INTERACTION OF PARTIALLY REDUCED POLYURIDYLIC ACID WITH POLYADENYLIC ACID

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Received January 14, 1966

Recently dihydrouridine (H_2U)[1] has been discovered as a minor component in t-RNA (1), and in appreciable quantities in a nucleoprotein (1a). Enzymatic incorporation of dihydrouridylic acid (H_2Up) and dihydrouridine-5'-triphosphate into RNA has been demonstrated (2,3). Whereas, most minor nucleotides exhibit close chemical and biophysical similarity to the major nucleotide from which they are derived, pronounced differences exist for the pair H_2U/U. These differences may well be reflected in their functional roles. Significant changes in functional behavior upon saturation of the 5,6-double bond of the pyrimidine ring are found in the altered template activity of partially reduced poly U (4) and poly U after UV-irradiation (5) (which results predominantly in hydration of the uridine 5,6-double bond). These findings have stimulated current interest in the binding properties of polymers containing dihydropyrimidine residues.

The discovery of a convenient photochemical method of preparing partially reduced poly U (poly U/H_2U) of high molecular weight (6) facilitates a direct experimental examination of this point in a well-studied polynucleotide system.

Poly U (from Miles Laboratories) was exposed to photoreductive conditions ($NaBH_4/hv$) at pH 9.5 and 50° C for different lengths of time in a

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[1] Abbreviations: H_2U, dihydrouridine; H_2Up, dihydrouridylic acid; poly U, polyuridylic acid; poly A, polyadenylic acid; poly U/H_2U (X:1), copolymer of Up and H_2Up (base-ratio given in parenthesis).

TABLE 1

Characterization of poly U/H_2U preparations

	total P_i μmoles/ml	residual Up μmoles/ml	hydrolyzable ribose[1] μmoles/ml	Ureido-groups μmoles/ml	base ratio U:H_2U
I	15.81	14.97	0.74	0.66	19:1
II	10.70	9.29	1.28	1.33	6.7:1
III	12.42	8.94	3.28	3.14	2.6:1

[1] A correction is made for the Orcinol-reaction exhibited by unreduced poly U.

N_2-atmosphere. The light source was a mercury low pressure lamp, filtered by dilute acetic acid. Excessive $NaBH_4$ was destroyed immediately after the irradiation was terminated with dilute hydrochloric acid and the pH adjusted to 4. The solutions were desalted on Sephadex G25 (elution with 10^{-3} M tris-acetate, pH 7.15) and concentrated by lyophilization. To remove short fragments the residues were taken up in a small amount of water brought to 2 M in salt with KCl, precipitated with 0.3 vol. 95% ethanol (-20° C). After washing three times with 80% ethanol in the cold the precipitates were taken up in deionized water and dialyzed for three hours against water (0° C). The preparations were characterized by measuring the residual absorbance at 260 mμ (pH 2, ϵ = 9800), the amount hydrolyzable ribose (Orcinol-assay), the ureido-groups present (Archibald-assay) after acidic cleavage of the glycosidic bond, alkaline opening of the dihydrouracil ring, and by determination of the total polymer phosphate (Table 1). Whereas photochemical water-addition and dimerization of the Up residues seem to be negligible under the outlined photoreductive conditions, we find minor contamination with a product of further reduction (which gives an Archibald test without prior hydrolysis), especially in preparations with a relatively high extent of reduction (> 30%).

The binding capability of poly U/H_2U was examined by means of quantitative infrared spectroscopy of aqueous solutions. The interpretation of the spectra was based upon previous studies of the poly A-poly U system (7) and upon the spectra of H_2Up and other dihydrouracil nucleoside model compounds (Fig. 1).

1-Glucosyldihydrouracil[2] has two strong bands at 1703 cm^{-1} and 1668 cm^{-1}, which we attribute to C=O stretching modes of the C_2 and C_4 carbonyl groups (8). Uridine and poly U have strong bands at 1692 cm^{-1} and 1657 cm^{-1} and a weak band at 1618 cm^{-1} which is absent in the dihydro-compounds.

[2] H_2Up is somewhat less satisfactory as a model compound than 1-glycosyl-dihydrouracil since the latter compound is crystalline whereas the former is not. They both have essentially the same infrared spectrum in the region of double bond stretching vibrations as do other 1-substituted 5,6-dihydro model compounds.

