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Energetics of Amino Acid Synthesis in Hydrothermal Ecosystems

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Thermodynamic calculations showed that the autotrophic synthesis of all 20 protein-forming amino acids was energetically favored in hot (100°C), moderately reduced, submarine hydrothermal solutions relative to the synthesis in cold (18°C), oxidized, surface seawater. The net synthesis reactions of 11 amino acids were exergonic in the hydrothermal solution, but all were endergonic in surface seawater. The synthesis of the requisite amino acids of nine thermophilic and hyperthermophilic proteins in a 100°C hydrothermal solution yielded between 600 and 8000 kilojoules per mole of protein, which is energy that is available to drive the intracellular synthesis of enzymes and other biopolymers in hyperthermophiles thriving in these ecosystems.

Recently, Woese (1) suggested that the ancestor of all life on Earth was not a discrete entity but rather a community of cells with a shared physical history. Over time, as the universal tree of life radiated outward from the root, three primary domains of organisms arose. Although interpretations of the complete genomes of over a dozen microbes have raised questions regarding the classification of various organisms within this phylogenetic tree (2), general properties of members belonging to the deepest branches of the Bacteria and Archaea lineages indicate that the earliest life was autotrophic not heterotrophic, relied on chemosynthesis rather than photosynthesis, and required high temperatures for growth (3). On the basis of these findings, ancient hydrothermal systems have been proposed as likely sites for the origin of life (4-6). This view is consistent with the results of hydrothermal experiments that were aimed at identifying the primordial chemosynthesis reactions for life's origin (7).

In the speculative arena of the origin and the early evolution of life, quantification of the energetics of biosynthesis reactions in microorganisms belonging to the deepest branches in the phylogenetic tree is of interest. However, the determination of these energetics in hydrothermal systems on early Earth (4, 8, 9) is hindered somewhat by poorly constrained chemical and physical properties of the earliest oceans, including the redox

state, pH, temperature, and concentrations of CO₂, NH₄⁺, and H₂S. On the other hand, ample data are available from active hydrothermal ecosystems, which are hosts to the deepest branches of thermophilic, chemoautotrophic Archaea and Bacteria. Analyses of seawater and vent fluids together with reliable equations of state for aqueous organic and inorganic compounds permit well-constrained calculations of the energetics of biosynthesis reactions in hydrothermal ecosystems. Once such a framework for evaluating the energetics of biosynthesis is in place, analogous calculations can be carried out to account for likely conditions on early Earth.

Chemical disequilibria in hydrothermal ecosystems provide substantial amounts of energy, which can drive anabolic reactions in thermophilic and hyperthermophilic chemoautotrophs (8, 10). Furthermore, the formation of many aqueous organic compounds is favored at high temperatures over low temperatures (11-13). We calculated the overall Gibbs free energies (ΔG_r) of net amino acid synthesis reactions (r) for hydrothermal systems and contrasted them with the energy requirements of synthesis reactions in surface seawater. We then used these calculations to explore the ramifications for the synthesis of thermophilic proteins, and we considered the implications for early life.

Amino acid synthesis pathways in extant microorganisms, although highly diverse, share two basic features: (i) the nitrogen of α -amino groups in amino acids originates from NH₄⁺ and (ii) the sources of skeletal carbons are intermediates of the tricarboxylic acid cycle and the other major metabolic

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pathways that are ultimately linked, in autotrophs, to the assimilation of inorganic carbon in the form of CO₂. These features allow us to write net reactions for the autotrophic synthesis of the 20 naturally occurring amino acids (Table 1). Implicit in all the calculations presented here is the assumption that the appropriate enzymes for each step in the amino acid synthesis pathways are present and active.

The net energetics of amino acid synthesis can be determined by combining standard-state Gibbs free energies of synthesis reactions (ΔG_r°) with products of intracellular activity. Although the intracellular concentrations of the essential reactants and products are poorly known, we can place constraints on the amounts of energy released or required during amino acid synthesis from CO₂, H₂, NH₄⁺, and H₂S by autotrophic microbes in natural ecosystems using extracellular activity products. The diffusion of neutral molecules and the transfer of ions by transport proteins establish chemical links across cell membranes between the host aqueous environment and the intracellular fluid. The energetic consequences of differences between the extracellular and intracellular activities of the reactants and products can be assessed when the energetic demands that organisms place on their geochemical environments are established. Even if the activities of free amino acids, for example, are orders of magnitude higher within the cell than in the natural environment, values of ΔG_r for their synthesis can be computed.

