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Loss of Coding Properties of the Oligonucleotide Adenylyluridylylguanosine after Photoreduction or Hydration

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Madison & Holley (1965) first established the existence of 5,6-dihydrouridylic acid in s-RNA* and evidence obtained by Huang & Bonner (1965) and by Carr & Grisolia (1964) suggests that it may be a component of other types of RNA. Mokrasch & Grisolia (1959) discovered dihydropyrimidine dehydrogenases that reversibly interconvert uridylic acid and dihydrouridylic acid. These observations indicate that dihydrouridylic acid may have a special role in protein synthesis.

Brown, Lee & Metz (1966) have suggested that the presence of one or more dihydrouridylic acid residues in a viral messenger RNA together with the existence in the virus-infected cell of a nucleotide-sequence-specific dihydrouridine dehydrogenase might constitute a translational control mechanism for viral protein synthesis. This postulated inter-conversion of uridylic acid and dihydrouridylic acid in messenger RNA would be expected to have a functional effect because the properties of uridylic acid are altered considerably when the 5,6-double bond is saturated to give either dihydrouridylic acid

or the closely similar 5-hydro-6-hydroxyuridylic acid. It has been shown that dihydrouridylic acid residues in poly(U,H₂U) copolymers are unable to form a helical structure with polyA (Cerutti, Miles & Frazier, 1966), and Rottman & Cerutti (1966) and Grossman (1963) have demonstrated that the partial conversion of uridine into dihydrouridine or into 5-hydro-6-hydroxyuridine in polyU decreased the incorporation of phenylalanine. However, though Smrt, Škoda, Lisý & Šorm (1966) found that the trinucleoside diphosphate GpUpU was unable to bind valyl-s-RNA if any or both of the uridine residues were replaced by dihydrouridine, the results of Rottman & Cerutti (1966) suggested that the binding of phenylalanyl-s-RNA was not sensitive to the amount of dihydrouridylic acid in copolymers of uridylic acid and dihydrouridylic acid.

It has recently been established that *N*-formyl-methionine acts as a chain initiator for protein synthesis in *Escherichia coli* cells (Clark & Marcker, 1966; Adams & Capecechi, 1966; Webster, Engelhardt & Zinder, 1966) and it is therefore of particular interest, in relation to possible control mechanisms, to investigate the effect of altering the codon for *N*-formylmethionine. The effect of u.v. irradiation on the codon is also of interest in the context of radiation damage. In this paper we show that when the uridylic acid in ApUpG, which is a coding triplet for *N*-formylmethionine (Clark & Marcker, 1966; Kellogg, Doctor, Loebel & Nirenberg, 1966), is replaced by dihydrouridylic acid, or the hydrate, it is no longer capable of specifically binding *E. coli*

* Abbreviations: s-RNA, 'soluble' or transfer RNA; A, adenosine; U, uridine; G, guanosine; H₂U, 5,6-dihydrouridine; (H₂O)U, 5-hydro-6-hydroxyuridine. Oligonucleotides have been written with a p to the right to indicate a 3'-phosphate, e.g. ApH₂UpG for adenylyl-(3'-5')-dihydrouridylyl-(3'-5')-guanosine, and codons have been abbreviated to the nucleosides in sequence. The constituent nucleosides have been used to designate polyribonucleotides, e.g. polyU for polyuridylic acid, poly(U,H₂U) for a copolymer of uridylic acid and dihydrouridylic acid of random sequence.

methionyl-s-RNA on ribosomes. Methionyl-s-RNA was not formylated because it has been shown that formylation does not affect the s-RNA binding properties (Clark & Marcker, 1966).

The trinucleoside diphosphate ApUpG was enzymically synthesized and purified by the method of Thach & Doty (1965). ApUpG was converted into ApH₂UpG by u.v. irradiation in 50mm-borate buffer, pH 9.5, containing sodium borohydride (10mm), by using a Chromatolite lamp (Cerrutti, Ikeda & Witkop, 1965). When the loss in extinction at 260m μ indicated that the reaction was complete, this solution and a similar unirradiated control solution of ApUpG were acidified and loaded on to Whatman 3MM paper. Excess of borate was eluted with 10% (v/v) ethylene glycol in ethanol, and the oligonucleotides were subjected to electrophoresis at pH 4.9 (Sanger, Brownlee & Barrell, 1965). The ApH₂UpG and ApUpG bands were eluted in water and freeze-dried.

The hydrated oligonucleotide Ap(H₂O)UpG was prepared by u.v. irradiation of ApUpG in 10mm-tris-acetate buffer, pH 7.2, for 55min., by using a Chromatolite lamp, the course of reaction being followed by the loss of extinction at 260m μ . The hydrate was reconverted into uridylic acid by heating at 86° for 1hr. at approx. pH 9.5 and then neutralizing (Logan & Whitmore, 1966). The volume and buffer concentration of the Ap(H₂O)UpG and of a control preparation of unirradiated ApUpG were adjusted to maintain exact equivalence of all three solutions.

E. coli s-RNA was charged with a mixture of [³⁵S]methionine (specific activity 143mc/m-mole) and 19 unlabelled amino acids to give an activity of 1.27m μ moles/mg. of s-RNA (Clark & Marcker, 1966). *E. coli* ribosomes were prepared by following the method of Clark & Marcker (1966), but omitting deoxycholate.

