Prebiotic synthesis of α-amino acids and orotate from α-ketoacids potentiates transition to extant metabolic pathways

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The Strecker reaction of aldehydes is the pre-eminent pathway to explain the prebiotic origins of α-amino acids. However, biology employs transamination of α-ketoacids to synthesize amino acids which are then transformed to nucleobases, implying an evolutionary switch—abiotically or biotically—of a prebiotic pathway involving the Strecker reaction into today’s biosynthetic pathways. Here we show that α-ketoacids react with cyanide and ammonia sources to form the corresponding α-amino acids through the Bucherer–Bergs pathway. An efficient prebiotic transformation of oxaloacetate to aspartate via N-carbamoyl aspartate enables the simultaneous formation of dihydroorotate, paralleling the biochemical synthesis of orotate as the precursor to pyrimidine nucleobases. Glyoxylate forms both glycine and orotate and reacts with malonate and urea to form aspartate and dihydroorotate. These results, along with the previously demonstrated protometabolic analogues of the Krebs cycle, suggest that there can be a natural emergence of congruent forerunners of biological pathways with the potential for seamless transition from prebiotic chemistry to modern metabolism.

Amino acids and nucleobases are among the most elementary prebiotic and metabolic building blocks. It is believed that on the early Earth, amino acids were available by the Strecker reaction of aldehydes with cyanide and ammonia (Fig. 1a). While the heterotrophic models suggest cyanide and formaldehyde were robust prebiotic source molecules, extant biochemistry utilizes α-ketoacids as the building blocks primarily via transamination (where the amino group is transferred from the amino acid to the recipient α-ketoacid, generating the complementary α-ketoacid and amino acid; Fig. 1b) for the synthesis of α-amino acids—which in turn give rise to the nucleobases, implying that there must be an evolutionary switch between the prebiotic and biotic chemistries. However, the mechanisms of such transformations and such transitions are not clear. An alternative autotrophic approach aims to reproduce the biological pathways from the very beginning but has inadequate experimental support. Discovering chemistries that would be compatible with prebiotic constraints and at the same time allow for the transition to biological pathways can resolve this conundrum. To address this issue, we therefore began exploring the reaction of simple activated carboxylic acids such as α-ketoacids (pyruvate and glyoxylate) and dicarboxylic acids (malonate) and have shown that their inherent reactivity does translate to reactions that give rise to protometabolic pathways (Supplementary Information, Scheme 1). Building on these observations we have recently shown that the reactions of these α-ketoacids and malonate in the presence of cyanide enable a series of transformations that harbours the potential for a reductive glyoxylate cycle of reactions. Here we take this system of reactions further and demonstrate that with the inclusion of various amino acid sources, these very same sets of reactions with α-ketoacids begin to naturally produce the next-generation products—the corresponding α-amino acids and the precursors to the canonical pyrimidine nucleobases—in a manner that can enable transition to the types of pathways that are observed in extant metabolism. The conversion of α-ketoacids to α-amino acids takes place via an overall reductive amination, where the ammonia and cyanide react with an α-ketoacid to give a (carboxy) aminonitrile intermediate. The next step of decarboxylative reduction gives the corresponding hydanotins which then hydrolyse to form the α-amino acids, while the novel appearance of N-carbamoyl aspartate intermediate offers a pathway to the pyrimidine nucleobases. The natural emergence of such pathways that seem to have coincidence with extant biology suggests that the transition from prebiotic to biotic chemistries can be more straightforward.

