Hydrogen peroxide thermochemical oscillator as driver for primordial RNA replication

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This paper presents and tests a previously unrecognized mechanism for driving a replicating molecular system on the prebiotic earth. It is proposed that cell-free RNA replication in the primordial soup may have been driven by self-sustained oscillatory thermochemical reactions. To test this hypothesis, a well-characterized hydrogen peroxide oscillator was chosen as the driver and complementary RNA strands with known association and melting kinetics were used as the substrate. An open flow system model for the self-consistent, coupled evolution of the temperature and concentrations in a simple autocatalytic scheme is solved numerically, and it is shown that thermochemical cycling drives replication of the RNA strands. For the (justifiably realistic) values of parameters chosen for the simulated example system, the mean amount of replicant produced at steady state is 6.56 times the input amount, given a constant supply of substrate species. The spontaneous onset of sustained thermochemical oscillations via slowly drifting parameters is demonstrated, and a scheme is given for prebiotic production of complementary RNA strands on rock surfaces.

1. Introduction

Molecular self-replication is a fundamental process of all living organisms, and its initiation in the environment provided by the early earth must have been essential for the emergence of life itself. In modern cells, DNA replication is accomplished isothermally at moderate temperatures by a working army of enzymes using the free energy of adenosine triphosphate hydrolysis, and the strongly driven non-equilibrium condition is maintained by respiration and ion transport across cell membranes. However in the primordial soup, there were no cells and no protein enzymes. The ‘RNA world’ hypothesis holds that cell-free RNA communities grew on solid surfaces and replicated, before the evolution of DNA [1]. What energy source may have driven RNA replication in such an environment?

It does seem that thermal cycling would be required to drive RNA replication in the absence of cellular (or other) machinery, because heat is required to dissociate double-stranded or multiplex RNA and a cooler phase is necessary for replication and annealing, given a supply of substrate template. This fact is often overlooked in hypotheses about the origin of life, as pointed out by Kováč et al. [2]. Exponential replication of RNA duplexes, fed by hairpins derived from an alanine tRNA and driven by externally imposed thermal cycling between 10 and 40°C, was achieved by Krammer et al. [3]. They proposed that, in the primordial soup, thermal cycling may have been provided by laminar convection between hot and cold regions in millimetre-sized rock pores [4], and that the substrate oligonucleotides could be concentrated by thermophoretic trapping.

It has been suggested by Matatov [5] that chemical energy also may have played a role in prebiotic evolution, because, in the laboratory, heat liberated in the decomposition of aqueous hydrogen peroxide (H₂O₂) supported formation of amino acids from simpler precursors. In this work, we propose that a natural mechanism for driving self-sustained thermal cycling is a...
thermochemical oscillator, and demonstrate in a specific case that the temperature cycling for driving the amplification of template RNA in environments where early life may have evolved can be provided by a thermochemical oscillator driven by exothermic reactions of H2O2.

Exothermic reactions of aqueous H2O2 are well known to give rise to robust, self-sustained thermochemical oscillations with frequencies around 0.02–0.005 s\(^{-1}\) [6–9]. As our specific example, we have chosen, with some justification expressed below, the oxidation of thiosulfate ion (\(\text{S}_2\text{O}_3^{2-}\)) by H2O2, which is fast and highly exothermic:

\[
\text{Na}_2\text{S}_2\text{O}_3 + 2\text{H}_2\text{O}_2 \rightarrow \frac{1}{2}\text{Na}_2\text{S}_2\text{O}_8 + \frac{1}{2}\text{Na}_2\text{SO}_4 + 2\text{H}_2\text{O}. \quad (R\ 1)
\]

The thermokinetics and thermochemistry of (R\ 1) have been well studied, and experimental data from the literature are collected in table 1. The reaction is first order in both reactants.

