PREBIOTIC CHEMISTRY

From soup to peptides

Proteins are biosynthesized from α-amino acids using hefty biological machinery, but the origin of this process on the early Earth is unclear. Now, a bottom-up approach for forming peptides, taking place under mild, prebiotically-plausible conditions, has been developed. This strategy uses α-aminonitrile precursors, bypassing α-amino acids entirely.

Robert Pascal and Irene A. Chen

In 1953, the Miller–Urey experiment demonstrated the formation of α-amino acids in a mixture of water and gases under simulated lightning[1], kicking off the field of prebiotic chemistry. The primary role of α-amino acids is as subunits of proteins, and therefore a prebiotically plausible method to convert monomers to peptides has been sought over the intervening decades. Chemical clues found in the Miller–Urey experiment implicated the well-known Strecker reaction — the formation of energy-rich α-aminonitriles from aldehyde, ammonia and cyanide, which can be subsequently hydrolysed to amino acids[2]. Direct polymerization of α-aminonitriles has long been an attractive hypothesis for the origin of peptides[3], given the seemingly roundabout alternative of hydrolysis to amino acids, followed by reactivation for polymerization. However, despite several attempts by different researchers (including one of us[4]), experimental conversion of α-aminonitriles to peptides was not found, and attention shifted toward activation of amino acids using *N*-carboxyanhydrides (NCAs) and related compounds. Yet NCA polymerization also presents significant challenges for prebiotic chemistry, such as controlling side reactions, and the problem of peptide synthesis remained without a conclusive solution.

Now, in a recent report in *Nature*, Matthew Powner and co-workers[5] reboot the α-aminonitrile hypothesis to produce quantitative yields of peptides in a gentle aqueous procedure. Remarkably, the reaction is successful with all 20 proteinogenic side chains without protecting groups. A peptide chain is initiated by quantitative acylation of an α-aminonitrile (Fig. 1a), which activates the nitrile for an iterative, one-pot, three-step ligation cycle (Fig. 1b, c): first, thiolysis of the nitrile to produce the corresponding thioamide; second, hydrolysis of the thioamide to the thioacid; and third, oxidation of the thioacid and reaction with an incoming α-aminonitrile monomer, leaving a nitrile available to undergo another ligation cycle. A conceptual difference between this scheme and NCA chemistry is the fact that the elongating peptides, not the monomers, are electrophilically activated. Important features of each of these steps contribute to the high efficiency of the overall process.

In the first step of the cycle, a simple reagent (H_2S) converts the α-amidonitrile to the α-amidothioamide. It is worth noting that the initial acylation is crucial, as the corresponding reaction involving non-acylated aminonitriles is much less efficient. Presumably, the electron-withdrawing character of the acetamido group strongly facilitates the nucleophilic reaction of sulfide with the nitrile.

A technical breakthrough occurs in the second step of the cycle, hydrolysis of the thioamide. In stark contrast to α-aminothioamides, the hydrolysis of which