Results and discussion

Recently, an α-keto analogue of the reductive citric acid pathway (rTCA) was shown to emerge from reacting two of the simplest α-ketoacids, glyoxylate and pyruvate, under metal-free conditions (Supplementary Information, Scheme 1). The α-ketoacids are converted biotically to α-amino acids by two main pathways: (1) by reductive amination wherein the corresponding imine derivative of the α-ketoacid is formed by reacting with ammonia and then reduced to an amine by an external reductant; or (2) by transamination reaction with another α-amino acid where the amino group is transferred to the recipient α-ketoacid, generating the complementary α-amino acid. Abiotic reductive amination of α-ketoacids under various conditions (for example, iron minerals with ammonium, light and ZnS, and metallic Fe(0) with hydrazine, or hydroxylamine) has been explored. Abiotic transamination of the α-ketoacids pyruvate and α-ketoglutarate with glycine produced...
Fig. 1 | Comparison of the prebiotic and biotic routes to \( \alpha \)-amino acids. a, Strecker reaction starting from aldehydes where the \( \alpha \)-aminoonitriile intermediate is hydrolysed to form \( \alpha \)-amino acid. b, A simplified representation of the biochemical transamination pathway in extant metabolism mediated by aminotransferases and the cofactor pyridoxal phosphate, where the amino group is transferred from the amino acid to the \( \alpha \)-ketoacid ('transamination') generating the complementary \( \alpha \)-ketoacid and amino acid. c, The proposed ‘decarboxylative’ Strecker transformation of \( \alpha \)-ketoacid to \( \alpha \)-amino acid (reductive amination) in the presence of an ammonia source (DAP) and cyanide where the hydrolysis of cyanide of I is mediated by DAP followed by the decarboxylation of the diacid II to form an \( \alpha \)-amino acid. d, The actually observed (Bucherer–Bergs reaction) pathway for the reaction of an \( \alpha \)-ketoacid with various ammonia sources and cyanide (reductive amination) where a stable hydantoin intermediate is formed, which is then hydrolysed to the corresponding \( \alpha \)-amino acid.

The Bucherer–Bergs (and not the ‘decarboxylative Strecker’) reaction to form \( \alpha \)-amino acids. With this expectation, we investigated the reaction of a pyruvate I with various equivalents of DAP and cyanide (Fig. 2) over a range of concentrations (0.1–0.35 M), pHs (6–9) and temperatures (room temperature–80 °C) in unbuffered and buffered (phosphate and carbonate–bicarbonate) aqueous solutions and monitored the reactions by \( ^1 \)H and \( ^13 \)C NMR spectroscopy (Supplementary Figs. 5–21 and 24–35). At room temperature, the cyanoacrydact I and \( \alpha \)-aminonitriile 4 (formed via the imine of pyruvate) were observed, which with time (days) or upon heating (50–80 °C, 24 h) converted (with varying efficiency), first to the 5-methylhydantoin 6 and finally to alanine 8 as the major product (Fig. 2). An NMR time-course study of the reaction and spiking with authentic compounds confirmed the formation of 5-methylhydantoin 6, which was hydrolysed to alanine via N-carbamoyl alanine 7 (Fig. 2). Thus, the reaction seems not to proceed as anticipated via the classic Strecker reaction (Fig. 1c) but via a Bucherer–Bergs pathway through the corresponding hydantoin 29–31, which hydrolyses to the \( \alpha \)-amino acid (Fig. 1d). While the formation of aminonitriile intermediate 4 was expected, the ease of hydantoin formation in water or phosphate buffer was surprising, since it implied efficient in situ trapping of the CO2 generated by decarboxylation. A mechanistic study involving a series of control reactions including \( ^13 \)C- and \( ^15 \)N-labelled compounds in both water and \( ^13 \)C-bicarbonate buffer revealed that in the absence of added bicarbonate, the C(2)-carbonyl moiety of hydantoin 6 originates from the CO2H group of the \( \alpha \)-ketoacid (Supplementary Figs. 14–19 and 26–35). The cyanide C becomes the C(4)-carbonyl moiety of hydantoin 6 and ends up as the CO2H group of the \( \alpha \)-amino acid 8. Reactions in degassed phosphate buffer at room temperature showed little or no formation of hydantoin 6, while heating led to production of 6 and acetate indicating that the initial CO2 originates from the 5-methylhydantoin 6.
from the oxidative decarboxylation of the pyruvate (by the traces of oxygen present in water) to acetate. The inefficient reaction in degassed solutions at room temperature could be 'rectified' by adding CO2 (either as bicarbonate or providing a CO2 atmosphere) or using undegassed solutions, suggesting that it is the availability of CO2 that is important for initiating the reaction (Supplementary Figs. 20–25). For example, the presence of gaseous CO2 in the reaction vessel atmosphere was enough to produce 60% of the hydantoin 6a (Supplementary Figs. 24–25). The available CO2 reacts with aminonitrile 4 to form N-carboxy aminonitrile 5, which is hypothetically (similar to the Bucherer–Bergs pathway) to undergo an intramolecular cyclization to form a putative cyclic α-carboxy imino-oxazolidinone 5a and 5b that rearranges (via a putative isocyanate intermediate 5c) to hydantoin 6 as a stable intermediate, as elucidated by 13C and 15N labelling studies and the reaction dependence on CO2 availability. The green arrows indicate the potential for CO2 generated by the in situ decarboxylation processes at various levels of the reaction to participate (via HCO3−) in the reaction. However, given the pervasive abundance of CO2 (and HCO3−) on early Earth, the impact of the CO2 released by decarboxylation in these pathways may not be noteworthy. The carbon atom derived from cyanide is shown in red, the carbon atom derived from CO2 is shown in green and the nitrogen derived from ammonia is shown in blue.