Oscillatory thermoconversion is typical of highly energetic, thermally sensitive liquids such as many of the peroxides [16]. The physical basis is as follows: in such liquids (and also in some vapour- and gas-phase substances), which have high specific heat capacity, the heat of reaction can be absorbed by the many intermolecular vibrational modes and intramolecular rotational modes. So the temperature rises but slowly as reaction proceeds, until these modes become saturated and consequently the temperature spikes—the heat cannot be absorbed any more by quantized excitations. But locally the reactant is depleted, so the temperature falls to a minimum before reactant accumulation allows reaction to occur and heat to be released, and the cycle begins again. With a steady supply of reactant, these self-sustained thermal oscillations can continue indefinitely.

### 2. Model, data and methods

For the reactive system in a single rock pore, we employ a spatially homogeneous flow model; in other words, a continuous stirred tank reactor (CSTR) paradigm. An \textit{a posteriori} assessment of the rationale and validity of this model is given in §4.1. The following dynamical system models the coupled evolution of the reactant concentrations in (R\ 1) and the temperature:

\[
\frac{dC_y}{dt} = -Vk_1(T)c_y c_w + F(c_v, -c_v),
\]

\[
\frac{dC_w}{dt} = -V2k_1(T)c_v c_w + F(c_w, -c_w)
\]

\[
\frac{dC_T}{dt} = (-\Delta H_1)k_1(T)c_v c_w - FC(T - T_c) - L(T - T_a),
\]

where the reaction rate constant \(k_1(T) = A_1 \exp(-E_1/(RT))\), \(c_y\) is the concentration of thiosulfate and \(c_w\) is the concentration of H2O2. The symbols and notation used in these and following equations are defined in table 2.

We used the following minimum subset of reactions from [3] that can produce duplex RNA by autocatalysis:

\[
X + Y + XY \xrightarrow{b_{23}} Z \quad \text{(R\ 2)}
\]

\[
Z \xrightarrow{b_{12}} 2XY \quad \text{(R\ 3)}
\]

where X and Y represent RNA complementary single strands.
the RNA duplex XY is the replicator and Z is the RNA quadruplex, the form of which is cartooned in figure 1. In the reaction volume, these species evolve as

\[ V \frac{dc_{x+y}}{dt} = -V 2k_2(T)c_x c_y c_{xy} + V k_1(T)c_x c_y c_{xy} + F(c_{x+y} - c_{x+y}), \]  

(2.4)

\[ V \frac{dx}{dt} = -V k_2(T)c_x c_y c_{xy} + V k_1(T)c_x c_y c_{xy} + F(c_x c_y f + c_{x+f} c_{y+f}), \]  

(2.5)

and

\[ V \frac{dc_{z}}{dt} = V k_2(T)c_x c_y c_{xy} - V k_1(T)c_z c_{z} - F c_z, \]  

(2.6)

where the notations \( c_{x+y} \) and \( c_{x+f} c_{y+f} \) are shorthand for \( c_x + c_y \) and \( c_{x+f} c_{y+f} \), \( k_2(T) = A_2 \exp(-E_x/(RT)) \) and \( k_3(T) = A_3 \exp(-E_z/(RT)) \). (These are to be considered as empirical rate constants; (R 2) as written is not intended to imply that an elementary three-body collision occurs.)

The enthalpy balance includes contributions from all reactions

\[ V C \frac{dT}{dt} = (-\Delta H_1) V k_1(T)c_x c_y c_{xy} + (-\Delta H_2) V k_2(T)c_x c_y c_{xy} + (-\Delta H_3) V k_3(T)c_z c_{z} - F C (T - T_0) - L (T - T_0). \]  

(2.7)

We derived the activation energies, pre-exponential factors and reaction enthalpies from the rate data in [3] (see appendix A). Equations (2.1)–(2.3) (no RNA supply) and equations (2.1), (2.2) and (2.4)–(2.7) (RNA single strands and duplexes supplied) were integrated using a stiff integrator from reasonable initial conditions. The stability of steady-state solutions was assessed over a range of the inflow temperature, \( T_0 \), by solving the corresponding eigenvalue problem and flagging points where an eigenvalue changed sign. These bifurcation points then were followed over a range of the thermal conductance \( L \) to obtain stability maps. Numerical values of the fixed parameters are given in table 2 and are discussed further in appendix A.

