A EUROPEAN JOURNAL OF CHEMICAL BIOLOGY

CHEMBIOCHEM

SYNTHETIC BIOLOGY & BIO-NANOTECHNOLOGY

Accepted Article

Title: The effects of pressure (0.1 - 200 MPa) and pH on the hydrolysis of cytosine at 373 K: implications for nucleotide stability around deep-sea black smokers

Authors: Christopher P Lepper, Martin AK Williams, David Penny, Patrick JB Edwards, and Geoffrey Brind Jameson

To be cited as: *ChemBioChem* 10.1002/cbic.201700555

Link to VoR: http://dx.doi.org/10.1002/cbic.201700555

A Journal of

ChemPubSoc Europe

WILEY-VCH

www.chembiochem.org

10.1002/cbic.201700555

WILEY-VCH

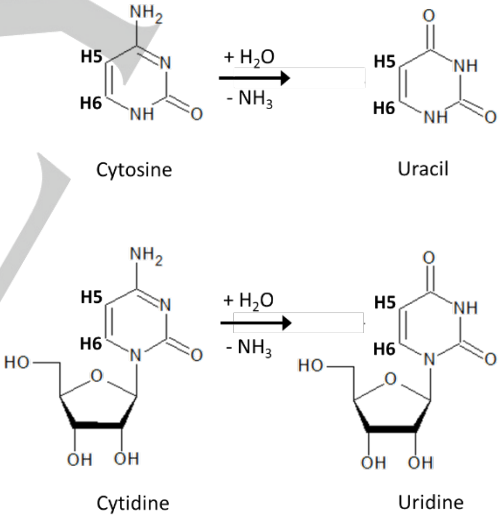
COMMUNICATION

The effects of pressure (0.1 – 200 MPa) and pH on the hydrolysis of cytosine at 373 K: implications for nucleotide stability around deep-sea black smokers

Christopher P. Lepper,[b] Martin A.K. Williams,[c] David Penny,[b] Patrick J.B. Edwards,[b] and Geoffrey B. Jameson*[a]

Abstract: The relatively low chemical stability of cytosine compared to other nucleobases is a key concern in origin-of-life scenarios, but the effect of pressure on the rate of hydrolysis of cytosine to uracil has remained unknown. We have determined by in situ NMR measurements that the half-life of cytosine at 373.15 K decreases from 18.0 (±0.7) days at ambient pressure (0.1 MPa) to 8.64 (±0.18) days at high pressure (200 MPa). This yields an activation volume for hydrolysis of -11.8 (±0.5) $cm^3 mol^{-1}$, a decrease that is similar to the molar volume of water (18.0 cm^3 mol^{-1}) and consistent with a tetrahedral 3,3-hydroxyamine transition-state/intermediate species. Very similar behavior was observed also for cytidine. At both ambient and high pressures, the half-life of cytosine decreases significantly as pH is dropped from 7.0 to 6.0. These results provide scant support for the notion that RNA-based life forms originated in high-temperature, high-pressure, acidic environments.

There are many, varied and hotly debated scenarios for the origin of life on earth. Many scenarios have RNA-based life forms preceding the extant protein-based life forms[1], or co-evolution of RNA alongside DNA.[2] However, these scenarios are vigorously disputed in favour of protein-first FeS-templated origins.[3] There are acid hot-start[4] *versus* alkaline warm-start[5] *versus* cold-start[6] scenarios with hot-start scenarios popularistically featuring the energy- and chemically-rich deep-ocean black smokers where hyperthermophiles from the kingdom Archaea, often taken as the closest living descendants of the first protein-based life forms,[7] thrive at temperatures between 80 °C and 110 °C and often at high pressure (> 50 MPa). A single point of agreement in current (*i.e.*, since ~2012) origin-of-life scenarios invokes a role for FeS species in redox reactions. Separately, it has long been known that, at least at ambient pressure of one atmosphere (0.1 MPa), folded RNA structures denature at temperatures less than 70 °C *in vitro*.[8] With respect to chemical stability, studies at mesophilic conditions[9] have revealed the susceptibility of cytosine and cytidine to hydrolytic deamination, both as individual molecules and when incorporated into ~5000 base-pair bacteriophage DNA.[10] Subsequently, Levy and Miller[11] found that at 100 °C and ambient pressure the nucleobase cytosine hydrolysed to uracil (Scheme 1) with a half-life of just 19 days, compared to 340 years at 25 °C. This rate of hydrolysis is at least 15 times faster than the rates of decomposition of the other nucleobases (the half-life of guanine and adenine are, respectively, 0.8 and 1 yr at 100 °C). These studies have been augmented very recently by Lewis *et al.*,[12] who reported rate constants and an enthalpy of activation of ~23 kcal mol^{-1} for deamination of cytosine and various derivatives at pH 2.4 and 7.0 over the temperature range ~90–~200 °C.