We calculated values of ΔG_r for all 20 net amino acid synthesis reactions in submarine hydrothermal solutions with a temperature (100°C) between that of cold (2°C) seawater and very hot (350°C) vent fluid; this temperature was achieved by mixing these end members in the subsurface. The amounts of energy associated with the reactions in Table

1 can be calculated from the expression

$$\Delta G_r = \Delta G_r^\circ + RT \ln Q_r \quad (1)$$

where ΔG_r and ΔG_r° are as defined above; R and T represent the gas constant and temperature in kelvin, respectively; and Q_r denotes the activity product. The values of Q_r that are required to evaluate ΔG_r with Eq. 1 can be determined from

$$Q_r = \prod a_i^{v_{i,r}} \quad (2)$$

where a_i stands for the activity of the i th species and $v_{i,r}$ represents the stoichiometric reaction coefficient of the i th species in reaction r , which is negative for reactants and positive for products (14).

The standard-state term (ΔG_r°) can be computed at any temperature and pressure by combining the apparent standard Gibbs free energies of formation (ΔG_i°) of the i th species in r (15) at these conditions in accord with the expression

$$\Delta G_r^\circ = \sum v_{i,r} \Delta G_i^\circ \quad (3)$$

The values of ΔG_i° at the temperature and pressure of interest for the aqueous species in the reactions given in Table 1 are readily calculated using thermodynamic properties and parameters for the revised Helgeson-Kirkham-Flowers equation of state (16) together with the SUPCRT92 software package (17). For all 20 reactions, the values of ΔG_r° at 250 bar and at temperatures from 0° to 150°C are negative and exhibit substantial temperature dependencies, increasing with increasing temperature (Fig. 1).

Evaluating $RT \ln Q_r$ (Eq. 1) for the reactions in Table 1 requires the activities of the reactants and products under the environmental conditions of interest. For example, a 100°C hydrothermal solution can be generated if ~2.55 kg of 2°C seawater are mixed with 1 kg of 350°C vent fluid (10). The calculated activities of the aqueous species

CO₂, H₂, H₂S, H⁺, and NH₄⁺ are given in Table 2 for a 100°C mixed hydrothermal solution and surface seawater at 18°C. The activities of the free amino acids in these two fluids were determined with the same mixing model, assuming, however, that the only contribution was from free amino acids in seawater (Table 3) (18).

The value of ΔG_r in the 100°C mixed solution for each net amino acid synthesis reaction can now be calculated (Table 3). As an example, we describe the approach used for the synthesis of alanine, represented by the first reaction in Table 1. At 100°C and 250 bar (see Fig. 1 and text above), ΔG_r° is equal to -155.34 kJ mol⁻¹, and Q_r , determined from

Table 2. Activities of aqueous inorganic species in mixed hydrothermal solution and surface seawater.

Aqueous species	Activity in 100°C hydrothermal solution*	Activity in 18°C surface seawater
CO ₂	2.2 × 10 ⁻³	1.0 × 10 ^{-4†}
NH ₄ ⁺	2.9 × 10 ⁻⁶	5.0 × 10 ^{-8‡}
H ₂	3.4 × 10 ⁻⁴	2.0 × 10 ^{-9‡}
H ⁺	1.9 × 10 ⁻⁶	5.0 × 10 ^{-9†}
H ₂ S	1.6 × 10 ⁻³	b.d.§

*Calculated by McCollom and Shock (10) using chemical analyses for deep seawater and hot vent fluid as given by Von Damm (24), except that the value for NH₄⁺ was determined in this study with activities in deep seawater and hot vent fluid equal to 5.0 × 10⁻⁸ and 1.0 × 10⁻⁵, respectively (25). †Calculated in this study at the temperature in this table from the pH and total CO₂ given in (26). ‡(25). §Below detection, b.d.; a value of 10⁻¹⁵ was used to perform the calculations described in the text.

Table 1. Net amino acid synthesis reactions (23).