The hydantoin intermediate is stable and usually requires forcing conditions to hydrolyse to the amino acid33,34. However, under these reaction conditions where ammonia is formed by the hydrolysis of DAP, heating at 80 °C was enough to hydrolyse 6 to alanine, as confirmed by the relatively rapid hydrolysis of 5-methylhydantoin 6 in the presence of added DAP versus its absence (Supplementary Fig. 36). The stability of the hydantoin intermediates may also be viewed as a way to 'store the amino acids' in a precursor form and has been suggested by Pascal and Commeyras as a novel way for the emergence of prebiotic peptides32,35. Nevertheless it also points out to the need for prebiotic catalysts and conditions that can enable the hydrolysis of hydantoins under milder conditions34. 

31P and 15N NMR spectra confirmed that DAP was hydrolysed and is acting as a source of NH3 with the phosphate playing no central role (contrary to what was envisioned in Fig. 1c). With this realization, we investigated the reaction of pyruvate 1 with various other ammonia sources (NH3OH, NH4Cl, cyanamide, urea and cyanide hydrolysis products such as formamide and ammonium formate) and observed comparable yields of amino acids at pH 8.5 (Supplementary Table 1), demonstrating the flexibility of the system with regards to the nitrogen source.
We chose DAP as the ammonia source for investigating conditions for optimization of yields, since (1) DAP was found to be more efficient at lower pH ranges (due its lower $pK_a$ of 5.5) among the various ammonia sources studied, and (2) it was found to be a convenient 'in situ' source of ammonia. As expected, use of aqueous bicarbonate as the solvent increased the yield of 5-methylhydantoin and alanine. Thus, reacting pyruvate under optimal conditions (2 equiv. of DAP, 1.2 equiv. of NaCN, pH 8.5, 0.5 M bicarbonate buffer, at room temperature) yielded 88% of 5-methylhydantoin 6, which was hydrolysed at 80 °C to alanine 8 (78%) (Fig. 3a and Supplementary Figs. 37–38). Applying these optimized conditions to $\alpha$-ketoglutarate 9 with DAP and cyanide generated the corresponding hydantoin 10 in 96% yield (Fig. 3b), which was hydrolysed to glutamate 12 (19%, with 72% of 10 remaining; Supplementary Fig. 51–53). The yield of 12 can be increased to 28% by changing the conditions to pH 9 in phosphate buffer at 80 °C (Supplementary Fig. 55).