Scheme 1. The hydrolysis of cytosine and cytidine to uracil and uridine, respectively. The atom labelling scheme is shown for NMR-monitored protons H5 and H6.

Assuming that earliest life forms were rather inefficient and not adapted and stripped down for speedy turnover, in contrast to many extant Prokarya and Archaea, these results on the diminished chemical stability of cytosine at elevated temperatures and the physical instability of folded RNA structures have rendered unlikely a hot-start scenario for RNA-based life forms. In this context, it must also be noted that although phosphodiester bonds have extreme chemical stability, with a half life for hydrolysis at 25 °C and pH 7 of ~130,000 to 30,000,000 years,[13] in the presence of strong Lewis-acid species, featuring usually

[a] Professor Dr Geoffrey B Jameson
Institute of Fundamental Sciences, the MacDiarmid Institute and the Riddet Institute
Massey University
Palmerston North
New Zealand
E-mail: g.b.jameson@massey.ac.nz
[b] Dr Christopher P Lepper, Professor Dr David Penny, Dr Patrick JB Edwards
Institute of Fundamental Sciences
Massey University
Palmerston North
New Zealand
[c] Professor Dr Martin (Bill) AK Williams
Institute of Fundamental Sciences, the MacDiarmid Institute and the Riddet Institute
Massey University
Palmerston North
New Zealand

Supporting information for this article is given via a link at the end of the document.

dizinc(II), dinickel(II) complexes or lanthanide species, hydrolysis rates are orders of magnitude higher than those for the deamination of cytosine.[14] Modern cytidine deaminases, including the APOBEC3 family of single-stranded DNA mutator proteins all feature a Zn(his)(cys)$_2$ active site.[15]

However, these studies on the stability of cytosine have not explicitly addressed the effect of pressure, and whether or not high pressure in the absence of strong Lewis-acid metal ions might counter or exacerbate the deleterious effects of high temperatures on RNA's physical and chemical stability. It has been argued that high pressure may have had an important role in the development of life on earth,[6, 16], even though the syntheses of the building blocks of life, nucleotides, carbohydrates, and amino acids, have recently been reported to be facilitated by ultraviolet radiation, which is conspicuously absent at depth.[17] We report here the effects of pressure, and of pH, on the chemical stability of cytosine and cytidine at 100 °C.

The hydrolysis of cytosine (Scheme 1) was monitored at 373 K and at pressures of 0.10, 50, 100, 150 and 200 MPa (Figure 1a). In Figure S1, the corresponding data for hydrolysis of cytidine at 373 K and 0.10 and 150 MPa are shown. The process is pseudo-first order in all cases, as expected from ambient pressure measurements,[9, 11-12, 18]. All plots of -ln[cytosine] *vs.* time are linear over the practically accessible observation times. The NMR spectra, especially of cytidine, were closely inspected for evidence of decomposition additional to the deamination to uracil. No evidence was found by way of peaks that were not assignable to either uracil (uridine) or cytosine (or cytidine). Moreover, the summed integrals of the H5 and H6 proton signals for uracil and cytosine (or cytidine), normalised to that of reference TMSP, were constant within 4.0% over the time course of the experiment. Representative NMR spectra at the start and end of the reaction are shown in Figure S2 at both 0.10 MPa and 200 MPa (see Supporting Information).

