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Thermodynamics imprinting reveals differential binding of metals to α-synuclein: Relevance to parkinson's disease

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Abstract

The aggregation of α-synuclein is a hallmark feature of Parkinson's disease (PD) and other synucleinopathies. Metals are the significant etiological factors in PD, and their interaction with α-synuclein affect dramatically the kinetics of fibrillation *in vitro* and are proposed to play an important and potential neurodegenerative role *in vivo*. In the present study, we investigated the stoichiometry of binding of copper [Cu (II)] and iron [Fe (III)] with α-synuclein (wild recombinant type and A30P, A53T, E46K mutant forms) using isothermal titration calorimetry (ITC). α-Synuclein monomer (wild and mutant forms) titrated by Cu (II), showed two binding sites, with an apparent K_B of 10^5 M and 10^4 M, respectively. But, α-synuclein (wild type and mutant forms) titrated with Fe (III) revealed a K_B of 10^5 M with single binding site. The present investigation uncovers the detailed binding propensities between metals and α-synuclein and has biological implications in PD.

Keywords: α-Synuclein; Copper; Iron; Wild type; Mutant forms; Thermodynamics; Isothermal titration calorimetry; Binding sites

Parkinson's disease (PD) is characterized by the aggregation of the α-synuclein peptide as insoluble fibrils (as Lewy bodies), rich in cross-β sheet structure [1–3]. α-Synuclein is a 140 amino acid, abundant presynaptic protein, which reversibly associates with membranes via a series of amphipathic α-helices located in its N-terminal region (residues 1–60) [4–7]. Additionally, two mutations (A53T and A30P) in the gene encoding α-synuclein have been linked to familial early-onset PD [8–12]. Recently a third missense mutation E46K, in α-synuclein is observed in an inherited form of dementia with Lewy bodies [13–15].

The mechanism of α-synuclein aggregation and the factors that favor aggregation under *in vivo* conditions still remain elusive. Li et al. [16,17] showed that mutants aggregate at a faster rate compared to wild type (WT). Hence, it has been shown that both familial PD mutations increase the rate of α-synuclein oligomerization [18]. The rate of mature fibril formation was increased by one mutation (A53T) and decreased by the other A30P mutation [16,17,19–21]. High resolution NMR data reveal that A30P mutation abolished a significant helical propensity found in an N-terminal stretch of the WT protein suggesting that it preferentially adopts a helical conformation in the WT and A53T protein, but not in the A30P mutant [22]. Recently, Li et al. [16,17] showed that A30P and A53T mutant α-synuclein might be more prone to form β-sheet structure than WT α-synuclein.

Metals have been proposed as major factors favoring α-synuclein aggregation [19–21]. α-Synuclein fibrillar deposits contain high concentration of redox active metals such as Fe, Cu and Zn, and their binding to the α-synuclein is reported [19–21]. Metals like Fe, Cu and Zn can promote or hinder fibrillogenesis [23–27]. The stoichiometry of binding of metals, its effect on both WT and mutant forms of α-synuclein in PD is still relatively unexplored.

To the best of our knowledge, there is no analysis of α-synuclein–metal interaction by isothermal titration calorimetry (ITC). Thus, no data on the reaction enthalpy

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and entropy are available which could clarify the role of α-synuclein–metal interactions in the etiology of neurodegenerative diseases. Here, we report for the first time, a thermodynamic study of α-synuclein–metal [Cu (II) and Fe (III)] interaction by isothermal titration calorimetry.

Materials and methods

α-Synuclein (WT) and the mutants (A30P, A53T and E46K) were purchased from rPeptides, USA and purity of peptide was ascertained by Mass Spectral data. $FeCl_3$ and $CuCl_2$ from Sigma Co. USA. Tris and Hepes buffers were purchased from Bangalore Genei, India.

