contained only nonadsorbing anions such as F^-. If an adsorbing anion (such as Cl^- or Br^-) (*12*, *17*) were present, it would adsorb at positive applied potentials, yielding a cation permselective membrane. Although the anion would not adsorb at sufficiently negative applied potentials, the metal would have excess electron density at such potentials, and again, cation permselectivity would be observed. Hence, in the presence of an adsorbing anion, cation permselectivity will be observed at all applied potentials (Fig. 5, open circles).

Anion adsorption can be prevented by adsorbing a monolayer of a strongly adherent thiol molecule to the Au surfaces (*18*, *19*). We used 1-propanethiol (PT) because the Au nanotubules can still be wetted with water after adsorption of the PT monolayer (*19*). The E_m versus applied potential curves for an untreated and PT-treated Au-nanotubule membrane, with KBr solutions present on either side of the membrane, are shown in Fig. 5. The untreated membrane shows only cation permselectivity, but the permselectivity of the PT-treated membrane can be switched, exactly as was the case with the nonadsorbing electrolyte (Fig. 4).

We have demonstrated that these metal nanotubule membranes can be cation permselective, anion permselective, or nonselective, depending on the potential applied to the membrane (*20*). These membranes can be as permselective as the commercially relevant Nafion polymer and should have applications in both fundamental and applied electrochemistry. In addition, because the Au tubules have radii that approach molecular dimensions, these membranes might have applications in chemical separations, for example, industrial gas separations (*21*).

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Prebiotic Synthesis of 5-Substituted Uracils: A Bridge Between the RNA World and the DNA-Protein World

Michael P. Robertson and Stanley L. Miller*

Under prebiotic conditions, formaldehyde adds to uracil at the C-5 position to produce 5-hydroxymethyluracil with favorable rates and equilibria. Hydroxymethyluracil adds a variety of nucleophiles, such as ammonia, glycine, guanidine, hydrogen sulfide, hydrogen cyanide, imidazole, indole, and phenol, to give 5-substituted uracils with the side chains of most of the 20 amino acids in proteins. These reactions are sufficiently robust that, if uracil had been present on the primitive Earth, then these substituted uracils would also have been present. The ribozymes of the RNA world would have included many of the functional groups found in proteins today, and their catalytic activities may have been considerably greater than presently assumed.

The discovery of catalytic RNA (*1*) gave credibility to prior suggestions that the first living organisms were RNA molecules with catalytic activity (*2*), a situation that is called the RNA world (*3*). However, the catalytic activities of RNA known so far are mostly limited to phosphodiester reactions. This limitation may be due to the small number of functional groups available in RNA—hydroxyls, phosphates, and the amino and imidazole parts of the bases. It has also been proposed that metal ions are the catalytic agents rather than the RNA functional groups and that the role of the RNA is to hold the metal ions in the correct position (*4*).

It is usually assumed that proteins largely replaced ribozymes because the additional functional groups available gave proteins greater versatility. There are many modified nucleosides in transfer RNA (tRNA), some of which have functional groups of potential catalytic importance (*5*). These nucleosides may be molecular fossils from the RNA world. We show here that, under potentially prebiotic conditions, RNA, particularly the 5-substituted uracils in RNA, would have had many of the functional groups that are available in proteins, and that, as a result, the catalytic activities of the ribozymes of the RNA world may have been considerably greater than presently assumed.

Cytosine and uracil can be efficiently synthesized prebiotically from cyanoacetaldehyde and urea, and a similar reaction with thiourea gives 2-thiocytosine and 2-thiouracil (*6*). Formaldehyde (HCHO) is generally believed to have been present on the early Earth because it is synthesized in good yield under reducing conditions with ultraviolet light and electric discharges (*7*). Both uracil and cytosine and their nucleosides are subject to electrophilic addition of formaldehyde at the C-5 position. We have concentrated on uracil because cytosine, 2-thiouracil, and uridine all react at 1/27 of the rate at which uracil acts, and cytidine reacts at 1/140 of uracil's rate. The pH rate

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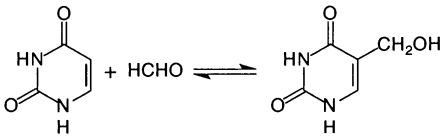
profile for the formation of 5-hydroxymethyluracil (HMU) is shown in Fig. 1 (8):



