Glycine to oligoglycine via sequential trimetaphosphate activation steps in drying environments

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Abstract

Polyphosphate-mediated peptide bond formation is central to protein synthesis in modern organisms, but a simpler form of activation likely preceded the emergence of proteins and RNA. One suggested scenario involves trimetaphosphate (TP), an inorganic phosphate that promotes peptide condensation. Peptide bond formation can also be promoted by high pH and drying, but the interaction of these factors with TP has yet to be characterized kinetically. We studied the formation of glycine oligomers formed under initially alkaline conditions in the presence of TP during the process of drying. Oligopeptide products sampled over 24 hours were analyzed by functionalization and high-performance liquid chromatography with ultraviolet absorption (UV-HPLC). As they dried, two different pH-dependent mechanisms dominated during different stages of the process. The first mechanism occurs in alkaline solutions and activates monomer amino acids to form dimers while reducing the pH. Our results then become consistent with a second mechanism that proceeds at neutral pH and consumes dimers to form longer products. The possibility that a series of reactions might occur where the first reaction changes the environment to favor the second, and so on, may have broader implications for prebiotic polymerization. Studying how the environment changes during time-varying conditions, like drying, could help us understand how organic polymers formed during the origin of life.

Introduction

Early in the origin of life, short peptides probably performed essential functions analogous to the roles filled by proteins in modern life (Frenkel-Pinter et al. 2020). The wide range of possible functions that peptides can adopt, including catalysis, secondary structure organization, and template-guided polymerization, suggests that they played a significant role in the emergence and development of life (Ruiz-Mirazo et al. 2014). Although the chemistry that led to the origin of life remains a topic of much speculation, amino acids are relatively easy to form through prebiotic routes (Frenkel-Pinter et al. 2020). In contrast, peptide bond formation does not proceed favorably in water (Danger et al. 2012), which poses a key question of how amino acids polymerized into peptides, and how those peptides avoided hydrolysis prior to translation or regulated catalysis.

Various methods for forming peptide bonds in possible prebiotic conditions have been proposed, as reviewed previously (Frenkel-Pinter et al. 2020; Ruiz-Mirazo et al. 2014; Danger et al. 2012). Virtually all methods work through one or both of two mechanisms: creating a dehydrating environment and activating functional groups. Some approaches use the solvation effects of salts or minerals to create a dehydrating environment in the presence of bulk water (Lahav et al. 1978; Rode 1999), whereas others use drying to physically remove water (Ross and Deamer 2016; Campbell et al. 2019). Drying is easily justified as a prebiotic process that could occur naturally due to tidal cycles, day/night cycles, or weather variation. Nonetheless, simply drying amino acids in water produces negligible peptide yields. Rather specific environmental conditions are needed for drying to promote effective polymerization (Lahav et al. 1978; Rodriguez-Garcia et al. 2015; Kitadai and Maruyama 2018; Rode 1999; Napier and Yin 2006). One benefit of drying is that the concentration of non-volatile reactants significantly increases as the solvent

evaporates, which can force dilute species to interact with each other and increases the rate of some reactions (Ross and Deamer 2016; Mamajanov et al. 2014). In addition to affecting the yield, this can also allow longer or more diverse peptides to form.

Another way to promote prebiotic peptide bond formation is to add an 'activating agent' – a material that interacts with amino acids or peptides to decrease the energy barrier for the condensation reaction to occur (Danger et al. 2012). One such material is trisodium trimetaphosphate (TP), a cyclic triphosphate that is known to promote peptide bond formation (Rabinowitz et al. 1969; Sibilska et al. 2017, 2018; Ying et al. 2018). Polyphosphates are key activators in many modern biological processes, including protein synthesis, which makes them interesting candidates for activating molecules in the origins of life (Lohrmann and Orgel 1973; Pasek et al. 2017). TP has relatively high solubility in water compared to other forms of phosphate (Yamagata et al. 1991), and the ring strain on the O-P-O bonds causes it to be especially reactive (Britvin et al. 2021). TP is considered prebiotically available because a pathway through which it could be formed by volcanic reactions has been proposed (Yamagata et al. 1991), and tetrametaphosphate, a closely related cyclophosphate, has been found in nature (Britvin et al. 2021). TP has been used extensively in studies of prebiotic polymerization due to the relatively high yields of peptides that it supports (Hill and Orgel 2002; Yamagata and Inomata 1997; Sibilska-Kaminski and Yin 2021). Mechanisms for TP-activated peptide bond formation have been published by several groups (Sibilska et al. 2017; Chung et al. 1971; Yamanaka et al. 1988; Inoue et al. 1993).

Although reaction mechanisms have been explored before, an account of how they act during the drying process has not yet been published. To understand how TP-activated peptide bond formation proceeds in a drying environment, we tracked the polymerization of glycine through a 24-hour drying period. We observed the samples going through two distinct phases, each consistent with a different reaction mechanism. We suggest that the shift from one mechanism to another is based on pH change, as protons are produced by the early polymerization steps. Improving our understanding of how dynamic reaction conditions such as drying produce complex molecules can give us insight into how the precursors to biological polymers may have emerged on the early Earth.