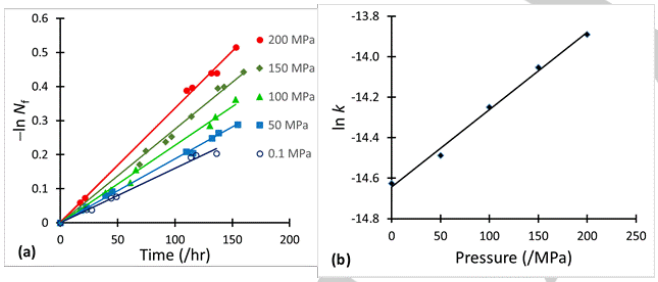


Figure 1. (a) Fraction of cytosine remaining, N_t = [cytosine]$_t$/[cytosine]$_0$, plotted as –ln N_t *vs* time (t) at 373 K and pH 7.0 for hydrolysis of cytosine at various pressures (dark blue empty circles 0.1 MPa, blue filled squares 50 MPa, green filled triangles 100 Pa, orange filled diamonds 150 MPa and red filled circles 200 MPa). The pH is corrected for the effects of pressure. The rate constant for hydrolysis is the slope of the line. (b) Plot of the rate constant k for the hydrolysis of cytosine at 373 K *vs.* pressure p.

The rate constants and associated estimated standard deviations, derived as the slope of these plots, are tabulated in Table S1 (Supporting Information), alongside the half-lives, for hydrolysis of cytosine (or cytidine). The half-life decreases monotonically as the pressure increases from 0.1 to 200 MPa, from (19.3 ± 0.7) days at 0.10 MPa to (8.64 ± 0.19) days at 200 MPa. The half- lives for cytidine at 0.10 MPa and 150 MPa and 373 K are (18.2 ± 0.7) days and (9.3 ± 0.4 days), respectively. A similar small enhancement in decomposition rate at 373 K for cytidine compared to cytosine was noted by Lewis *et al*.[12]

The rate constants for hydrolysis of cytosine at 373 K are plotted in Figure 1b as ln k *vs.* pressure p. From equation 1

$$\frac{\partial \ln k}{\partial p} = -\frac{\Delta V^{\ddagger}}{RT} \qquad \text{Equation 1}$$

where R is the gas or universal constant and T is the absolute temperature in K, volume of activation, $\Delta V^{\ddagger}$, was calculated to be: for cytosine (–11.8 ± 0.5) cm^3 mol^{-1}, and for cytidine, –14.6 cm^3 mol^{-1}. Given the changing isothermal compressibility of water with pressure, linearity of this plot was not necessarily to be expected.

A brief examination of the pH dependence of cytosine hydrolysis was carried out at pressures of 0.1 and 150 MPa (Figure 2). It is seen in both curves that the rate of hydrolysis is the slowest in the region of pH 7, and increases significantly, especially at ambient pressure, on dropping the pH to 6.0. Initial measurements made at pH 4.8 and 373 K (data not shown) showed further enhancement of the rate of hydrolysis compared to that at pH 7.0 and pH 6.0. Rates of hydrolysis at pH 8.0 were only marginally greater than those at pH 7.0.

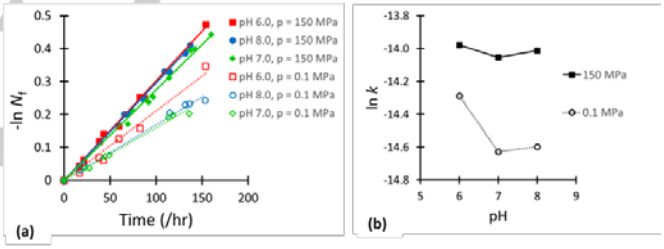


Figure 2. (a) Fraction of cytosine remaining, N_t = [cytosine]$_t$/[cytosine]$_0$, plotted as –ln N_t *vs* time (t) at 373 K and pH 6.0 (red), 7.0 (green) and 8.0 (blue) at 0.1 MPa (empty shapes and dotted lines) and 150 MPa)filled shapes and solid lines). The pH is corrected for the effects of pressure and temperature. The rate constant for hydrolysis of cytosine is the slope of the line. (b) The pH dependence of the rate constants for the hydrolysis of cytosine at 50 MPa (red) and 200 M Pa.

The chemical instability of biomolecules has long been recognised as a weakness in the argument for a hot start origin of life theory[19]. The relatively quick rate of hydrolysis of cytosine to uracil at 100 °C and ambient pressure (half-life of 19 days), compared to geological time scales, has previously been cited, on the one hand, as a limiting factor in the evolution of life[11] and, on the other hand, as a driver of evolution.[12]. Moreover, Hazen *et al.* posited that the instability of RNA and its components at high temperatures may be offset by high pressures.[16] Because of these factors we have examined the effect of pressure and pH on this hydrolysis, given that the environment around black smokers is highly acidic, while that around warm haline springs is moderately alkaline. Moreover, sources of both energy and molecular building blocks can be found at high hydrostatic pressures.