The rate of addition, k, at pH 7 is given by

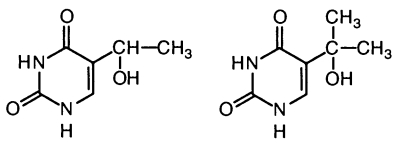
$$\log k\ (M^{-1}\ s^{-1}) = 11.75 - 5488/T$$

(where T is temperature) giving a half-life $t_{1/2}$ of 4.8 years at 0°C and 36 days at 25°C with 1M HCHO and proportionally less at lower HCHO concentrations. The equilibrium constant, K, is given by

$$\log K = -1.36 + 1794/T$$

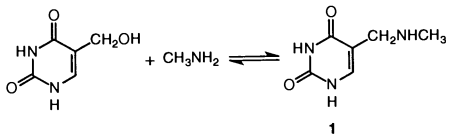
giving K = 161,000 at 0°C and 45,000 at 25°C. This very favorable equilibrium constant suggests that a considerable fraction of the uracil in the prebiotic ocean would have been converted to HMU, which can be considered the uracil analog of serine.

Acetaldehyde and acetone also react with uracil to form:



The rate of reaction with acetaldehyde is 1/400 of that of formaldehyde, and the equilibrium constant is also less favorable. Even so, this reaction, which forms a threonine analog, is potentially prebiotic. A similar reaction would have taken place with most aldehydes. This reaction also occurs with acetone, but the rate is only 1.5×10^{-5} of that of formaldehyde, and so this reaction is probably not prebiotic.

HMU reacts readily with a number of nucleophiles. Amines such as ammonia, methylamine (CH_3NH_2), and glycine react reversibly to give the amino adduct as:



1

Yields, relative rates, and amino acid analogs are given in Table 1 for selected reactions. The Mannich reaction of uracil with formaldehyde and methylamine or glycine is also effective (9). The Mannich reaction, as well as the mechanism for substitution of the hydroxyl of HMU, is shown in Fig. 2. Methylaminomethyluracil (1) and carboxymethylaminomethyluracil (2) are modified bases that occur in tRNA, generally in the wobble position of the anticodon.

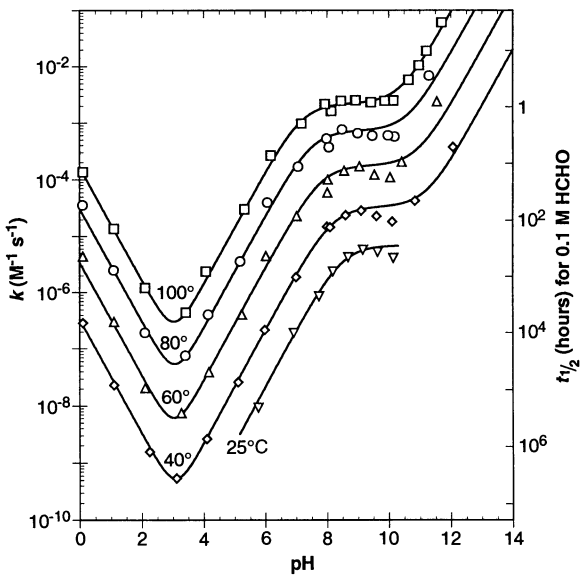
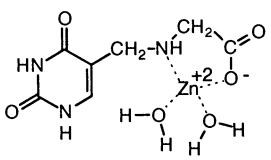


Fig. 1. The pH rate profile for the formation of HMU at a variety of temperatures. The rate constants are the second-order values for rate = k(HCHO)(uracil).

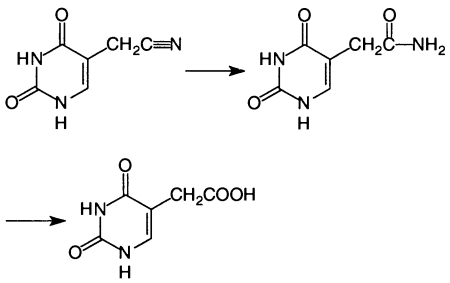
Table 1. Products and yields from the addition of various nucleophiles to HMU at pH 7 and 100°C. The nucleophile concentrations were 0.1 M except for HCN, H_2S, and indole, which were 0.01 M. The yields are based on HPLC analysis of product and starting material (15). The relative rates were measured in comparison to NH_3/NH_4^+ at pH 7 and 100°C.