### Table 2. Nomenclature and numerical values.

<table>
<thead>
<tr>
<th>symbol (units), definition</th>
<th>quantity</th>
<th>no RNA</th>
<th>with RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>( A ) (M(^{-1}) s(^{-1})), pre-exponential factor</td>
<td>( A_1 )</td>
<td>( 1.63 \times 10^{10} )</td>
<td>( 1.69 \times 10^{12} )</td>
</tr>
<tr>
<td>( A_2 )</td>
<td>n.a.</td>
<td>( 1.0 \times 10^{19} )</td>
<td></td>
</tr>
<tr>
<td>( A_3 )</td>
<td>n.a.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( c ) (M), concentration</td>
<td>( c_{x+f} )</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>( c_{x+y} )</td>
<td>1.35</td>
<td>1.35</td>
<td></td>
</tr>
<tr>
<td>( c_{x+f+y+f} )</td>
<td>0</td>
<td>5.0 \times 10^{-7}</td>
<td></td>
</tr>
<tr>
<td>( c_{x+y} f )</td>
<td>3.5 \times 10^{-8}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( C ) (J K(^{-1}) l(^{-1})), volumetric specific heat</td>
<td>( C )</td>
<td>3400</td>
<td>3400</td>
</tr>
<tr>
<td>( E ) (kJ mol(^{-1})), activation energies</td>
<td>( E_1 )</td>
<td>68.12</td>
<td>68.12</td>
</tr>
<tr>
<td>( E_2 )</td>
<td>n.a.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( E_3 )</td>
<td>n.a.</td>
<td>260.14</td>
<td></td>
</tr>
<tr>
<td>( F ) (( \mu ) l s(^{-1})), flow rate</td>
<td>( F )</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>( \Delta H ) (kJ mol(^{-1})), reaction enthalpies</td>
<td>( \Delta H_1 )</td>
<td>n.a.</td>
<td>-169.6</td>
</tr>
<tr>
<td>( \Delta H_2 )</td>
<td>n.a.</td>
<td>260.14</td>
<td></td>
</tr>
<tr>
<td>( \Delta H_3 )</td>
<td>n.a.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( L ) (W K(^{-1})), wall thermal conductance</td>
<td>( n ), sum of species reaction orders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( T ) (K), temperature</td>
<td>( T_0 )</td>
<td>283</td>
<td>283</td>
</tr>
<tr>
<td>( V ) (( \mu ) l), pore volume</td>
<td>( V )</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>( a ), pertaining to (R 1), (R 2), (R 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( a ), of the ambient or wall temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( f ), of the flow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( v ), ( w ), of thiosulfate, ( H_2O_2 )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( x ), ( y ), ( z ), of single strands, duplex, quadruplex</td>
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</table>

3. Results

First, we examined the behaviour of the standalone \( H_2O_2/ \) thiosulfate system. Figure 2a shows the computed stability map. The regime of threefold multiplicity mapped by the locus of saddle-node bifurcations is included for completeness, but this regime is largely irrelevant for our purposes because the temperature is too low to drive RNA replication. At each point within the Hopf bifurcation loop, the stable solution is a limit cycle. The time series for a selected point in the loop is shown in figure 2b.
Figure 1. The quadruplex RNA species $Z$. The duplex replicator species are $XY$ and $x y$, which for the purposes of the simulation are equivalent. See [3] for more details. (Online version in colour.)

When reactions (R 2) and (R 3) are included, the stability map and temperature time series are indistinguishable numerically from those in figure 2 because the contributions of the second and third terms on the right-hand side of equation (2.7) are relatively insignificant.