Materials & Methods

Materials

All chemicals were of analytical grade purity and used without further purification. Materials were obtained from suppliers as follows: glycine, diglycine, triglycine, pentaglycine, trisodium trimetaphosphate, and trifloroacetic acid from Sigma-Aldrich, tetraglycine from Bachem, sodium hydroxide from Fisher Scientific, acetone from Alfa Aesar, 9-fluorenylmethoxycarbonyl chloride (FMOC) from Creosalus, acetonitrile from VWR Chemicals, and sodium tetraborate anhydrous from Acros Organics. Reactions were carried out in 1.5 mL low-retention Eppendorf tubes.

Experiment Setup

Unless otherwise specified, all samples contained 0.1 M glycine, 0.1 M TP, and 0.15 M NaOH. All samples had an initial volume of 1 mL and were placed, with their caps open, in a heat block preheated to 90^{o}C. The ratio of TP to amino acids, starting pH (10.5–11), and heating temperature were determined based on what conditions were most favorable to peptide bond formation in Sibilska et al. (2018). Data was collected using at least three independent experimental replicates at each time point.

Prior to analysis, samples heated with open caps were rehydrated with milliQ water to replace what was lost during evaporation, bringing them back to their original volume (1 mL). To determine the amount of water to replace in samples, six samples were weighed to determine the mass of the 1.5 mL tube plus the sample contents. These weights only varied by $\pm$0.01 g. After heating, each sample was individually weighed, and its weight was subtracted from the average initial mass to determine how much water was needed to reach the original volume, assuming a water density of 1 g/mL. Samples were vortexed (Pulsing Vortex Mixer, Fischer Scientific) until there were no longer any visible solids remaining in the sample, usually about 60–90 seconds on maximum speed for fully dried samples. The pH of the samples was measured using an Apera Instruments PH8500-MS Portable pH microelectrode. pH measurements were performed after the sample was replenished and vortexed to ensure there was a large enough sample volume to measure the pH.

Samples were analyzed using FMOC derivatization and UV-HPLC. FMOC was used to increase the retention time and signal strength of peptide analytes. For the FMOC derivatization procedure, 25 µL of sample was diluted with 75 µL milliQ water to put the large monomer peaks in a quantifiable range. Each sample was then mixed with 100 µL 0.1 M sodium tetraborate buffer for pH control. Finally, 800 µL 3.125 mM FMOC dissolved in acetone was added to each sample. For a sample of 0.1 M amino acid, this results in an equal concentration of FMOC and amino acid, and a slight excess of FMOC in any samples where peptide bond formation had occurred. We were able to recover near-linear calibration curves for all species with this approach (Fig. S1a-e), which were used to estimate concentrations from the integrated absorbance values of the HPLC peaks.

Many FMOC procedures suggest performing an extraction procedure to remove excess FMOC-OH (Jámbor and Molnár-Perl 2009), however, we found this was unnecessary as the noise peaks associated with FMOC in the UV-HPLC chromatogram were sharp and did not interfere with any of the peaks associated with our measured species. Samples were allowed to react with FMOC for at least one minute at room temperature, though most reacted longer while queued in the autosampler of the HPLC.

Samples were analyzed with a Shimadzu Nexera HPLC with a C-18 column (Phenomenex Aeris XB-C18, 150 mm x 4.6 mm, 3.6 µL). Products were measured at 254 nm. UV-HPLC analysis was performed using Solvent A: milliQ water with 0.01% v/v trifluoroacetic acid (TFA) and Solvent B: acetonitrile with 0.01% v/v TFA. The following gradient was used: 0–4 min, 30% B, 4–12 min, 30–100% B, 14–15 min, 100 – 30% B, 15–17 min, 30% B. The solvent flow rate was 1 mL/min. Peak integration was performed using LabSolutions with the ‘Drift’ parameter set to 10000.

Results

Amino acid condensation is promoted by TP, alkaline conditions (presence of NaOH), and drying (Sibilska et al. 2018). To clarify their roles in activating peptide bond formation, we left out each condition – drying, TP, or NaOH – one by one and measured the resulting concentrations of glycine homopolymers over 24 hours (Fig. 1). As expected, the samples that were treated with TP, drying, and high initial pH had the highest peptide yields. The most significant differences were in the yields of trimer (G_3) and tetramer (G_4) glycine polymers – the samples including all three conditions had higher yields than the other treatments (Fig. 1c, d). In contrast, the dimer (GG) yield of the samples treated with all three conditions was matched by the dimer yield of the samples that contained TP and started at high pH, but were not allowed to dry out (Fig. 1b). The similarity of the diglycine yields from samples using TP and high pH, regardless of whether or not they were dried, is explained by the observation that the vast majority of diglycine formed within the first two hours of heating. At that point, most of the bulk water was still present even in the samples being dried, so any reactions taking place had to be able to proceed in water (Fig. 1a). A small amount of trimer formation also occurs in the absence of drying. Collectively, these results indicate that almost all dimer (and some trimer) formation in alkaline samples containing TP occurs through a relatively fast reaction which does not require dehydration to proceed.