Nucleophile	Reaction time (hours)	Yield (%)	Relative rates	Amino acid analog
NH_3	24	31	1.0	Lysine
CH_3NH_2	1	63	0.6	Sarcosine
Glycine	1	56	3	Iminodiacetic
Guanidine	25	2.2	0.001	Arginine
HCN	25	99	1.2	Asn, Asp, Gln, Glu
H_2S	25	99	25	Cysteine
Imidazole	25	95	9	Histidine
Indole	25	99	62	Tryptophan
Phenol	25	~90	3	Tyrosine

The glycine adduct should be an excellent chelator of metals. Other amino acids such as iminodiacetic, cysteine, and ethylenediamine have the potential for selective chelation of metals. Thus, if the role of the ribozyme is to hold metals (such as zinc) and complexed water in the proper position (4), these uracil adducts offer a wider variety of chelating groups than those in contemporary RNA to accomplish this goal:



Guanidine adds to HMU, although less efficiently than amines because of its high pK of 13.5. Hydrogen cyanide reacts with HMU to form uracil-5-acetonitrile. Hydrolysis produces uracil-5-acetamide followed by uracil-5-acetic acid:



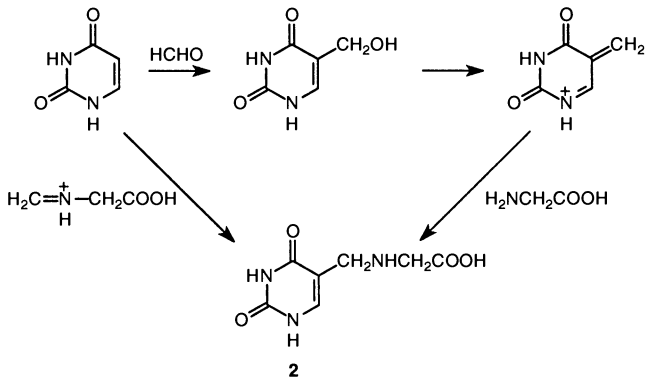
Hydrogen sulfide (H_2S) reacts rapidly with HMU to form the sulfhydryl adduct (Fig. 3). It is expected that CH_3SH reacts similarly to form the methyl derivative.

HMU can be reduced effectively with formate to thymine (Fig. 3). This is related to the Leuckart reaction (10). The reaction rate is given by

$$\text{rate} = k(HCOO^-)(HMU)$$

and is independent of pH between 4 and 9. The reaction is efficient only in concentrated formate solutions and thus would be important in the concentrated

Fig. 2. Mechanism for the substitution of the hydroxyl group of HMU.



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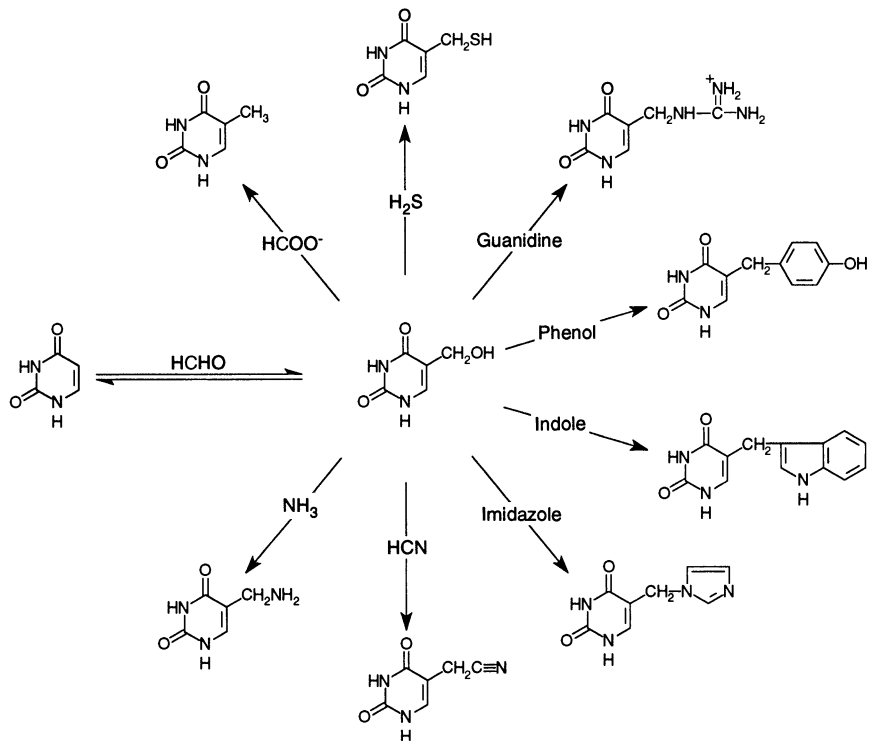
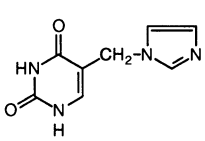


