2s^4UDP) 9.44×10^{-4} M). Poly s^2s^4U was isolated by gelfiltartion using Sephadex G-200 and exhibited a sedimentation coefficient of $S^{c=0}_{20,w}$ of 12.5 S as found by sedimentation velocity analysis in a Beckman Model E ultracentrifuge. The ultraviolet absorption spectrum of poly s^2s^4U shown in fig. 1 largely deviates from that of s^2s^4UMP. Enzymatic hydrolysis

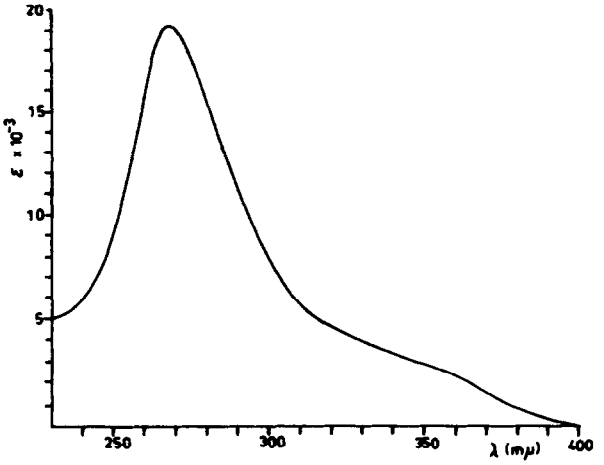

Fig. 1. Ultraviolet absorption spectrum of poly s^2s^4U in 0.05 M sodium cacodylate pH 7.0.

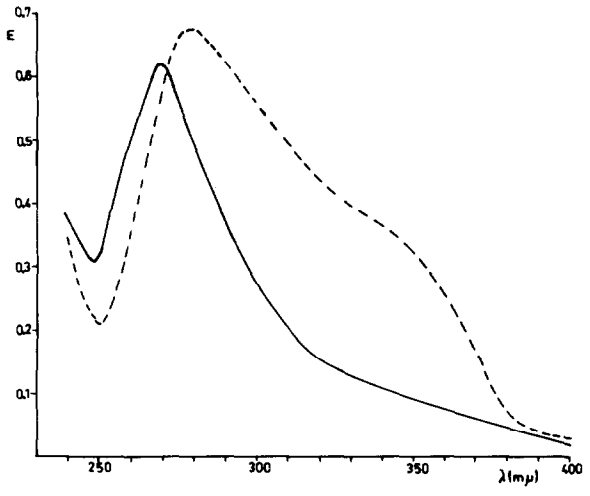

Fig. 2. Enzymatic hydrolysis of poly s^2s^4U. Ultraviolet absorption spectra of poly s^2s^4U (1.5 A_{270}-units in 2.5 ml 0.1 M citrate buffer pH 6) before (——) and after (- - - -) treatment with 2.5 enzyme units spleen phosphodiesterase. Enzymatic hydrolysis was carried out directly in a cuvette for 1 hr at 37°.

of poly s^2s^4U by spleen phosphodiesterase revealed the existence of a very large hyperchromycity in the absorption spectrum (see fig. 2). The apparent pK of poly s^2s^4U was determined spectroscopically as 9.2 (s^4Urd:pK 7.5) [4]. Since poly s^2s^4U was not phosphorolysed by polynucleotide phosphorylase, and only slowly degraded by pancreatic ribonuclease, a secondary structure of poly s^2s^4U was suspected. Indeed, the ultraviolet absorption-temperature profiles of poly s^2s^4U both at 288 and 340 mμ showed sharp sigmoidal transitions with identical T_m-values of 81° (fig. 3). The transitions were accompanied by large hyperchromicities, as can be seen from fig. 4. Even at very low ionic strength, e.g. 1 mM NaCl, poly s^2s^4 possessed a transition midpoint of 74°.

Various attempts to obtain a helical complex between poly s^2s^4U and poly A were unsuccessful. Mixing of poly s^2s^4U and poly A did not lead to the appearance of any detectable hyperchromicity in the absorption spectrum as shown by spectroscopic titration and difference spectroscopy. The ultraviolet absorption temperature profile of a 1:1 mixture of poly s^2s^4U and poly A showed only the helix-coil transition of poly s^2s^4U. The same result was obtained when both components were mixed at 90°, and the mixture slowly cooled to 20°.