After four hours of heating, the rate of formation of trimers and tetramers increased in samples that were drying (Fig. 1c, d). The simplest explanation for these increases follows from the decreasing volume of water and corresponding shift towards the condensation reaction per Le Chatelier's principle, plus increasing reactant concentrations. However, if trimer and tetramers were forming through the same mechanism as dimers, then the diglycine concentration should also rise due to drying, since there is still a large amount of monomer remaining in all conditions. Instead, the dimer concentration drops as the yields of trimer and tetramer rise, presumably due to conversion into longer polymers and some quantity of 2,5-diketopiperazine (DKP) (Table S1). These results suggest that in the samples being dried, trimers and tetramers were formed through a different mechanism than what formed dimers during the first two hours, and that the reactions that formed the longer peptides mostly proceeded after drying was nearly complete.

It is noteworthy that it also took about four hours for any notable peptide formation to occur in samples that were dried and contained TP but had no additional sodium hydroxide added, and therefore started with neutral pH conditions (pH 7) (Fig. 1c, d). When peptides eventually formed in these conditions, the dimer yield was low, but the yield of trimers relative to the amount of available dimer reactant was high. This suggests that the mechanism driving peptide bond formation in dry, neutral pH conditions favors trimer formation, which offered a possible explanation for the accelerated trimer formation after four hours in the samples treated with TP, drying, and NaOH.

We suspected pH might be changing over the course of the experiment. In the samples treated with TP, drying, and high pH, we found that the pH dropped dramatically during the first hour then continued to drop roughly linearly for another four hours. Therefore, although the pH is initially alkaline, even samples

treated with NaOH have a relatively neutral pH for most of the experiment (Fig. 2). At the time when samples including all three conditions begin to promote trimer and tetramer formation, at about four hours, they have a similar pH to the samples that started at neutral conditions. This may suggest that the initial presence or absence of NaOH does not significantly affect the rate of formation of trimers and tetramers for the last 20 hours of the experiment. Instead, the effect of NaOH in promoting total trimer and tetramer formation is likely due to having a higher concentration of diglycine available at four hours, when drying-induced condensation begins.

Effect of Solid Formation

The highest rates of trimer and tetramer formation coincide with the time when solids begin to form, but peptide formation largely stops once the samples are fully dried. This brief period of increased peptide formation could result from samples having very high reactant concentrations while still having enough solvent to avoid restricting the molecules' mobility, a limitation that might exist in the fully solid state. We examined the relationship between the solute mass fraction, the formation of solids, and the rates of peptide formation to better understand the effects of drying.

The first consistent appearance of solids occurs at the same time as the rates of longer peptide formation begin to increase. The solids we observed were a translucent but clearly visible separate phase that did not immediately dissolve when the samples were filled back to their original volume, but would eventually dissolve when the samples were subjected to vortex mixing. Samples heated for 4 hours consistently formed solids at the bottom of the tube, despite about 20% of the original water still being present. The highest rates of trimer and tetramer formation occur just afterwards, after 5 and 6 hours of heating and corresponding to solute mass fractions of 0.4 and 0.8, respectively (Fig. 3). The solute mass fraction changed rapidly during this time because the sample was mostly dry, but it appeared that longer peptides formed the fastest when the solute mass fraction was neither particularly high nor particularly low. Further drying beyond 6 hours, the last point where there was still a measurable amount of solvent remaining, stops peptide bond formation almost entirely. After 8 hours, the samples were considered fully dried, and the rate of peptide bond was negligible in all the conditions tested. This suggests that further reactions are inhibited while the sample is completely dry.

We conclude that although we did not observe significant peptide bond formation after establishing the dry solid phase, the process of approaching the dry solid phase still has a significant role in promoting trimer and tetramer formation. The different ratios of dimers to trimers and tetramers forming at different times in the experiment appears to be driven by the pH shift, but completely dehydrating the sample is required to drive forward the reactions that form longer peptides.

Discussion

Mechanisms

A key result of our study was the identification of two distinct phases of TP-activated peptide formation that correlated with changes in the pH and hydration conditions of the samples. The two phases we observed correlate well with two different mechanisms of TP-activated peptide formation, both of which were previously described by Yamanaka et al. (1988) (Fig. 4). The first mechanism proceeds through activation of the N-terminus, the second proceeds through activation of the O-terminus.

Mechanism 1 likely accounts for the rapid increase in diglycine observed during the first hour in samples containing TP at alkaline conditions. First proposed by Chung et al. (1971), Mechanism 1 is generally accepted for TP-activated peptide elongation in alkaline conditions (Yamanaka et al. 1988; Inoue et al. 1993). This mechanism creates a phosphoryl-carboxyl mixed anhydride, a five-membered ring intermediate. The high reactivity of the mixed anhydride allows this reaction to occur in solution without dehydration. However, this mechanism releases hydronium ions but requires alkaline conditions to proceed, creating a negative feedback loop – as the reaction continues, it increasingly hinders itself.