As the reductive amination process of $\alpha$-ketoacids described above produces $\alpha$-amino acids, the question naturally arises as to whether these $\alpha$-amino acids themselves can begin to play a role by interacting with the $\alpha$-ketoacids under the reaction conditions (Extended Data Fig. 1). To check this, we reacted glycine (or glycinamide) with pyruvate under the optimized conditions at room temperature in bicarbonate buffer (Supplementary Figs. 56–59).
and observed only the formation of the corresponding amino acid–nitrile adduct (4a, Extended Data Fig. 1) with no further reaction to form the corresponding substituted hydantoin derivative (5f) and the carboxymethyl-substituted alanine derivative (5g). This observation suggested the critical role of the hydrogen atom on the endocyclic nitrogen in intermediates 5a/5b, which is important for the formation of the proposed isocyanate intermediate 5e en route to the hydantoin 6 (Fig. 2) as noted by Pascal and Commeiras. When starting with an α-amino acid, glycine, the corresponding putative carboxylated intermediate 5e has no free hydrogen atom on the endocyclic nitrogen but is substituted with an alkyl group instead (Extended Data Fig. 1). This substitution would prevent the formation of the corresponding isocyanate intermediate and account for the absence of formation of substituted hydantoin 5f and, therefore, also the absence of the expected corresponding substituted amino acid product (5g). We then considered the scenario where we let ammonia and glycine (1:1) compete for pyruvate under optimized conditions and observed that although the adduct 4a was formed, with time the reaction with ammonia continued to proceed forward to 5-methylhydantoin 6 (34%) with continuous decrease in the amount of adduct 4a (9%) in 14 days (Supplementary Figs. 60 and 61). Together, these observations suggest that any α-amino acid formed under these reaction scenarios need not adversely interfere with the Bucherer–Bergs reductive amination process.

The Bucherer–Bergs reaction of α-ketoacids raises an interesting contrast with the Strecker reaction of aldehydes in that both of them proceed through an α-aminoisobutirile intermediate but are processed further via different pathways (Fig. 1a versus Fig. 1d). Interestingly, when authentic 2-aminopropionitrile was reacted under the optimized conditions in 0.5 M bicarbonate buffer it produced the 5-methylhydantoin 6 in 80% yield in 14 days (Supplementary Figs. 62 and 63), reflecting the reports from Commeiras and co-workers who observed 5-methylhydantoin formation from 2-aminopropionitrile. However, to have a reasonable juxtaposition, we need to compare the same Bucherer–Bergs reactions starting from acetaldehyde with cyanide and ammonia in bicarbonate buffer. Thus, we conducted a reaction where pyruvate was replaced with acetaldehyde under the optimized Bucherer–Bergs conditions (ammonia, cyanide in 0.5 M bicarbonate buffer at room temperature) and observed comparable efficiencies of 5-methylhydantoin formation at 7 days, 41% for acetaldehyde versus 46% for pyruvate (Supplementary Fig. 64). This implies that the canonical Strecker reaction usually performed in the absence of CO₂ (bicarbonate) may not be an accurate description of how α-amino acids were formed on early Earth where CO₂ was pervasive. The formation of α-amino acids in the presence of CO₂ will progress through the corresponding hydantoin intermediate rather than a traditional Strecker pathway. These observations suggest that both aldehydes and α-ketoacids can give rise to hydantoins that can be converted to α-amino acids via the same Bucherer–Bergs reaction by accumulation of the corresponding precursor hydantoins. In this context, it is noteworthy that many hydantoins have been found in meteorites and the Bucherer–Bergs chemistry described here and by others can account for their formation.