Experimental design

Isothermal titration calorimetry studies. ITC experiments were carried out to determine the enthalpy changes of the effect of metal ions [Cu (II) and Fe (III)] on α-synuclein (WT and A30P, A53T, E46K mutant forms) with a VP-ITC instrument (MicroCal, Amherst, MA). The α-synuclein (monomer) concentration was maintained at 0.5 mg/ml in Hepes buffer (pH 7.4). This buffer was chosen because it has been shown to have a negligible interference with Cu (II) and Fe (III). In the experimental set-up, the cell was filled with α-synuclein (monomer) and the syringe was filled with metal ions. The same buffer (Hepes buffer, pH 7.4) was used in both the syringe and the cell. In another experimental set-up, the cell was filled with α-synuclein (monomer) and the syringe filled with Hepes buffer (pH 7.4) solution. Experiments with 29 injections of 10 μl with an initial delay of 60 s, duration of 20 s, spacing of 180 s were performed. To avoid the spontaneous aggregation of α-synuclein, no salt was used in the syringe.

Before the curve-fitting process, a background titration, consisting of the identical metal ion solution but only the buffer solution in the sample cell, subtracted from each experimental titration to account for the heat of dilution [28]. From the non-constrained fitting to the plot of heat evolved/mol of metal ions injected versus the molar ratio of metal ions to α-synuclein monomer, the binding stoichiometry (N), the binding enthalpy (ΔH) and the entropy (ΔS) were determined. The integration of the calorimeter signals, base-line corrections, and normalization with respect to α-synuclein and metal concentrations were done using MicroCal ORIGIN 6.0 software.

The corrected binding isotherms were fitted using least squares regression to obtain the association constant (K), the Cu (II) and Fe (III) binding to α-synuclein; stoichiometry-'N' and the enthalpy change associated with the interaction (ΔH) and the entropy (ΔS) [28]. All parameter uncertainties were evaluated at the 90% confidence level.

Results

Isothermal titration calorimetry, a calorimetric technique provides direct proof on thermodynamics of Cu (II) and Fe (III) binding to α-synuclein. Fig. 1 showed ITC measurements of α-synuclein (WT and mutant forms) monomer titrated by Cu (II) at 25 °C. Cu (II) titrations to α-synuclein monomer were accompanied by exothermic reaction. The enthalpy change as a function of the molar ratios of Cu (II) to α-synuclein monomer showed considerable binding. The binding constant (K_B) of Cu (II) reveals two binding sites with two binding constant values. K_B of α-synuclein (WT), is 5.9×10^5 and 7.5×10^4; A30P, 4.5×10^5 and 4.9×10^4; A53T, 3.7×10^5 and 6.1×10^4; and E46K is 3.9×10^5 and 4.2×10^4. The binding enthalpy, ΔH_1 and ΔH_2 are −1843 and −111 kcal/mol (WT); −4804 and −211 kcal/mol (A30P); −4355 and −410 kcal/mol (A53T); and −3715 and −276 kcal/mol (E46K). The entropy, ΔS_1 and ΔS_2 for α-synuclein (WT), A30P, A53T and E46K with Cu (II) reveals 7.6 and 10.5; 9.7 and 17.5; 10.5 and 20.5; 8.9 and 11.9 cal/mol K. The ITC data of Cu (II) with α-synuclein both WT and mutant forms possess two binding sites (Fig. 1A–D).

Fig. 2 revealed that titration of Fe (III) to α-synuclein was also accompanied by exothermic reaction. The enthalpy change as a function of the molar ratios of Fe (III) to α-synuclein monomer increased without a clear inflection point. ΔH values were extraordinarily large, which corresponds to an extremely exothermic process. Further, large negative entropy compensates for the exothermic enthalpy. The stoichiometry of binding, K_B of α-synuclein to Fe (III) were 4.9×10^5 (WT); 3.4×10^5 (A30P); 4.5×10^5 (A53T) and 3.3×10^5 (E46K). The binding enthalpy ΔH of WT, A30P, A53T, and E46K are −6.5; −8.4; −7.3 and −9.2 kcal/mol, respectively. The entropy ΔS were 14.6; 9.8; 20.8 and 6.3 cal/mol K for α-synuclein WT, A30P, A53T, E46K, respectively (Fig. 2A–D). The ITC data of Fe (III) with α-synuclein depicts a single binding site.

The results were fit best with a model in which the binding to each site has equal energy, using MicroCal ORIGIN 6.0 software.

