ESRF	**Experiment title:** POLYMERIZATION OF UNACTIVATED AMINO ACIDS BY COMBINING PRESSURE AND ZEOLITE CONFINEMENT: A NEW ROUTE FOR PEPTIDE SYNTHESIS	**Experiment number**: CH-5235
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Report:

Objective:

The aim of this experiment was to realize the first linear peptide chains of amino acids by the synergic effect of pressure and geometrical constrains imposed by zeolitic frameworks. The effective condensation of unactivated amino whitin 1D zeolite channels, avoiding the need of activators and catalysts, could open a synthesis route never experienced before.

The investigation here proposed fits into two frontiers topics of the scientific research: from one side, the formation of the peptides in primitive Earth addressing as an essential process in the origin of life and in prebiotic chemistry; from the other side, a new way for a "green chemistry" route in peptides synthesis.

Experimental:

A Na-mordenite sample (Na-MOR) was activated and pre-loaded with two amino-acids (glycine and α-alanine). The two amino acid-loaded samples (Na-MOR+gly and Na-MOR+αala) were held under controlled Ar atmosphere in a glove-box to avoid sample rehydration. The ambient condition XRPD data collections were performed after packing and sealing the powders in boron capillaries. Both samples were analysed in two in situ high pressure (HP) XRPD experiments, the samples were loaded in a membrane diamond anvil cell (DAC), using DAPHNE oil as non-penetrating PTM (DAC loading performed inside a glove box with a controlled Ar atmosphere) and cryo-loaded argon as penetrating PTM (DAC loading using a high-pressure gas-load device). The one-dimensional diffraction patterns were obtained in the 2θ range 0-21° by integrating the bi-dimensional images with the software DIOPTAS. The possibility to combinate Raman spectroscopy measurments with the XRPD collection during the HP experiments was prevented by technical issues on the Raman instrumentation.

Results of the study:

Na-MOR+gly sample:

The inspection of the difference Fourier map allowed localizing glycine molecules inside the 12MR channel. The glycine atoms occupy five crystallographycally independent partially occupied sites, all lying on the (100) plane. On the basis of the occupancy factor of these atoms (~0.32) and of the multiplicity of the positions (4) it is possible to calculate that 1.3 molecules p.u.c are hosted in Na-MOR. Each glycine molecule coordinates one Na cation via the carboxyl group and a framework oxygen atom via the amino group (Figure 1-left). The structural refinement shows a disposition of glycine molecules promising for the promotion of the condensation reaction. In fact, considering a full occupancy of each site and the steric hindrance of the atoms, two glycine molecules could be hosted in each channel of a unit cell (figure 1-right), accounting for a total of 4 glycine molecules p.u.c. In this configuration, the distance between two equivalents glycine molecules in the channels is suitable for the peptide formation. However, as stated above, the amount of glycine inside the channels after vapor loading is about 1.3 molecules p.u.c., this means that the probability of finding two neighbor glycine molecules simultaneously present in the same channel is too low.

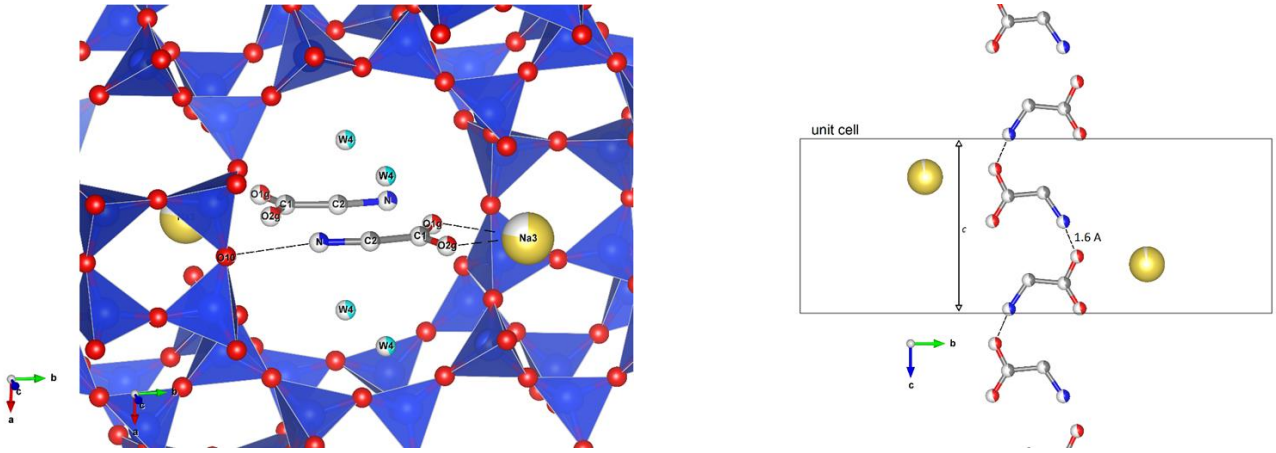

Figure 1: Left) View of the 12MR channel containing the glycine molecules. Right) Disposition along the MOR channel of the glycine molecules assuming a full occupancy of the sites. MOR framework is not shown for sake of clarity. Yellow atoms are Na3 cations.