Reactions of oxaloacetate and glyoxylate provide simultaneous access to pyrimidines. Unlike pyruvate and α-ketoglutarate, transamination of oxaloacetate to aspartate is challenging since 13 decarboxylates in solution, producing pyruvate 1° leading to alanine. Under the optimized conditions at room temperature, oxaloacetate was converted to the corresponding hydantoin 14 (69%), which at 80 °C provided a prebiotically plausible access to aspartate 16 (68%, Fig. 3c,d) via N-carbamoyl aspartate 15 as an intermediate (Supplementary Figs. 65–72). This formation of aspartate in high yield starting from oxaloacetate is, once again, to be contrasted with previous attempts either via non-enzymatic transamination or even other reductive amination reactions of oxaloacetate, which has yielded at best trace amounts of 16. The formation of N-carbamoyl aspartate provides a natural path to dihydroorotate (DHO) 17 paralleling the biosynthesis of orotate, which is the precursor to the canonical pyrimidines. A simple heating in solution (50 °C, pH 4.5) or wet–dry cycling (pH 4.5, 50 °C) of 15 led to the formation of hydantoin 14 (up to 49%), aspartate 16 (up to 13%) and DHO 17 (up to 13%; Supplementary Table 2 and Supplementary Figs. 79–83). DHO has been shown to be converted abiotically to orotate.

Similarly, employing the optimized conditions at pH 7, glyoxylate was converted to hydantoin 20, which at 80 °C produced glycine (14–61%) along with 21–32% of N-carbamoylglycine 21 (Fig. 3f). This reaction of glyoxylate giving rise to hydantoin becomes noteworthy in the context of the recent prebiotic synthesis of orotate from the reaction of hydantoin with glyoxylate. And thus, not unexpectedly, traces (~1%) of orotate were also observed in the reaction mixture (Supplementary Figs. 74–78). The Bucherer–Bergs chemistry of α-ketoacids documented in Fig. 3a–d suggests a purely chemical reason as to why the pyrimidines are a natural outcome of this protometabolic set of reactions (which also seems to be reflected in the extant metabolic pathways).

The natural emergence of interconnected pathways between protometabolic pathways. The above reactions provide a natural extension of the previous work where aldol condensation of just the α-ketoacids pyruvate and glyoxylate gives rise to α-ketoglutarate and the corresponding α-ketoacid analogues, the constituents of the Krebs cycle (Supplementary Information, Scheme 1). Recently, in a related work, we have shown that reaction of these α-ketoacids or their condensation products with cyanide alone leads to selective reductive transformations reminiscent of the tRCA pathway (Supplementary Information, Scheme 2). Therefore, it was of interest to know what these α-ketoacids and their condensation products would produce in the presence of ammonia sources alone. When the primary condensation products from the reaction of pyruvate and glyoxylate were exposed to various prebiotic sources of ammonia, 12–20% of α-ketoglutarate was formed (Fig. 4a) as opposed to the 2–4% yield in their absence (Supplementary Table 3 and Supplementary Figs. 84–87). Thus, the presence of amines/ammonia enables a more efficient transformation of the pyruvate-glyoxylate condensation products to the stable α-ketoglutarate via a retro–Claisen reaction and/or by enhancing the cross–Cannizzaro reaction.

As an example of how naturally productive such systems-chemistry can be, we subjected a mixture of malonate and glyoxylate in the presence of urea (as an ammonia surrogate) in phosphate buffer to heating and/or wet–dry cycles (Fig. 4b), which led to the formation of hydantoin 14 (32%), aspartate 16 (10%) and DHO 17 (14%) along with malate (~2%) (Fig. 4c and Supplementary Figs. 88–90). The formation of aspartate and DHO independent of oxaloacetate as the source material may be consequential since the prebiotic provenance of oxaloacetate (due to its instability) is not resolved.

Implications for transitioning to extant metabolic pathways. The formation of α-amino acids via the well-known Strecker reaction starting from aldehydes and the Bucherer–Bergs reaction starting from α-ketoacids demonstrated here enhance the spectrum of plausible prebiotic systems-chemistry scenarios. For example, a recent work starting from reduction of CO₂ leading to aldehydes and then corresponding α-amino-nitriles—the precursors of α-amino acids—is an example of the Strecker reaction in a systems-chemistry context. Our observations reported here start from the α-ketoacids which also have been shown recently to be produced from the CO₂ reduction process. And we have shown that starting from acetaldehyde, the same Bucherer–Bergs reaction pathway also leads to...
5-methylhydantoin, the precursor of alanine. Thus, utilizing the same starting molecules and reactants there can be diverse (Strecker and Bucherer–Bergs) pathways that can generate biomolecules, however, with only some starting molecules and reactions harbouring the potential to transition to extant metabolic pathways—such as the natural emergence of the connection between amino acid and nucleobase synthesis as exemplified by the formation of N-carbamoyl aspartate and its conversion to DHO (Fig. 3c).

