

## Prebiotic Peptide (Amide) Bond Synthesis Accelerated by Glycerol and Bicarbonate under Neutral to Alkaline Dry-Down Conditions.

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**Introduction:** Past studies of prebiotic peptide bond synthesis have generally been carried out in the acidic to neutral pH range [1, 2]. Here we report a new process for peptide bond (amide) synthesis in the neutral to alkaline pH range that involves simple dry-down heating of amino acids in the presence of glycerol and bicarbonate. Glycerol was included in the reaction mixture as a solvent and to provide hydroxyl groups for possible formation of ester intermediates previously implicated in peptide bond synthesis under acidic to neutral conditions [1]. Bicarbonate was added to raise the reaction pH to 8-9.

**Results:** Our early studies showed that dry-down condensation at 90°C of  $\alpha$ -L-alanine in the presence of glycerol and bicarbonate gave high yields of dialanine and cyclic dialanine with a small amount of alanine trimer and tetramer. Both glycerol and bicarbonate were required for peptide bond synthesis. Apparently, the synthesis of long peptides by  $\alpha$ -alanine is blocked by efficient intramolecular aminolysis via a 6-membered ring to give the cyclic dimer. In contrast, new HPLC results indicate that oligomerization of the L-2-aminobutyric acid, also an  $\alpha$ -amino acid, yields primarily linear oligomers.

Condensation of the  $\beta$ -amino acids ( $\beta$ -alanine and isoserine) under the same conditions produced long linear peptides (9-mer & larger) indicating chain elongation was not blocked by cyclic dimer formation (Fig. 1). Unlike  $\alpha$ -L-alanine,  $\beta$ -alanine and isoserine also oligomerized at neutral pH without bicarbonate. Analysis by LC/MS, full spectrum MS, and MS/MS confirmed the synthesis of (a)  $\beta$ -alanine oligomers, and (b)  $\beta$ -alanine +1Da oligomers, a result indicating partial replacement of the terminal amino group of oligomers by an hydroxy group. Oligomerization occurred at 65°C at a much slower rate. Both  $\beta$ -alanine and isoserine are products of model prebiotic synthesis reactions. In addition co-oligomerization of  $\alpha$ - and  $\beta$ -amino acids is supported by HPLC analysis that shows additional product peaks not detected in the analysis of the homo-condensation reactions of the two reacting amino acids. The simplicity of this peptide synthesis process suggests that it could have played a role in molecular evolution on the early Earth.

[1] Forsythe JG et al. (2015) *Angewandte Chemie International Edition* 54:9871-9875. [2] Danger G, Plasson R and Pascal R (2012) *Chemical Society Reviews* 41:5416-5429.

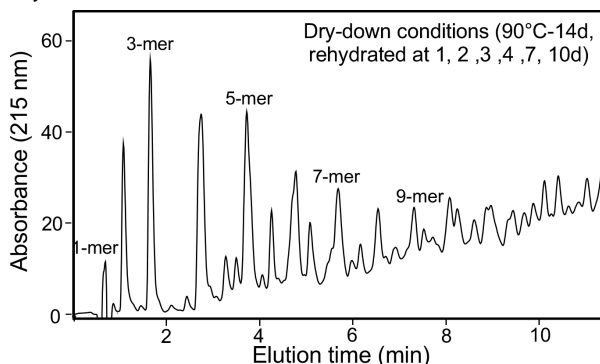


Figure 1. Chromatogram of products of  $\beta$ -alanine dry-down oligomerization promoted by glycerol and bicarbonate.