Mechanism 1 primarily consumes monomers to produce dimers. The mixed anhydride intermediate can only form from amino acids, so at least one reactant must be a monomer. The nucleophile attacking the mixed anhydride can be a longer peptide instead of an amino acid, so it is possible for this mechanism to form peptides longer than dimers, but the excess of monomer here favors dimer formation. The formation of longer products via this mechanism is further limited by the stability of N-phosphorylated diglycine in alkaline conditions (Yamanaka et al. 1988). In N-phosphorylated diglycine, the amine group is blocked by phosphate and unable to act as a nucleophile. If diglycine reacts with TP to become N-phosphorylated instead of attacking a mixed anhydride, then it is essentially excluded from further extension while the sample is at high pH. N-phosphorylated diglycine hydrolyzes back into diglycine at neutral conditions, allowing it to potentially react again (Yamanaka et al. 1988). However, Mechanism 1 does not significantly proceed at a neutral pH because amino acids have protonated amine groups and are unable to perform the nucleophilic attack on TP.

Mechanism 2, originally proposed by Yamanaka et al. (1988), proceeds in neutral conditions through an O-phosphorylated peptide that is attacked by the deprotonated amine of another peptide (Fig. 4b). This reaction mechanism favors the formation of trimer and tetramer in neutral pH conditions. It requires one nucleophilic attack by an amino acid or peptide with a deprotonated amine group, which at neutral pH is rare. However, it is much more common among peptides than glycine monomers due to significant differences between the basic dissociation constants for the amine groups (pK_b) of glycine and oligoglycine. The pK_b of glycine is 9.60, while the pK_b values of diglycine and triglycine are 8.13 and 7.94, respectively (Yamanaka et al. 1988; Settimo et al. 2014). The pK_b values of diglycine and triglycine are low enough that these species will have non-negligible quantities of both protonated and unprotonated amine groups at pH 7, and the deprotonated species can act as nucleophiles. Glycine is much further below its pK_b at pH 7, so virtually no glycine will be able to act as a nucleophile. Therefore, species already containing a peptide bond are proportionately more likely to participate in Mechanism 2, resulting in increased trimer and tetramer formation.

Significance of Drying

Although they studied conditions that favored Mechanism 2, Yamanaka et al. (1988) only observed negligible yields of triglycine and no tetraglycine from reactions starting with monomer glycine because their samples were never dried. The change in pH and resulting shift in reaction mechanism explains why longer peptides form favorably later in the experiment, but the results clearly demonstrate the important role of dehydration. Samples that were permitted to dry into a solid had distinctly higher yields of tri- and tetraglycine than those that did not. Mechanism 2 proceeds only to a limited extent in bulk water, which is further supported by samples that started at neutral conditions yielding no detectable peptides until most of the bulk water had evaporated (Fig. 1).

Drying increases the rate of peptide condensation by removing water and increasing amino acid and peptide concentrations. However, reaction rates depend on mobility as well as concentration (Ross and Deamer 2016). Molecules in the solid phase have a limited ability to diffuse and rotate, which can slow or stop their reactivity. As samples dry to the solid phase, it would be reasonable to expect reaction rates to increase, then abruptly slow or completely stop due to lack of mobility. In practice, this is not always the case - in some proposed prebiotic reaction conditions, peptide bond formation occurs mostly in the solid state (Napier and Yin 2006; Campbell et al. 2019), and there is some evidence suggesting peptides form slowly after drying in TP-activated samples (Sibilska et al. 2017). However, for the experiments described in this paper, peptide bond formation in the solid phase was negligible.

Condensation into longer peptides likely proceeds best when the system has very low water activity, but has not dried completely. Low water activity shifts the equilibrium towards polymerization and allows longer polymers to form without being hydrolyzed. Once the longer polymers have formed, they do not immediately hydrolyze when rehydrated. Fluctuations between the solid and dissolved states were explored in Campbell et al. (2019) using deliquescent salts, and those systems were found to produce comparable yields of peptides even in the absence of activating agents. We believe our system temporarily reaches similar levels of water activity in the period between 4 and 8 hours, when Mechanism 2 dominates. Understanding these details may be useful for finding systems that produce larger peptides with more potential for complex behavior.

We should also acknowledge the possibility that other physical properties of the dry state may contribute to the increased reaction rates as the sample approaches the solid phase. For example, glycine polymers are known to aggregate into a variety of ordered structures when dried (Yanagawa et al. 1984), and it is possible that some structures align molecules in a manner that promotes peptide bond formation. Subtle mechanistic effects like this are not possible to distinguish with our current methods but we can observe a clear connection between drying and the formation of longer oligopeptides.

Environmental Conditions in Prebiotic Chemistry

An interesting feature in the trimetaphosphate system is that two different mechanisms of peptide bond formation occur at different environmental conditions, and the first mechanism directly contributes to

creating conditions favorable for the second mechanism. The fact that these reaction mechanisms have been known for many years and there has been limited appreciation for the link between them suggests that it may be worthwhile to pay greater attention to the effects of proposed prebiotic reactions on their environment, in addition to the effects of the environment on the reactions.

