The production of hydrogen peroxide during early-stage protein aggregation: a common pathological mechanism in different neurodegenerative diseases?

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Abstract

By means of an ESR spin-trapping method, we have shown that Aβ (amyloid β), α-synuclein and various toxic forms of the prion protein all appear to generate H2O2 in vitro. A fundamental molecular mechanism underlying the pathogenesis of cell death in several different neurodegenerative diseases could be the direct production of H2O2 during the early stages of protein aggregation.

Introduction

The formation of amyloid fibrils from a range of different proteins and peptides is a common feature of numerous different ‘protein conformational’ diseases. In these diseases, normally, soluble proteins or their proteolytic fragments are deposited extracellularly in the form of approx. 10 nm fibrils with a characteristic cross-β-pleated sheet protein structure. In the systemic amyloidoses, these amyloid deposits are found in many different tissues and organs throughout the body. Localized amyloid deposits are found in some other diseases, including several important neurodegenerative diseases [1]. A key example of the latter is Alzheimer’s disease, one of the hallmark features of which is the accumulation in the brain of amyloid fibrils composed of Aβ (amyloid β). In some neurodegenerative diseases, fibrillar protein deposits are found inside neurons or glial cells. Examples of such deposits are the neurofibrillary tangles found in Alzheimer’s disease and the Lewy bodies found in Parkinson’s disease, which contain aggregated forms of tau and α-synuclein respectively. Aggregated forms of many amyloidogenic proteins are toxic to cells, implying a possible direct link between protein aggregation and pathological damage to the tissues in which they are found. Recent studies have suggested that intermediate ‘soluble oligomers’ could be a common toxic form of several different types of amyloid (see for example [2, 3]). However, the mechanism by which these oligomeric forms of various amyloidogenic proteins are toxic to cells is not clear. The most extensive studies on amyloid-mediated toxicity have been carried out with Aβ, and cell death caused by exposure to aggregated Aβ appears to be due to calcium influx and the induction of oxidative free radical damage. Several different hypotheses have been put forward to explain this toxic effect, including the formation of ion channels in cell membranes, the spontaneous fragmentation of Aβ to generate peptidy radicals [4] and the direct formation of H2O2 by the peptide [5]. According to the latter hypothesis, Aβ generates H2O2 from molecular oxygen by electron transfer involving bound redox-active metal ions [5].

Investigation into the generation of peptidyl radicals or H2O2 by ESR spectroscopy

The most commonly employed method for the detection of organic free radicals is ESR spectroscopy. This technique has high sensitivity and the correct interpretation of the hyperfine structure observed in the resulting spectra allows the nature of the radical(s) present to be clearly established. ESR spectroscopy is often employed in conjunction with the ‘spin-trapping’ technique in which the initial short-lived radicals react with either a nitrore or a nitroso compound to form a more stable nitroxy radical adduct. In order to investigate the possibility that free radicals might be generated spontaneously during the incubation of Aβ, Butterfield and co-workers [4] conducted experiments involving the prolonged incubation, over several days, of Aβ in the presence of the spin-trap PBN (N-tert-butyl-α-phenylnitrone). In these experiments, they detected the weak 4-line ESR spectrum of a true PBN adduct, but of tert-butylhydroaminoxyl, a product of PBN hydrolysis and oxidation. Our own experiments, using high purity PBN, have confirmed this result [6]. However, PBN in aqueous solution is prone to oxidation and hydrolysis and so these experiments could reflect the formation of H2O2 (a strong oxidizing agent) by the peptide, rather than the formation of peptidyl radicals. Alternatively, PBN would trap any hydroxyl radicals derived from the H2O2 generated by Aβ (see below) and the resulting PBN

Key words: Alzheimer’s disease, H2O2, neurodegeneration, Parkinson’s disease, prion, protein aggregation

Abbreviations used: Aβ, amyloid β; DMPO, S,S-dimethyl-1-pyrroline-N-oxide; PBN, N-tert-butyl-α-phenylnitrone; PHP, prion protein

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adduct is unstable and is readily transformed into tert-butylhydroaminoxyl. Thus these data using PBN do not unequivocally support the idea that Aβ can spontaneously generate peptidyl radicals [6].

In our laboratories, we have developed a sensitive ESR technique for detecting the generation of H₂O₂ from amyloidogenic proteins [7]. This technique involves the conversion of H₂O₂ into hydroxyl radicals by the addition of Fe(II) (i.e. the Fenton reaction):

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\text{Fe}(II) + \text{H}_2\text{O}_2 \rightarrow \text{Fe}(III) + \text{OH}^- + \text{OH}^-.
\]

The hydroxyl radicals are subsequently trapped by DMPO (5,5-dimethyl-1-pyrroline-N-oxide) and the characteristic DMPO hydroxyl radical adduct (DMPO-OH) is detected by ESR spectroscopy. Using this technique, we have shown recently that Aβ [7], α-synuclein [7] and certain toxic fragments of the PrP (prion protein; implicated in the transmissible spongiform encephalopathies) [8,9] can all liberate hydroxyl radicals following incubation in vitro, upon the addition of small amounts of Fe(II) (see Table 1). Under the same experimental conditions, the non-toxic peptides Aβ(1–40) Met35Nle and Aβ(40–1) lacked this ability, as did β-synuclein and γ-synuclein. The activity of α-synuclein seems to reside in the N-terminal half of the central 'NAC' (non-Aβ component of α-synuclein (residues 60–95)) region, which correlates very well with toxicity studies [12]. The PrP (106–126) peptide only generated an ESR spectrum in the presence of Cu(II) ions, which again is in accord with published toxicity data [8]. In the case of the PrP (121–231) fragment, only mutant forms associated with inherited prion disease were active in our ESR experiments [9]. Thus, so far, we have found that the ability of the various proteins and peptides under investigation to generate H₂O₂ correlates very well with their pathogenic and cytotoxic properties.

We have noted, in all of our experiments in which H₂O₂ is formed, that the variation in the relative intensity of the DMPO-OH ESR spectrum with peptide incubation time always follows a characteristic profile. There is a short delay time before the spectrum is first observed, after which its intensity rapidly increases to a maximum, before slowly decreasing back to zero. In the case of Aβ, our data clearly indicate that the generation of H₂O₂ occurs during the early
stages of the aggregation process, largely preceding the formation of mature amyloid fibrils. Ultrastructural studies have revealed the presence of structures resembling ‘soluble oligomers’ or ‘protofibrils’ during this early phase of H2O2 formation (M. German, unpublished work). Mature amyloid fibrils derived from Aβ did not generate H2O2.

**Conclusion**

Based on our results, we have concluded that the formation of H2O2 by an amyloidogenic protein, predominantly during its early stages of aggregation, could be a common mechanism of cell death in several different neurodegenerative diseases (Figure 1), and possibly other protein conformational diseases [10–12]. H2O2 is toxic to cells in its own right, but if formed in the vicinity of metal ions, such as Fe(II), would be converted via the Fenton reaction into the even more toxic and highly reactive hydroxyl radical. Exposure of cells to reactive oxygen species, such as H2O2 and hydroxyl radicals, would result in oxidative stress, and it is well established that this can lead to cell death. Metals have been observed at the sites of brain lesions in various neurodegenerative diseases and there is mounting evidence for oxidative damage to proteins, lipids and nucleic acids in affected areas of the brain in patients suffering from these diseases.

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**References**


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