Electronic Supplementary Material (ESI) for Metallomics.


# Supporting Information

## Peptide bond cleavage in the presence of Ni-containing particles

Nina E. Wezynfeld, *[a,b] Tomasz Frączyk,[a, c] Arkadiusz Bonna,[d] and Wojciech Bal *[,a]

[a] Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5a, 02-106 Warsaw, Poland

[b] Faculty of Chemistry, Warsaw University of Technology, Noakowskiego 3, 00-664, Warsaw, Poland

[c] Department of Immunology, Transplantology and Internal Medicine, Medical University of Warsaw, Nowogrodzka 59, 02-006 Warsaw, Poland

[d] Department of Biochemistry, University of Cambridge, Tennis Court Road, Downing Site, Cambridge CB2 1QW, United Kingdom

## Experimental section

### Materials

N-α-9-Fluorenylmethyloxycarbonyl (Fmoc) amino acids were purchased from Novabiochem (Merck). Trifluoroacetic acid (TFA), piperidine, O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU), triisopropylsilane (TIS), and N,N-diisopropylethylamine (DIEA) were purchased from Merck. Acetic anhydride was purchased from Sigma-Aldrich. TentaGel S RAM resin was obtained from Rapp Polymer GmbH. Nickel(II) nitrate hexahydrate, 99.999% trace metal basis, was obtained from Sigma-Aldrich. HEPES was obtained from Carl-Roth. Pure sodium hydroxide was obtained from Chempur. HEPES (≥99.5%) was purchased from Carl Roth GmbH.

Metallic Ni particles (Ni Pulver, No. 5643.1) was obtained from Carl Roth. Nickel oxide black (No. 244031), nickel oxide green (nickel(II) oxide metal basis, No. 481793), and nickel oxide nanoparticles (nickel oxide nanopowder, No. 637130) were purchased from Sigma Aldrich.

### Characterization of Ni-containing particles

Ni-containing particles were analyzed using Scanning Electron Microscope (SEM) Quanta 200 (FEI) equipped with energy dispersive X-ray spectroscopy (EDX) signal detection, at accelerating voltage of 25−30 kV, at the high vacuum mode. SEM images were done for the suspensions of particles in $H_2O$ and for the tablets from the surface of particles, whereas the EDX analysis of the particle composition was performed using the tablets from the surfaces of particles.

### Peptide synthesis

The synthesis of Ac-GGASRHWKF-am was performed in the solid phase according to the Fmoc protocol using automatic peptide synthesizer (Prelude, Protein Technology). The synthesis was accomplished on a TentaGel S RAM resin, using HBTU as a coupling reagent, in the presence of DIEA. The next steps were carried out manually. The 10% acetic anhydride solution in DCM as employed for the acetylation, while the mixture composed of 95% TFA, 2.5% TIS, and 2.5% water for the cleavage from the resin. The crude peptide was precipitated from cleavage mixtures after the addition of cold diethyl ether. Then, the peptide was dissolved in water and lyophilized. Finally, it was purified by the Waters HPLC/UV system. The eluting solvent A was 0.1% (v/v) TFA in water, and solvent B was 0.1% (v/v) TFA in 90% (v/v) acetonitrile. The identity of peptide was checked by the Q-TOF Premier ESI-MS spectrometer, Waters obtaining the expected m/z signals.

### The kinetics of the hydrolysis of Ac-GGASRHWKF-am in the presence of Ni-containing particles and the $Ni^{2+}$ release from particles

Samples of 1 mM Ac-GGASRHWKF-am and particles containing 5.87 g/l in 100 mM HEPES pH 7.4 were incubated at 37 °C, 300 rpm over one week. The samples without the peptide, containing only 5.87 g/l or 0.587 g/l Ni in 100 mM HEPES pH 7.4 were studied to check the influence of the peptide on $Ni^{2+}$ release and the samples containing 10 mM or 1 mM $Ni(NO_3)_2$, 1 mM Ac-GGASRHWKF-am in 100 mM HEPES pH 7.4 were used as controls to calculate the maximal theoretical rate constant for the Ac-GGASRHWKF-am hydrolysis. Procedures of hydrolysis monitoring and determination of released $Ni^{2+}$ were the same for the control samples and the samples containing both peptide and Ni-containing particles.