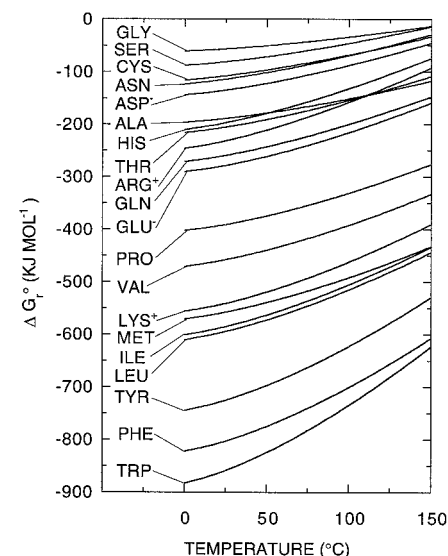
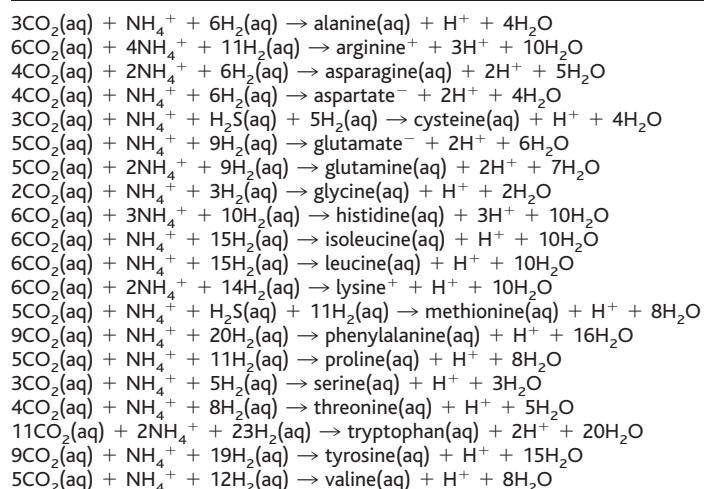


Fig. 1. Values of ΔG_r° of the net amino acid synthesis reactions (see Table 1) in aqueous solution as a function of temperature at 250 bar. This pressure was chosen to approximate conditions in deep-sea hydrothermal systems.

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$$Q_1 = \frac{a_{\text{alanine}} a_{\text{H}^+}}{(a_{\text{CO}_2})^3 a_{\text{NH}_4^+} (a_{\text{H}_2})^6} \quad (4)$$

using activities a given in Tables 2 and 3, is equal to 1.1×10^{20} . Combining these values, as in Eq. 1, gives $\Delta G_r = -12.12 \text{ kJ mol}^{-1}$. Therefore, the net synthesis of 1 mol of alanine in a 100°C mixed hydrothermal solution releases 12.12 kJ of energy, which is available to drive otherwise endergonic anabolic reactions such as peptide and protein synthesis.

The value of ΔG_r is negative for 11 of the 20 net amino acid synthesis reactions in a 100°C mixed hydrothermal solution that supports the growth of hyperthermophilic microbes (Table 3). The formation of these 11 amino

acids from inorganic precursors lowers the energy state of the system at the conditions that occur in this subsurface ecosystem. The net synthesis reactions for the remaining nine amino acids are endergonic and must be coupled to exergonic reactions in order to proceed. By comparison, the net synthesis reactions of all 20 amino acids are strongly endergonic in cooler (18°C), oxidized, surface seawater. The values of ΔG_r (Table 3) for the reactions in Table 1 under these conditions were generated with the values of ΔG_r° at 18°C and 1 bar together with activity products for seawater computed with data from Tables 2 and 3. Comparisons of ΔG_r for the two distinct environments show differences of 65 to 470 kJ mol^{-1} of amino acid produced.

If the concentrations of free amino acids were, for example, three orders of magnitude higher, values of ΔG_r (Table 3) would increase for each amino acid synthesis reaction by 21.43 and $16.72 \text{ kJ mol}^{-1}$ at 100° and 18°C , respectively. As a result, 9 instead of 11 net amino acid synthesis reactions would be exergonic in the 100°C hydrothermal solution. At millimolar concentrations, an increase in ΔG_r of 42.86 and $33.44 \text{ kJ mol}^{-1}$ can be calculated at the two temperatures. In this case, six net amino acid synthesis reactions have negative values of ΔG_r at 100°C .