Discussion

α-Synuclein fibrils are characterized by a cross-β-structure where β-strands are perpendicularly oriented to the axis of the fibrils [29]. Numerous *in vitro* studies have supported the concept that partially folded protein molecules are the precursors for the nucleation and growth of amyloid fibrils [30–32], even though the detailed molecular mechanism underlying fibril formation is not well defined. Kinetic studies showed that the α-synuclein formation is a nucleation-dependent reaction [33].

ITC is a sensitive method for the study of the heat effect of ligand binding to proteins or the study of protein association [34–38]. The shape of the ITC curves depends characteristically on the binding mode, which can be either construed as partition equilibrium or as a specific binding with a limited number of saturable binding sites [39–41]. Accordingly, a partition coefficient or a binding constant can be obtained, which yields the free energy and entropy of the binding reaction [41]. The structural features of Cu (II) and Fe (III) suggest that the binding most likely occurs through hydrophobic interactions, electrostatic interactions, or both [23–27]. However, in the present study ITC data showed that the formation of α-synuclein–metal complex was enthalpically driven, excluding hydrophobic interactions. There is only one study on the thermodynamics of α-synuclein lipid interaction by Nuscher et al. [41]. Further, they revealed that in the binding of α-synuclein to lipid bilayer membrane, heat release and the ellipticity were linearly correlated with binding and helix folding [41].

ITC titration curves of Cu (II) with α-synuclein (WT and mutant forms) reveals two binding sites on α-synuclein.

