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At Low Concentrations, 3,4-Dihydroxyphenylacetic Acid (DOPAC) Binds Non-Covalently to α-Synuclein and Prevents Its Fibrillation

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Several studies have shown that catecholamines can inhibit the fibrillation of α-synuclein (α-Syn), a small presynaptic protein whose aggregation is believed to be a critical step in the etiology of Parkinson's disease and several other neurodegenerative disorders. However, the mechanism of this inhibition is uncertain. We show here that substoichiometric concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC), a normal product of the metabolism of dopamine, can inhibit the fibrillation of α-Syn, due to non-covalent binding of DOPAC to α-Syn monomer. Intriguingly, the presence of α-Syn accelerates the spontaneous oxidation of DOPAC, and the oxidized form of DOPAC (the quinone) is responsible for the fibrillation inhibition. In addition, the presence of DOPAC leads to the oxidation of the methionine residues of α-Syn, probably due to the H_2O_2 production as a by-product of DOPAC oxidation. The lack of fibrillation results from the formation of stable oligomers, which are very similar to those observed transiently at early stages of the α-Syn fibrillation. A possible explanation for this phenomenon is that DOPAC stabilizes the normally transient oligomers and prevents them from subsequent fibril formation. The analysis of the α-Syn Y39W variant suggests that DOPAC binds non-covalently to the same N-terminal region of α-Syn as lipid vesicles, probably in the vicinity of residue 39. In contrast to the compounds with 1,2-dihydroxyphenyl groups (DOPAC and catechol), their 1,4-dihydroxyphenyl isomers (hydroquinone and homogentisic acid) are able to modify α-Syn covalently, probably due to the less steric hindrance in the Michael addition.

Keywords: α-synuclein; DOPAC; DOPAC-stabilized oligomer; amyloid fibril; oxidative modification

Introduction

The abnormal movements associated with Parkinson's disease (PD) result from the death of the specific dopaminergic neurons in the substantia nigra. The oxidative instability of dopamine and its several metabolites creates potentially greater predisposition for oxidative stress in the dopaminergic neurons, which is assumed to be associated with the particular vulnerability of these neurons. Several investigations have shown that catecholamines can inhibit the fibrillation of α-synuclein (α-Syn), a protein whose aggregation is believed to be a critical step in the etiology of PD and several related neurodegenerative diseases.

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† Prof. Anthony L. Fink has passed away on March 2, 2008.

Abbreviations used: DOPAC, 3,4-dihydroxyphenylacetic acid; α-Syn, α-synuclein; PD, Parkinson's disease; MS, mass spectrometry; ThT, thioflavin T; EM, electron microscopy; AFM, atomic force microscopy; SEC, size-exclusion chromatography; ESI, electrospray ionization; NBT, nitroblue tetrazolium chloride; FRET, fluorescence resonance energy transfer; HQ, hydroquinone; HGA, homogentisic acid; DETAPAC, diethylenetriaminepentaacetic acid.

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The diagnostic hallmark of PD is the presence of intracellular fibrillar inclusions, Lewy bodies and Lewy neuritis in the surviving dopaminergic neurons.[1–3] α-Syn, a 140-residue presynaptic protein of unknown function, is the primary component of these fibrillar inclusions.[3] *In vitro*, far-UV CD, Fourier transform infrared, fluorescence and NMR spectroscopies, as well as small-angle X-ray scattering and several hydrodynamic technique, show that α-Syn is intrinsically disordered.[4–6] Intrinsic disorder refers to the lack of a fixed structure in proteins, and many biologically active proteins were shown to remain unstructured, or incompletely structured, under physiological conditions.[7–34] Intrinsic disorder has been reported both at a region and at a whole protein level. There are several crucial differences between amino acid sequences of intrinsically disordered proteins and regions and structured globular proteins and domains.[9,24,35] Intrinsically disordered proteins are highly abundant in nature and the overall amount of disorder in proteins increases from bacteria to archaea to eukaryota.[36–39] These proteins carry out numerous biological functions, many of which obviously rely on high level of flexibility and lack of stable structure. These functions are diverse and complement those of ordered proteins and protein regions.[27–29] Many intrinsically disordered proteins are associated with human diseases such as cancer,[40] cardiovascular disease,[41] amyloidoses,[42] neurodegenerative diseases, diabetes and others.[34] Based on these intriguing links among intrinsic disorder, cell signaling and human diseases, suggesting that protein conformational diseases may result not only from protein misfolding but also from misidentification and missignaling, the "disorder in disorders" or D^2 concept was recently introduced.[34]

