Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 8158–8160, August 1995
Chemistry

Rates of decomposition of ribose and other sugars: Implications for chemical evolution

(RNA world/pre-RNA world/ribose stability)

ROSA LARRALDE*, MICHAEL P. ROBERTSON†, AND STANLEY L. MILLER

Department of Chemistry and Biochemistry, University of California at San Diego, La Jolla, CA 92093-0317

Contributed by Stanley L. Miller, May 30, 1995

ABSTRACT **The existence of the RNA world, in which RNA acted as a catalyst as well as an informational macromolecule, assumes a large prebiotic source of ribose or the existence of pre-RNA molecules with backbones different from ribose-phosphate. The generally accepted prebiotic synthesis of ribose, the formose reaction, yields numerous sugars without any selectivity. Even if there were a selective synthesis of ribose, there is still the problem of stability. Sugars are known to be unstable in strong acid or base, but there are few data for neutral solutions. Therefore, we have measured the rate of decomposition of ribose between pH 4 and pH 8 from 40°C to 120°C. The ribose half-lives are very short (73 min at pH 7.0 and 100°C and 44 years at pH 7.0 and 0°C). The other aldopentoses and aldohexoses have half-lives within an order of magnitude of these values, as do 2-deoxyribose, ribose 5-phosphate, and ribose 2,4-bisphosphate. These results suggest that the backbone of the first genetic material could not have contained ribose or other sugars because of their instability.**

The discovery of catalytic RNA (1, 2) gave credibility to previous suggestions that the first living organisms were RNA molecules with catalytic activity (3–7), a concept known as the RNA world (8, 9). We take the RNA world to mean the period before the evolution of protein synthesis when RNA itself contained the genetic information and acted as a catalyst in biochemical reactions. This RNA would have contained a ribose-phosphate backbone with adenine, uracil, guanine, and cytosine (A, U, G, and C) as the bases. It is likely that there was a period before the RNA world, which we will call the pre-RNA world, when living organisms contained a backbone different from ribose-phosphate and possibly bases different from A, U, G, and C. These molecules would still have encoded genetic information and acted as catalysts.

There are a number of reasons to believe that the original backbone was not ribose-phosphate, although A, U, G, and C are likely candidates for the bases. First, the polymerization of formaldehyde to sugars, known as the formose or Butlerow reaction, produces almost all possible pentoses and hexoses, including branched-chain sugars, with no selectivity for ribose (10, 11). Also, while the formose reaction works under prebiotic conditions, it is not particularly efficient (12, 13). Another problem is that nucleoside synthesis from the heating of purines with ribose is inefficient and does not work at all with pyrimidines (14, 15). Even if nucleosides could be efficiently synthesized, the products would be racemic, leading to enantiomeric cross-inhibition difficulties in template polymerizations (16).

There is an additional problem as severe as any of the above—the stability of ribose. Sugars are well known to be unstable in acid and base (17–21), but there are few data for neutral solutions (22, 23). We find that ribose and other sugars have surprisingly short half-lives for decomposition at neutral pH, making it very unlikely that sugars were available as prebiotic reagents.

EXPERIMENTAL PROCEDURES

The sugars were all purchased from Sigma except for glucose (Fisher), gulose (Biospherics, Beltsville, MD), and ribose 2,4-bisphosphate (S. Pitsch, Eidgenössiche Technische Hochschule, Zurich).

The rates of decomposition were measured by ^1H NMR on a General Electric QE 300-MHz instrument. The samples were dissolved in aqueous 0.05 M deuterated phosphate [p^2H (pD) 6.4–8.4] or acetate (pD 4.4–5.4) buffer and sealed in NMR tubes. Each tube also contained an external reference typically consisting of 0.5% $CHCl_3$ in CCl_4 in a sealed capillary tube. The pH of the solutions was measured at 25°C on a Corning model 250 pH meter and these readings were converted to pD by the formula pD = pH + 0.4 (24). The pD was corrected for the temperature by $\Delta pD = \Delta pK$ (24). In the case of ribose and ribose 5-phosphate, the decomposition of the sugar was monitored by the decrease in the NMR signal of the proton on carbon 1 (1-H) of the α and β furanose forms relative to a $CHCl_3$ external reference. The 1-H of the furanose forms was chosen because of the relative isolation of their chemical shifts from those of water and the decomposition products. For the other sugars, the 1-H signals most separated from the water signal were used. No attempt was made to identify the decomposition products because of the complexity of the mixtures (17, 25).