Na-MOR+gly sample at high pressure:

The structural refinement was possible only on the patterns collected on Na-MOR+gly in the non-penetrating PTM Daphne oil at P_{amb} and after pressure release (Pmax 2.7 GPa). The pressure treatment only induces slight variations on the glycine molecules and framework position. Specifically, the pressure induces the deformation of the 12MR channel along the *b* axis and reduces the coordination distances between the carboxyl group of the glycine and the sodium cation, because of a slight shift of the molecules. Due to the bad quality of the data, consequence of a slight pressure-induced loss of crystallinity of zeolite during the pressure treatment, it was impossible to follow the structural evolution of the system at high pressure. However, it is possible to state that, whatever the structural modifications the system underwent at HP, they resulted quite reversible after the pressure release, in fact the amino acid molecules disposition is not far from the initial one. The variation of the c parameter, at the highest-investigated pressure, is about 3.6%.

Concerning the HP experiment using Ar as penetrating PTM, a higher electronic density was found in the channels, even in the first pressure point investigated (0.07 GPa) suggesting the penetration of Ar inside the pores. The amount of Ar increases with the pressure, hindering a straightforward interpretation of the difference Fourier maxima, due to the high scattering factor of the Ar atoms in comparison with that of the organic molecules. Because of the complexity of the system, the structural analysis on these X-ray diffraction data is really challenging and it not possible to evaluate if the penetration of Ar atoms in the zeolite porosity may induce an approach of the molecules or further hinders the diffusion of the glycine molecules.

The unit cell volume evolution in funcion of pressure are shown in figure 2 for both experiments.

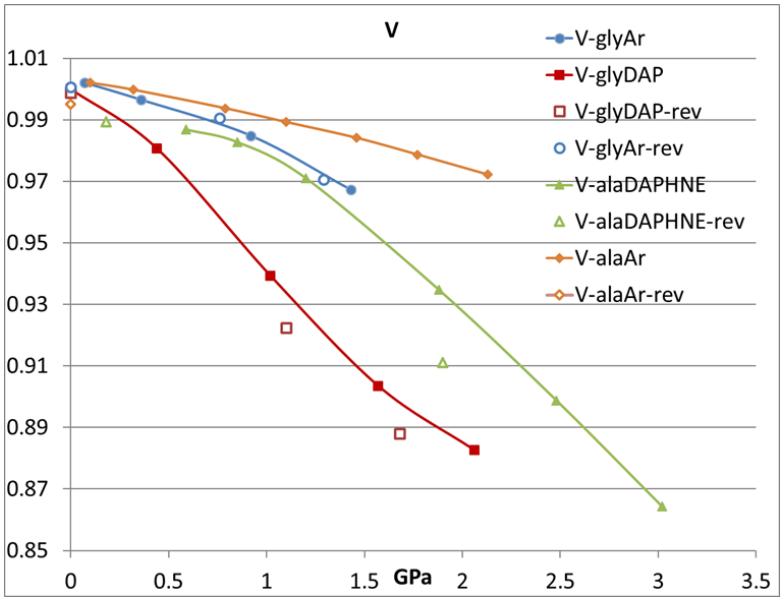

Figure 2: unit cell volume behaviour of the Na-MOR+gly and the Na-MOR+αala samples during the various HP experiments. Empty markers represent the steps during pressure release.

Na-MOR+αala sample

The Rietveld refinement of Na-MOR+αala sample was very challenging, due to the presence of maxima in the difference electron density map attributable to alanine molecules and/or to H_2O molecules strongly correlating each other. For the above-mentioned reasons, an accurate localization and quantification of the amino acid molecules was not possible. Overall, it is possible to asses, with a certain degree of confidence, that α-alanine molecules are located in the 12MR channel coordinating Na cations, similarly to the glycine molecule in the previous sample.

Na-MOR+αala sample at high pressure

The variation of the unit cell volume as a function of P are reported for both HP experiments in Figure 2. The data were refined with the Le Bail method for both experiments in the whole pressure range. When compressed in Daphne oil, Na-MOR+αala volume cell experiences a maximum variation at 3 GPa of 14.2%, while when compressed in Ar, the maximum variation at 2.1 GPa is 2.8%. Comparing the unit cell volume behaviour in the various HP experiments, the Na-MOR+gly sample results in both experiments more compressible than the Na-MOR+αala sample in the respective PTM. This behaviour well agrees with a higher degree of pores filling in Na-MOR+αala sample.