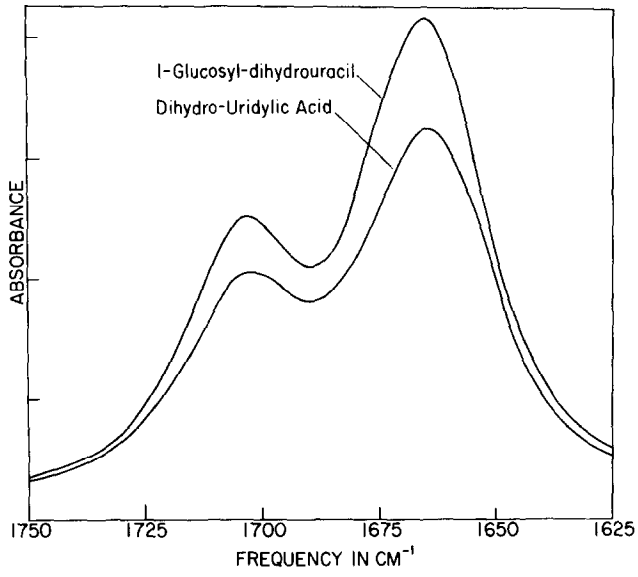


Fig. 1. Infrared spectra in D_2O solution of 1-glucosyldihydrouracil and of dihydrouridylic acid. There are no absorption bands between 1625 cm^{-1} and 1500 cm^{-1}.

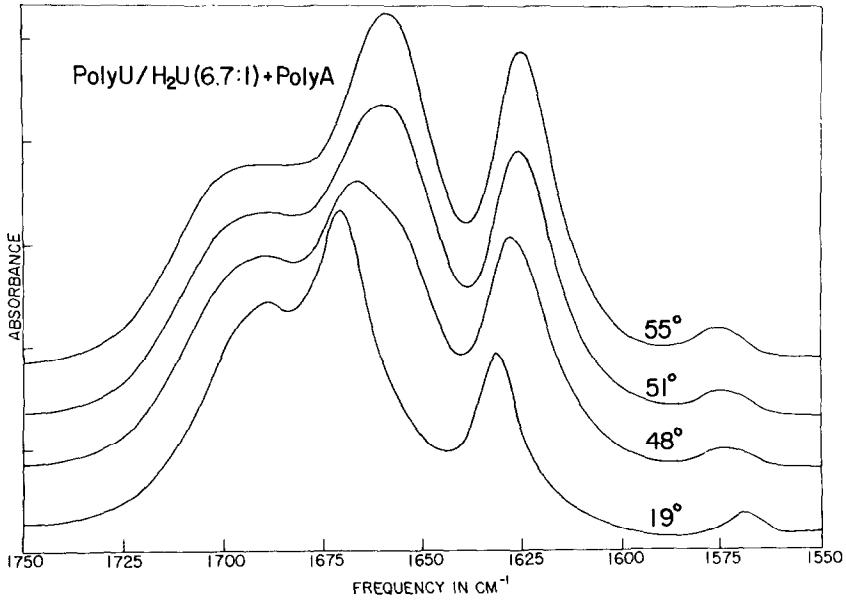


Fig. 2. Infrared spectra in D_2O solution of a mixture of poly A (0.04 M) and poly U/H_2U (6.7:1, preparation II; 0.04 M based on unreduced uridine content), 0.15 M Na^+, pD 7, path length 47.4μ, scale expansion 2.7 fold. Ordinate marks are 0.1 unit apart.

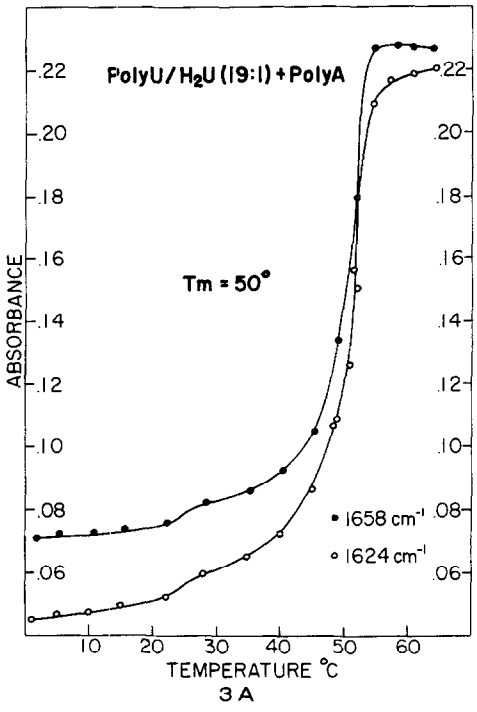
The IR-spectra of samples of poly U having a low extent of reduction are very similar to poly U. By more extensive photoreduction preparations are obtained which exhibit maxima at 1703 cm^{-1} and 1668 cm^{-1} in agreement with the data obtained from the monomeric model compounds.

