



The opposite effect of K^+ and Na^+ on the hydrolysis of linear and cyclic dipeptides

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ABSTRACT

Potassium and sodium are generally considered inert 'spectator' ions for organic reactions. Here, we report rate constants for the acid-promoted hydrolysis of the seven dipeptides of glycine (G) and alanine (A) and an unexpected pattern in how these rates differ in the presence of K^+ and Na^+ . The linear dipeptides hydrolyze 12–18% percent slower in the presence of KCl versus an equal concentration of NaCl, while the cyclic dipeptides hydrolyze 5–13% faster in the presence of KCl (all P-values < 0.025). We believe this is the first report of a general organic reaction—here, amide hydrolysis—for which some substrates react faster in the presence of K^+ and others in Na^+ . The results offer a potential reason for life's mysterious universal selection of intracellular potassium over sodium.

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Introduction

While all living systems on Earth enrich K^+ from their external environments—and the vast majority of cells have higher intracellular concentrations of K^+ than Na^+ —there exists no definitive explanation for this preference.^{1–3} Features that are ubiquitous in biology are likely to have evolved early in the development of life, perhaps as early as the first living system.⁴ Examples of potential biochemical 'fossils' from life's origin that have been the focus of origin-of-life research include lipid membranes,^{5,6} nucleic acids,^{7,8} proteins/polypeptides,^{9–11} and common metabolic cycles.^{4,12,13}

Another ubiquitous feature of life is the maintenance of ion gradients across cellular membranes.¹⁴ In modern organisms, K^+ and Na^+ ion gradients are critical for a number of functions.^{14,15} The high concentration of potassium in cells has led some to hypothesize that life may have developed in an environment with high levels of potassium.^{3,16} However, the observation that cells can expend up to a third of their energy budget on Na^+/K^+ -ATPase suggests the intracellular enrichment of potassium is important itself—not just a vestigial remnant of prebiotic conditions that would have faded as life evolved.¹⁷ Still, the role of intracellular K^+ is not obvious.¹⁸ Why did life choose K^+ when it could seemingly use intracellular Na^+ for the same purpose?

Few non-biological systems exist in which identical concentrations of potassium or sodium considerably change the properties of the system. Salts of K^+ and Na^+ often have different solubilities, and

the presence of equal concentrations of KCl and NaCl can affect the solubility of organic compounds in aqueous solutions.¹⁹ The only study we are aware of that reports a notable difference for an organic reaction in water in the presence of K^+ and Na^+ is for the coupling of glutamic acid mediated by carbonyldiimidazole.²⁰ Natochin and coworkers observed higher yields of oligo(glutamic acid) formation in the presence of K^+ versus Na^+ . Motivated by this report and the premise that universal features of biochemistry may have been important to the origin of life, we decided to measure the influence of K^+ and Na^+ on reactions of peptides.

Experimental design

In selecting conditions for model prebiotic reactions, decisions must be made to balance historical relevance with experimental convenience. This section explains our choices regarding the design of experiments and any simplifications or assumptions that went into these decisions.

Selection of reaction for study

We elected to examine amino acids and peptides for their (i) obvious relevance to biology, (ii) historical interest to prebiotic chemistry, and (iii) well-studied structure and reactivity.⁹ In aqueous solution, equilibrium favors the hydrolysis of peptides. And if we wish to understand the potential generation of protein biopolymers from the coupling of amino acids in water, we will have to understand the influence of hydrolysis as a competing deleterious side reaction.²¹

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Selection of substrates for study

We chose to focus our initial work on dipeptides in order to begin with the simplest system available. While a single amino acid can dimerize into only two dipeptides—the linear and cyclic forms—there are seven permutations when selection is expanded to two amino acids. The obvious candidates for study were dipeptides of glycine and alanine. Glycine (G) is the simplest amino acid. It is the smallest by mass, has no functional groups on its side chain, and is achiral. Alanine (A) is the next smallest amino acid by mass and has only a relatively inert methyl group for a side chain. G and A also appear to be the amino acids most relevant to prebiotic chemistry. A multi-factor analysis of 60 criteria by Trifonov concluded that G and A were the first two dominant amino acids in the chronology of evolution.²² G and A are synthesized in the highest yields in most reported prebiotic syntheses of amino acids, including spark-discharge experiments.^{9,23} G and A are also routinely the most abundant amino acids found on potential extraterrestrial impactors like meteorites, asteroids, and comets, which may have seeded early Earth from space.¹¹

Cyclic dipeptides (2,5-diketopiperazines or DKPs, see Scheme 1) are of special relevance to origin-of-life chemistry as potentially important species in the construction and destruction of oligopeptides. Although conditions have been reported where direct attack of DKPs by the free amine of an amino acid can extend a growing peptide chain by two residues,²⁴ the formation of DKPs via intramolecular attack of the peptide backbone is a principal degradation route of short oligopeptides.^{10,25} The absence of free amino and carboxyl groups in DKPs limit their participation in peptide coupling reactions unless they first hydrolyze, so DKPs are often invoked as “dead ends” or traps for amino acids that remove them from a pool of molecular building blocks.²⁶ High yields of DKPs—to the detriment of linear peptides—from amino acid precursors have been reported from a variety of experiments, including those with simulated prebiotic conditions.^{25,27}