RESULTS AND DISCUSSION

The rate of decomposition of ribose in H_2O at 100°C and pH 7.0 was compared with that in D_2O at 100°C and pD 7.4, by evaporating the H_2O and replacing it with D_2O after the reaction. The rates were identical within experimental error ($k_{H_2O}/k_{D_2O} = 1.0 \pm 0.2$).

There is considerable general base catalysis of the decomposition of ribose and the other sugars. At pD 7.4 and 100°C,

$$\text{rate } (s^{-1}) = 2.86 \times 10^{-3} + 9.36 \times 10^{-2}([DPO_4^{2-}] + [D_2PO_4^-]).$$

The decomposition products are acidic, and buffer was required to maintain the pH. To minimize catalysis by the buffer, the lowest buffer concentration that maintained the pH at a constant value was used (0.05 M phosphate or acetate).

The pD–rate profile for the decomposition of ribose is shown in Fig. 1. The rate is approximately proportional to the OD^- ion concentration in the pD range 4–6. The rate is faster than extrapolated from Fig. 1 at pD < 4 due to acid catalysis (data not shown). The curve levels off at higher pH values, suggesting that the ionization of an intermediate in the de-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: D, deuterium.

*Present address: Harvard University, Cambridge, MA 02138.
†Present address: Department of Chemistry, Indiana University, Bloomington, IN 47405.

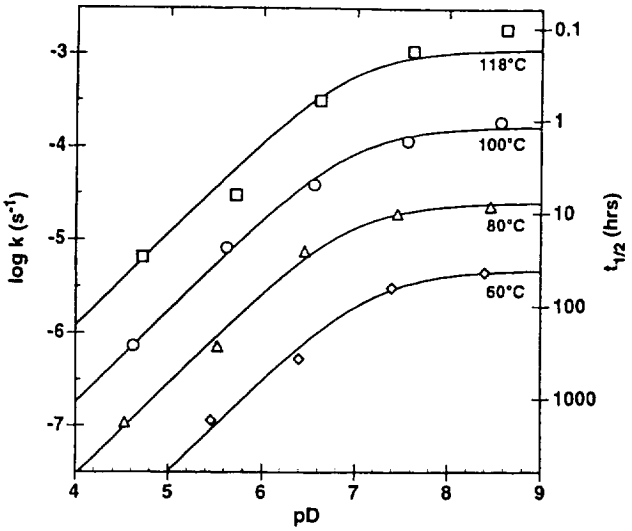

FIG. 1. Rates of ribose decomposition as a function of pD. The curves were fitted by assuming a functional form $k = k_0/(1 + [H^+]/K_a)$, where K_a and k_0 are adjusted constants.

composition is the rate-determining step, although it could also be due to general base catalysis by HPO_4^{2-} rather than OH^-.

Although phosphate is a minor component in the present ocean, there is considerable buffering by the 2.3×10^{-3} M HCO_3^-. The bicarbonate concentration would have been higher in a prebiotic ocean with a primitive atmosphere high in CO_2 (26, 27). We measured the rate of ribose decomposition at pD 7.4, using a 0.05 M bicarbonate buffer. The rate is 0.52 that observed with a 0.05 M phosphate buffer. We believe, therefore, that these results should be applicable to any reasonable primitive ocean model.

The half-life of ribose decomposition at 100°C and pD 7.4 is 73 min. Even if we assume a primitive ocean devoid of catalysis by general bases, ribose still decomposes so rapidly that it is difficult to see how it could have played a role in any high-temperature origin-of-life scenario unless it was utilized immediately after its prebiotic synthesis.

The temperature dependence of the rate of ribose decomposition follows an Arrhenius curve as shown in Fig. 2. The equation for pD 7.4 is given by