However, time-series data rendered in figure 3 for the concentrations of single strands, duplex and quadruplex show that the H$_2$O$_2$/thiosulfate thermochemical oscillator can indeed drive the RNA replication process. This time series was computed for a slightly lower value of $T_f$ because the contributions of the second and third terms on the right-hand side of equation (2.7) are relatively insignificant.

Figure 2. Computed data from equations (2.1)–(2.3): (a) Hopf (solid line) and saddle-node (dashed line) bifurcation loci; (b) time series for $T_f = 282$ K, $L = 2.4$ mW; the oscillation period is 108.7 s. (Online version in colour.)

The period is determined by the ratio $\phi = (CE_1)/(R_{xy,f}(\Delta H_1))$, and in general a large value of the numerator (high specific heat and activation energy) corresponds to longer cycling times and a large value of the denominator (high specific reaction enthalpy) corresponds to shorter period oscillations. The physical parameters $V/F$ (mean residence time), $T_D$, $T_a$ and $L$ also affect the period, as might be inferred from the locus of Hopf bifurcations in figure 2a [26]. The hydrogen peroxide oscillator has a variable period of the right order—around 80–110 s—to drive the replication of small RNAs. If the period is too long, the RNAs may decay faster than replication can amplify them. If the period is too short, the strands do not separate completely and replication may fail.

But the period is not tuned to the putative doubling time for replicant concentration as was the thermal cycling time in [3]. As our system is an open flow system operating at dynamic steady state, where it has ‘forgotten’ the initial conditions, the doubling time is a different quantity from that for the closed batch system in [3]. Here, it is defined as a time interval during which the cumulative replicant concentration at the outlet, $C_{xy,f} + C_x$, becomes equal to twice the inflow concentration $C_{xy,f}$. In equations (2.1), (2.2) and (2.4)–(2.7), the variables are evolved self-consistently and the doubling time is temperature-dependent, and therefore changes continuously. We can make use of average quantities though.

From computed datafiles, the replicant concentration $C_{xy,f} + C_x$ integrated over one period of 99.3 s (using the trapezoidal rule on 50 000 data points for high accuracy) is $2.281 \times 10^{-5}$ mol l$^{-1}$, which yields an instantaneous average concentration $C_{xy,f} + C_x = 2.297 \times 10^{-7}$ mol l$^{-1}$ and an average doubling time of 0.31 s, for $C_{xy,f} = 3.5 \times 10^{-8}$ M. This does not infer that replication is exponential, it simply means that on average it takes 0.31 s for the cumulative amount of replicant produced to become equal to twice the feed amount.

Another measure of the system’s performance is the ratio $C_{xy,f} + C_x / C_{xy,f} = 6.56$. This means that the system has been designed so that in steady flow state, for the ideal case, where no decay of replicant occurs within the reaction volume, the mean outlet replicant amount is 6.56 times the inlet duplex amount.

Let us consider the phase relationships in figure 3 over one period. Single strands are consumed as the temperature rises because duplex production by (R 3), which requires heat, increases. Correspondingly, the concentration of quadruplex falls almost to zero near the temperature maximum. Single strands begin to accumulate, but there is only a small bump before quadruplex production picks up as the temperature declines. The presence of this bump means that the oscillation is actually quasi-periodic and reminds us that the dynamical system of equations (2.1)–(2.2) and (2.4)–(2.7) is capable of complex periodic and even chaotic behaviour. This may lend additional, powerful capabilities to a molecular replicating system. For example, biperiodic temperature response is capable of replicating two different
RNA species, and nature may well have done exactly that in the primordial rock pores. Duplex concentration attains a broad maximum, then declines in phase with the temperature as reaction (R 2) is favoured. The inflow delivers more single strands, which accumulate even though they allow more production of quadruplex.

