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Towards understanding secondary structure transitions: phosphorylation and metal coordination in model peptides

Malgorzata Broncel, Sara C. Wagner, Kerstin Paul, Christian P. R. Hackenberger and Beate Koksch

Institut für Chemie und Biochemie, Freie Universität Berlin, Takustr. 3, 14195 Berlin, Germany

Analytical HPLC of purified CP and P (10,17)

Samples were analyzed on a VWR-Hitachi Elite LaChrome system (VWR International) with water/0.1% TFA (solvent A) and ACN/0.1% TFA (solvent B) gradient (20-70% B, 50 min) at 1 mL min^{-1}.

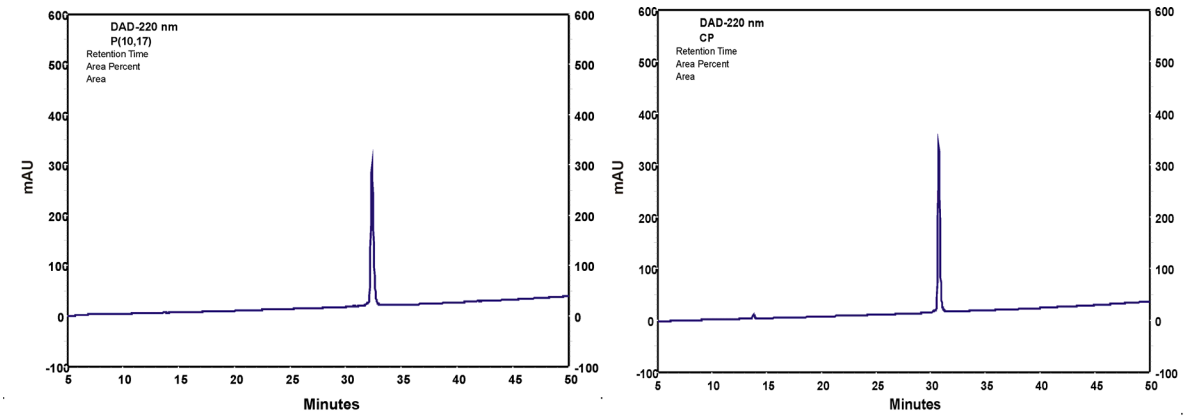

Fig. 1 Analytical HPLC chromatograms of P(10,17) and CP.

Size Exclusion Chromatography

SEC was performed to analyze the oligomerization state of CP, P (10,17), and P (10,17) in the presence of 2 mM $MgCl_2$. Peptides were analyzed on a VWR-Hitachi Elite LaChrome system (Pump L-2130, UV Detector L-2400) equipped with a Superdex 75 PC 3.2/30 column (Amersham Bioscienes) running isocratically (100 mM phosphate buffer, pH 7.4) at 0.025 mL min^{-1}. Peptide absorbance was registered at 220 nm. Molecular weight calibration was accomplished with the Gel Filtration Calibration Kit LMW (GE Healthcare Life Sciences). The retention times were corrected with internal and external references. Anthranilic acid labelled Gly was used as an internal reference, whereas peptides GCN4p1, GCN4pII, and GCN4pLI were applied as external standards for dimeric, trimeric and tetrameric coiled coils, respectively.[1]