These results support the argument that hydrothermal vent environments are well suited for organic synthesis (5, 11, 19). Even biomolecules such as amino acids can be synthesized exergonically in hydrothermal ecosystems. The environmental conditions that permit the exergonic formation of amino acids are entirely geochemically and geologically controlled. The temperature, pressure, and activities of reactants and products that exist in hydrothermal systems determine the energetics discussed above.

Although values of ΔG_r° for the reactions in Table 1 are strongly temperature dependent (see Fig. 1), it is an inescapable consequence of the reducing potential of mixed hydrothermal solutions that synthesis of the more reduced amino acids is favored over the synthesis of the more oxidized ones. In a 100°C hydrothermal solution, negative values of the mean nominal oxidation state of the carbon atoms (Z_c) (20) in the amino acids correspond to negative values of ΔG_r (Table 3). In fact, the net synthesis reactions are exergonic for the 10 most reduced (lowest values of Z_c) amino acids (Leu, Ile, Val, Lys⁺, Phe, Met, Pro, Tyr, Trp, and Ala) and are endergonic for 9 of the 10 most oxidized (highest values of Z_c) amino acids (His, Asn, Asp⁻, Gly, Cys, Ser, Gln, Arg⁺, and Thr). In contrast to the 100°C mixed hydrothermal solution, all values of ΔG_r in 18°C surface seawater are positive (endergonic). These observations support the notion that, under oxidizing near-surface conditions, the autotrophic synthesis of amino acids driven by photosynthesis requires a tremendous external energy source (solar radiation), which is not necessary in ecosystems of high or even moderate reducing potential.

The energy yield from the autotrophic synthesis of amino acids in a 100°C mixed hydrothermal fluid (see Table 3) may ultimately be used to overcome the energy requirements of protein synthesis. To calculate this energy yield, we combined sequence data for thermophilic proteins with the thermodynamic evaluation discussed above. For example, from the sequence of rubredoxin from the hyperthermophilic Archaeon *Pyrococcus furiosus* (53 residues) and the values of ΔG_r for all 20 amino acids (Table 3), we computed

Table 3. Activities of amino acids in surface seawater (27) and in mixed hydrothermal solution, values of ΔG_r for net amino acid synthesis reactions (Table 1) in these two fluids, and mean nominal oxidation states of the carbon atoms (Z_c) in the amino acids.

Amino acid	Concentration* in seawater (nm) (28)	Activity in 100°C hydrothermal solution	ΔG_r for 100°C hydrothermal solution (kJ mol^{-1})	ΔG_r for 18°C surface seawater (kJ mol^{-1})	Z_c
Alanine	3.9	2.8×10^{-9}	-12.12	113.66	0.00
Arginine ⁺	0.7	5.0×10^{-10}	197.52	409.46	0.33
Asparagine	1.1	7.9×10^{-10}	83.53	201.56	1.00
Aspartate ⁻	3.3	2.4×10^{-9}	32.78	146.74	1.00
Cysteine	0.5 [†]	3.6×10^{-10}	60.24	224.67	0.67
Glutamate ⁻	3.8	2.7×10^{-9}	-1.43	172.13	0.40
Glutamine	1.0	7.2×10^{-10}	44.03	223.36	0.40
Glycine	5.8 [‡]	4.2×10^{-9}	14.89	80.49	1.00
Histidine	1.2	8.6×10^{-10}	154.48	350.52	1.00
Isoleucine	0.7	5.0×10^{-10}	-96.40	213.93	-1.00
Leucine	0.8	5.7×10^{-10}	-105.53	205.03	-1.00
Lysine ⁺	1.0	7.2×10^{-10}	-28.33	258.56	-0.67
Methionine	2.8 [§]	2.0×10^{-9}	-174.71	113.22	-0.40
Phenylalanine	0.5	3.6×10^{-10}	-114.54	303.64	-0.44
Proline	0.5 [†]	3.6×10^{-10}	-38.75	192.83	-0.40
Serine	4.6	3.3×10^{-9}	69.47	173.73	0.67
Threonine	5.8 [‡]	4.2×10^{-9}	53.51	216.50	0.00
Tryptophan	0.6	4.3×10^{-10}	-38.99	431.17	-0.18
Tyrosine	0.9	6.5×10^{-10}	-59.53	334.20	-0.22
Valine	2.8 [§]	2.0×10^{-9}	-70.12	178.00	-0.80

*Owing to the low amino acid concentrations, activity coefficients equal to unity are used to convert to activity. [†]Measured values were not reported; those given here correspond to the lowest measured concentrations of the other amino acids. [‡]Glycine and threonine commonly coelute; the reported value is the maximum value. [§]Methionine and valine commonly coelute; the reported value is the maximum value.

