



	Experiment title: Metal-Substituted Polyoxometalates as Artificial Peptidases	Experiment number: 26-01-935
Beamline:	Date of experiment: from: 04/11/2011 to: 07/11/2011	Date of report: 15-01-2012
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Report:

Hydrolysis of different dipeptides was examined in the presence of the Zr-Wells-Dawson polyoxometalate $Zr(\alpha_2\text{-P2W17O61})_2$ by means of ^1H and ^{13}C NMR spectroscopy. The ^1H NMR spectra show a gradual intensity decrease of the dipeptide resonances and the appearance of resonances that are attributed to the free peptides, indicating that the cleavage of the peptide bond in the dipeptide occurred. The pH dependence of k_{obs} was measured in order to identify the catalytically active polyoxometalate species. Comparison of the rate profile with the concentration profile of Zr polyoxometalates suggests that the 1:1 $Zr(\alpha_2\text{-P2W17O61})$ and 2:2 $Zr_2(\alpha_2\text{-P2W17O61})_2$ are the hydrolytically active species, rather than the 1:2 $Zr(\alpha_2\text{-P2W17O61})_2$ species. The coordination mode of the dipeptides was studied by examining the effect of Zr-Wells-Dawson addition on the ^1H and ^{13}C NMR spectra of these dipeptides. In addition EXAFS measurements were performed to get more insight in the binding of the dipeptide to the polyoxometalate. Understanding the coordination of dipeptides to the Zr-Wells-Dawson species, active in the hydrolysis of dipeptide bonds, allows the identification of the mechanism for this novel reaction. In addition the gained knowledge will allow us to understand the mechanism of peptide and protein hydrolysis promoted by other oxyanions which we are currently investigating.

Solutions containing Zr-Wells-Dawson at different pH values in the range of pH 1 to 7 were prepared and used to optimize the experimental EXAFS setup for the Zr K-edge. All measurements were done in cryo-conditions (15K) on flashfrozen samples. At pH 4.6, the use of 2 mM Zr-Wells-Dawson polyoxometalate resulted in an optimal S/N and the polyoxometalate was present in its hydrolytically active form. Once the setup was calibrated and optimized, these solutions were used as blank measurements to study the Zr polyoxometalate species distribution in the presence of 100 mM of different dipeptides. For each sample 3-6 runs were measured. This resulted in an approximate measurement time of 3-6 h for each sample.

Cryo-XAS measurements at 15K in most cases are sufficient to prevent beam induced redox reactions. Unexpectedly, test samples containing Zr Wells-Dawson with a pH above 5 showed clear signs of beam

induced alteration as evidenced by a blue coloration were the x-ray beam hit the sample. The blue color is typical for reduced heteropolyacids, frequently called 'heteropoly blues'.

The cryo-XAS spectral series collected for Zr-Wells-Dawson heteropolyacids at pH below 5, will allow to verify the speciation of the heteropolyacid and provide important info on the coordination of the dipeptides to these Zr substituted POMS. This information should allow to better understand the mechanism of peptide and protein hydrolysis promoted by heteropolyacids.