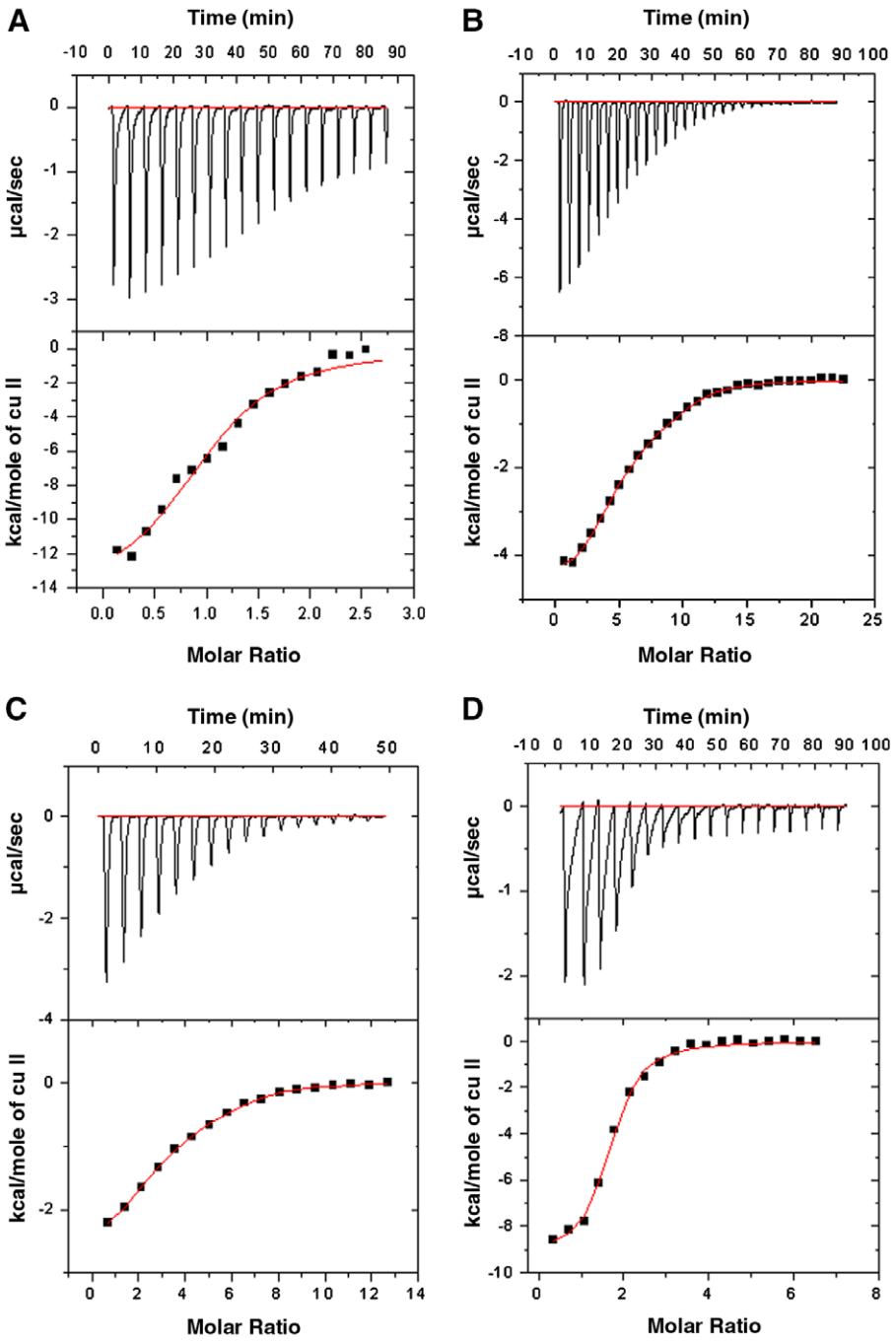

Fig. 1. (A,B) Binding of α-synuclein to Cu (II). The upper isotherm indicates ITC measurements at 25 °C of Cu (II) binding to α-synuclein. (A) α-Synuclein (WT); (B) A30P mutant. The lower sigmoidal curve symbolizes the molar heat values obtained by integration of individual heat flow signals as a function of the total metal/protein molar ratio in the calorimeter cell. Inset, binding isotherm. (C,D) Binding of α-synuclein to Cu (II). The upper isotherm indicates ITC measurements at 25 °C of Cu (II) binding to α-synuclein. (C) A53T mutant; (D) E46K mutant. The lower sigmoidal curve symbolizes the molar heat values obtained by integration of individual heat flow signals as a function of the total metal/protein molar ratio in the calorimeter cell. Inset, binding isotherm.

This is in accordance with the observations of Jankowska et al. [42,43] and Guilloreau et al. [43]. Further Jankowska and her coworkers [42–44] and Sung et al. [45] showed that the high-affinity binding site(s) for Cu (II) is located at the N-terminal domain, specifically the ^{1}–MDVFMKGLS–^{9} and ^{48}–VAHGV–^{52} regions. These new findings regarding the structural basis of Cu (II) interaction with α-synuclein provide a link between PD and other amyloid-related disorders and suggest that perturbations in metal homeostasis may constitute a widespread element in neurodegenerative disorders than previously recognized.

By comparing the two binding sites of Cu (II) to α-synuclein WT and mutant forms, it was also found that the high-affinity site has a smaller enthalpy but a larger