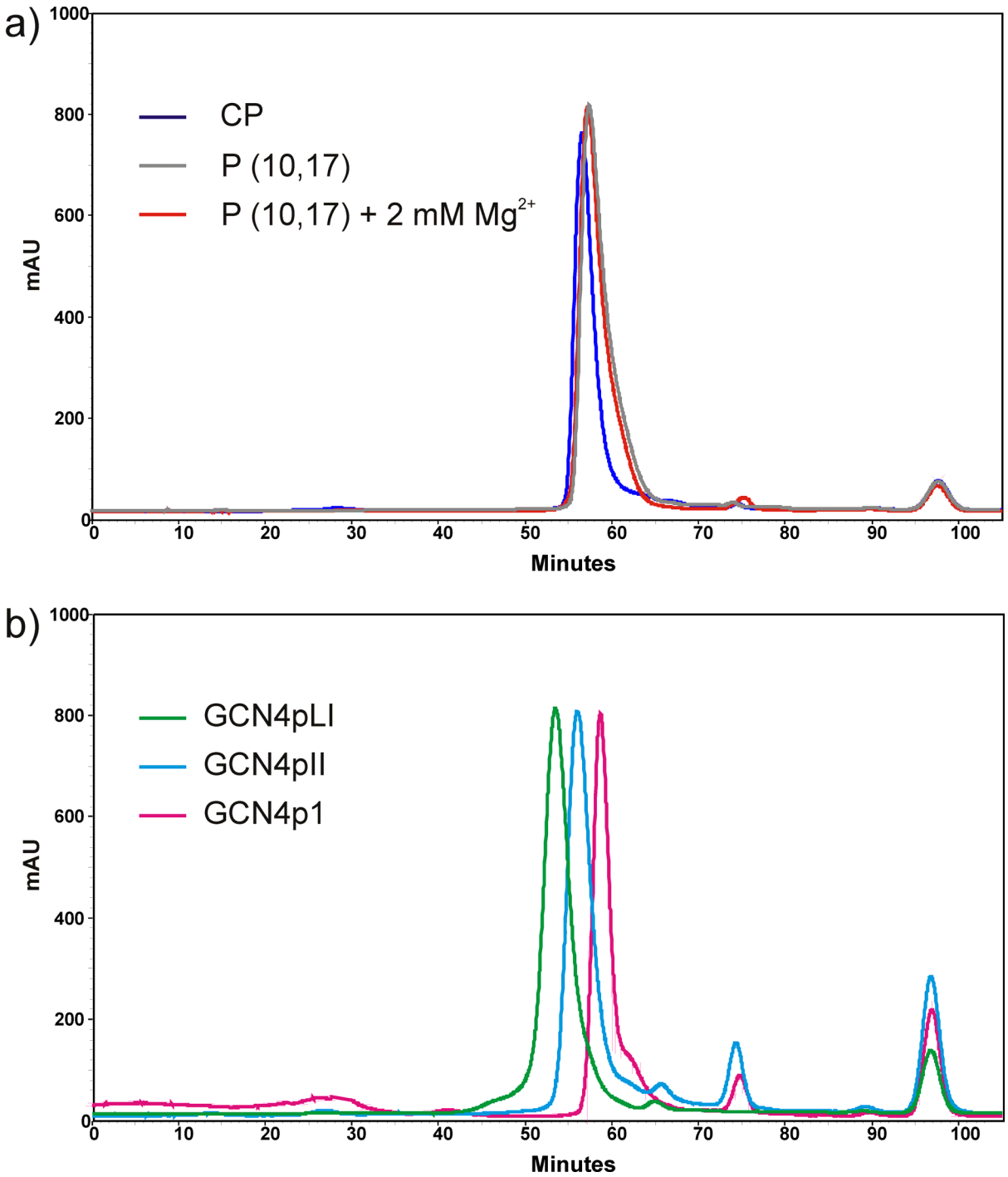

Fig. 2 SEC of a) CP, P (10,17), and P (10,17) containing 2 mM $MgCl_2$ at 100 μM concentration, and b) peptides GCN4p1, GCN4pII, and GCN4pLI. The peak at 97 min is the internal reference.

Our results show the presence of trimeric species (56 min) for all investigated peptides at 100 μM concentration. In addition, a small fraction of dimeric species (60 min) was present in P (10,17) with and without metal.

Thermal denaturation

Samples were prepared as described in the Experimental Part and incubated with 3 M urea for 15 min before the measurement. Thermal melts were carried out in 0.1 ºC intervals with a heating rate of 3 ºC min $^{-1}$.

Fraction unfolded (f_U) was calculated from the ellipticity at 222 nm using the expression:

$$f_U = ([\theta] - [\theta]_F) / ([\theta]_U - [\theta]_F)$$

where $[\theta]$ is the observed molar ellipticity at 222 nm, $[\theta]_U$ and $[\theta]_F$ are the ellipticities of the denatured and native states, respectively.