The capacity of ApUpG, ApH₂UpG and Ap(H₂O)UpG to effect the binding of methionyl-s-RNA to ribosomes was assayed by the procedure of Nirenberg & Leder (1964). It was established in preliminary experiments that the oligonucleotide concentration was below the value for ribosome saturation, so that the s-RNA binding was roughly proportional to the amount of oligonucleotide added, and the effect of any residual ApUpG in the irradiated samples was minimized. The oligonucleotides were incubated for 25min. at 24° with washed ribosomes and [³⁵S]methionine-labelled s-RNA, at a variety of concentrations. Typical results are given in Table 1. After incubation, the samples were diluted with 3ml. of cold buffer of the same composition as that used for the incubation, immediately poured on to pre-washed Millipore HAWP 0.45 μ filters and washed with three 3ml. volumes of cold buffer of the same composition as that used for the incubation. The samples were counted in a Packard Tri-Carb liquid-scintillation counter.

As shown in Table 1, the photoreduction of ApUpG destroyed its ability to bind methionyl-s-RNA. The complete loss of activity demonstrates that ApH₂UpG is inactive because, although the

Table 1. *Binding of methionyl-s-RNA to ribosomes in the presence of adenylyl-(3'-5')-dihydro-uridylyl-(3'-5')-guanosine and adenylyl-(3'-5')-5-hydro-6-hydroxyuridylyl-(3'-5')-guanosine*

In Expt. A, the oligonucleotide ApH₂UpG and the control sample of ApUpG were incubated with 230 μ g. of ribosomes and 38 μ g. of [³⁵S]methionine-labelled s-RNA in 170 μ l. of a solution comprising tris-HCl buffer, pH 7.2 (100mm), KCl (50mm) and magnesium acetate (10mm). In Expt. B, ApH₂UpG and ApUpG were incubated as in Expt. A except that the magnesium acetate concentration was 6mm. In Expt. C, equal amounts of Ap(H₂O)UpG, heated Ap(H₂O)UpG and ApUpG were incubated separately with 38 μ g. of [³⁵S]methionine-labelled s-RNA and 400 μ g. of ribosomes in 90 μ l. of a solution comprising tris-HCl buffer, pH 7.2 (130mm), KCl (50mm) and magnesium acetate (10mm). The amount of methionyl-s-RNA bound to the ribosomes was measured as described in the text.

	Concn. of Mg ²⁺ (mm)	Oligonucleotide	Oligonucleotide addition (m μ moles)	Methionine bound (μ m-moles)
Expt. A	10	ApUpG	2.5	3.15 $\pm$ 0.11
		ApH ₂ UpG	3.5	0.52
		None	—	0.33
Expt. B	6	ApUpG	2.5	1.55 $\pm$ 0.11
		ApH ₂ UpG	3.5	0.31
		None	—	0.28
Expt. C	10	ApUpG	0.36	1.73 $\pm$ 0.08
		Ap(H ₂ O)UpG	0.36	0.54
		Heated Ap(H ₂ O)UpG	0.36	1.78
		None	—	0.31

photoreduction procedure produces other minor components (Cerutti *et al.* 1966), the major reaction is the conversion of uridylic acid into dihydro-uridylic acid. The hydrated oligonucleotide offers the advantage, which was kindly pointed out to us by Professor D. Shugar, that the 5-hydro-6-hydroxyuridylic acid can be specifically reconverted into uridylic acid on heating (Sinsheimer, 1954). Table 1 shows that, not only is the s-RNA binding capacity of ApUpG destroyed by converting uridylic acid into 5-hydro-6-hydroxyuridylic acid, but the effect is completely reversed on heating, proving that the loss of activity is not due to other reactions or degradation of the oligonucleotide.

5-Hydro-6-hydroxyuridylic acid would be expected to have the same template properties as dihydrouridylic acid. Grossman (1963) has reported that u.v.-irradiated polyU, comprising uracil dimers, uridylic acid and 5-hydro-6-hydroxyuridylic acid, incorporates serine as well as having a decreased phenylalanine incorporation, which is in contrast with poly(U,H₂U), which only has a decreased incorporation of phenylalanine relative to polyU (Rottman & Cerutti, 1966). However, although Grossman (1963) attributed the serine incorporation to the 5-hydro-6-hydroxyuridylic acid, because of the behaviour of the copolymer after irradiation at different wavelengths, it is possible that the serine incorporation was due to chain scission (Logan & Whitmore, 1966), rather than a template difference between uridylic acid and its hydrate.

The experiments reported here show that when ApUpG is converted into either ApH₂UpG or Ap(H₂O)UpG the triplet loses its ability to bind methionyl-s-RNA. These results are compatible

with the idea that the interconversion of uridylic acid and dihydrouridylic acid in a chain-initiating coding triplet could be involved in the regulation of protein synthesis at the translational level.

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Some Properties of Two Extracellular β -Lactamases from *Bacillus cereus* 569/H

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Many bacteria produce β -lactamases that hydrolyse both benzylpenicillin and cephalosporin C, although the relative maximum rates of hydrolysis of these two substrates vary greatly with the source of the enzyme. Cephalosporinase has been used as an operational term to describe β -lactamases that

hydrolyse cephalosporin C at a significant rate (Abraham & Newton, 1956; Crompton, Jago, Crawford, Newton & Abraham, 1962; Fleming, Goldner & Glass, 1963; Sabath, Jago & Abraham, 1965).

The cephalosporinase activity of culture fluid of