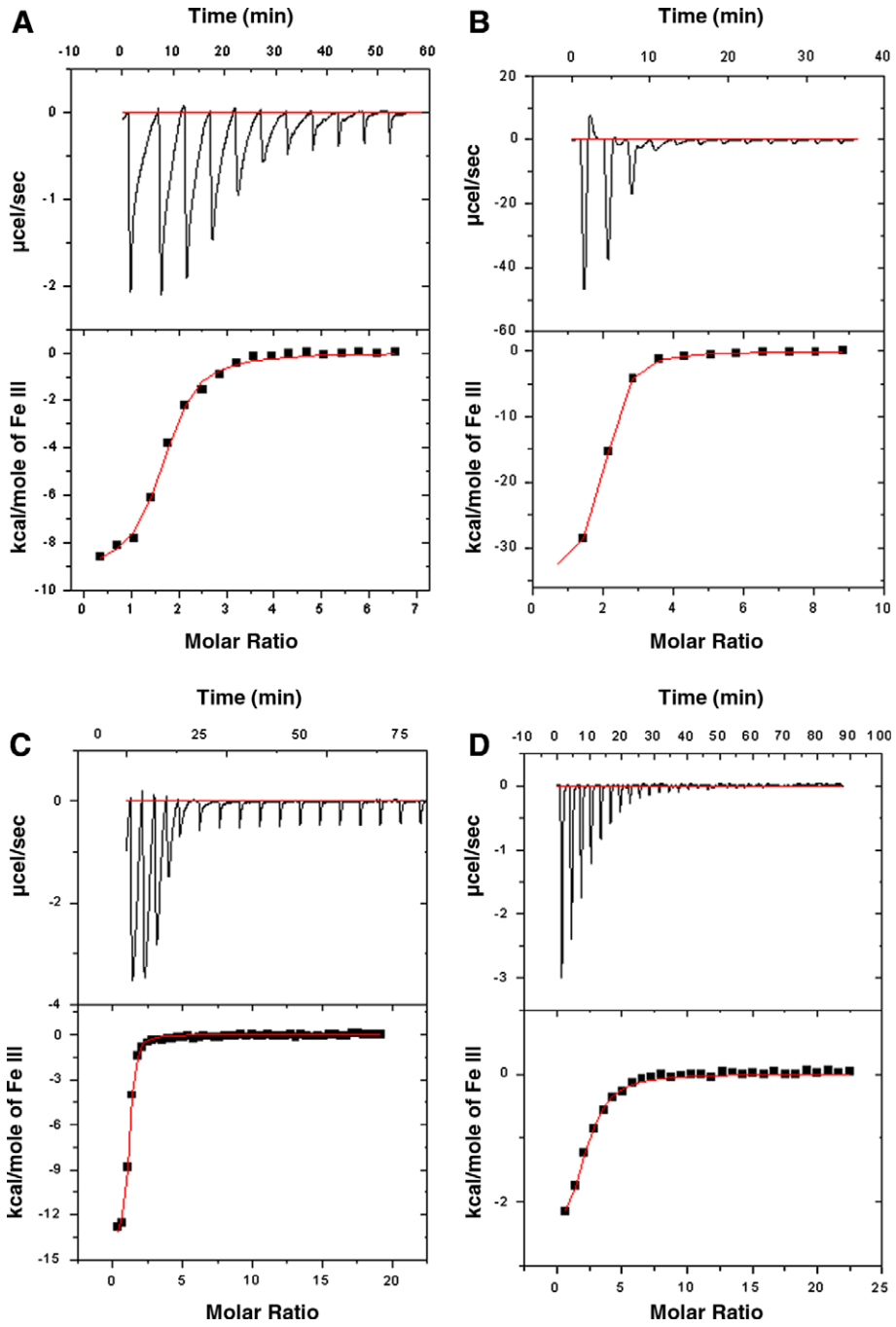

Fig. 2. (A,B) Binding of α-synuclein to Fe (III). The upper isotherm indicates ITC measurements at 25 °C of Fe (III) binding to α-synuclein. (A) α-Synuclein (WT); (B) A30P mutant. The lower sigmoidal curve symbolizes the molar heat values obtained by integration of individual heat flow signals as a function of the total metal/protein molar ratio in the calorimeter cell. Inset, binding isotherm. (C,D) Binding of α-synuclein to Fe (III). The upper isotherm indicates the ITC measurements at 25 °C of Fe (III) binding to α-synuclein. (C) A53T mutant; (D) E46K mutant. The lower sigmoidal curve symbolizes the molar heat values obtained by integration of individual heat flow signals as a function of the total metal/protein molar ratio in the calorimeter cell. Inset, binding isotherm.

entropic contribution. The higher entropic contribution of the binding of the first Cu (II) binding compared with that of the second could possibly be due to (i) release of more water molecules from the hydrated Cu (II) upon Cu (II)–α-synuclein complex formation; (ii) release of more water molecules from the peptide hydration shelf; (iii) conformational changes, i.e., lower degree of freedom upon binding of the second Cu (II), or (iv) entropic changes due to the protonation of the buffer [proton (s) originating from the ligand displaced by the Cu (II) binding].

There is limited data on the role of Fe (III) binding to α-synuclein. Golts et al. [46] reported that neuroblastoma BE-M17 cells treated with iron, induced α-synuclein aggregation and further proposed that α-synuclein aggregation

increases cellular iron content. However, the possible binding sites of Fe (III) on α-synuclein, which renders its aggregation and in turn cause cellular toxicity, needs to be explored.