3.1. Development of self-sustained oscillations

We have addressed also the question of how self-sustained H$_2$O$_2$ thermochemical oscillations may have arisen spontaneously in rock pores on the early earth. The fuzzy answer is that parameters may drift quasi-statically into an oscillatory régime, for instance the loop of Hopf bifurcations in figure 2a. A precise answer is provided by simulating various scenarios where one or more tuneable parameters $p$ in equations (2.1)–(2.3) is given the following increasing time dependence, such that $p$ approaches a constant value:

$$ p = p_0(1 - \alpha \exp(-\gamma t)). \quad (3.1) $$

A time series from equations (2.1)–(2.3) using (3.1) for one or more parameters is expected to show the temperature increasing slowly, then beginning to wobble, then settling into self-sustained oscillations. Some examples are shown in figure 4. Of course, such evolutions could proceed for millions of years before the onset of periodic behaviour, but we have set the time constant $\gamma$ in equation (3.1) more conveniently.

4. Discussion

4.1. Validity and relevance of continuous stirred tank reactor paradigm

The CSTR paradigm described by equations (2.1), (2.2) and (2.4)–(2.7) can be thought of in two ways.

On the one hand, it provides a recipe for a laboratory experiment in a microreactor cell. The flow rate $F$, inflow concentrations $c_{v,f}$, $c_{w,f}$, $c_{x+y,f}$ and $c_{xy,f}$, inflow temperature $T_f$
and wall or ambient temperature $T_w$ are tuneable by the experimenter, the wall thermal conductance $L$, and volume $V$ are set by design, and thermokinetic parameters and reaction enthalpies of the RNA reactions can be manipulated by engineering the ribonucleotide sequences and strand lengths. A variety of continuous-flow microreactors are already in use for various calorimetric applications in biotechnology, and the field is developing rapidly [27].

On the other hand, we need to address the adequacy of the CSTR as a model for reactions in a porous rock. In an actual physical situation we assume a flow through a porous rock structure comprising a large array of individual pores, having patterns of connection determined by the character of the rock structure. Provided that the system is sufficiently ergodic, and input flow is steady in volumetric velocity and chemical composition, it is reasonable to assume that the time behaviour in a single pore as reported above gives results at least qualitatively representative of the larger array over times longer than the period of the thermochemical oscillator.

Further, the use of a CSTR model for a pore implicitly assumes a typical residence time $\tau$ for the mixing time. Mixing is achieved mainly by convection, and in this respect thermoconvection times of 3–7.5 s were reported by Mast et al. [28]. The mean residence time in our system is 20 s. The perfect mixing assumption is considered to hold adequately when the residence time is around 5–10 times the mixing time, so the CSTR model can be a reasonably good approximation. The 15 s thermoconvection time reported by Braun & Libchaber [29] is too long for the perfect mixing assumption to hold, and in that case the appropriate model is a system of reaction–convection–diffusion equations and boundary conditions. In that case, reactive and convective thermal oscillations would couple in important and interesting ways, but that is for future study. We prefer, at this stage, to avoid the sheer ‘tyranny of numbers’ that still plagues the numerical analysis of such systems, and isolate the reactive thermal oscillations by studying a system for which the perfect mixing assumption holds.

In experiments, the mixing behaviour of the system would be characterized by using a tracer to measure the residence time distribution or by determining the period of time necessary for the system to achieve a desired level of homogeneity. We note that Imai et al. [30] confirmed experimentally the well-stirred condition for a flow microreactor that simulated a hydrothermal environment, in which elongation of oligopeptides was achieved at high temperature and pressure.

### 4.2. RNA stability to hydrogen peroxide

The response of RNA to $H_2O_2$ is a mixed story. Some small RNAs have been found to be very stable; for example, a 109-nucleotide RNA which is induced by oxidative stress in *E. coli* has an *in vivo* half-life of 12–30 min [31]. There is evidence for modern RNA dysfunction, but not degradation, caused by oxidation initiated by $H_2O_2$ [32]. In [33], it was found that small RNA (approx. 50 bases) of yeast is stable in the CSTR as a model for reactions in a porous rock. In an actual physical situation we assume a flow through a porous rock structure comprising a large array of individual pores, having patterns of connection determined by the character of the rock structure. Provided that the system is sufficiently ergodic, and input flow is steady in volumetric velocity and chemical composition, it is reasonable to assume that the time behaviour in a single pore as reported above gives results at least qualitatively representative of the larger array over times longer than the period of the thermochemical oscillator.