Selection of reaction conditions

It is always preferable to match experimental conditions to those presumed to exist in the prebiotic landscape, but some concessions must be made for experimental convenience. We selected a temperature of 70 °C for the reactions to match the temperature of the Archean ocean inferred from geochemical analysis of chert minerals that date to ~3.5 Ga,²⁸ though that analysis and the question of the temperature of the early ocean are the subject of great debate. The concentration of the substrate was set to 50 millimolar (mM) and the concentration of salt was set to 4 molal (m). This concentration of salt allowed us to observe the maximum effect of the ions on the rate of hydrolysis, as 4 m approaches the limit of solubility of KCl and NaCl at 70 °C. We report components with

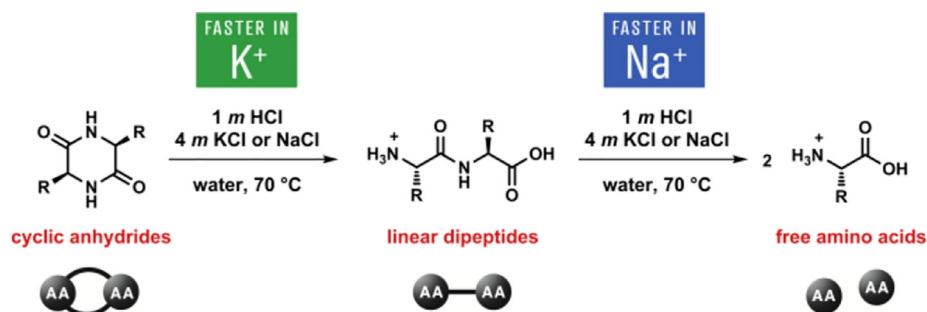
relatively high concentration in molality to maintain consistent ionic strength and concentration (i.e., 4 M NaCl and 4 M KCl are not the same ionic strength, while 4 m NaCl and 4 m KCl are the same ionic strength).²⁹ The addition of 1 m HCl allowed reasonable reaction rates, resulted in pseudo-first-order kinetics,^{30,31} and is more prebiotically relevant than alkaline conditions.^{32,33} While high concentrations of acid and salts are of limited pertinence to extant biology, they are directly relevant to the ‘drying lagoon’ model for prebiotic chemistry in which condensation and hydrolysis reactions transpire in trapped, evaporating bodies of water on Prebiotic Earth.³⁴ Though the hydrolysis of amides at room temperature and neutral pH is notoriously slow,³¹ conveniently, our hydrolysis experiments were complete in hours to days under these conditions.

While essentially no data exist regarding microenvironments on early Earth—including the possibility of environments that mimic the high acidity and heat of our study—there exists both geological and biological evidence that the early Earth was generally hotter and more acidic than today.^{32,33} Several thermoacidophilic microorganisms are known to thrive in very acidic microenvironments on modern Earth, including those of the genus *Picrophilus*, which can grow near pH 0 and up to 65 °C.³⁵ The ability of these archaea to thrive in inhospitable environments thought reminiscent of the hot, acidic, volcanic settings on the early Earth suggests these organisms may be “primordial relics from which more complex life evolved.”³⁵ There is also a theory that life evolved in an acidic, highly saline environment because the presumed “prebiotic set” of amino acids lacked amino acids with aromatic and basic side chains, and peptides composed of residues from this set likely restricted the possibility of folding to acidic and saline environments.^{11,36}

We previously used NMR spectroscopy to measure rate constants for the hydrolysis of thioesters as a function of pH.³⁷ Here, we measure pseudo-first-order rate constants for the acid-promoted hydrolysis (k_a) of each substrate at [HCl] = 1 m and [KCl] or [NaCl] = 4 m. We define the starting material to have hydrolyzed after any net hydrolysis of peptide bonds is observed. A typical kinetics experiment involved removing aliquots of the reaction mixtures at various time points, diluting them with an equal volume of deuterium oxide, and collecting an ¹H NMR spectrum of the sample. The relative concentration of hydrolyzed substrate was determined by comparing the integration values of signals corresponding to the reactants and products (Fig. 1). Experimental details are provided as Supporting Information.

Results and discussion

The histograms in Fig. 2a and b depict the rates of hydrolysis for the seven dipeptides of G and A in 1 m HCl at 70 °C in the presence of 4 m KCl, 4 m NaCl, or no added alkali salt. We compared the rates



Scheme 1. Cyclic dipeptides—thought to be “dead ends” in the prebiotic synthesis of polypeptides—hydrolyse to form linear dipeptides. In turn, these acyclic compounds hydrolyse to form free amino acids. In our experiments, we examined the hydrolysis of the seven dipeptides formed from glycine (G, R = H) and alanine (A, R = CH₃) at 70 °C in 4 m KCl or NaCl, promoted by 1 m HCl.

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