Impact of temperature on the time required for the establishment of primordial biochemistry, and for the evolution of enzymes

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All reactions are accelerated by an increase in temperature, but the magnitude of that effect on very slow reactions does not seem to have been fully appreciated. The hydrolysis of polysaccharides, for example, is accelerated 190,000-fold when the temperature is raised from 25 to 100 °C, while the rate of hydrolysis of phosphate monoester dianions increases 10,300,000-fold. Moreover, the slowest reactions tend to be the most heat-sensitive. These tendencies collapse, by as many as five orders of magnitude, the time that would have been required for early chemical evolution in a warm environment. We propose, further, that if the catalytic effect of a "proto-enzyme"—like that of modern enzymes—were mainly enthalpic, then the resulting rate enhancement would have increased automatically as the environment became cooler. Several powerful nonenzymatic catalysts of very slow biological reactions, notably pyridoxal phosphate and the ceric ion, are shown to meet that criterion. Taken together, these findings greatly reduce the time that would have been required for early chemical evolution, countering the view that not enough time has passed for life to have evolved to its present level of complexity.

activation energy | thermophilic organisms | pyridoxal phosphate | phosphate ester hydrolysis | amino acid decarboxylation

Whereas enzyme reactions ordinarily occur in a matter of milliseconds, the same reactions proceed with half-lives of hundreds, thousands, or millions of years in the absence of a catalyst (Fig. 1) (1). Yet life is believed to have taken hold within the first 25% of Earth's history (2). How could cellular chemistry, and the enzymes that make life possible, have arisen so quickly? Here, we show that because of an extraordinarily sensitive relationship between temperature and the rates of very slow reactions, the time required for early evolution on a warm earth was very much shorter than it might appear. That sensitivity also suggests some likely properties of an evolvable catalyst, and a testable mechanism by which its ability to enhance rates might have been expected to increase as the environment cooled.

Rapid substrate turnover is necessary to support the metabolism of an organism at the enzyme concentrations found in cells, but the same reactions, in the absence of enzymes, proceed vastly more slowly (Fig. 1). For example, the decarboxylation of orotidine 5′-phosphate (OMP), the final step in the biosynthesis of pyrimidines—and thus nucleic acids—proceeds with a half-life of 0.017 s at the active site of OMP decarboxylase. In neutral solution in the absence of the enzyme, the same reaction proceeds with a half-life of 78 million years (1). It is natural to ask how enzymes arose to meet so formidable a challenge.

The Time Required for Primordial Chemistry to Become Established

The rates of simple reactions, even if they are immeasurably slow at ordinary temperatures, can often be estimated by first determining their rates at elevated temperatures. Plots of the logarithm of the observed rate constants against the reciprocal of temperature (Arrhenius plots), which have been shown in some cases to be linear over many orders of magnitude (3), can then be used to estimate rate constants at ordinary temperatures by extrapolation. A reaction's heat of activation ($\Delta H^{\ddagger}$, usually expressed in kcal/mol) is directly related to the negative slope of the Arrhenius plot, furnishing a direct measure of the sensitivity of the reaction rate to changing temperature (Eq. **1**):

$$\Delta H^{\ddagger} = -R \cdot d(\ln k)/d(1/T) - RT, \qquad [1]$$

where k is the rate constant, R is the gas constant and T is the absolute temperature.

A familiar generalization—included in many textbooks and traceable to Harcourt's pioneering experiments on the "iodine clock" reaction (4)—holds that the rates of chemical reactions in water usually double when the temperature is raised from 20° to 30 °C ($\Delta H^{\ddagger} = 12$ kcal/mol), or 70-fold when temperature is raised from 25 to 100 °C.