Fig. 3. Formation of amino acid analogs by the reaction of HMU with various nucleophiles.

formate solutions of dried lagoons and beaches. Thymine can be considered the uracil analog of alanine. The analogs of the other aliphatic amino acids could have been produced by reduction of aldehyde adducts.

Imidazole, which is considered a prebiotic compound (*11*), adds to HMU at the nitrogen (**3**) with the identification based on nuclear magnetic resonance (NMR) spectroscopy. This is clearly the kinetic product, with the thermodynamic product being the C–C bond compound (**4**):



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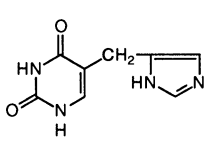
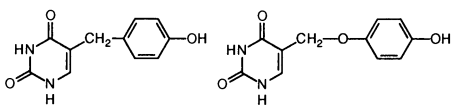


4

However, heating at 100°C for 10 days gave no indication of formation of the thermodynamic product. The kinetic product should be just as effective as the thermodynamic product in general acid-base catalysis.

Indole adds efficiently to HMU to give the tryptophan analog (Fig. 3). Indole can be synthesized by pyrolysis and electric discharge reactions of hydrocarbon and ammonia mixtures (*12*) and is one of the more effective prebiotic nucleophiles.

Phenol also adds efficiently to HMU:



Neither the *ortho* isomer nor the O adduct was detectable (<5%). Phenol is a reasonable prebiotic compound as it is easily produced from benzene by reaction with hydroxyl radicals (*13*).

The ease with which nucleophiles add to HMU suggests that even more functional groups were available in the RNA world than are available in modern proteins. These additional RNA functional groups may have been used in amino acids in the early DNA-protein world and subsequently discarded or produced by posttranslational modifications. Examples of compounds that could react with HMU to give additional functional groups include secondary and tertiary amines, H_2Se, mercaptans, pyrrole, sugars, and parts of contemporary coenzymes such as pyridoxal.

Some reasons that have been offered for the selection of the 20 protein amino acids include prebiotic abundance and stability (*14*). The results presented here suggest an alternative basis for the selection: The side chains of the 20 amino acids were originally attached to the C-5 of uracil. As the functions performed by the RNA were taken over by proteins, the functional groups would have been needed in the new protein polymers to carry out the necessary catalytic functions. Thus, the 5-substituted uracils would have been a bridge between the RNA world and the DNA-protein world.

We have not dealt with the problem of getting the modified base into the correct position in a ribozyme. In modern tRNA and ribosomal RNA, this is done by posttranscriptional modifications catalyzed by protein enzymes. In the RNA world, this process may have been carried out by ribozymes or by catalysis of adjacent nucleotides. An initial solution could have been the addition of the modified bases at random. If the catalytic effect of the modified base is sufficiently high, the catalytic efficiency of the collection of such ribozymes may have been sufficient for early metabolic processes.

The above data indicate the ease of synthesis of HMU from uracil as well as the other 5-substituted uracils. These reactions are very robust, and it is evident that, if conditions on the primitive Earth were favorable for cytosine and uracil synthesis, then HMU and the amino acid analogs would also have been synthesized. In other words, the 5-substituted uracils are as prebiotic as uracil itself. They would have been present in the early RNA world regardless of whether the modified nucleosides in modern tRNA are molecular relics of an earlier time.