The negative activation volumes, $\Delta V^{\ddagger}$, for the hydrolysis of cytosine and cytidine at 100 °C, respectively, are similar to the molar volume of a water molecule (18.0 cm^3 mol^{-1}) and consistent with an associative mechanism. A tetrahedral 3,3-hydroxyamine

intermediate or near-transition-state species had earlier been deduced from $^{15}N/^{14}N$ kinetic isotopic effects for the deamination of cytidine (both uncatalysed and catalysed).[18] Activation volumes for biochemical processes typically fall within the range +50 to -50 cm^3 mol^{-1}[20] and for the hydrolysis of weakly related carboxamide species under acid or base catalysis values of -9.4 to -16.9 cm^3 mol^{-1} have been compiled.[21] Our results are incompatible with the hypothesis that the instability of RNA and its components at high temperatures may be offset by high pressures in a high-temperature/high-pressure theory[16].

The half-life determined at 0.10 MPa and 100 °C in our measurements of 19.3 ± 0.7 days compares well with that of 19 days determined by Levy and Miller at 0.10 MPa 100 °C for cytosine at pH 7 under the same conditions (0.05 M phosphate buffer at an ionic strength of 0.2 M). An average half-life of ~10 days for individual cytosines on a primitive genomic strand of 5000 nucleotides, one quarter of which are cytosine, corresponds to a spontaneous mutation every couple of hours for each strand. In the absence of editing and repair mechanism. This is not conducive to preservation of genomic information in a Model T Ford of inefficient, slowly reproducing early life forms. At pressures corresponding to depths in sea water of 3790 m (current average depth on earth [22]) and 10920 m (maximum current depth on earth [23]), the half-life of cytosine hydrolysis at 38.2 MPa is calculated to be, respectively, 16.8 days and 12.9 days. Compared to the half-life at 0.1 MPa (atmospheric pressure) of 19.3 days, the value of the half-life at 3790 m or 38.2 MPa is only 13% lower. The effects of pressure on the hydrolytic stability of cytosine in earth's oceans are significant but modest.

The pH dependence of the hydrolysis of cytosine is less marked at 150 MPa than at ambient pressure (Figure 2b). At both 0.1 and 150 MPa, the rate constant for hydrolysis is smallest near pH 7. At atmospheric pressure this result is to be expected since the nucleobases are the most stable at pH 7.[9a] It is significant that this pH dependence has been maintained at high pressure, provided that pH measured is corrected for the effects of pressure. The notably faster rate of hydrolysis at acidic pH, especially noticeable at 0.1 MPa is not supportive of an acidic environment for evolution of RNA-based life forms. Our results here are in apparent conflict with the report by Lewis et al.[12] that the activation enthalpy remains the same at 23 kcal mol^{-1} not only over a wide temperature range but remarkably also at pH 2.4 and 7.0. However, this reported activation enthalpy would strictly appear to be an internal energy of activation $\Delta U^{\ddagger}$, since measurements appear to have been made at the essentially constant volume of sealed quartz tubes. Thus, assuming little change in the Arrhenius pre-exponential factor over this pH range, pressure effects on constant volume measurements over a range of temperature would appear to fortuitously cancel pH effects on the internal energy of activation.

Both continental crust and oceans were established in Hadean times (up to ~4.1 bya),[24] and conditions amenable to chemical synthesis of complex molecules developed remarkably rapidly,[25], with the average temperature of water being less than 100 °C by 4.2 bya .[26], The sequestration of carbon dioxide from a dense atmosphere ($p(CO_2)$ to ~25 bar for 120 °C and ~30 °C at 1 bar)[27] was also occurring rapidly, leaving a remarkably short period of just 1-20 My where surface temperatures exceeded 100 °C. Indeed, it is posited that extant hyperthermophiles and all modern life descended from hyperthermophilic survivors of ocean-boiling asteroid impacts that occurred at the end of the Hadean epoch ~3.9 Gya.[25], although this Late Heavy Bombardment has been questioned strongly.[28] This leaves open the question of environment for origin of life. These considerations and the work presented here do not support a hyperthermophilic, hyperbaric or acidic environment for an origin of RNA-based life in the period ~4.2 to 3.9 Gya, at least with cytosine