Except for a few rapid reactions, that generalization is seriously misleading (Table 1). With the recent accumulation of observations on spontaneous reaction rates at elevated temperatures, it has become apparent that the sensitivity of most uncatalyzed reactions to temperature is actually much more pronounced than that of the iodine clock. For example, the rate of uncatalyzed amide, peptide and urea hydrolysis ($t_{1/2} \sim 500$ y at 25 °C, $\Delta H^{\ddagger} = 23$ kcal/mol), increases ~3,000-fold when the tem-

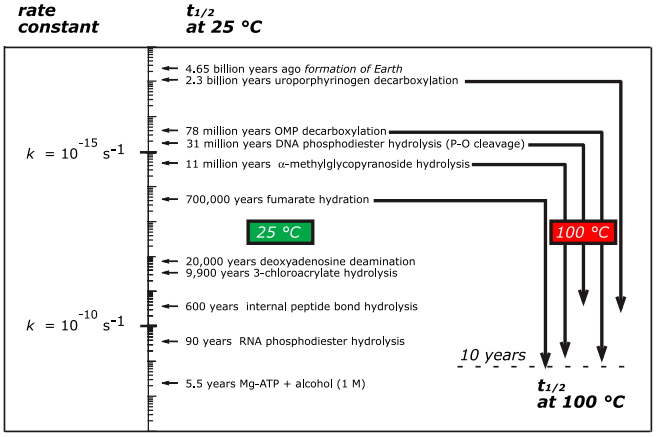

Fig. 1. Half-lives ($t_{1/2}$) and first order rate constants (k) of some biological reactions proceeding spontaneously in the absence of a catalyst in neutral solution at 25 °C and 100 °C. For references, see (3).

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Table 1. Temperature effects on the rates of biological reactions in the absence of a catalyst, compared with temperature effects on k_{cat} for a typical enzyme reaction (in italics, from Table 4)

	$t_{1/2}$ (25 °C)	$\Delta H^{\ddagger}$	$k_{100°}/k_{25°}$	$k_{0°}/k_{25°}$
Phosphomonoester hydrolysis (7)	1.1×10^{12} yr	47.0	10,300,000	0.0006
Uroporphyrinogen decarboxylation (21)	2.3×10^{9} yr	41.2	1,440,000	0.0016
Amino acid decarboxylation (14)	1.1×10^{9} yr	38.4	560,000	0.0024
Anhydrocellobiitol hydrolysis (6)	1.1×10^{8} yr	35.2	190,000	0.0040
Phosphodiester hydrolysis (22)	3.1×10^{7} yr	29.5	27,000	0.0096
Fumarate hydration (23)	7×10^{5} yr	28.9	22,000	0.0105
Chloroacrylate dehalogenation (24)	1.0×10^{3} yr	26.7	10,500	0.015
Peptide hydrolysis(internal) (25)	600 yr	22.9	2,900	0.027
Mg-ATP + glucose, 1 M (19)	6 yr	25.6	2,600	0.019
Bicarbonate dehydration (26)	27 sec	16.2	220	0.075
Typical enzyme reaction (Table 4)		*12.2*	*70*	*0.14*

perature is raised from 25 to 100 °C (5). The rate of hydrolysis of O-glycoside bonds [$t_{1/2} \sim 18 \times 10^{6}$ y at 25 °C, $\Delta H^{\ddagger} = 35$ kcal/mol (6)] increases ~190,000-fold when the temperature is raised from 25 to 100 °C; while the hydrolysis of aliphatic phosphate monoester dianions [$t_{1/2} \sim 1.1 \times 10^{12}$ y at 25 °C, $\Delta H^{\ddagger} = 47$ kcal/mol (7)] is accelerated ~10,000,000-fold when the temperature is raised from 25 to 100 °C.

In addition to the startling magnitudes of these rate accelerations, Table 1 shows that *the slowest reactions are most sensitive to temperature*. These combined tendencies result is a marked "leveling" of rates, so that even very slow reactions become measurable at temperatures not far removed from the boiling point of water (vertical arrows, Fig. 1). It is therefore of special interest that the earliest branches of the tree of life—as indicated by analysis of molecular sequences and structures—are largely populated by thermophilic organisms (8). Even now, temperatures near 100° are present near submarine vents and in hot springs that support organisms of widely differing phylotypes (9).

If life originated under these conditions, an idea that has been widely [(10) and references cited therein], but not universally (11), accepted, then the time required for prebiotic chemistry to become established would have been greatly abbreviated (by as many as five orders of magnitude at 100 °C) compared with the time required at 25 °C. Conversely, primordial chemistry would have been far more difficult at 0° than at 25 °C (Table 1, final column), aggravating the problem of understanding how chemical evolution could have occurred within a reasonable period of time.

