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A new, model-free calculation method to determine the coordination modes and distribution of copper(II) among the metal binding sites of multihistidine peptides using circular dichroism spectroscopy

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ABSTRACT

A new calculation method to determine microscopic protonation processes from CD spectra measured at different pH and Cu(II):ligand ratios was developed and used to give the relative binding strengths for the three histidines of hsPrP(84–114), a 31-mer polypeptide modeling the N-terminal copper(II) binding region of human (homo sapiens) prion protein. Mutants of hsPrP(84–114) with two or one histidyl residues have also been synthesized and their copper(II) complexes studied by CD spectroscopy. The 1-His models were analyzed first, and the molar CD spectra for the different coordination modes on the different histidines were calculated using the general computational program PSEQUAD. These spectra were deconvoluted into the sum of Gaussian curves and used as a first parameter set to calculate the molar spectra for the different coordination modes (3N and 4N coordination) and coordination positions (His85, His96 and His111) of the 2-His peptides. The calculation method therefore does not require the direct use of CD spectra measured in the smaller peptide models. This is a significant improvement over earlier calculation methods. In the same runs, the stepwise deprotonation pK_{mic} values were refined and the pH-dependent distribution of copper(II) between the two histidines was determined. The results revealed the high, but different copper(II) binding affinities of the three separate histidines in the following order: His85 << His96 ⩽ His111. The calculation also showed that molar CD spectra which belong to the same coordination mode and coordination position in different ligands have very similar transition energies but different intensities. For this reason, direct transfer of molar CD spectra between different ligands may be a source of error, but the pK_{mic} values and the copper(II) binding preferences are transferable from the 2-His peptides to the 3-His hsPrP(84–114).

1. Introduction

Prion diseases are a group of neurodegenerative pathologies including Creutzfeldt-Jakob disease in humans, scrapie in sheep and bovine spongiform encephalopathy (mad cow disease) in cattle, all leading to death of the infected species [1–3]. They represent a peculiar case in biology due to the "protein only" hypothesis which explains the formation of these diseases not by an infection of a virus or bacterium but by a conformational conversion of the normal prion protein, PrP^{c}, to the disease-related scrapie isoform, PrP^{sc} [4].

Increasing evidence indicates that PrP is a copper-binding protein [5–15]. For this reason, it is very important to study and understand the copper(II) binding properties of the peptide, or that of shorter peptide fragments responsible for the copper(II) binding ability of the whole peptide. These interactions may also have a role in the formation of prion diseases.

Considerable work has been devoted to studying the binding sites, their affinities and the coordination environment in the full-length prion protein and its smaller fragments responsible for the copper(II) coordination [11,12,16–29].

Literature data show that visible CD is a great technique to investigate metal-peptide interactions [7,14,19,21,22,27,30–41]. Evaluation of d-d bands of visible CD is one of the best methods to differentiate between the different histidines with the same or very similar coordination modes in colored metal–peptide complexes. Other spectroscopic techniques for copper(II) complexes (such as UV–vis and EPR) are sensitive mainly to the direct coordination environment of the central copper(II) ion and – in the case of peptide complexes – to the number and types of nitrogens in the coordination sphere. These are the same in prion peptide fragments, no matter whether the stepwise amide deprotonation starts from His85, His96 or His111. The similarity of His96 and His111 is even more clear because the sizes of the chelate rings formed with

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these two histidines is also exactly the same. Nevertheless, CD spectroscopy was found to be an excellent method to distinguish the copper(II) ions coordinated to these three histidines at pH above six. First of all, free copper(II) ion d-d transitions are CD silent. Secondly, although the coordination modes starting from His85, His96 and His111 are similar or identical, their CD spectra are relatively strong and very different because of the different non-coordinating amino acid side chains on the coordinated amide functions. Thirdly, there are also some empirical rules for understanding the CD effects of side chains and distortions in the coordination geometries [37].

The calculation method presented here does not require the direct use of CD spectra measured in the smaller or other, different peptide models and avoids the errors originating from the fact that a model is almost never as perfect as the molecule being modelled. This imperfection of model peptides is clearly shown by some interesting new results about the copper(II) binding of two peptides with very similar binding possibilities [39]. PrP(90–126) and PrP(91–115) – both containing His96 and His111 of the prion protein – have very different measured CD spectra in the presence of copper(II) at a given pH and copper(II) to ligand ratio, in contrast to the expected very similar or identical spectra. The difference between these systems is assumed to originate from the different relative preferences of the two histidines toward copper(II).

In our previous paper [42], soluble polypeptide fragments of the prion N-terminus encompassing histidine residues within and outside the octarepeat domain were studied by various spectroscopic techniques. The coordination modes and coordination positions as well as reliable affinity constants of the copper(II) species were determined. For this purpose, the three-His 31-mer prion N-terminus model, hsPrP(84–114), four one-His fragments (hsPrP(84–91), hsPrP(94–97), hsPrP(109–112) and hsPrP(106–114)) and four two-His mutants of hsPrP(84–114) (hsPrP(84–114)His85Ala, hsPrP(84–114)His96Ala, hsPrP(84–114)His111Ala and hsPrP(91–115)) were synthesized. The measurements showed that the relative copper(II) binding preferences of the three histidines of hsPrP(84–114) are as follows: His85 << His96 $\leqslant$ His111.