The possibility has been contemplated for an origin of life that does not involve cytosine as a nucleobase, where genetic information is reduced to a two-letter code of just adenine and uracil, or adenine and inosine, or where the cytosine-guanosine pair has been substituted for an alternate more stable base pair (isoguanine and isocytosine, diaminopurine and uracil, diaminopyrimidine and xanthine [11]). Numerical simulations indicate that a model containing only adenine and uracil does not lead to the unique stable folded RNA structures necessary for catalytic functions [8, 29]. However, this important requirement if proteins had yet to be used for catalytic function appears to have been met in a two-nucleotide ribozyme reported by Reader and Joyce.[30]

As observed before, the rate of hydrolysis of cytosine at 100°C is relatively short on the geological time scale. Both cytosine and cytidine have increasingly faster rates of hydrolysis as pressure is increased from ambient to 250 MPa. In addition, the rates of hydrolysis of cytosine at 100 °C are significantly faster at pH 6 than at pH 7-8, especially at ambient pressure. Our results on the stability of cytosine point away from a high-temperature/high-pressure origin-of-life scenario in favour of a low-temperature/low pressure environment for the emergence of the first RNA-based life forms.

What still remains unknown is the effect that specific adjuvants, such as amino acids, short peptides, Mg^{2+} ions and molecular crowding may have on the chemical stability of cytosine and its derivatives while under pressure. There is also the question of the chemical stability of cytosine within folded RNA/DNA molecules under pressure. We will report in due course on the chemical and physical stability of RNA/DNA molecules.

Experimental Section

High pressure NMR apparatus. A high-pressure NMR cell (zirconia tube, inner diameter 3 mm, outer diameter 5 mm) was purchased from Daedalus Innovations and attached to home-designed aluminum manifold, connected to long stainless steel tube, connected to a remote hand pump. Pressure is applied to the aqueous sample in the NMR cell *via* an immiscible hydraulic fluid (low viscosity paraffin oil). This set up allows the pressure on the sample to be set at any value between 0.1 and 250 MPa (measured with HiP Bourdon Gauge). A specially designed rig allowed the NMR cell to be reproducibly positioned in the spectrometer and to be safely moved under pressure from an external oil bath at 373 K to the spectrometer. The cell was interfaced with a Bruker Avance-500 NMR spectrometer operating at 500.13 MHz for ^1H. The sample was positioned in a commercial Bruker TXI 5 mm ^1H-detection inverse probe with a *z*-field gradient coil.

Samples. A stock solution of cytosine (or cytidine both from Sigma-Aldrich), 3-(trimethylsilyl)-2,2',3,3'-tetradeuteropropionicacid (TMSP-d_4) (Merck) and sodium azide was added to a phosphate buffer in H_2O/D_2O (90:10 v/v for NMR lock) to give final solution concentrations of 1.00 mM cytosine, 0.050 M phosphate buffer, 0.020 mM sodium azide (to ensure no bacterial growth over long time period of measurements) and 0.200 mM TMSP-d_4. The ionic strength of all solutions was adjusted to 0.2 M with

NaCl. The TMSP-d_4 was added to act as both a NMR reference and as an internal concentration reference. Due to the effect of pressure on pH in a phosphate buffer, the pH values at atmospheric pressure for the high-pressure samples were corrected to give the target pH at 298 K while under pressure[31]. The pH values used are (with values in parentheses at 0.1 MPa): at 0.1 MPa - 6.00, 7.00 and 8.00; at 50 MPa – 7.00 (7.20); at 100 MPa – 7.00 (7.39); at 150 MPa - 6.00 (6.56), 7.00 (7.56) and 8.00 (8.56); at 200 MPa – 7.00 (7.71). Experiments for cytidine were conducted at 373 K and 0.1 and 150 MPa. As all measurements were conducted at 373 K, no correction to pH for temperature was made.

