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Probing the quaternary structure of metal-bridged peptide oligomers

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ABSTRACT

The oligomerisation of many proteins and peptides is known to be influenced by the binding of transition metal ions such as divalent copper. To investigate the oligomeric state of model peptides related to the N-terminus of α-synuclein (αSyn) in the presence of Cu(II), electron paramagnetic resonance (EPR) spectroscopy and isotopic labelling were recently used to conclude that Cu(II) occupies N-terminal bridging positions within closed-chain αSyn dimers and trimers with a Cu/peptide stoichiometry of 1:1. Herein, a statistical correction is identified and the consequences are evaluated. The analysis reveals that αSyn forms Cu-bridged antiparallel dimers and closed-chain trimers that coexist with Cu(II)-bound monomers (including a "macrochelate") and, depending on metal stoichiometry and protein environment, with open-chain Cu-bridged oligomers and heterodimers. The results demonstrate that the Cu(II) ion can be exploited as a probe of protein quaternary structure, with the potential to delineate heterogeneous oligomeric populations.

1. Introduction

Divalent copper is known to mediate the aggregation pathways of intrinsically-disordered peptides and proteins such as β-amyloid (Aβ) and α-synuclein (αSyn) [1,2,3,4,5,6,7,8,9,10]. The Cu(II) coordination sphere is therefore of clear importance and previous studies using low frequency electron paramagnetic resonance (EPR) and site-specific isotopic labelling have provided valuable information about the Cu(II) coordination of Aβ [11] and αSyn [12]. By resolving and simulating the metal–ligand hyperfine structure within the EPR spectrum, it is possible to determine the ligands present in the first coordination sphere of tetragonal Cu(II) complexes. Although it is acknowledged that such ligands can be supplied by different monomers (ie. intermolecular Cu^{2+} bridges are present) [13], Cu-bound monomers are typically assumed because, under ordinary circumstances, EPR cannot easily distinguish between these two possibilities.

To characterise the oligomeric state of N-terminal peptides spanning the first 56 residues of synculein (αSyn56), the isotopic labelling method was recently applied to heterogeneous mixtures of αSyn56 containing a ^{15}N label on either one of two different nitrogen ligands. EPR spectroscopy was then used to identify Cu(II) ions that were simultaneously coordinated by two ^{15}N ligands [12]. Since two ^{15}N ligands can only be possible through the participation of two different labelled monomers, this approach provided an effective means to identify $[Cu(\alpha Syn)]_n$ oligomers. In the previous study, the probability of forming different types of αSyn oligomers was considered in order to analyse the experimental EPR spectra of the mixed-labelled system. Herein, this previous analysis is re-evaluated and the experimental spectra reinterpreted to provide evidence for a heterogeneous population of Cu-bound monomers (including macrochelate) and both open- and closed-chain Cu-bridged αSyn oligomers.

2. Theoretical

Detailed spectroscopic studies have demonstrated that the Cu(II) binding of αSyn is dominated by a $\{NH_2^{M1}, N^{-\ D2}, COO^{-\ D2}, N_{Im}^{H50}\}$ coordination sphere in aqueous solution at physiological pH [14,15,16]. This coordination (referred to as "mode II") accounts for >70% of the bound Cu(II) at pH 7.4 and >95% of the bound Cu(II) at pH 8.5 [12,17] and the following discussion deals only with this species. The $\{NH_2^{M1}, N^{-\ D2}, COO^{-\ D2}, N_{Im}^{H50}\}$ coordination sphere is even formed in a heterogeneous mixture of N-terminally acetylated synuclein (Ac-αSyn56) and synuclein lacking His50 (αSyn56(H50N)) [12]. This observation clearly indicated that the first coordination sphere can be assembled from different monomers and hence that intermolecular Cu(II) bridging can occur. Note that full length αSyn1–140 is ubiquitously N-terminally acetylated in the brain [18] and although N-truncations occur to yield a free amino terminus [19], these truncations also remove the aspartate at P2 and thus prevent the terdentate 5,6-membered chelate formed by the non-acetylated, full length protein. Therefore, the peptides used herein should be viewed as a model system to which the ensuing methodology can be applied.