It is concluded from the above reported results that poly s^2s^4U forms a stable helical structure by itself, but is unable to undergo complex formation with poly A. It is reasonable to assume that hydrogen-bonding in helical poly s^2s^4U is identical with that observed in 2,4-dithiouracil [5] and 2,4-dithiouridine crystals [6]. The remarkable stability of the poly s^2s^4U helix compared with those from poly U and other poly U analogs could be explained by the low pK of the N^3-proton (7.5) and the considerable hydrophobicity of the 2,4-dithiouracil residue. A reasonable explanation for the lack of helix formation be-

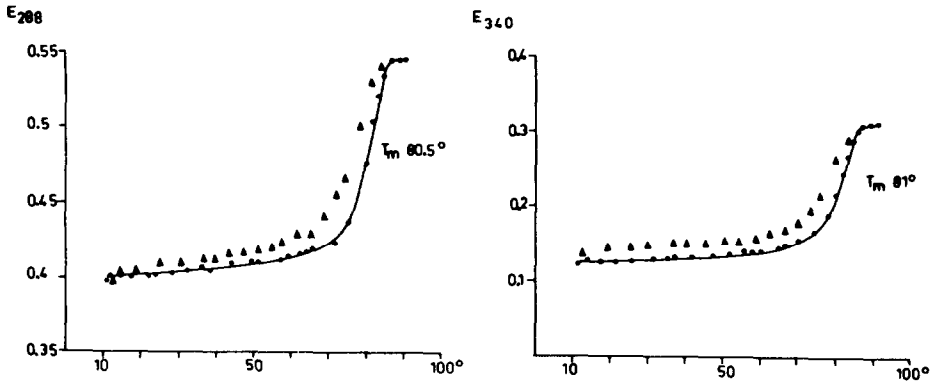

Fig. 3. Ultraviolet absorption temperature profile of poly s^2s^4U. Solvents: 0.05 M sodium cacodylate pH 7; ▲: transition observed during cooling.

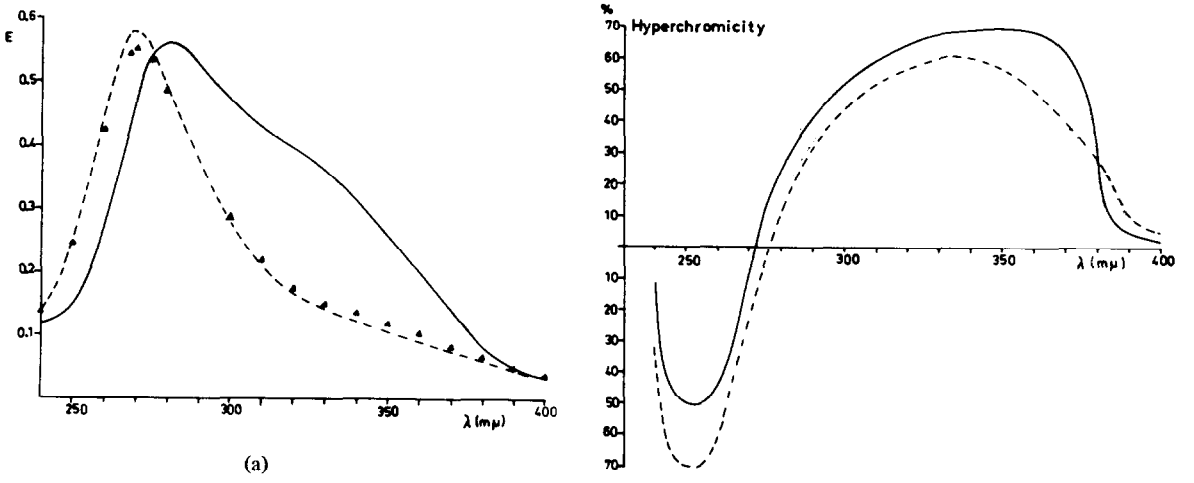

Fig. 4. Hyperchromicity of poly s^2s^4U.

Fig. 4a. Ultraviolet absorption spectrum of poly s^2s^4U in 0.05 M sodium cacodylate pH 7 at 16° (- - -), 90° (——) and after cooling (▲).

Fig. 4b. Hyperchromicity spectrum of poly s^2s^4U thermal denaturation (- - -); enzymatic hydrolysis (——).

tween poly s^2s^4U and poly A would be that the (poly s^2s^4U) (poly A)-helix is thermodynamically less stable than the poly s^2s^4U helix.

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