Hydrolysis measurements. Samples were inserted into the high-pressure cell and brought up to pressure. A ^{1}H NMR spectrum was recorded and the sample was then placed in an oil bath at 373 K for a period of at least 135 hours while maintaining the pressure at the target value (no leaks were observed over periods of weeks). During this time further ^{1}H NMR spectra were recorded at 298 K after which the sample was promptly restored to 373 K. All NMR spectra were recorded with 64 scans with a relaxation time of 10 s with a pre-saturation pulse to suppress the water peak. The integrals of the NMR peaks corresponding to protons H5 and H6 for both cytosine and uracil (see Figure 1) were measured for each spectrum using the TMSP-d_4 peak as a reference. Sample spectra are provided in Supporting Information as Figure S2. From this a plot of -ln [cytosine]$_t$/[cytosine]$_{t=0}$ *vs*. time was used to determine the rate constant for hydrolysis, *k*, for each sample. From the pressure dependence of the rate constant an activation volume was calculated using Equation 1.

Acknowledgements

We gratefully acknowledge funding from the Marsden Fund of the Royal Society of NZ (grant 09-MAU-140) for a PhD scholarship to CPL and the Allan Wilson Centre for Molecular Ecology and Evolution to purchase the high-pressure/high-temperature NMR cell. We acknowledge helpful discussions with Associate Proessor Vyacheslav V Filichev.

Keywords: Cytosine hydrolysis • high pressure • high temperature • NMR spectroscopy • origin of life

[1] a) J. W. Szostak, *Angew Chem Int Ed Engl* **2017**; b) J. A. Baross, W. F. Martin, *Cell* **2015**, *162*, 13-15; c) G. F. Joyce, *Nature* **2002**, *418*, 214-221; d) G. F. Joyce, *Cold Spring Harb Symp Quant Biol* **2009**, *74*, 17-23; e) W. Ma, C. Yu, W. Zhang, S. Wu, Y. Feng, *BMC evolutionary biology* **2015**, *15*, 272; f) W. F. Martin, F. L. Sousa, N. Lane, *Science* **2014**, *344*, 1092-1093.

[2] J. V. Gavette, M. Stoop, N. V. Hud, R. Krishnamurthy, *Angew Chem Int Ed Engl* **2016**.

[3] a) G. Wachtershauser, *Life (Basel, Switzerland)* **2014**, *4*, 1050-1091; b) B. R. Francis, *Life (Basel, Switzerland)* **2015**, *5*, 467-505.

[4] A. H. Segerer, S. Burggraf, G. Fiala, G. Huber, R. Huber, U. Pley, K. O. Stetter, *Origins of life and evolution of the biosphere : the journal of the International Society for the Study of the Origin of Life* **1993**, *23*, 77-90.

[5] W. Martin, M. J. Russell, *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **2007**, *362*, 1887-1925.

[6] I. Daniel, P. Oger, R. Winter, *Chem Soc Rev* **2006**, *35*, 858-875.

[7] M. C. Weiss, F. L. Sousa, N. Mrnjavac, S. Neukirchen, M. Roettger, S. Nelson-Sathi, W. F. Martin, *Nature microbiology* **2016**, *1*, 16116.

[8] V. Moulton, P. P. Gardner, R. F. Pointon, L. K. Creamer, G. B. Jameson, D. Penny, *J. Mol. Evol.* **2000**, *51*, 416-421.

[9] a) E. R. Garrett, J. Tsau, *J Pharm Sci* **1972**, *61*, 1052-1061; b) R. Shapiro, R. S. Klein, *Biochemistry* **1966**, *5*, 2358-2362; c) T. Lindahl, B. Nyberg, *Biochemistry* **1974**, *13*, 3405-3410.

[10] L. A. Frederico, T. A. Kunkel, B. R. Shaw, *Biochemistry* **1990**, *29*, 2532-2537.

[11] M. Levy, S. L. Miller, *Proc Natl Acad Sci U S A* **1998**, *95*, 7933-7938.

[12] C. A. Lewis, Jr., J. Crayle, S. Zhou, R. Swanstrom, R. Wolfenden, *Proc Natl Acad Sci U S A* **2016**, *113*, 8194-8199.

[13] a) G. K. Schroeder, R. Wolfenden, *Biochemistry* **2007**, *46*, 13638-13647; b) A. Radzicka, R. Wolfenden, *Science* **1995**, *267*, 90-93.