To determine the proportion of $\{NH_2^{M1}, N^{-\ D2}, COO^{-\ D2}, N_{Im}^{H50}\}$ that is formed by intermolecular Cu-bridged αSyn oligomers, one may study a solution of αSyn with some monomers having their naturally abundant ^{14}N nuclei replaced with a ^{15}N label at Met1 and others with a ^{15}N label

Abbreviations: αSyn, α-synuclein; EPR, electron paramagnetic resonance.
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at His50. The above mixture of labelled peptides then introduces four unique possibilities for the ligands:

$$\begin{Bmatrix} {}^{15}NH_2{}^{M1},\ N^{-}{}^{D2},\ COO^{-}{}^{D2},\ {}^{14}N_{Im}{}^{H50} \end{Bmatrix}$$
$$\begin{Bmatrix} {}^{14}NH_2{}^{M1},\ N^{-}{}^{D2},\ COO^{-}{}^{D2},\ {}^{15}N_{Im}{}^{H50} \end{Bmatrix}$$
$$\begin{Bmatrix} {}^{14}NH_2{}^{M1},\ N^{-}{}^{D2},\ COO^{-}{}^{D2},\ {}^{14}N_{Im}{}^{H50} \end{Bmatrix} \quad (1)$$
$$\begin{Bmatrix} {}^{15}NH_2{}^{M1},\ N^{-}{}^{D2},\ COO^{-}{}^{D2},\ {}^{15}N_{Im}{}^{H50} \end{Bmatrix}.$$

In each instance, the coordination is geometrically equivalent, but magnetically distinct owing to the different $^{14}N/^{15}N$ content of the ligands, which can be experimentally distinguished by the different patterns of hyperfine peaks within an EPR spectrum. If Cu(II) binding is strictly intramolecular then only the first two coordination spheres are possible; however, the latter two coordination spheres are additionally possible if intermolecular Cu(II) bridging takes place.

In order to derive information pertaining to the quaternary structure of a Cu-bridged oligomeric species, one must first determine the probability of encountering a first coordination sphere containing zero, one, or two ^{15}N-labelled ligands. Denoting αSyn56(^{15}N-Met1) as "peptide A" and αSyn56(^{15}N-His50) as "peptide B", one seeks to know the probability of forming an n^{th} order Cu-bridged oligomer of the form $Cu_nA_jB_k$, where j and k represent the number of times peptide A and B appear in the oligomer and $j + k = n$. Since the peptides are in exchange with the metal ion,[1] then the problem reduces to that of taking n random samples with replacement from an infinite population of A and B.

When an equal concentration of peptides A and B is present, all 2^n permutations of an open-chain oligomer $Cu_mA_jB_k$ ($j + k = n$, $m = n - 1$) are distinguishable and occur with equal probability of $(1/2)^n$. By examining each permutation, one can establish that each of the four possible Cu(II) coordination spheres in Eq. (1) occurs an equal number of times (Fig. 1a,b) and the relative speciation of all four coordination spheres occurs with the ratio $^1/_4 : {}^1/_4 : {}^1/_4 : {}^1/_4$ (Table 1).

The problem for closed-chain (cyclic) oligomers can most easily be understood simply by taking the open-chain structures and joining the free ends together with an additional bridging Cu(II) ion (compare Fig. 1a,b with Fig. 1c,d). This results in some permutations that are no longer structurally distinct. For example, open-chain metal-bridged dimers Cu_2AB and Cu_2BA are distinguishable (Fig. 1a), whereas the corresponding cyclic (antiparallel) metal-bridged dimers are related by a trivial rotation in space and thus indistinguishable (Fig. 1c). Nevertheless, each instance must be counted. Mathematically, the problem is analogous to calculating the statistical effect in forming a ternary metal complex CuA_jB_k of coordination number $n = j + k$ by two distinct ligands [20], except that one is interested in closed-chain oligomers Cu_nL_n rather than mononuclear metal centres CuL_n. The probability $P_n(j,k)$ of forming an n^{th} order closed-chain oligomer $Cu_nA_jB_k$ is given by the binomial expression:

$$P_n(j,k) = \binom{n}{j,k}(p_A)^j(p_B)^k \quad (2)$$

where $p_A = p_B = 1/2$ for an equal concentration of peptides A and B and the binomial coefficient accounts for the fact that the statistical probability of forming $Cu_nA_jB_k$ is $\binom{n}{j,k} = \frac{n!}{j!k!}$ times more likely than forming Cu_nA_n and Cu_nB_n. For example, for antiparallel dimers there are $\frac{2!}{1!1!} = 2$ equally probable Cu_2AB structures (Fig. 1c) and for closed-chain trimers there are $\frac{3!}{2!1!} = 3$ equivalent Cu_3AB_2 structures of equal probability and three equally probable Cu_3A_2B structures (Fig. 1d). If each distinguishable closed-chain oligomer is drawn only once, then its probability must be weighted by the statistical factor. When this is done, enumeration of each type of first coordination sphere (Fig. 1) shows once again that their relative speciation is $^1/_4 : {}^1/_4 : {}^1/_4 : {}^1/_4$ (Table 1).