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A Reevaluation of the Ozone Budget with HALOE UARS Data: No Evidence for the Ozone Deficit

P. J. Crutzen,* J.-U. Grooß, C. Brühl, R. Müller, J. M. Russell III

Recently, additional ozone production mechanisms have been proposed to resolve the ozone deficit problem, which arises from greater ozone destruction than production in several photochemical models of the upper stratosphere and lower mesosphere. A detailed ozone model budget analysis was performed with simultaneous observations of O_3, HCl, H_2O, CH_4, NO, and NO_2 from the Halogen Occultation Experiment (HALOE) on the Upper Atmosphere Research Satellite (UARS) under conditions with the strongest photochemical control of ozone. The results indicate that an ozone deficit may not exist. On the contrary, the use of currently recommended photochemical parameters leads to insufficient ozone destruction in the model.

One of the goals of stratospheric modeling is to reproduce observed O_3 concentrations, a prerequisite for the prediction of future O_3 trends. Several model studies (*1–7*) have used experimental data to simulate stratospheric chemistry; most (*1–5*) have concluded that O_3 production by O_2 dissociation is significantly smaller than its destruction in the upper stratosphere and lower mesosphere. This discrepancy is called the "ozone deficit problem." To account for it, additional autocatalytic O_3 production mechanisms involving vibrationally or electronically excited O_2 molecules produced from O_3 photolysis have been proposed (*8–11*). One of the proposed mechanisms, the photodissociation of vibrationally excited O_2 as a source of O_3, has been discarded because of efficient deactivation of high vibrational levels (*6*, *12*). However, the formation of highly vibrationally excited O_2 (where the vibration state $v \geq 26$) from O_3 photolysis at a wavelength of 226 nm, followed by the reaction $O_2 (v \geq 26) + O_2 \rightarrow O + O_3$, has recently been proposed as a significant source of O_3 (*9*).

Here, we revisit this issue, adopting sunset and sunrise observations of HALOE (*13*), together with a photochemical, gas phase–only, box model with an exact numerical package for stiff differential equations (*14*). The model integrates a comprehensive photochemical set of reactions, using currently recommended rate coefficients, absorption cross sections (*15*), and solar fluxes (*16*). Starting from zonally averaged sunrise or sunset HALOE measurements of O_3, H_2O, CH_4, HCl, NO, and NO_2, the model calculates the diurnal variabilities of the concentrations of the HALOE gases and several important compounds not measured by HALOE, in particular $O(^1D)$ and $O(^3P)$, H, OH, HO_2 and H_2O_2, Cl, ClO, HOCl, $ClONO_2$ and Cl_2O_2, and NO_3, N_2O_5, HNO_3, and HNO_4. Photolysis rates are calculated at 15-min intervals. The model is applied to the stratosphere between about 25 and 55 km, at 23°N during July and 23°S during January, when conditions of strongest photochemical control on ozone and stratospheric chemistry in general are found. The observations were reset at 24-hour intervals, and the model was run over full diurnal cycles for 40 days to ensure convergence in chemical species concentrations between successive days. The difference between our modeling results and those of several previous studies will be discussed in the section on sensitivity studies.

In Fig. 1A, we show the percentage changes in O_3 concentrations, r_3, calculated during the final day of integration. Except for a small region with slight negative deviations near 40 km in one case (12 January 1994, 23°S, sunset), all cases show an increase in calculated O_3 concentrations, corresponding to an O_3 surplus (*17*) instead of a deficit, especially above the 3-hPa (1 hectopascal = 100 Pa) pressure level (≈40

Table 1. The reactions most relevant to the O_3 budget.

Reaction			$\Delta(O_x)$
$O + ClO$	→	$Cl + O_2$	$-2\ (D_{Cl})$
$O + NO_2$	→	$NO + O_2$	$-2\ (D_N)$
$O + O_3$	→	$2\ O_2$	$-2\ (D_O)$
$O + OH$	→	$H + O_2$	$-1\ (D_H)$
$O_3 + H$	→	$OH + O_2$	$-1\ (D_H)$
$O_3 + OH$	→	$HO_2 + O_2$	$-1\ (D_H)$
$O_3 + HO_2$	→	$OH + 2O_2$	$-1\ (D_H)$
$O + HO_2$	→	$OH + O_2$	$-1\ (D_H)$
$O(^1D) + H_2O$	→	$2\ OH$	$-1\ (D_H)$
$HO_2 + NO$	→	$NO_2 + OH$	$+1\ (P_N)$
$HO_2 + OH$	→	$H_2O + O_2$	0
$ClO + NO$	→	$Cl + NO_2$	0
$O_2 + h\nu$	→	$O + O$	$+2\ (P_O)$

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