Influence of amino acids sequence on metal binding properties of selected pentapeptides - fluorescence and UV-Vis spectroscopy studies

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DOI: 10.31708/spi3.18/makdo.cns18

Introduction

Interactions between peptides and metal ions are the subject of constant investigations, as they are considered to be of great significance. For instance, it has been shown that several ions, e.g. Cu^{2+} and Zn^{2+} , are involved in the amyloid- β peptide aggregation process, which presumably plays the main role in Alzheimer's disease (Nair, Perry, Smith, & Reddy, 2010). From among all amino acids present in the A β sequence, histidine and tyrosine side chains are proven to have a distinct affinity for Cu^{2+} and other metal cations (Murariu et al., 2018). The aim of our work was to investigate potential applications of fluorescence and UV-Vis spectroscopy for research into interactions between several metal cations (Mn^{2+} , Fe^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} and Zn^{2+}) and four selected peptides (EYHHQ, EHYHQ, EHHQY and KYHHE).

Materials and Methods

The peptides: EHHQY, EHYHQ, EYHHQ and KYHHE were synthesized using a procedure described previously (Makowska, Bagińska, Liwo, Chmurzyński, & Scheraga, 2008). Their stock solutions (0.1 mM) were prepared by dissolving proper amount of peptides in 5 mM MES buffer (pH 6.0). Inorganic salts: $\text{Cu}(\text{II})$, $\text{Zn}(\text{II})$, $\text{Mn}(\text{II})$, $\text{Co}(\text{II})$, $\text{Ni}(\text{II})$ nitrates and $\text{Fe}(\text{II})$ chloride were purchased from Sigma-Aldrich and dissolved in the 5 mM MES buffer (pH 6.0) to concentration of 16 mM. All steady-state fluorescence measurements were performed at room temperature on a Cary Eclipse Varian spectrofluorometer. The excitation and emission wavelengths were set to 275 nm and 305 nm, respectively. Absorption spectra essential to inner-filter corrections were recorded on a Perkin Elmer Lambda 650 UV-Vis spectrophotometer at room temperature.

Results

Fluorometric titrations were conducted for all 24 peptide-metal combinations. No significant changes in fluorescence intensity were observed in all samples containing Mn^{2+} , Fe^{2+} , Co^{2+} and Zn^{2+} ions. Titrations of selected peptides against Cu^{2+} ions induced significant and rapid changes in fluorescence intensity, therefore it was possible to determine the stoichiometry

(1 mole of Cu^{2+} per 2 moles of peptide in all cases), as exemplified in Fig. 1, and stability constants of obtained complexes (results not shown).

Fig. 1. The plot of $(F_0 - F)/F_0$ and absorbance (at 275 nm) against the titrant to analyte (Cu^{2+} to EHHQY) molar concentration ratio. Line-line intersections represent the stoichiometry of the formed Cu^{2+} -EHHQY complex.

Investigations of interactions between peptides and Ni^{2+} ions provided surprising results. Fluorescence intensity of EYHHQ and EHYHQ peptides was not affected by adding further portions of Ni^{2+} ions. Contrarily, EHHQY- Ni^{2+} and KYHHE- Ni^{2+} interactions led to a slow but indisputable decrease of fluorescence intensity.

Discussion

The fluorometric titration is a useful method for monitoring Cu^{2+} -peptide interactions. The stoichiometry (1:2) and stability constants of formed complexes were determined with satisfactory accuracy. However, this experiment is not applicable to study interactions between selected peptides and other chosen metal ions, namely Mn^{2+} , Fe^{2+} , Co^{2+} and Zn^{2+} . Ni^{2+} -peptide interactions will be investigated further. Described differences between properties of the selected peptides (EYHHQ, EHYHQ vs. EHHQY, KYHHE) have proven existence of a connection between a peptide sequence and its metal binding properties. It is probable that the Glu-His-His (and opposite) arrangement of amino acids is responsible for observed distinctions. However, with

no doubt it needs further examinations. It was observed that EYHHQ and EHYHQ peptides interact exclusively and instantly with the Cu²⁺ ions (any interactions with other selected metals were insignificant when investigated by fluorescence spectroscopy). This fact might imply the application of EYHHQ and EHYHQ peptides as Cu²⁺ ions sensors. Our hypothesis requires subsequent measurements to be confirmed.

Acknowledgments

This work was financially supported by the Polish National Science Centre (NCN) under Grant No. 2016/23/D/ST4/01576.

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