Although this study is limited in scope, the idea that dynamic reaction environments can increase yields and allow more complex molecules to form is well established (Ross and Deamer 2016; Damer and Deamer 2015; Varfolomeev and Lushchekina 2014; Walker et al. 2012). Finding a path to more complex peptides would be significant since the conditions tested here may be too limiting to create peptides with more complex interactions. Glycine is the most reactive amino acid, but the longest peptides identified in this experiment were only six amino acids long, with the hexamer being present in such low abundance that it was difficult to consistently measure. This is enough polymerization occurring within 24 hours for the system to be intriguing, especially since there is some evidence that peptides as short as dimers may have catalytic activities (Gorlero et al. 2009; Plankensteiner et al. 2005). However, the nature and length of peptides that may have contributed to the origin of life is still very poorly understood (Van der Gulik et al. 2009; Raggi et al. 2016), and the peptides we observed are still far shorter than what is generally used in engineered systems used to study auto-catalytic peptides (Yao et al. 1998; Rout et al. 2018).

Our experiments use drying, one of the simplest to implement and most common dynamic environmental conditions studied in prebiotic chemistry, to demonstrate mechanistically how such conditions can allow longer peptides to form. Experiments with more diverse reactants and longer sequences of environmental conditions, such a wet-dry cycling and reactant replenishment, may be required to obtain peptides with greater length and complexity. Nevertheless, it seems reasonable to suggest that there may be other combinations of environmental conditions and reaction mechanisms that overlap in ways which facilitate the formation of larger organic molecules and build up reaction networks that occur in series, where the environmental conditions are partially controlled by the organic reactions taking place. There are many parallels between such scenarios and the cycles or cascades of reactions that constitute modern biology. A few examples of how reactions could influence the surrounding environmental conditions include pH changes, the creation of various by-products and intermediates, temperature changes caused by endothermic and exothermic reactions, and phase separation owing to the accumulation of various intermediates. Understanding these relationships would be extremely useful for discovering how chemical systems could develop enough complexity coupled with enough specificity to take on life-like behaviors.

Conclusion

We investigated TP-activated glycine homopolymer formation in drying conditions and described the results in the context of the known mechanisms for this process. There are two mechanisms for TP-activated peptide formation, which are active in different pH and concentration conditions, and favor different peptide lengths. Alkaline samples of glycine and TP naturally proceed through both mechanisms in sequence as they dry. The first mechanism forms dimers and lowers the pH, which allows

the second mechanism to proceed as the sample dries. The second mechanism favors trimer and tetramer formation, further polymerizing the dimers formed during the first reaction. This particular sequence of reactions enables the formation of longer glycine polymers.

Production of longer peptides is significant because it indicates that the system can achieve a higher level of molecular complexity, which may have been useful in the development of early life. The observation that longer peptides can arise from a naturally occurring sequence of reactions suggests the possible importance of dynamic reaction conditions in developing complex molecules. Studying different prebiotic reactions, the environments they occur in, and the effect that they have on the surrounding environment may suggest routes through which longer biopolymers could have developed on the early Earth.

Declarations

Ethics Approval No approvals required.

Conflict of Interest The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Data Availability The data shown in this study is available from J. Y. upon reasonable request.

Authors' Contributions Both authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by HB. The first draft of the manuscript was written by HB and both authors contributed to subsequent versions of the manuscript.