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In experiments, the mixing behaviour of the system would be characterized by using a tracer to measure the residence time distribution or by determining the period of time necessary for the system to achieve a desired level of homogeneity. We note that Imai et al. [30] confirmed experimentally the well-stirred condition for a flow microreactor that simulated a hydrothermal environment, in which elongation of oligopeptides was achieved at high temperature and pressure.
produce complementary strands preferentially. Polynucleotides more than 50-mers can be synthesized on montmorillonite [38], which also facilitates homochiral selection [39], and this would seem a stronger hypothesis.

A supply of complementary template oligomers could be provided by a variation on the surface-promoted replication procedure in [40], when competitive pathways are included for consumption and production of complementary free RNA species X and Y as indicated in figure 5. Here, too, replication may be driven by an H2O2 thermochemical oscillator, because strand separation requires heating and annealing, ligation and immobilization of single strands is promoted by cooling.

In another prebiotic scenario, lipid molecules in a dehydrated environment promoted condensation of nucleic acid monomers, and an alternating wet phase replenished the monomers [41]. Such a system also would be enhanced by the H2O2 thermochemical oscillator, which would provide periodic heating to drive the dehydrations and cooling to allow rehydrations.

4.4. Effects of other reactions involving H2O2
Hydrogen peroxide may undergo other competing reactions; for example, the Fenton reaction in the presence of Fe²⁺/Fe³⁺ discussed above, which is also exothermic. In this case, there would be an extra reaction rate term in the species balance for hydrogen peroxide and in the thermal balance. Such a system has an enhanced propensity towards quasi-periodic oscillations.

It is noteworthy that the Cu(II)-catalysed H2O2/thiosulfate ion reaction displays pH oscillations isothermally [42], implying that the chemistry alone is sufficiently nonlinear to permit oscillatory dynamics, and in any case more complex than (R1) suggests. Spontaneous pH cycling could well be an assistant driver for RNA replication as it would provide scope for favourable conformation changes.

5. Summary and conclusion
In summary, we have proposed and explored a previously unrecognized mechanism for driving a replicating system on the prebiotic earth. The chances of a hydrogen peroxide thermochemical oscillator arising spontaneously on the early earth in the presence of nucleotide precursors are perhaps very small. However, the earth is large, rock pores are innumerable and there was plenty of time on the early earth for improbable events to happen.

Braun & Libchaber [29] are strong proponents of an interdisciplinary approach between biochemistry and geophysics for understanding the origin of life at the molecular level.

Appendix A. Choices of numerical values
— The weighted average volumetric specific heat \( \tilde{C} = 3400 \text{J K}^{-1} \text{mol}^{-1} \) is considerably lower than that of pure water. The volumetric specific heat of seawater is about 3850 J K⁻¹ mol⁻¹ and that of H₂O₂ is about 2620 J K⁻¹ mol⁻¹, from which we arrive at the given value of 3400 J K⁻¹ mol⁻¹.

— The activation energies and pre-exponential factors of the RNA reactions were obtained from the data in [3] using

\[
E = \frac{R \ln \left( k(T_2)/k(T_1) \right)}{1/T_1 - 1/T_2},
\]

then \( A = k \exp(E/RT) \). The activation energy for the association reaction (R2) is zero in [3] but we used the small negative value of 10 kJ mol⁻¹ in the calculations to reflect the fact that the rates of association reactions of biological macromolecules often decrease with temperature, because owing to thermal motion a smaller fraction of energetically favourable collisions result in reaction. The data in [3] gave a pre-exponential factor for reaction (R3) in the main article of 10⁴⁰ s⁻¹. On the basis of only two data points, this should not be taken too literally; in practice, we had to reduce it to 10³⁹ s⁻¹ to couple the reaction to the thermochemical oscillator.

References