α-Syn assembles into filaments and fibrils after incubation under physiological conditions *in vitro*. Morphologies of these filaments and fibrils are similar to those extracted from the diseased brain.[43–46] A variety of factors, such as low pH, high temperature, naturally occurring polyamines and environmental PD risk factors (including heavy metals, pesticides and herbicides), accelerate fibrillation of α-Syn *in vitro*.[5,47–51] Substantial evidence indicates that α-Syn aggregation is a critical step in the etiology of PD,[51,52] as well as in a number of other neurodegenerative diseases, collectively known as synucleinopathies.[51] However, the question on whether the mature fibrils, protofilaments, protofibrils, specific oligomers or some folding intermediates are the neurotoxic species responsible for the cell death in these diseases is still a subject of great controversy.[53–58] Oligomers of α-Syn with globular, annular or chain-like conformations have been observed.[59,60] Some of these oligomers were suggested to form pores that can permeabilize membranes.[60–64] Since it is primarily dopaminergic neurons that appear to be affected in PD, there has been considerable speculation regarding the role of dopamine and its metabolites in the disease.

Although the normal function of α-Syn is still unknown, this protein is believed to be involved in regulation of the dopamine neurotransmission via effects on vesicular dopamine storage and trafficking.[65–69] Interestingly, dopamine and related compounds that have vicinal dihydroxy groups were shown to be the effective inhibitors of the α-Syn fibrillation.[70–74] It was proposed that α-Syn is able to form α-Syn–dopamine-quinone adducts with oxidized dopamine and that these adducts block the α-Syn fibrillation and stabilize the potentially most toxic α-Syn oligomers or protofibrils.[70] However, the mechanism of fibrillation inhibition by α-Syn–dopamine-quinone adducts remains uncertain because the yield of adducts is very low as observed by the lack of characteristic signals from the dopamine adducts detectable by mass spectrometry (MS).[70]

A better understanding of the mechanism of inhibition of α-Syn fibrillation by catechols is of particular interest for a number of reasons, including an attempt to understand whether the resulting oligomers are toxic.[75] It has been shown that the neurotoxicity of α-Syn is dopamine dependent[76] and that α-Syn also facilitates the toxicity of oxidized catechol metabolites.[77] SDS-PAGE analysis of α-Syn incubated with dopamine and other catechol compounds revealed a ladder of SDS-stable oligomers that suggested covalently cross-linked species. However, these effects were at concentrations of catechol compounds (0.18–2 mM) far greater than those occurring *in vivo*.[78] Recently, it has been shown that intracellular catechols such as dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) have the ability to modulate α-Syn aggregation in cultured human cells.[79] In particular, an increase in cytosolic catechol levels was associated with a decrease in α-Syn-containing inclusions, possibly through the formation of catechol-induced oligomeric intermediates.

Here, we take advantage of the good water solubility of DOPAC, a normal product of the dopamine metabolism, to show that (a) lower concentrations of DOPAC than those used in previous studies are sufficient to inhibit fibrillation of α-Syn, (b) DOPAC binds to α-Syn non-covalently at low concentration but the covalent modifications of α-Syn occur at higher concentrations, (c) DOPAC can oxidize methionine groups of α-Syn and (d) in the absence of DOPAC, the formation of transient oligomers preceded the α-Syn fibrillation. Although the α-Syn oligomers were also formed in the presence of low DOPAC concentrations, these oligomers did not assemble into fibrils.

Results

Unless otherwise noted, the conditions for α-Syn incubation were as follows: α-Syn was incubated in 20 mM phosphate, pH 7.4, and 100 mM NaCl, at 37 °C, with 70 μM α-Syn and agitation. Under these conditions, α-Syn formed fibrils with a lag phase of ~20 h, and fibrillation was complete by 35–40 h; the fibrillation kinetics were very sensitive to small changes in the rate of agitation.