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Figures

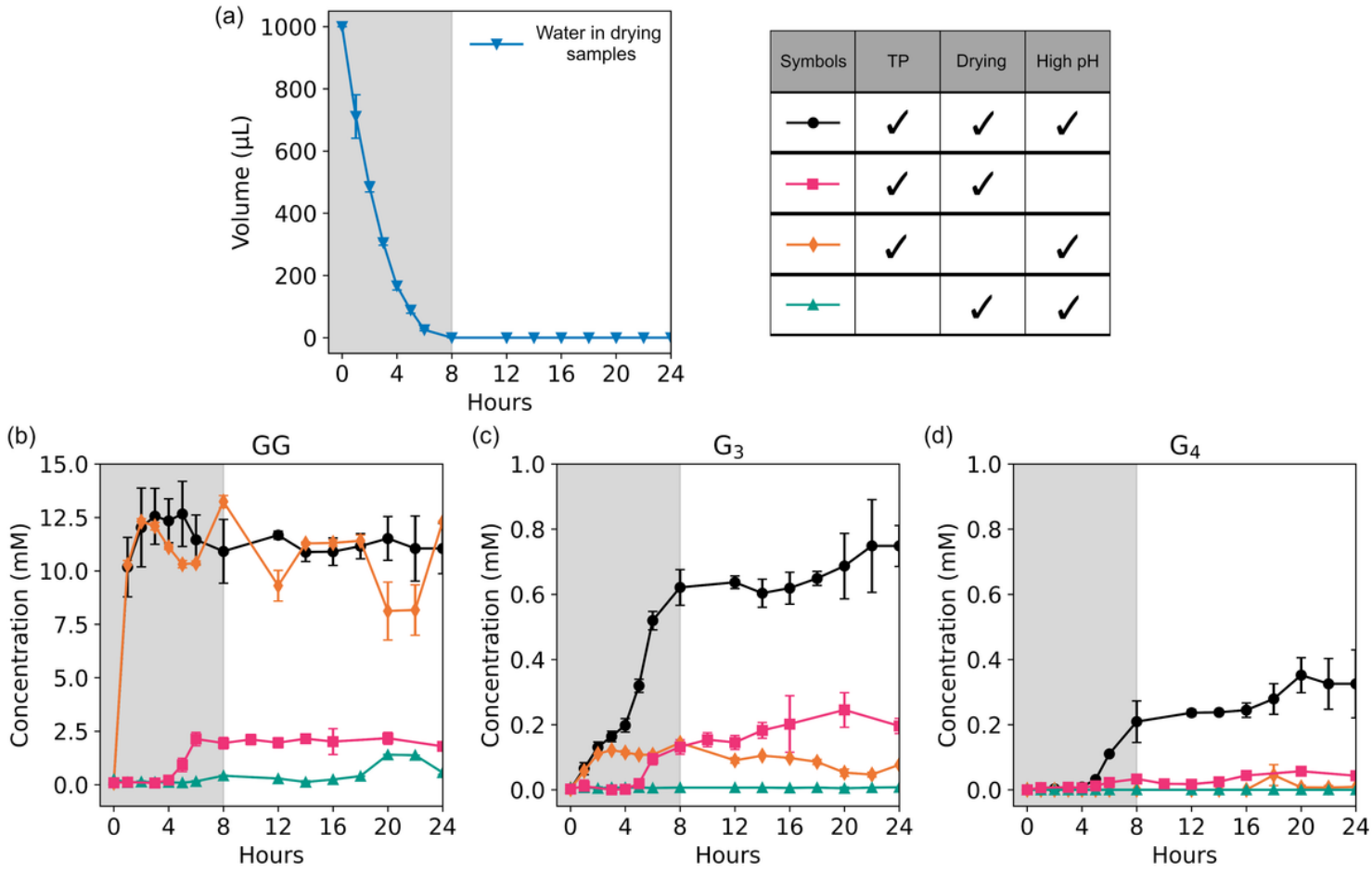

Figure 1

Di-, tri-, and tetraglycine yields depend on different combinations of three treatments known to promote peptide bond formation. (a) Volume remaining in drying samples. Samples that are not dried maintain a constant volume throughout the experiment. (b) Diglycine concentrations, (c) Triglycine concentrations, and (d) Tetraglycine concentrations. The shaded region from 0 to 8 hours highlights the relationship between sample drying and peptide formation. Error bars represent sample standard deviations calculated from independent experimental triplicates.