The abiotic conversion of α-ketoacids to amino acids using the same type of cyanide and ammonia sources as for the Strecker reaction potentiates the prospect of using α-ketoacids—which are central to many biological pathways—in a prebiotic scenario. If the prebiotic availability of ketoacids on early Earth can be substantiated, then this overlap can enable a more seamless transition from primordial cyanide/ammonia-based chemistry to the next step in the presumed evolutionary pathway of using the amino acids themselves as the nitrogen source for transamination of reactions that can transition to protobiological systems. For example, the hydantoin produced from the Bucherer–Bergs reaction of glyoxylate reacts further with glyoxylate to produce not only orotate but also pyruvate. Pyruvate can then react with glyoxylate to give rise to the α-keto analogue of the rTCA pathway, which itself produces α-ketoglutarate. These two α-ketoacids, pyruvate and α-ketoglutarate, can undergo the Bucherer–Bergs reaction to yield the corresponding α-amino acids, alanine and glutamate. The formation of α-amino acids can enable a scenario of non-enzymatic transamination using the starting α-ketoacids, the efficiency of which may depend on the presence of organocatalysts such as peptides. In this context, the demonstration that DAP, used for decarboxylative reductive aminations reported in this work, is also able to synthesize short peptides starting from the amino acids suggests a model scenario where peptides could naturally emerge to function as organocatalysts to enable the switch to amino acids as the nitrogen and hydrogen source via transamination of α-ketoacids (Fig. 1b), thus replacing cyanide and ammonia in the Bucherer–Bergs reaction (Fig. 1d).

Since the transamination of α-amino acids would result in regeneration of α-ketoacids, this sets up a feedback mechanism (Fig. 1b)—making it self-sustaining and weaning the system away from the external cyanide and ammonia sources. Such a process seems to have been exploited by biology, as evidenced by how central and universal this reversible transamination process is in extant biochemistry. It is of interest to note that biology still utilizes the mechanisms that could lead to a chemical evolutionary network of reactions that can transition to protobiological systems. For example, the hydantoin produced from the Bucherer–Bergs reaction of glyoxylate reacts further with glyoxylate to produce not only orotate but also pyruvate. Pyruvate can then react with glyoxylate to give rise to the α-keto analogue of the rTCA pathway, which itself produces α-ketoglutarate. These two α-ketoacids, pyruvate and α-ketoglutarate, can undergo the Bucherer–Bergs reaction to yield the corresponding α-amino acids, alanine and glutamate. The formation of α-amino acids can enable a scenario of non-enzymatic transamination using the starting α-ketoacids, the efficiency of which may depend on the presence of organocatalysts such as peptides. In this context, the demonstration that DAP, used for decarboxylative reductive aminations reported in this work, is also able to synthesize short peptides starting from the amino acids suggests a model scenario where peptides could naturally emerge to function as organocatalysts to enable the switch to amino acids as the nitrogen and hydrogen source via transamination of α-ketoacids (Fig. 1b), thus replacing cyanide and ammonia in the Bucherer–Bergs reaction (Fig. 1d).