ITC data on Fe (III) reveals that it possesses single binding site on α-synuclein. But the specific sequence for Fe (III) binding towards α-synuclein is still elusive. These analyses demonstrate the ability of ITC to characterize the ensemble of binding efficacy and the stoichiometry of binding of α-synuclein to metals in PD. The study is provocative in providing the information related to the thermodynamics of α-synuclein fibril formation in the presence of metals. In this study, we sought to delineate the effects of Cu (II) and Fe (III) on the native state of α-synuclein, and found the primary difference in the energies consumed to accelerate the kinetics of α-synuclein fibril formation. In the present case, the starting reagents are Cu (II)/Fe (III) and α-synuclein and the complex Cu (II)–α-synuclein or Fe (III)–α-synuclein complexes are the final product. But the differential energy conserved on binding of metals with the WT and the mutant forms still remains subtle.

Altogether, the data prove conclusively that Cu (II) has preferentially two model sites of binding to α-synuclein. Fe (III) possess single binding site and have high binding efficiency and Cu (II) is enthalpically driven with large entropy than Fe (III). Presumably, these findings might lead to a new conceptual scheme to understand the hierarchy of metal–α-synuclein interactions, based on the principles of thermodynamics, thus reflect a better mode to understand the binding properties of α-synuclein with metals in PD.

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References

[1] M. Goedert, Alpha-synuclein and neurodegenerative diseases, Nat. Rev. Neurosci. 2 (2001) 492–501.
[2] L. Maroteaux, J.T. Campanelli, R.H. Scheller, Synuclein: a neuron-specific protein localized to the nucleus and presynaptic nerve terminal, J. Neurosci. 8 (1998) 2804–2815.
[3] L.C. Serpell, J. Berriman, R. Jakes, M. Goedert, R.A. Crowther, Fiber diffraction of synthetic alpha-synuclein filaments shows amyloid-like cross-beta conformation, Proc. Natl. Acad. Sci. USA 97 (2000) 4897–4902.
[4] C.C. Jao, A. Der-Sarkissian, J. Chen, R. Langen, Structure of membrane-bound alpha-synuclein studied by site-directed spin labeling, Proc. Natl. Acad. Sci. USA 101 (2004) 8331–8336.
[5] J.M. George, The synucleins, Genome Biol. 3 (2002) 1.
[6] A. Iwai, E. Masliah, M. Yoshimoto, N. Ge, L. Flanagan, H.A. de Silva, A. Kittel, T. Saitoh, The precursor protein of non-A beta component of Alzheimer's disease amyloid is a pre-synaptic protein of the central nervous system, Neuron 14 (1995) 467–475.
[7] M.C. Irizarry, W. Growdon, T. Gomezisla, K. Newell, J.M. George, D.F. Clayton, B.T. Hyman, Nigral and cortical Lewy bodies and dystrophic nigral neurites in Parkinson's disease and cortical Lewy body disease contain alpha-synuclein immunoreactivity, J. Neuropathol. Exp. Neurol. 57 (1998) 334–337.
[8] R. Jakes, M.G. Spillantini, M. Goedert, Identification of two distinct synucleins from human brain, FEBS Lett. 345 (1994) 27–32.
[9] G. Vogel, Gene discovery offers tentative clues to Parkinson's, Science 276 (1997) 1973.