These observations lend credence to the view that life originated on a warm earth, and they counter the view that insufficient time has passed for life to have evolved to its present level of complexity.

Evolution of Proto-Enzymes: a Hypothesis

In a warm environment in which these chemical transformations were proceeding without great difficulty, even a modest catalyst would have produced useful rates of reaction. In a cooler environment, however, many reactions would have become so sluggish that they would have required the action of much more powerful catalysts. For a reaction such as the decarboxylation of OMP, with a $t_{1/2}$ of 10^{8} years at 25 °C (1), a proto-enzyme that reduced that half-life by a factor of 10^{3}, 10^{6}, or even 10^{9}, would have conferred no significant competitive advantage on the host organism. How, then, did cells develop the ability to produce useful rates of reaction at ordinary temperatures?

Suppose that a small molecule (perhaps a metal ion or an organic acid or base) was once present in a warm aqueous environment where it produced a small but significant acceleration of a particular reaction. In principle, that increase in rate might have been achieved either by lowering $\Delta H^{\ddagger}$ or by raising the value of $T\Delta S^{\ddagger}$ (the entropy of activation) (Fig. 2) (for thermodynamic definitions, see *SI Appendix*). If that primitive catalyst enhanced the rate of reaction entirely by raising the value of $T\Delta S^{\ddagger}$, then the rate enhancement it produced would have been unaffected by changing temperature (Fig. 2*A*). But if that catalyst enhanced the reaction rate by lowering $\Delta H^{\ddagger}$, then the rate enhancement that it produced would have *increased automatically as the surroundings cooled* (Fig. 2*B*), quite apart from any selective pressures that might also have been at work.

The plausibility of this latter scenario for enzyme evolution depends on whether primitive nonbiological catalysts tended to lower activation barriers by reducing the value of $\Delta H^{\ddagger}$, or by raising the value of $T\Delta S^{\ddagger}$. To address that question, we turned to the behavior of nonenzymatic catalysts acting in aqueous solution.

Do Primitive Catalysts Act by Lowering $\Delta H^{\ddagger}$? A Test

Substantial rate accelerations have been reported for a variety of nonenzymatic catalysts acting in water (including metal ions, simple organic molecules, antibodies, and other proteins) (Table 2), but there appears to be relatively little information about the temperature dependence of these catalytic effects. We decided to examine the action of two molecules catalyzing particularly difficult reactions, phosphate ester hydrolysis (the Ce^{IV} ion) and amino acid decarboxylation (pyridoxal phosphate, or PLP). By comparing the temperature dependence of k_{cat} for the catalytic complex with that of k_{non} for the substrate alone, we sought to determine whether these nonenzymatic catalysts act by reducing $\Delta H^{\ddagger}$ or by enhancing $T\Delta S^{\ddagger}$. The results, reported below, were unequivocal.

The hydrolysis of phosphate esters is strongly catalyzed by soluble complexes of dinuclear Ce^{IV} (12, 13)—the most abundant

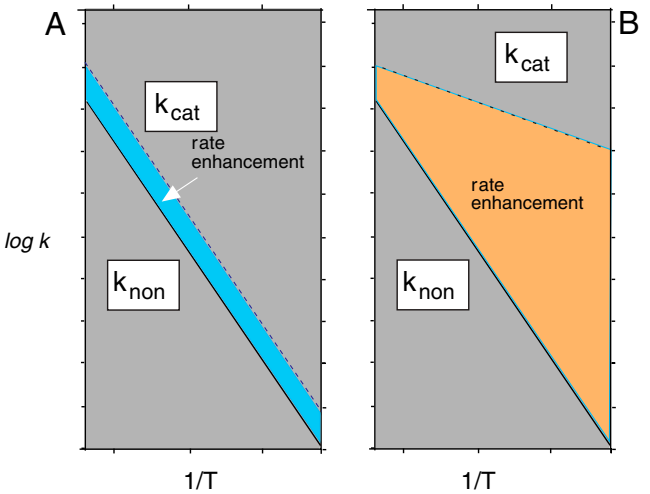

Fig. 2. Expected variation with temperature of the rate enhancement (k_{cat}/K_m) produced by two catalysts, acting (*A*) by enhancing a reaction's entropy of activation, or (*B*) by reducing a reaction's enthalpy of activation.