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Fig. 5 | The emergence of the various pathways with incremental systems-chemistry complexity. The reaction of glyoxylate with malonate and cyanide (blue arrow pathway) affords a simplified reductive glyoxylate pathway19 devoid of higher-order α-ketoacids, while the reaction of glyoxylate with pyruvate produces the α-keto analogues of the rTCA pathway17 with α-ketoglutarate as a central intermediate (black arrow pathway). Introduction of nitrogenous compounds (amines/ammonia, ‘NH₃’) to this α-keto analogue pathway enables higher conversion to α-ketoglutarate, while the reaction of carboxy malate with urea leads to aspartate derivatives and the pyrimidine precursor, DHO (green arrow pathway). In the presence of cyanide and ammonia sources, the α-ketoacids begin to produce the respective α-amino acids and also the pyrimidine nucleobase, orotic acid (red arrow pathway). The brown and red boxes indicate the new reactions reported in this work mediated by amines/ammonia (‘NH₃’) and cyanide + (‘NH₃’), respectively. The pink arrows represent previously reported transformations. di-DHKG, di deoxy hydroxy ketoglutarate.
reductive amination pathway starting with α-ketoglutarate and ammonia (catalysed by glutamate dehydrogenase with NADPH acting as the reductant) to synthesize glutamate. And glutamate serves as the starting point for synthesizing all other α-amino acids by transamination.

In this context the release of CO₂ at various stages of the reaction pathway and the possibility for the CO₂ to come back to participate in the reaction cycle (Fig. 2), although interesting, may not be impactful in a prebiotic context. We did demonstrate that in a degassed aqueous solution the release of CO₂ from the α-ketoacid pyruvate, by heating, was crucial in initiating the reaction by capture of CO₂ by the aminonitrile intermediate 4 enabling the hydrolysis of the cyanide to form the 5-methylhydantoin, thus emphasising the need for the presence of CO₂. Moreover, comparing the efficiency of product (5-methylhydantoin, N-carbamoyl alanine and alanine) formation under various conditions (room temperature versus 50 °C versus 80 °C in degassed versus undegassed versus 1 equiv. HCO₃⁻ added 0.5 M phosphate buffer; Supplementary Figs. 20–23) showed the beneficial role of increasing amounts of CO₂, implicating the CO₂ feedback shown in Fig. 2. However, given the abundance of CO₂ on the early Earth, which would permeate any given aqueous environment (as bicarbonate at practically plausible pH ranges7,8), it is doubtful that the CO₂ released within the reaction pathways—especially from the hydrolysis of hydantoin or from the CO₂ released from the putative intermediate 5a—would have a meaningful impact when compared to the magnitude of CO₂ available from the atmosphere9. For example, we observed that the presence of CO₂ atmosphere in the reaction flask is enough to form the hydantoin in over 60% yield (Supplementary Figs. 24 and 25). More importantly, in order for the hydantoin to release the CO₂, relatively high temperatures (for example, 80 °C) are needed, conditions under which the α-ketoacids or intermediates 5a also decarboxylate to release CO₂ (long before the formation of hydantoin). Thus, the CO₂ released at various points in the reaction pathway will certainly add to the bicarbonate that is already present, although the amount of CO₂ available from the early Earth environment will exceed the CO₂ produced from the reaction pathway.

Further contemplating the potential for the chemistries to transition to extant metabolic pathways, hydantoins 14 and 20 are the only intermediates capable of transiting to DHO and orotate, respectively, which is indicative of the natural emergence and the potential connection between such protometabolic pathways and pyrimidine nucleobases—coinciding with the synthesis of ornitc acid from aspartate (via DHO) in extant biology. And such transformations40, which appear closer to biological pathways when compared to the prebiotic generation of ornitc acid starting only from cyanide41,42, hint at the potential for the appearance of inherent forerunners of biological pathways from prebiotic chemistry. Thus, the apparent divide between heterotrophic and autotrophic scenarios connecting prebiotic chemistry and biology need not be incompatible, at least in some cases, so as to invoke a drastic and discontinuous switch from one chemistry to the another—leading to a better understanding of where such transitions are feasible and, more importantly, are implausible43,44,45,46.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41557-022-00999-w.