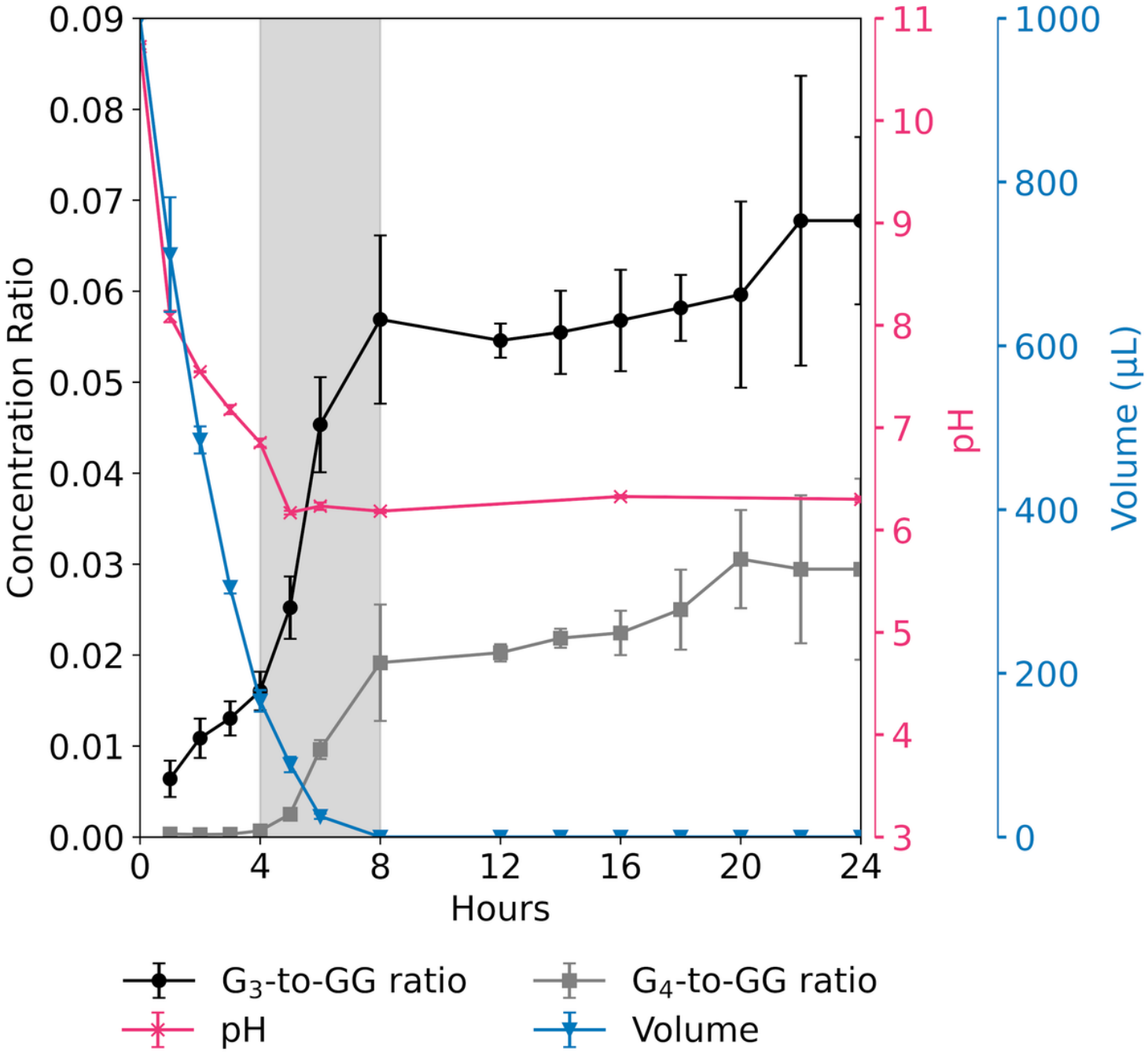

Figure 2

The relative concentrations of tri- and tetraglycine increase during drying at neutral pH. Relative concentrations are calculated by dividing by the diglycine concentration at each point. The remaining water volume and pH are shown by the right-hand y-axes. The shaded area highlights the period of rapid tri- and tetra-glycine formation that occurs after 4 hours. Results at 0 hours were excluded due to near-zero numbers producing high variability. Samples were treated with trimetaphosphate, drying, and started at alkaline pH. Error bars represent sample standard deviations calculated from independent experimental triplicates.