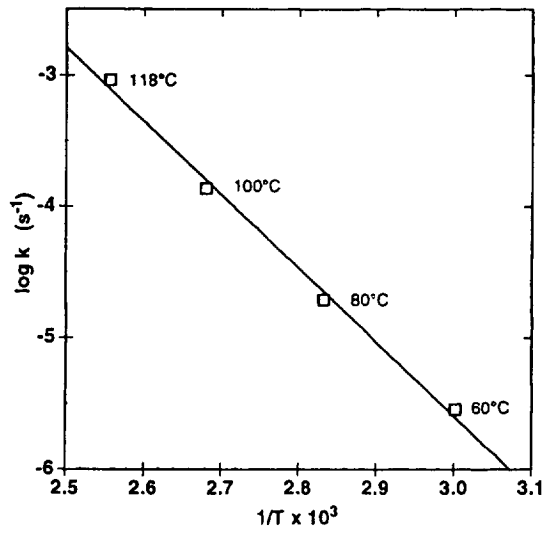

FIG. 2. Arrhenius plot of the rate constants of ribose decomposition at pD 7.4 in 0.05 M phosphate buffer. The experimental points have been adjusted for the change in the buffer pD with temperature.

$$\log k\ (s^{-1}) = 11.23 - 5608/T,$$

with a heat of activation of 25.7 kcal·mol^{-1}. The half-life at pD 7.4 extrapolates to 300 days at 25°C and to 44 years at 0°C. Even with correction for the buffer concentration, this is a very short period of geological time, and it is difficult to see how ribose could have been available for prebiotic use, even at low temperatures.

The stability of other sugars is of interest because of their involvement in many biological processes and because they may have been utilized earlier than ribose. Fig. 3 shows the rate constants for the decomposition of the four aldopentoses and eight aldohexoses which are approximately proportional to the percent of free aldehyde (28). The major uncertainty lies in the values of the free aldehyde, which were measured at 20°C rather than the temperature of 100°C used for the decomposition measurements. All of the sugars decompose with half-lives within an order of magnitude of the ribose value. Unless the temperature coefficients of these decompositions differ greatly from that of ribose, it is clear that these other sugars are as unlikely as ribose to have been available in the prebiotic world.

2-Deoxyribose decomposes at pD 7.4 and 100°C with a half-life of 225 min (2.6 times slower than ribose). Ribose 5-phosphate decomposes with a half-life of 7 min (12 times faster than ribose). These differences in rate are relatively minor, so that the same considerations apply to these ribose derivatives as to ribose itself.

Ribose 2,4-bisphosphate has been synthesized with considerable selectivity from glycolaldehyde phosphate and formaldehyde (29), and it may have been a prebiotic reagent. In addition, it has been shown to be much more stable than ribose in 2 M NaOH. Our measurements of the decomposition of ribose 2,4-bisphosphate at pD 7.4 show that it decomposes with a half-life of 31 min at 100°C. Although ribose 2,4-bisphosphate is quite stable in base, it is clear that it is not substantially more stable at pD 7.4 than other aldopentoses and aldohexoses.

These data probably underestimate the rate of decomposition of sugars under plausible prebiotic conditions. The pH of the present ocean is 8.2 rather than 7.0, which would increase

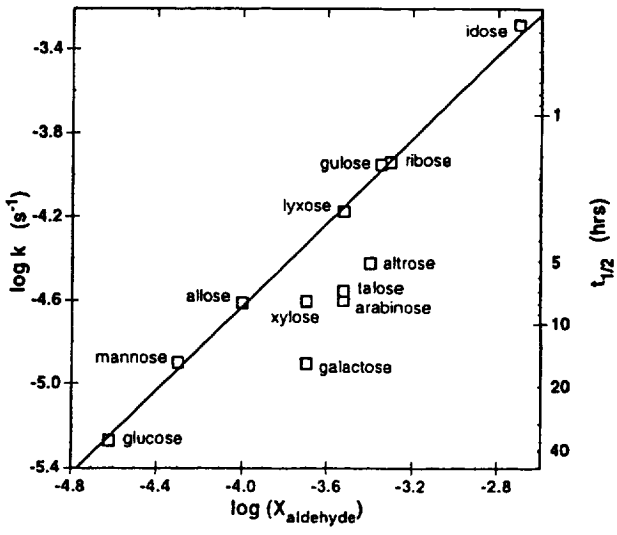

FIG. 3. Rate of decomposition of aldoses at pD 7.4 and 100°C in 0.05 M phosphate buffer vs. the mole fraction (X) of the free aldehyde at 20°C. The line assumes proportionality between k and $X_{aldehyde}$ and was drawn through ribose. The amount of free aldehyde of aqueous gulose is not known, so this point has been arbitrarily placed near the line. The value for glucose has been extrapolated from its decomposition rate at 80°C in 1 M phosphate buffer.

the rate by about 50% (Fig. 1). The reaction of amino acids with sugars—the Maillard, or browning, reaction—is a rapid and irreversible decomposition pathway (30, 31). In addition, aldoses are easily oxidized to sugar acids and they react readily with low concentrations of HCN (Kiliani reaction) to form the next higher sugar acid (32, 33). These and other decomposition pathways make it unlikely that sugars played a role in early prebiotic syntheses. These pathways may also account for the absence of sugars in the Murchison meteorite (34). Carbonaceous chondrites such as the Murchison meteorite were formed on an asteroid in an environment considered to be a reasonable model of prebiotic synthesis on the early Earth.