[10] M.H. Polymeropoulos, C. Lavedan, E. Leroy, S.E. Ide, A. Dehejia, A. Dutra, B. Pike, H. Root, J. Rubenstein, et al., Mutation in the alpha-synuclein gene identified in families with Parkinson's disease, Science 276 (1997) 2045–2047.
[11] N. Heintz, H. Zoghbi, Alpha-synuclein–a link between Parkinson and Alzheimer diseases? Nat. Genet. 16 (1997) 325–327.
[12] R. Kruger, W. Kuhn, T. Muller, D. Woitalla, M. Graeber, S. Kosel, H. Przuntek, J.T. Epplen, L. Scols, O. Riess, Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease, Nat. Genet. 18 (1998) 106–108.
[13] E.A. Greenbaum, C.L. Graves, A.J. Mishizen-Eberz, M.A. Lupoli, D.R. Lynch, S.W. Englander, P.H. Axelsen, B.I. Giasson, The E46K mutation in alpha-synuclein increases amyloid fibril formation, J. Biol. Chem. 280 (2005) 7800–7807.
[14] N. Pandey, R.E. Schimdt, J.E. Galvin, The alpha-synuclein mutation E46K promotes aggregation in cultured cells, Exp. Neurol. 197 (2006) 515–520.
[15] W. Choi, S. Zibaee, R. Jakes, L.C. Serpell, B. Davletov, R.A. Crowther, M. Goedert, Mutation E46K increases phospholipid binding and assembly into filaments of human alpha-synuclein, FEBS Lett. 576 (2004) 363–368.
[16] J. Li, V.N. Uversky, A.L. Fink, Effect of familial Parkinson's disease point mutations A30P and A53T on the structural properties, aggregation, and fibrillation of human alpha-synuclein, Biochemistry 40 (2001) 11604–11613.
[17] J. Li, V.N. Uversky, A.L. Fink, Conformational behavior of human alpha-synuclein is modulated by familial Parkinson's disease point mutations A30P and A53T, Neurotoxicology 4 (2002) 553–567.
[18] O.M. El-Agnaf, A.M. Bodles, D.J.S. Guthrie, P. Harriot, G.B. Irvine, The N-terminal region of non-A beta component of Alzheimer's disease amyloid is responsible for its tendency to assume beta-sheet and aggregate to form fibrils, Eur. J. Biochem. 258 (1998) 157–163.
[19] J.C. Rochet, K.A. Conway, P.T. Lansbury Jr., Inhibition of fibrillization and accumulation of prefibrillar oligomers in mixtures of human and mouse alpha-synuclein, Biochemistry 39 (2000) 10619–10626.
[20] K.A. Conway, J.D. Harper, P.T. Lansbury Jr., Accelerated in vitro fibril formation by a mutant alpha-synuclein linked to early-onset Parkinson disease, Nat. Med. 4 (1998) 1318–1320.
[21] L. Narhi, S.J. Wood, S. Stevenson, Y. Jung, G.M. Wu, D. Anafi, S.A. Kaufman, F. Martin, K. Sitney, P. Denis, J.C. Louis, J. Wypych, A.L. Biere, M. Citron, Both familial Parkinson's disease mutations accelerate alpha-synuclein aggregation, J. Biol. Chem. 274 (1999) 9843–9846.
[22] R. Bussell Jr., D. Eliezer, Residual structure and dynamics in Parkinson's disease-associated mutants of alpha-synuclein, J. Biol. Chem. 276 (2001) 45996–46003.
[23] R.M. Rasia, C.W. Bertoncini, D. Marsh, W. Hoyer, D. Cherny, M. Zweckstetter, C. Griesinger, T.M. Jovin, C.O. Fernandez, Structural characterization of copper (II) binding to alpha-synuclein: Insights into the bioinorganic chemistry of Parkinson's disease, Proc. Natl. Acad. Sci. USA 102 (2005) 4294–4299.
[24] V.N. Uversky, J. Li, K. Bower, A.L. Fink, Synergistic effects of pesticides and metals on the fibrillation of alpha-synuclein: implications for Parkinson's disease, Neurotoxicology 4-5 (2002) 527–536.