Table 2. Estimated rate enhancements produced by nonenzymatic catalysts acting in water

catalyst (or solvent)	reaction	k_{cat}/k_{non}
Transfer to vapor phase (27)	$OH^- + CH_3Br$	1.3×10^{18}
Ce^{IV} (28)	$H_2O + CH_3OPOO^{-2}$	1.0×10^{14}
Transfer to cyclohexane (solvent effect) (29)	$H_2O + CH_3OPOO^{-2}$	2.5×10^{12}
Pyridoxal phosphate (PLP) (28)	2-aminoisobutyric acid (AIB) decarboxylation	2.3×10^{9}
HMPA (solvent effect) (30)	Kemp elimination	9×10^{7}
Metal complexation (*cis*-$Co(en)_2^{+3}$) (25, 31)	OH^- + gly-gly	9×10^{7}
Computer-aided enzyme design (32)	Kemp elimination	1.2×10^{6}
Catalytic antibody (33)	aldol cleavage	3.6×10^{5}

lanthanide in the earth's crust and the only lanthanide that is stable in the +4 oxidation state. The hydrolysis of unactivated phosphate monoesters—a common event in metabolism and cell signaling—is intrinsically one of the slowest biochemical reactions known (estimated $t_{1/2} = 1.1 \times 10^{12}$ y at 25 °C) (7). When we examined the temperature dependence of the effect of Ce^{IV} on the hydrolysis of two phosphate monoesters (*SI Appendix*), the results were unequivocal. The rate enhancement by Ce^{IV}, estimated by comparing the rate constant for the catalyzed reaction (k_{cat}) with the rate constant for the uncatalyzed reaction (k_{non}), was achieved entirely by reducing the enthalpy of activation, while the effect of Ce^{IV} on $T\Delta S^{‡}$ was actually unfavorable (Table 3).

The decarboxylation of amino acids, another reaction that is intrinsically very slow [$t_{1/2} \sim 10^9$ y in neutral solution at 25 °C (14)] is catalyzed by enzymes that ordinarily use PLP as a cofactor. PLP by itself is known to be an effective catalyst of this reaction, accounting for a major fraction of the rate enhancement produced by the enzyme (15). Examining the temperature dependence of the PLP rate enhancement of the decarboxylation of 2-aminoisobutyrate (*SI Appendix*), we observed once again that catalysis arises entirely from a reduction in the enthalpy of activation for the catalyst-substrate complex (Table 2).

These findings are not unprecedented. Enthalpic effects have also been shown to predominate in systems in which catalysis is much less pronounced, including general base catalysis of the bromination of acetoacetate by glycolate (16), in the covalent catalysis of the hydrolysis of 4-nitrophenyl acetate by imidazole (17), in general base catalysis of the aminolysis of carboxylic esters by alkylamines (18), and in the Mg^{II}-catalyzed methanolysis of ATP (19).

Table 3. Effects of nonenzymatic catalysts on thermodynamics of activation (kcal/mol, where $\Delta G^{‡} = \Delta H^{‡} - T\Delta S^{‡}$)

	k (s^{-1}) (25 °C)	$\Delta G^{‡}$ (25 °C)	$\Delta H^{‡}$	$T\Delta S^{‡}$
Phosphate ester hydrolysis *(1)*				
4-npP^{-2}	3.4×10^{-9}	29.0	27.6	−1.4
4-npP:Ce^{IV}	5.2×10^{-5}	23.2	12.1	−11.1
Rate enhancement	$\mathbf{1.5 \times 10^{4}}$	*−5.8*	**−15.5**	**−9.7**
(2)				
MeP^{-2}	2×10^{-20}	44.3	47.0	2.7
MeP^{-2}:Ce^{IV}	1.9×10^{-6}	25.2	6.3	−18.9
Rate enhancement	$\mathbf{9.6 \times 10^{13}}$	*−19.1*	**−40.7**	**−21.6**
Amino acid decarboxylation *(3)*				
AIB	1.1×10^{-18}	41.8	39.9	−1.9
AIB:PLP	2.6×10^{-9}	29.1	21.3	−7.8
Rate enhancement	*4.4 x 10^{11}*	*−12.7*	*-18.6*	*-5.9*

In each case, the effect of the catalyst on $\Delta H^{‡}$ is favorable, but its effect on $T\Delta S^{‡}$ is unfavorable. For experimental details, see *SI Appendix*.