The means by which ribose became incorporated into the genetic material is not clear and is difficult to discuss without knowing the precursor to the ribose-phosphate backbone. Although ribose and other sugars are very unstable, it is possible that stable prebiotic derivatives of ribose were present in the primitive ocean that could have been converted to ribose by one or two enzymatic steps. For example, ribose and other sugars produced in the formose reaction are quickly converted to the next higher sugar acid by the Kiliani reaction. The conversion back to ribose would involve an enzymatic step to ribulose and a nonenzymatic isomerization to ribose (Fig. 4). Another potential pathway would have been to oxidize ribose to the aldonic acid immediately after its prebiotic synthesis. It could then be converted back to ribose in a single enzymatic step from the lactone with NADH.

Glycolysis has long been thought to have been one of the earliest energy sources in biology, although a case has been made against this (35). The instability of the pentoses and hexoses supports the relatively late appearance of glycolysis, since the sugars would not have been available near the time of the origin of life.

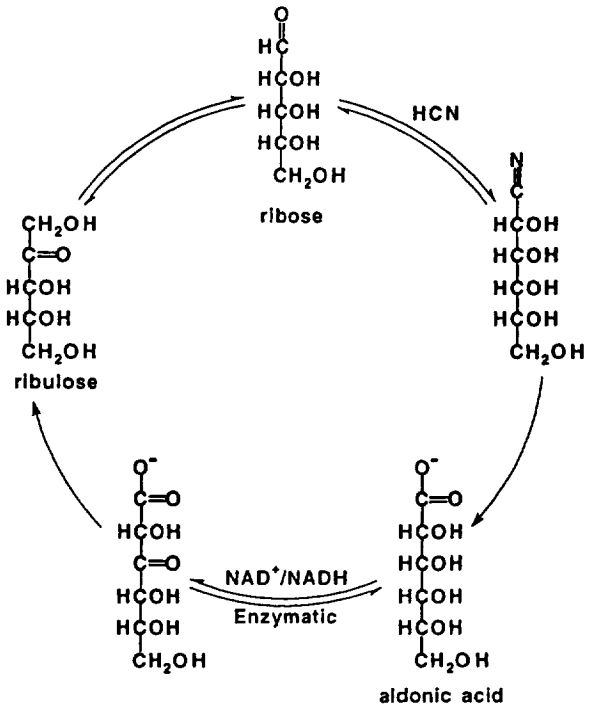

FIG. 4. A possible prebiotic storage scheme for ribose. Hydrogen cyanide reacts with aldoses to form the cyanohydrin, which is rapidly hydrolyzed to the lactone and the aldonic acid (that of allose is shown here, but the aldonic acid of altrose is also formed). The aldonic acids are very stable and could have been converted back to ribose by a single enzymatic step when ribose was needed at the beginning of the RNA world. These reactions are related to the first steps of the pentose phosphate pathway, but use nonphosphorylated sugars.

CONCLUSION

The above results show that stability considerations preclude the use of ribose and other sugars as prebiotic reagents except under very special conditions. It follows that ribose and other sugars were not components of the first genetic material and that other possibilities, such as the peptide nucleic acids (36) and other non-sugar-based backbones, should be examined.

We thank Dr. Stephan Pitsch for the sample of ribose 2,4-bisphosphate, Antonio Lazcano and Jason Dworkin for helpful comments, and the National Aeronautics and Space Administration Specialized Center for Research and Training in Exobiology at the University of California, San Diego, for grant support.