To monitor the hydrolysis of the peptide in the presence of Ni-containing particles, 30 µl aliquots of the reaction mixture were periodically collected and added to 200 µl of 0.2% TFA to stop the hydrolysis, centrifuged for 5 min, 14 000 rpm at 8 °C, and 200 µl of the supernatant was stored at 4 °C until the analysis. The obtained samples were separated by the Waters HPLC/UV system and their identity was checked on the Q-TOF Premier ESI-MS spectrometer, Waters. The rate constants were determined using equations for the first order reactions, Equations S1, S2, and S3.

$$S(t) = S_0 \times e^{-k_1 \times t} \qquad \textit{Eq.S1}$$

$$IP(t) = \frac{k_1 \times S_0}{k_2 - k_1} \times (e^{-k_1 \times t} - e^{-k_2 \times t}) \qquad \textit{Eq.S2}$$

$$P(t) = S_0 \times (1 - e^{-k_1 \times t}) \qquad \textit{Eq.S3}$$

$S_0$ denotes the initial amount of the substrate; *S(t), IP(t), P(t)* denote the amounts of the substrate, intermediate product, and product at a given time t, respectively; $k_1$ and $k_2$ are rate constants. The kinetic parameters were calculated using the Origin software.

To monitor the kinetics of $Ni^{2+}$ release, 30 µl aliquots of the reaction mixture were periodically collected and added to 200 µl of water, centrifuged for 5 min, 14 000 rpm at 8 °C, and 200 µl of the supernatant was stored at room temperature until the analysis. Ni concentration was determined by a colorimetric method described in detail in the next section and verified by ICP-OES measurements of selected samples.

**UV-vis titration of $Ni(NO_3)_2$ and Ac-GGASRHWKF-am**

The sample containing 0.95 mM Ac-GGASRHWKF-am and 0.9 mM $Ni(NO_3)_2$ was titrated with small portions of concentrated NaOH in the pH range of 4.5−11.5, at 25 °C. The changes in the UV-vis spectra were monitored on the LAMBDA 950 UV/vis/NIR spectrophotometer (PerkinElmer) in the range of 330−850 nm.

The changes in absorption at 455 nm for Ni(II)-Ac-GGASRHWKF-am were compared with the species distribution for the previously studied Ni(II)/Ac-GASRHWKFL-am complex [*Kopera et al., Inorg. Chem*. 2010, 49, 6636-6645], calculated for the concentrations of the UV-vis experiment (0.95 mM peptide and 0.9 mM Ni(II)).

**Table S1.** Molar fractions of nickel ($X_{Ni}$) and oxygen ($X_O$) in nickel-containing particles used in metal-dependent hydrolysis studies determined by EDX microanalysis

| **Ni particle** | **$X_{Ni}$** | **$X_O$** |
|---|---|---|
| Ni metal | 0.98 | 0.02 |
| NiO black | 0.56 | 0.44 |
| NiO green | 0.55 | 0.45 |
| NiO nano | 0.49 | 0.51 |

## Determination of $Ni^{2+}$ concentration by colorimetric method

The concentration of Ni(II) ions was measured colorimetrically utilizing their previously described propensity to form complexes with dithiothreitol (DTT) with *d-d* band at 460 nm [*Krężel et al*., *J. Inorg. Biochem.* 2001, 84, 77 –88]. The measurements of 1-50 µM nickel ions were performed in 10 mM DTT, 50 mM phosphate buffer, pH 7.0. We tested the stability of the absorbance at 460 nm after mixing all the reaction constituents (Fig. S1). Some samples contained the ATCUN-type product of the hydrolysis reaction (His residue in the third position), able to bind Ni(II) tightly, that could interfere with the measurement by competing for Ni(II) ions. Thus, we measured also the absorbance at 460 nm in the presence of a model ATCUN-type peptide – Gly-Gly-His (Fig. S1). The optimal time for measurement of absorbance was chosen to be 30 minutes.