The present work uses the same prion models and reports a new calculation method which is able to determine the microscopic stability constants of the different copper(II) complex isomers formed, which enables the monitoring of the distribution of copper(II) between the three histidines: His85 (from the octarepeat domain), His96 and His111 as a function of pH. The new method does not require the direct use of any spectroscopic or equilibrium data of smaller model peptides, but it does require the knowledge of the possible coordination modes of copper(II) to the peptide. Previous information on the molar spectra and the stepwise microscopic deprotonation constants at the different histidines helps to decrease computation time necessary by providing a very good starting parameter set for the calculation. This information is, however, not indispensable for the calculation: in its absence, the number of colored (CD active) species in the system can be estimated by matrix rank analysis of the pH- and concentration dependent spectra. However, it is important to keep in mind that this (and any CD-based) calculation itself gives only the individual spectra and pK_{mic} values, and not the corresponding coordination modes.

It is essential to have good ideas about all the possible coordination positions and coordination modes in the metal–peptide system before starting the calculation, but this is basically enough. Further information from smaller peptides about the pK_{mic} values or individual CD spectra helps decrease the calculation time by improving the starting parameter set. After gathering this information, a mathematical equation can be written for the measured CD spectra at any metal to ligand ratio and any pH containing the concentrations of the components (Cu(II), Ligand and H^+) as variables, and the molar CD spectra (in the form of a sum of Gaussian curves), the stepwise deprotonation constants and the % distribution of copper(II) among the histidines as parameters to be refined.

2. Experimental

2.1. Materials

One-, two- and three-His containing peptide fragments of homo sapiens prion protein (hsPrP) were synthesized using the solid-phase peptide synthesis strategy on a Pioneer™ Peptide Synthesizer. Detailed synthesis results, yields, purities and indentification results for the peptides are given elsewhere [42]. The peptide fragments used for the calculations with the CD spectra were four 1-His fragments (hsPrP(84–91) containing His85, hsPrP(94–97) containing His96, hsPrP(109–112) containing His111 and hsPrP(106–114) also containing His111) and four 2-His fragments (hsPrP(91–115) containing His96 and His111, hsPrP(84–114)His85Ala containing His96 and His111, hsPrP(84–114)His96Ala containing His85 and His111 and hsPrP(84–114)His111Ala containing His85 and His96 together). The sequences of all these peptides are shown in Fig. 1.

The $CuCl_2$ stock solution was prepared from analytical grade reagent and its concentration was checked by gravimetry via the oxinate precipitate [43].

2.2. Potentiometric titrations

pH-potentiometric titrations were used to determine the ligand concentration of the peptide stock solutions with an accuracy better than ±1%. The pH-potentiometric titrations were performed in 3 cm^3 samples at approximately 1×10^{-3} M ligand concentration and strong acid (HCl excess) in the samples to make sure that all basic groups are fully protonated at the beginning of the titrations.

When preparing the solutions for the titrations, not only their volume was measured by a pipette (typically between 0.6 mL and 1.4 mL), but also the weights of the solutions using an analytical balance. This made it possible to calculate the concentrations of the components (ligand and hydrogen ion) with high accuracy.

During the titration, argon was bubbled through the samples to ensure the absence of oxygen and carbon dioxide. The samples were stirred by a VELP Scientific magnetic stirrer. All pH-potentiometric measurements were carried out at 298 K and at a constant ionic strength of 0.2 M KCl. pH measurements were made with a MOLSPIN pH-meter equipped with a 6.0234.100 combination glass electrode (Metrohm) and a MOL_ACS microburette controlled by a computer. pH-potentiometric titrations were performed using a carbonate-free (carbonate content less than 0.1%) KOH solution of approximately 0.2 M concentration, that had been exactly determined. The KOH solution was stored under argon atmosphere. The KOH and carbonate concentrations of the titrant solution were calculated from the potentiometric titration of a 0.0500 M potassium hydrogen phthalate solution by this KOH titrant.

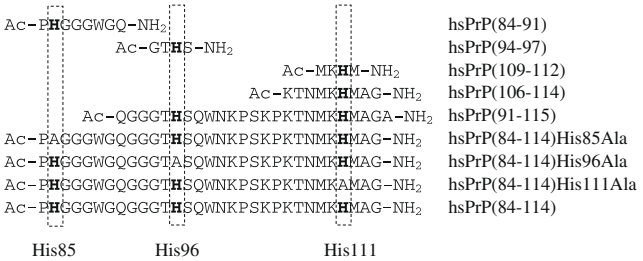

Fig. 1. Amino acid sequences of the peptides investigated.