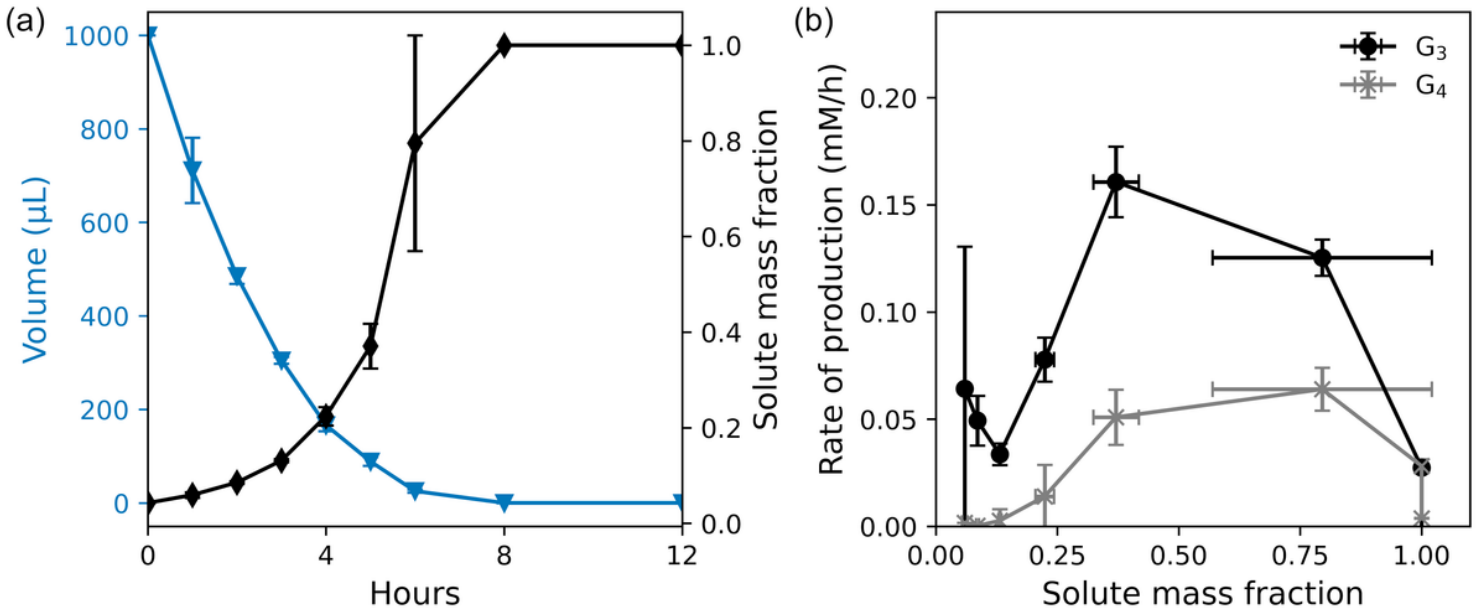

Figure 3

The highest rates of trimer and tetramer formation occur at intermediate solute mass fractions. (a) Volume of water remaining in samples and solute mass fraction over time. For simplicity, solute mass is assumed to be constant and equal to the theoretical mass based on concentrations and molar masses. (b) Rates of G_3 and G_4 formation. Rates were estimated using the three-point central difference formula. Error bars represent sample standard deviations calculated from independent experimental triplicates. Details on the calculation of the solute mass fraction and error propagation can be found in the Supplementary Information (Section S1).

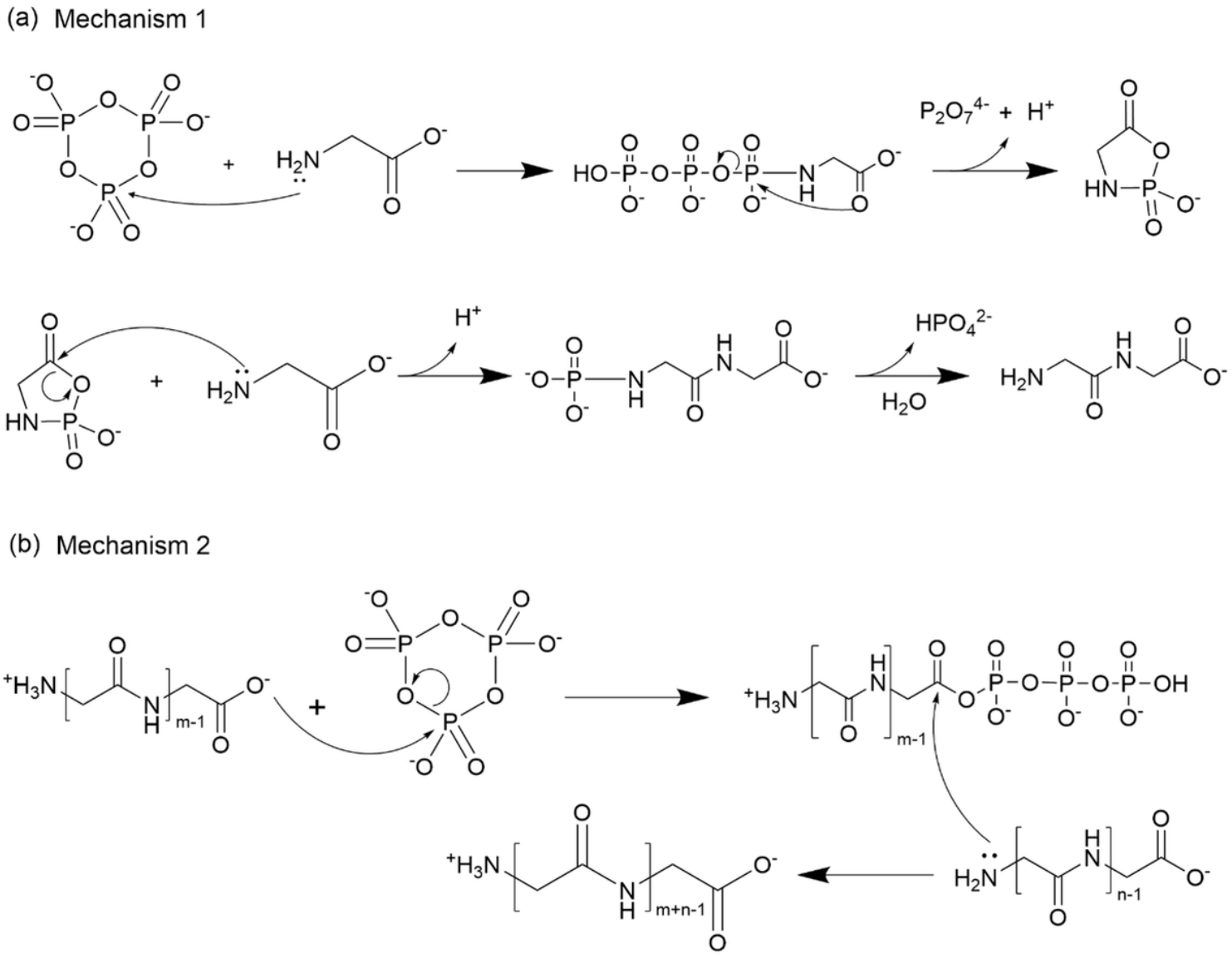

Figure 4

Mechanisms for TP-activated peptide bond formation. Adapted from Yamanaka et al. (1988). (a) Mechanism 1 – Dimer formation in alkaline conditions. (b) Mechanism 2 – Bond formation between peptides of arbitrary length at neutral pH.

Supplementary Files

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- 20220705Gly24SupplementaryInformationFinal.docx