Table 4. Values of $\Delta H^{‡}$ for some biological reactions in the presence (k_{cat}) and absence (k_{non}) of an enzyme at neutral pH (literature references in parentheses)

Bond cleaved	Enzyme	$\Delta H^{‡}$ (k_{cat})	$\Delta H^{‡}$ (k_{non})
C — C	OMP decarboxylase	11.0 (34)	37.1 (1)
C — N	Dihydroorotase	12.3 (35)	24.7 (35)
	Cytidine deaminase	14.9 (36)	22.1 (36)
	Urease	9.9 (37)	22.9 (5)
	Trypsin + casein	12.0 (38)	22.9 (25)
C — O	Invertase	11.5 (39)	27.3 (40)
P — O	Alkaline phosphatase	12.7 (41)	47.0 (7)
	Staphylococcal nuclease	10.8 (42)	29.5 (22)
	Hexokinase	11.6 (19)	12.8 (19)
S — O	Alkyl sulfatase	12.4 (43)	24.6 (44)
C — H	Mandelate racemase	15.4 (45)	31.9 (46)
	Fumarase	13.9 (47)	28.9 (48)
C — Cl	Chloroacrylate dehalogenase	9.4 (24)	26.7 (24)
	average	**12.2**	**28.0**

It is interesting to consider the properties of present-day enzymes in the light of these findings. The final entry at the bottom of Table 1 indicates the average behavior of k_{cat} for 12 different enzymes shown in Table 4. Despite differences in mechanism, the heats of activation are remarkably similar to each other, with $\Delta H^{‡} \sim 12$ kcal/mol. If $\Delta H^{‡}$ values for k_{cat} of these highly evolved enzyme reactions are compared with $\Delta H^{‡}$ values for the uncatalyzed reactions in Table 1, it is evident that all these enzymes act by lowering the value of $\Delta H^{‡}$.* Thus, the corresponding increase in affinity, as the enzyme-substrate complex in such reactions passes from the ground state to the transition state, is mainly enthalpic in origin, consistent with chemical mechanisms that typically involve the formation of new electrostatic and hydrogen bonds in the transition state (20).

From an evolutionary standpoint, it is unlikely that the common enthalpy-lowering effect of present-day enzymes is fortuitous. As the environment cooled, a primitive catalyst that reduced $\Delta H^{‡}$ would have offered a selective advantage over a catalyst that raised $T\Delta S^{‡}$ by an equivalent amount (Fig. 2). An entropic catalyst, in contrast, would have been useful only for reactions with intrinsically low enthalpic barriers in which a large rate enhancement is unnecessary and substrate specificity is of special importance.† We propose that enthalpy-lowering mechanisms became common *because* they are so temperature-dependent; and because there is almost no limit—at least in principle—to the benefit that might arise from the action of a purely "enthalpic" catalyst. Natural selection has presumably resulted in the evolution of enzymes toward greater catalytic power and specificity, but at the most fundamental level—the thermodynamics of substrate activation—PLP and the Ce^{IV} ion resemble modern-day enzymes and furnish plausible models for their evolution.

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*In all known cases, K_m values increase with increasing temperature. Thus, the $\Delta H^{‡}$-lowering effect of enzymes becomes even more striking if k_{cat}/K_m, rather than k_{cat}, is used as a basis for comparison.

†The only known exception, the peptidyl transferase center of the ribosome, produces a relatively small rate enhancement (10^7-fold) that arises entirely from a more favorable $T\Delta S^{‡}$. This entropic effect is believed to arise from physical desolvation and juxtaposition of the two substrates, rather than from chemical catalysis in the usual sense. The enthalpic barrier to uncatalyzed peptidyl transfer ($\Delta H^{‡} = 8$ kcal/mol in water)—which is much smaller than those of the reactions considered here, actually *increases* within the active site of the ribosome (49).

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