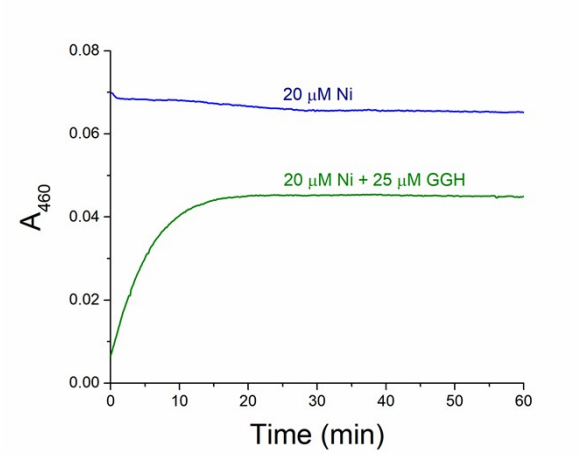


**Fig. S1.** The stability of the absorbance at 460 nm in the mixture of 10 mM DTT, 50 mM phosphate buffer, pH 7.0, 20 µM $Ni(NO_3)_2$, in the absence (blue) or the presence (green) of 25 µM Gly-Gly-His peptide (the peptide was preincubated with the Ni solution for 24 hours, in order to account for slow binding kinetics of ATCUN-type peptides at pH 7).

Because of the observed interference of ATCUN-type peptides during the nickel concentration measurements, we prepared two separate calibration curves, one for the absence and the second for the presence of equimolar Gly-Gly-His peptide (Fig. S2). The dependence of absorbance on Ni(II) concentration can be described by the equations S4 and S5, in the absence and presence of an ATCUN-type peptide, respectively:

$$y = 0.0024 \times x^{1.3207} \qquad \textit{Eq.S4}$$

$$y = 0.000789 \times x^{1.3397} \qquad \textit{Eq.S5}$$

where y stands for absorbance at 460 nm, and x stands for a micromolar nickel concentration.

The measurements were made in three replicates. The results of the DTT method were positively verified by ICP-OES for selected samples.

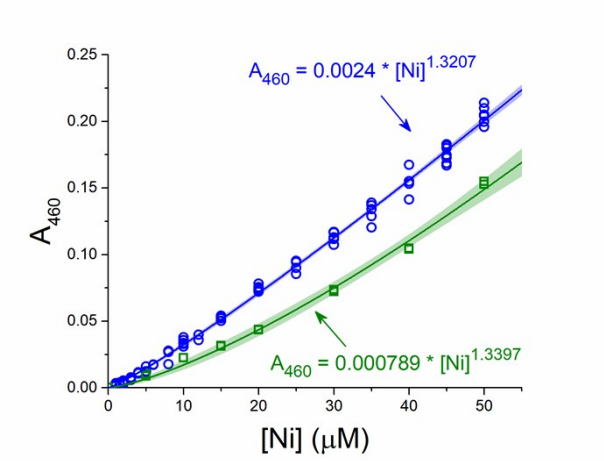


Fig. S2. The calibration curves for Ni concentration measurement. The absorbance at 460 nm was measured after 30 minutes of preparing the mixture of 10 mM DTT, 50 mM phosphate buffer, pH 7.0, 1-50 μM $Ni(NO_3)_2$, in the absence (blue) or the presence (green) of equimolar Gly-Gly-His peptide (the peptide was preincubated with the Ni solution for 24 hours due to slow binding kinetics to ATCUN-type peptides at pH 7). The fits of general equation $y = ax^b$ are shown, with 99% confidence bands.