Received: 23 September 2021; Accepted: 14 June 2022; Published online: 28 July 2022

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Methods

General experimental. Reagents and solvents were purchased from commercial sources: Sigma-Aldrich, VWR International, Fischer Scientific, Acros, Oakwood, TCI Chemicals, Alfa Aesar, Spectrum and Combi Blocks, and were used without further purification. 1-14C-labelled glycine (99%), contains glycine acid (3%) and oxalic acid (1.5 %); 2-14C-labelled sodium pyruvate (99%), 1-13C-labelled sodium pyruvate (99%), 2-13C-labelled sodium pyruvate (99%), 13C-labelled NaCN and 13C-labelled NaHCO3 were obtained from Cambridge Isotope Laboratories. 15N-labelled NH3 solution (95% in H2O) was purchased from Sigma-Aldrich. [2-15N]-Diamidophosphate (DAP) was prepared according to the reported procedure. The ultrapure water used throughout the experiments was obtained from a Milli-Q laboratory water purifier. Degased solutions were used for all of the experiments unless otherwise stated. The ‘degassed solutions’ were prepared by purging with argon gas into the ‘deionized water or buffer’ for 10 min and then subjecting them to house-vacuum for 10 min. This purging-and-vacuuming process was repeated three times. pH was measured using a Accumet Research AR25 pH meter. NMR spectra were recorded at 298 K on a Bruker DRX600 or AV-600 (600 MHz for 1H and 150 MHz for 13C). 31P NMR (H-decoupled) spectra were acquired using a Bruker AV-400 (162 MHz). 15N NMR spectra were acquired using a Bruker AV-500. Mass spectra were recorded with an Agilent ESI-TOF or ThermoElectron Finnigan LTQ ion trap mass spectrometer. Absolute yields of products in the reaction mixture were calculated by 1H NMR analysis by NMR to monitor the progress of the reaction after the first wet–dry cycle (Supplementary Figs. 68–69). Mass spectrometry (total 48 h). After that, 2.0 ml of Milli-Q H2O was added to the dried reaction mixture (a 100 µl aliquot of the reaction mixture was taken at this time and analysed by NMR to monitor the progress of the reaction after the first wet–dry cycle), and the reaction mixture was left uncapped for the second wet–dry cycle. This wet–dry process was repeated for a total of four wet–dry cycles (Supplementary Figs. 88–90).

Data availability

The authors declare that all data supporting the findings of this study are available within the paper and its Supplementary Information.

Acknowledgements

This work was jointly supported by NSF and the NASA Astrobiology Program under the NSF Center for Chemical Evolution, CHE: 1504217, NASA Exobiology grant, 80NSSC18K1300 (to R.K.) and a grant from the Simons Foundation, 327124FY19 (to R.K.). We thank L. Leman and J. Peretò for feedback on the manuscript.

Author contributions

R.K. proposed the project. G.S. and R.K. designed and supervised research. S.P. and M.Y. designed and performed the experiments and collected the data. S.P., M.Y., G.S. and R.K. analysed data. R.K. wrote the manuscript with feedback and edits from S.P., M.Y. and G.S.

Competing interests

The authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41557-022-00999-w.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41557-022-00999-w.

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Peer review information Nature Chemistry thanks George Cody and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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Extended Data Fig. 1 | Non-interference from α-amino acids in the Bucherer-Bergs reaction. The α-amino acids formed as products have the potential to compete and react with the starting α-ketoacids under the Bucherer-Bergs reaction conditions. Starting from pyruvate and cyanide, in the presence of glycine, only the adduct 4a formed (top pathway), and the substituted hydantoin 5f is not observed (Supplementary Figs. 56–57). When ammonia is present, the reaction is channelled towards the formation of 5-methylhydantoin 6 (bottom pathway, Supplementary Figs. 60–61). This is because while the aminonitrile adduct 4 is able to form the isocyanate intermediate 5c, the amino acid–nitrile adduct 4a cannot form the corresponding isocyanate intermediate leading to hydantoin 5f since the obligate intermediate 5e lacks the hydrogen atom necessary for the ring opening reaction (when compared to 5b).