[14] a) V. G. Stepanov, J. Nyborg, *Extremophiles* **2002**, *6*, 485-490; b) Y. W. Ren, J. X. Lu, B. W. Cai, D. B. Shi, H. F. Jiang, J. Chen, D. Zheng, B. Liu, *Dalton transactions (Cambridge, England)* **2011**, *40*, 1372-1381; c) S. J. Franklin, *Curr Opin Chem Biol* **2001**, *5*, 201-208.

[15] a) K. M. Chen, E. Harjes, P. J. Gross, A. Fahmy, Y. Lu, K. Shindo, R. S. Harris, H. Matsuo, *Nature* **2008**, *452*, 116-119; b) L. Betts, S. Xiang, S. A. Short, R. Wolfenden, C. W. Carter, Jr., *J Mol Biol* **1994**, *235*, 635-656.

[16] R. M. Hazen, N. Boctor, J. A. Brandes, G. D. Cody, R. J. Hemley, A. Sharma, H. S. Y. Jr, *J. Phys.: Condens. Matter* **2002**, *14*, 11489.

[17] a) D. Ritson, J. D. Sutherland, *Nature chemistry* **2012**, *4*, 895-899; b) B. H. Patel, C. Percivalle, D. J. Ritson, C. D. Duffy, J. D. Sutherland, *Nature chemistry* **2015**, *7*, 301-307; c) C. Meinert, I. Myrgorodska, P. de Marcellus, T. Buhse, L. Nahon, S. V. Hoffmann, S. d'Hendecourt Lle, U. J. Meierhenrich, *Science* **2016**, *352*, 208-212; d) S. Ranjan, D. D. Sasselov, *Astrobiology* **2016**, *16*, 68-88.

[18] M. J. Snider, L. Reinhardt, R. Wolfenden, W. W. Cleland, *Biochemistry* **2002**, *41*, 415-421.

[19] R. H. White, *Nature* **1984**, *310*, 430-432.

[20] V. V. Mozhaev, K. Heremans, J. Frank, P. Masson, C. Balny, *Proteins* **1996**, *24*, 81-91.

[21] C. A. Lewis Jr., R. Wolfenden, *J. Am. Chem. Soc.* **1973**, *95*, 6685-6688.

[22] The World's Ocean in *The Columbia Encyclopedia*, 6th ed., Columbia University Press, **2007**.

[23] IHO-IOC GEBCO Gazetteer of Undersea Feature Names, August 2011 version.GEBCO, **2011**.

[24] S. A. Wilde, J. W. Valley, W. H. Peck, C. M. Graham, *Nature* **2001**, *409*, 175-178.

[25] N. H. Sleep, K. Zahnle, P. S. Neuhoff, *Proc Natl Acad Sci U S A* **2001**, *98*, 3666-3672.

[26] S. J. Mojzsis, T. M. Harrison, R. T. Pidgeon, *Nature* **2001**, *409*, 178-181.

[27] N. H. Sleep, D. K. Bird, E. C. Pope, *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **2011**, *366*, 2857-2869.

[28] P. Boehnke, T. M. Harrison, *Proc Natl Acad Sci U S A* **2016**, *113*, 10802-10806.

[29] D. Penny, A. Poole, *Current opinion in genetics & development* **1999**, *9*, 672-677.

[30] J. S. Reader, G. F. Joyce, *Nature* **2002**, *420*, 841-844.

[31] M. E. Bruins, A. M. Matser, A. E. M. Janssen, R. M. Boom, *High Pressure Res.* **2007**, *27*, 101-107.

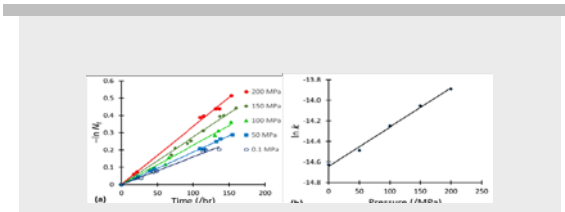
10.1002/cbic.201700555

WILEY-VCH

Entry for the Table of Contents (Please choose one layout)

Layout 2:

COMMUNICATION



*Author(s), Corresponding Author(s)**

Page No. – Page No.

Title

The relatively low chemical stability of cytosine compared to other nucleobases is a key concern in origin-of-life scenarios, but the effect of pressure on the rate of hydrolysis of cytosine to uracil has remained unknown. At high (200 MPa) pressures the half-life of cytosine at 100 ºC is halved to 8.6 days compared that at ambient pressure.