Table 4. Values of ΔG_r in a 100°C mixed hydrothermal solution for the net synthesis of the amino acids that constitute nine proteins from thermophilic and hyperthermophilic Archaea and Bacteria. This is not the net energy for protein synthesis. Numbers in brackets are the number of amino acid residues. GAPDH, glyceraldehyde phosphate dehydrogenase; OMP, orotidine 5'-phosphate.

Thermophilic organism	Protein	ΔG_r (kJ per mole of protein)
<i>Pyrococcus furiosus</i>	Rubredoxin [53] (29)	-722
<i>Thermococcus litoralis</i>	Ferredoxin [59] (30)	-664
<i>Pyrococcus furiosus</i>	Ferredoxin [66] (30)	-931
<i>Thermotoga maritima</i>	GAPDH [332] (31)	-3171
<i>Thermotoga maritima</i>	OMP α [380] (32)	-5022
<i>Thermotoga maritima</i>	Elongation factor-Tu [400] (33)	-4237
<i>Thermus thermophilus</i>	Elongation factor-Tu [406] (33)	-2438
<i>Thermotoga maritima</i>	Glutamine synthase [439] (34)	-5335
<i>Pyrococcus woesei</i>	Glutamine synthase [439] (34)	-7892

that, in a 100°C mixed hydrothermal solution, the net synthesis of the amino acids constituting 1 mol of this protein releases 722 kJ. The values of ΔG_r for the net amino acid synthesis of this and eight other thermophilic proteins (Table 4) are all negative (exergonic). Combined with the conclusion that peptide bond formation is energetically favored with increasing temperature (12), an argument can be made that thermophilic chemoautotrophs, such as those occupying the deepest branches in the universal tree of life, expend considerably less energy for the synthesis of macromolecules, such as proteins, than do their mesophilic counterparts. Depending on the amino acid composition of the protein, the synthesis of the monomers from CO₂, H₂, and other inorganic precursors in hot, reduced aqueous solutions may provide substantial surplus energy that can be harnessed to drive intracellular synthesis of enzymes and other polymers.

Our results might start to explain the phenomenal rates of biomass production around hydrothermal vents (21) and also how hyperthermophilic Archaea in natural or laboratory high-temperature systems are able to synthesize all required intracellular biomolecules in time periods ranging from minutes to hours as their population doubles. Our calculations can be used as a template in concert with constraints on the flow of energy through early hydrothermal systems to determine the potential of such systems as environments where amino acid and protein synthesis, primitive metabolisms, and even the universal ancestor of all extant life emerged. Calculations representing early Earth hydrothermal systems must reflect the differences in geochemistry and geophysics from active analogs. For example, sensitivity tests show that lower O₂ concentrations in seawater and ultramafic host rocks enhance the potential for hydrothermal organic synthesis (13), and the same should be expected for amino acid and protein synthesis.

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Crystal Structure of the Catalytic Domain of Human Plasmin Complexed with Streptokinase

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Streptokinase is a plasminogen activator widely used in treating blood-clotting disorders. Complexes of streptokinase with human plasminogen can hydrolytically activate other plasminogen molecules to plasmin, which then dissolves blood clots. A similar binding activation mechanism also occurs in some key steps of blood coagulation. The crystal structure of streptokinase complexed with the catalytic unit of human plasmin was solved at 2.9 angstroms. The amino-terminal domain of streptokinase in the complex is hypothesized to enhance the substrate recognition. The carboxyl-terminal domain of streptokinase, which binds near the activation loop of plasminogen, is likely responsible for the contact activation of plasminogen in the complex.

The activation of human plasminogen (Plg) to plasmin (Pm) in blood plasma is the central event that results in the dissolution of the fibrin clot by proteolysis. Human Plg, a single-chain protein of 791 residues, contains five kringle domains and a serine protease

domain (1). Plg activation by physiological activators, for example, tissue-type plasminogen activator (TPA), is accomplished by the hydrolysis of the Arg⁵⁶¹-Val⁵⁶² bond in Plg (2). Plg activators, TPA and streptokinase (SK), are widely used as thrombolytic agents