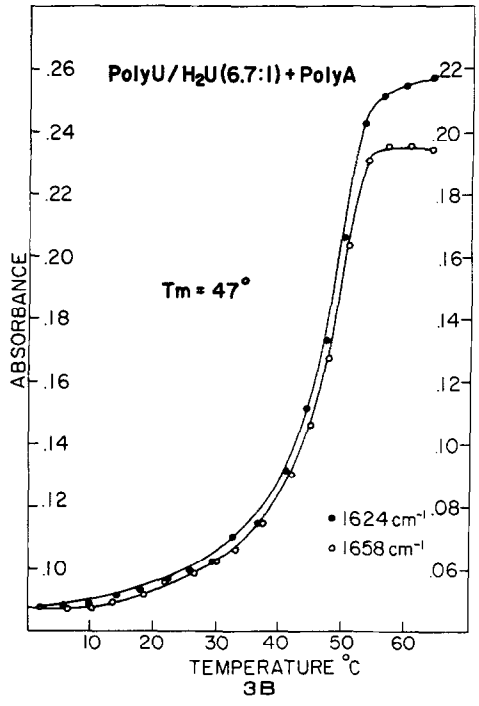
The ability of poly U/H_2U to bind to poly A was examined by observing a mixture of these polymers under conditions known to lead to strong interaction in the case of poly A and poly U (7). The infrared spectra indicated that two-stranded helices had formed under the conditions of our experiment (7), and the spectra changed with temperature as shown in Fig. 2 for the case poly A-poly U/H_2U (6.7:1). We observed that all poly A-poly U/H_2U complexes showed large increases in viscosity compared to poly (A+U), even at low extents of reduction.

A comparison of the binding characteristics of poly U/H_2U with poly A as a function of the H_2U content shows that the affinity of the two polymers decreases progressively with the extent of reduction of poly U (Fig. 3). Thus at 5% (I) and 13% (II) reduction the melting is still sharp and the T_m about 6 and 10° lower than that of poly (A+U) under comparable conditions. When 28% (III) of the uridine residues are reduced the interaction with poly A is only weak. A very broad melting curve and a T_m of roughly 23° are observed.[3] We conclude from these experiments that the H_2U residues do not bind to poly A and that they effectively diminish the ability of the residual uridine residues to do so. To state the matter from a different perspective, it appears that H_2U is not able to form a helical structure with poly A, nor, apparently, can it be "carried" into helical regions by the normal interaction of the residual uridine in poly U.

Possible reasons for the failure of interaction may be the fact that the hydrogen atoms on the tetrahedal carbons C_5 and C_6 in H_2U project slightly above and below an otherwise largely planar ring and so interfere

[3] Experiments with a number of other poly U/H_2U preparations follow the same general trend of lowering of stability of the complex with poly A as the extent of reduction increases.





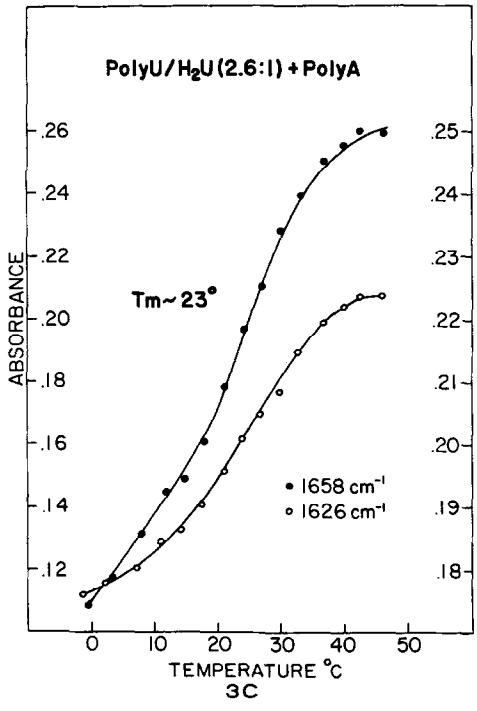


Fig. 3. Melting curves from infrared spectra in D_2O solution of mixtures of poly A (0.04 M) and poly U/H_2U (0.04 M, based on content of unreduced uridine), 0.15 M Na^+, pD 7. Path length of cells 47.4μ.

with close stacking of the bases. The loss of aromaticity and the shortening of the Π-electron system upon saturation of the 5,6-double bond in uridine weaken the London-dispersion forces between the heterocyclic rings and may further diminish poly A - poly U/H_2U interaction. The possibility of extensive enolization of dihydrouridine may be ruled out on the basis of the infrared spectrum of a keto model compound (1-glucosyl-3-methyl-dihydrouracil, υmax 1707 cm^{-1}, ϵ = 450 and 1656 cm^{-1}, ϵ = 975 (8)) and cannot, therefore, provide an explanation for the instability of the complexes formed between poly A and poly U/H_2U. The increase in pK_a upon reduction may also have some influence upon binding capacity, though possibly less than the foregoing factors.

While we do not have definitive evidence on the secondary structure of the H_2U regions of the helices the abnormally high viscosity of these complexes suggests that some of these unbonded regions may link different helices together, resulting in three dimensional gel formation. For t-RNA molecules, which have a very low H_2U content, we suggest on the basis of our experiments that a biological function of H_2U might be to terminate regions of base pairing and by that to expose certain base sequences of special functional significance.

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