[25] G. Yamin, C.B. Glaser, V.N. Uversky, A.L. Fink, Certain metals trigger fibrillation of methionine-oxidized alpha-synuclein, J. Biol. Chem. 278 (2003) 27630–27635.
[26] V.N. Uversky, J. Li, A.L. Fink, Stabilization of partially folded conformation during alpha-synuclein oligomerization in both purified and cytosolic preparations, J. Biol. Chem. 276 (2001) 44284–44296.
[27] S.R. Paik, H.J. Shin, J.H. Lee, Metal-catalyzed oxidation of alpha-synuclein in the presence of Copper (II) and hydrogen peroxide, Arch. Biochem. Biophys. 378 (2000) 269–277.
[28] T. Wiseman, S. Williston, J.F. Brandts, L.-N. Lin, Rapid measurement of binding constants and heats of binding using a new titration calorimeter, Anal. Biochem. 179 (1989) 131–137.
[29] L.C. Serpell, C.C. Blake, P.E. Fraser, Molecular structure of a fibrillar Alzheimer's A beta fragment, Biochemistry 39 (2000) 13269–13275.
[30] H.A. Lashuel, B.M. Petre, J. Wall, M. Simon, R.J. Nowak, T. Walz, P.T. Lansbury Jr., Alpha-synuclein, especially the Parkinson's disease-associated mutants, forms pore-like annular and tubular protofibrils, J. Mol. Biol. 5 (2002) 1089–1092.
[31] M.J. Volles, P.T. Lansbury Jr., Zeroing in on the pathogenic form of alpha-synuclein and its mechanism of neurotoxicity in Parkinson's disease, Biochemistry 26 (2003) 7871–7878.
[32] D.M. Walsh, D.J. Selkoe, Oligomers on the brain: the emerging role of soluble protein aggregates in neurodegeneration, Protein Pept. Lett. 11 (2004) 213–228.
[33] M. Stefani, C.M. Dobson, Protein aggregation and aggregate toxicity: new insights into protein folding, misfolding diseases and biological evolution, J. Mol. Med. 81 (2003) 678–699.
[34] J. Kardos, K. Yamamoto, K. Hasegawa, H. Naini, Y. Goto, Direct measurement of the thermodynamic parameters of amyloid formation by isothermal titration calorimetry, J. Biol. Chem. 279 (2004) 55308–55314.
[35] Y.S. Kim, T.W. Randolph, M.C. Manning, F.J. Steven, J.F. Carpenter, Congo red populates partially unfolded states of an amyloidogenic protein to enhance aggregation and amyloid fibril formation, J. Biol. Chem. 278 (2003) 10842–10850.
[36] M.M. Lopez, D.H. Chin, R.L. Baldwin, G.I. Makhatadze, The enthalpy of the alanine peptide helix measured by isothermal titration calorimetry using metal-binding to induce helix formation, Proc. Natl. Acad. Sci. 99 (2002) 1298–1302.
[37] S. Govindarajan, R.A. Goldstein, On the thermodynamic hypothesis of protein folding, Proc. Natl. Acad. Sci. USA 95 (1998) 5545–5549.
[38] G. Hummer, S. Garde, A.E. Garcia, M.E. Paulaitis, L.R. Pratt, The pressure dependence of hydrophobic interactions is consistent with the observed pressure denaturation of proteins, Proc. Natl. Acad. Sci. USA 95 (1998) 1552–1555.
[39] M.M. Pierce, C.S. Raman, B.T. Nall, Isothermal titration calorimetry of protein–protein interactions, Methods 19 (1999) 213–221.
[40] K. Sasahara, H. Naiki, Y. Goto, Exothermic effects observed upon heating of beta2-microglobulin monomers in the presence of amyloid seeds, Biochemistry 45 (2006) 8760–8769.
[41] B. Nuscher, F. Kamp, T. Mehnert, S. Odoy, C. Haass, P.J. Kahle, K. Beyer, Alpha-synuclein has a high affinity for packing defects in a bilayer membrane: a thermodynamics study, J. Biol. Chem. 279 (2004) 21966–21975.
[42] T.K. Jankowska, A. Rajewska, K. Wisniewska, Z. Grzonka, J.J. Jezierska, Coordination abilities of N-terminal fragments of alpha-synuclein towards copper (II) ions: a combined potentiometric and spectroscopic study, J. Inorg. Biochem. 99 (2005) 2282–2291.
[43] T.K. Jankowska, A. Rajewska, E. Jankowska, K. Wisniewska, Z. Grzonka, Products of Cu (II)-catalyzed oxidation of the N-terminal fragments of alpha-synuclein in the presence of hydrogen peroxide, J. Inorg. Biochem. 100 (2006) 1623–1631.
[44] L. Guilloreau, L. Damian, Y. Coppel, H. Mazarguil, M. Winterhalter, P. Faller, Structural and thermodynamical properties of Cu (II) amyloid-beta16/28 complexes associated with Alzheimer's disease, J. Biol. Inorg. Chem. 8 (2006) 1024–1038.
[45] Y.H. Sung, C. Rospigliosi, D. Eliezer, NMR mapping of copper binding sites in alpha-synuclein, Biochim. Biophys. Acta 1764 (2006) 5–12.
[46] N. Golts, H. Snyder, M. Frasier, C. Theisler, P. Choi, B. Wolozin, Magnesium inhibits spontaneous and iron-induced aggregation of alpha-synuclein, J. Biol. Chem. 277 (2002) 16116–16123.