The molar fraction of unfolded peptide was plotted against temperature. For a comparison of the stability, the melting temperature (T_M), at which 50% of the peptide remains folded, was calculated.

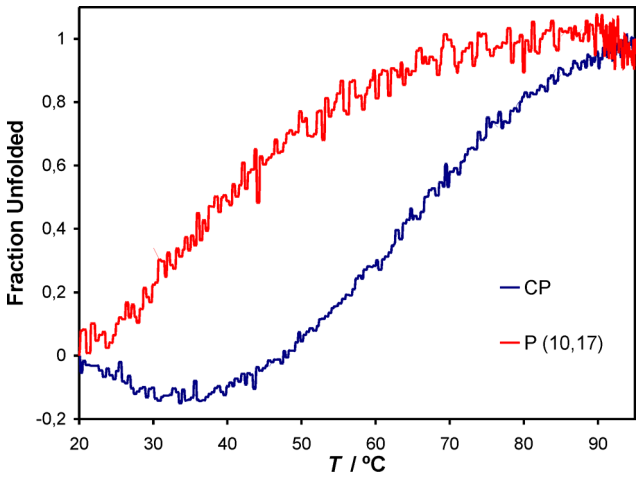

Fig. 3 Thermal unfolding of 40 μM peptides in 10mM Tris/HCl buffer, pH 7.4 in the presence of 3 M urea. Melting temperature was determined from 50% fraction folded **(67 ºC for CP and 41 ºC for P (10,17))**.

Salt screening

Samples were prepared as described in the Experimental Part and incubated for 15 min with NaCl (0.2 M) before measurement.

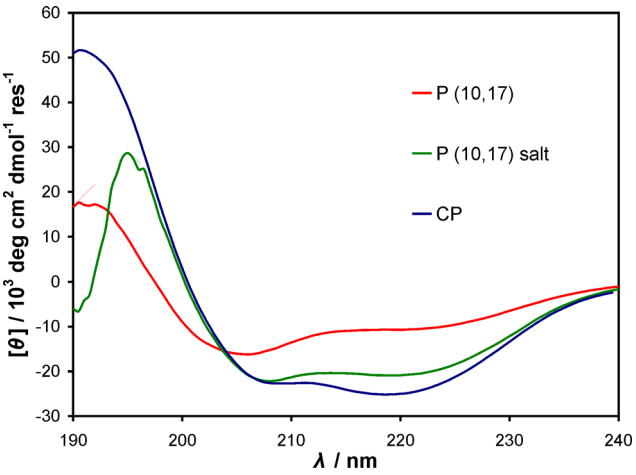

Fig. 4 CD spectra of 100 µM P (10,17) in 10 mM Tris/HCl buffer, pH 7.4 in the presence of 0.2 M NaCl.

Metal titration of CP

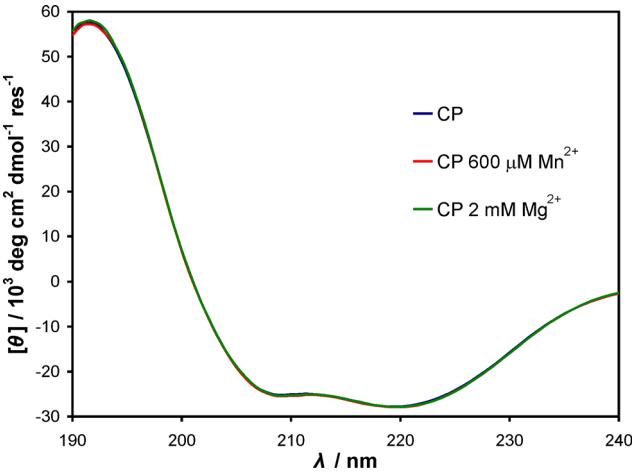

Fig. 5 Metal titration of 100 µM CP in 10 mM Tris/HCl buffer, pH 7.4 monitored by CD spectroscopy. For clarity reasons only the highest metal concentrations are shown.

References

1 P. B. Harbury, P. S. Kim and T. Alber, *Nature*, 1994, **371**, 80-83.