The previous study of Cu/A/B 1:½:½ demonstrated experimentally that the relative speciation of the four coordination spheres was consistent with the ratio $^1/_3 : {}^1/_3 : {}^1/_6 : {}^1/_6$ (Table 1), which was attributed to closed-chain dimers and/or trimers [12]; however, the aforementioned statistical weighting factor was neglected in that study. Therefore, there is a need to re-evaluate the physical interpretation of the experimentally derived speciation.

3. Discussion

In the following analysis, one again considers Cu/αSyn56 1:1 at pH 8.5, because under these conditions the {NH_2^{M1}, $N^{-}{}^{D2}$, $COO^{-}{}^{D2}$, N_{Im}^{H50}} coordination mode accounts for >95% of the Cu-bound species. By studying αSyn56 peptides containing ^{15}N-labels on either Met1, Asp2 or His50, the metal–ligand hyperfine couplings have been determined by simulation of the low-frequency (S-band) EPR spectra [12]. With these parameters, it is possible to simulate the spectra characterising each of the four coordination spheres listed in Table 1, as shown in Fig. 2a. It is clear that the first two spectra in Fig. 2a (corresponding to coordination spheres containing one ^{15}N ligand) display maxima at the position where the latter two spectra (corresponding to coordination spheres containing zero or two ^{15}N ligands) exhibit minima. Therefore, compared with the spectrum produced by a macrochelate, intermolecular Cu(II) bridging would be expected to produce less prominent hyperfine peaks due to destructive interference (vide infra).

3.1. A macrochelate cannot be the sole species present in solution at pH 8.5

If αSyn monomers are non-interacting, then a solution containing 50% αSyn56(^{15}N-Met1) and 50% αSyn56(^{15}N-His50) permits only {$^{14}NH_2^{M1}$, $^{14}N^{-}{}^{D2}$, $COO^{-}{}^{D2}$, $^{15}N_{Im}^{H50}$} and {$^{15}NH_2^{M1}$, $^{14}N^{-}{}^{D2}$, $COO^{-}{}^{D2}$, $^{14}N_{Im}^{H50}$} as first coordination spheres (Table 1). However, an equal weighting of the pH 8.5 spectra of Cu/αSyn56(^{15}N-Met1) 1:1 and Cu/αSyn56(^{15}N-His50) 1:1 does not reproduce the experimental spectrum of Cu/αSyn56(^{15}N-Met1)/αSyn56(^{15}N-His50) 1:½:½ (Fig. S1). This is also evident from the corresponding macrochelate simulations shown in Fig. 2b, where the stark difference in the pattern of hyperfine peaks indicates that intermolecular interactions must contribute to the Cu(II) binding.

3.2. A Cu-bridged oligomer is not the sole species present in solution at pH 8.5

For metal-bridged oligomers with any quaternary structure, the Cu(II) binding of αSyn is comprised of equal contributions from all four possible first coordination spheres (Table 1). This scenario was considered previously [12] and, like the macrochelate, the simulations predict a very different hyperfine pattern as compared with experiment (Fig. 2). Since this simulation is valid for all oligomeric structures, including closed-chain dimers and/or trimers, this finding represents a major difference from the previous study, which erroneously concluded that closed-chain dimers and/or trimers alone were responsible for the experimental peak pattern [12].

3.3. A mixture of Cu-bound macrochelate and Cu-bridged oligomer is consistent with experiment

Noting the relative speciation of the four possible coordination spheres is $^1/_2 : {}^1/_2 : 0 : 0$ for a macrochelate (monomer) and $^1/_4 : {}^1/_4 : {}^1/_4 : {}^1/_4$ for a Cu(II)-bridged oligomer, the only satisfactory

[1] Rapid freezing of samples in liquid nitrogen for analysis by EPR spectroscopy takes a "snapshot" of the instantaneous population.