1. Kruger, K., Grabowski, P. J., Zaug, A. J., Sands, J., Gottschling, D. E. & Cech, T. R. (1982) *Cell* **31**, 147–157.
2. Guerrier-Takada, C., Gardiner, K., Marsh, T., Pace, N. & Altman, S. (1983) *Cell* **35**, 849–857.
3. Woese, C. R. (1967) *The Genetic Code* (Harper & Row, New York), pp. 179–195.
4. Crick, F. H. C. (1968) *J. Mol. Biol.* **38**, 367–379.
5. Orgel, L. E. (1968) *J. Mol. Biol.* **38**, 381–393.
6. White, H. B., III (1976) *J. Mol. Evol.* **7**, 101–104.
7. Visser, C. M. & Kellogg, R. M. (1978) *J. Mol. Evol.* **11**, 163–169.
8. Gilbert, W. (1986) *Nature (London)* **319**, 618.
9. Sharp, P. A. (1985) *Cell* **42**, 397–400.
10. Decker, P., Schweer, H. & Pohlmann, R. (1982) *J. Chromatogr.* **244**, 281–291.
11. Shapiro, R. (1988) *Origin Life Evol. Biosphere* **18**, 71–85.
12. Gabel, N. W. & Ponnamperuma, C. (1967) *Nature (London)* **216**, 453–455.
13. Reid, C. & Orgel, L. E. (1967) *Nature (London)* **216**, 455.
14. Fuller, W. D., Sanchez, R. A. & Orgel, L. E. (1972) *J. Mol. Biol.* **67**, 25–33.
15. Fuller, W. D., Sanchez, R. A. & Orgel, L. E. (1972) *J. Mol. Evol.* **1**, 249–257.
16. Joyce, G. F., Schwartz, A. W., Miller, S. L. & Orgel, L. E. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4398–4402.
17. Evans, W. L. (1942) *Chem. Rev.* **31**, 537–560.
18. Khym, J. X., Doherty, D. G. & Cohn, W. E. (1954) *J. Am. Chem. Soc.* **76**, 5523–5530.
19. Isbell, H. S., Frush, H. L., Wade, C. W. R. & Hunter, C. E. (1969) *Carbohydr. Res.* **9**, 163–175.
20. Pigman, W. & Anet, E. F. L. J. (1972) in *The Carbohydrates: Chemistry and Biochemistry*, eds. Pigman, W. & Horton, D. (Academic, New York), 2nd Ed., Vol. 1A, pp. 165–194.
21. El Khadem, H. S., Ennifâr, S. & Isbell, H. S. (1987) *Carbohydr. Res.* **169**, 13–21.
22. Borenfreund, E. & Dische, Z. (1957) *Biochim. Biophys. Acta* **25**, 215–216.
23. Mopper, K., Dawson, R., Liebezeit, G. & Ittekkot, V. (1980) *J. Mar. Chem.* **10**, 55–66.
24. Bates, R. G. (1964) *Determination of pH: Theory and Practice* (Wiley, New York), 2nd Ed.
25. Browne, C. A. & Zerban, F. W. (1941) *Physical and Chemical Methods of Sugar Analysis* (Wiley, New York), pp. 645–659.
26. Walker, J. C. G. (1983) *Nature (London)* **302**, 518–520.
27. Kasting, J. F. (1993) *Science* **259**, 920–926.
28. Angyal, S. J. (1984) *Adv. Carbohydr. Chem. Biochem.* **42**, 15–68.
29. Müller, D., Pitsch, S., Kittaka, A., Wagner, E., Wintner, C. E. & Eschenmoser, A. (1990) *Helv. Chim. Acta* **73**, 1410–1468.
30. Waller, G. R. & Feather, M. S., eds. (1983) *The Maillard Reaction in Foods and Nutrition* (Am. Chem. Soc., Washington, DC).
31. Baynes, J. W. & Monnier, V. M., eds. (1989) *The Maillard Reaction in Aging, Diabetes, and Nutrition* (Liss, New York).
32. Hudson, C. S. (1945) *Adv. Carbohydr. Chem.* **1**, 1–36.
33. Serianni, A. S., Nunez, H. A. & Barker, R. (1980) *J. Org. Chem.* **45**, 3329–3341.
34. Cronin, J. R., Pizzarello, S. & Cruikshank, D. P. (1988) in *Meteorites and the Early Solar System*, eds. Kerridge, J. F. & Matthews, M. S. (Univ. of Arizona Press, Tucson), pp. 819–857.
35. Clarke, P. H. & Elsden, S. R. (1980) *J. Mol. Evol.* **15**, 333–338.
36. Nielsen, P. E. (1993) *Origin Life Evol. Biosphere* **23**, 323–327.