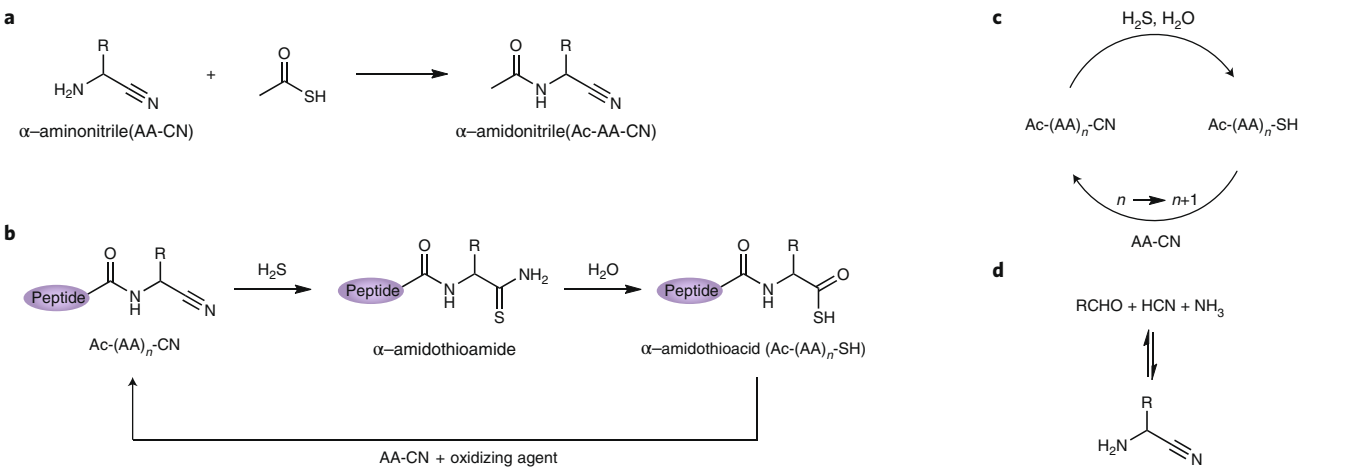

Fig. 1 | Gentle, prebiotically plausible peptide ligation using α-aminonitriles. a, Acetylation of an α-aminonitrile by thioacetic acid creates an activated monomer that initiates a peptide chain. **b**, The three-step, iterative ligation cycle of α-acetamidonitriles via the thioamide and thioacid with addition of α-aminonitriles, demonstrated by Powner and colleagues[5]. **c**, Simplified depiction of the overall cycle emphasizing elongation (*n* to *n*+1). **d**, Racemization of α-aminonitriles at equilibrium with their precursors raises prospects for chiral enrichment.

yields a mixture of products, hydrolysis of the α-acetamidothioamide selectively produces a *C*-terminal thioacid. This surprising facility, which highlights the importance of the *N*-terminal modification, realizes a decades-old suggestion of Liu and Orgel[6]. The thioacid can subsequently be converted into an acyl transfer agent by oxidation with ferricyanide or electrophilic activation.

Last but not least, the third step, elongation through nucleophilic attack by α-aminonitriles, exploits the low pK_a (about 5.3) of protonated aminonitriles to achieve selectivity. At an intermediate pH (~5–9), the aminonitriles outcompete stronger (more basic) amine nucleophiles that might otherwise terminate the polymer. The team further show that a single elongation step could add more than one monomer if the nucleophile itself were a peptidonitrile. These features enable the creation of oligomers of significant length.

What is behind the success of this peptide-forming chemistry using α-aminonitriles? The nitrile group combines thermodynamic instability and kinetic stability, creating a system held 'far from equilibrium by kinetic barriers' that might undergird the self-organization of life[7]. However, the sluggish kinetics of α-aminonitrile reactions (for example, hydrolysis) also constitutes an obstacle, such that use of efficient reagents (H_2S) or catalysts is paramount. High yields along each step of this pathway combined to produce excellent overall yield, and wise avoidance of the amino acid, a thermodynamic trap, meant that relatively gentle conditions were adequate for activation.

Any study of prebiotic chemistry must contend with questions of yield and prebiotic plausibility. These reactions proceed with impressively high yield (nearly quantitative in most cases) and high selectivity. In terms of prebiotic plausibility, the reactants (α-aminonitriles, H_2S, ferricyanide) would be considered reasonable by most workers in the field. A common issue is the need for interventions by the chemist, who purifies intermediates and adds reagents at the proper times. In this case, the ligation cycle occurred efficiently in a one-pot reaction without purification of intermediates. However, H_2S and ferricyanide must be added sequentially during the cycle to avoid mutual reaction. Understanding how such dynamic chemical and energetic inputs might drive prebiotic chemistry remains an important area of consideration for the field as a whole.

This highly efficient peptide chemistry raises the possibility of addressing several interesting features. Chirality, and specifically the emergence of a homochiral system, is a central puzzle for the origin of peptides. Electrophilic activation of the *C*-terminus typically results in epimerization of the α-carbon through chirally unstable intermediates. In contrast, Powner and colleagues show that stereochemistry of the electrophile is conserved during the gentle ligation cycle. Thus, the chirality of the peptides is inherited from the α-aminonitrile substrates. A tantalizing question is whether the chirality of the electrophile favours a specific chirality of the nucleophile. At moderate pH values, α-aminonitriles exist at equilibrium with their precursors (Fig. 1d), a process that causes racemization. If peptide elongation is stereo-selective and slow, relative to the α-aminonitrile equilibrium, racemization of the unreacted α-aminonitrile pool could potentially lead to runaway enantiomeric excess[8]. Mechanistic questions are also of interest, such as why hydrolysis of the α-amidothioamide to the corresponding thioacid is so efficient. General features of this pathway, including surprising tolerance to side chains, one-pot aqueous conditions and high yields may motivate synthetic peptide chemists to explore these reactions further.

An important lesson from this and other seminal work is that, although the fundamental strategies may be preserved[9], details of biochemistry may mislead us about prebiotic chemistry. Much like the metaphor of 'my grandfather's axe'[10], the ancient origin of peptides may not resemble the modern biochemistry of protein translation in seemingly major ways. Amino acids, now used as substrates in protein translation, may be a red herring, much like the biosynthesis of ribonucleotides through ribose[11]. Nevertheless, bottom-up pathways must eventually reconnect to their biological descendants. Now, given the starting point described by Powner and co-workers, discovering such connections will surely create a fascinating scientific journey toward synthesizing life. ❐

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