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Functional characterization of alpha-synuclein protein with antimicrobial activity

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ABSTRACT

Alpha-synuclein (α-Syn), a small (14 kDa) protein associated with Parkinson's disease, is abundant in human neural tissues. α-Syn plays an important role in maintaining a supply of synaptic vesicles in presynaptic terminals; however, the mechanism by which it performs this function are not well understood. In addition, there is a correlation between α-Syn over-expression and upregulation of an innate immune response. Given the growing body of literature surrounding antimicrobial peptides (AMPs) in the brain, and the similarities between α-Syn and a previously characterized AMP, Amyloid-β, we set out to investigate if α-Syn shares AMP-like properties. Here we demonstrate that α-Syn exhibits antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. In addition, we demonstrate a role for α-Syn in inhibiting various pathogenic fungal strains such as *Aspergillus flavus*, *Aspergillus fumigatus* and *Rhizoctonia solani*. We also analyzed localizations of recombinant α-Syn protein in *E. coli* and *Candida albicans*. These results suggest that in addition to α-Syn's role in neurotransmitter release, it appears to be a natural AMP.

1. Introduction

Alpha-Synuclein (α-Syn) is a small soluble protein that is expressed in vertebrate neuronal cells and other tissues including the heart, skeletal muscle, pancreas and placenta [1–4]. It is an acidic protein of 140-amino acids with 3 distinct regions. The highly conserved N-terminal region (1–65) contains KTKEGV repeats, which fold into two amphipathic α-helical lipid-binding motifs that are quite similar to the lipid-binding domain of apolipoproteins [5]. The central region (66–95) is hydrophobic and referred to as the non-amyloid-β component of plaques. Finally, the less conserved C-terminal region (96–140) is rich in proline and the acidic amino acids, glutamic and aspartic acids, and is known to mediate many of α-Syn's protein-protein interactions [6–8]. α-Syn is a known component of Lewy bodies in Parkinson's disease (PD), Multiple System Atrophy (MSA) and is predominantly expressed in the nucleus of mammalian brain neurons with both soluble and membrane-bound forms [6,9,10]. Although the precise function of α-Syn is still unclear, several lines of evidence demonstrate its propensity to interact directly with negatively charged phospholipid vesicles or mitochondria-associated membranes. Through these interactions, α-Syn is believed to regulate lipid and calcium homeostasis [11–14]. α-Syn's propensity to interact with membranes is believed to be central to its role in neurotransmitter release [15,16] and synaptic vesicle trafficking [17–19].

During pathogenesis, α-Syn is commonly found in plaques with another small protein, amyloid-β. Amyloid-β, a membrane-binding protein with a host of known ligands in the brain, is regulated by environmental stressors and is capable of inducing an innate immune response. Recently, Soscia et al. reported that amyloid-β protein, a key mediator of Alzheimer's disease (AD), also serves as an antimicrobial peptide (AMP) with potent, broad-spectrum killing activity against several microorganisms [20]. This AMP

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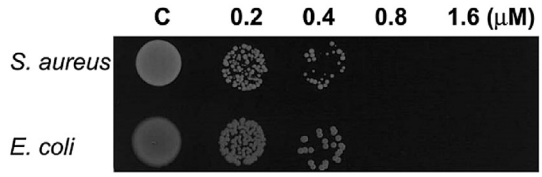

Fig. 1. Antibacterial activity of α-Syn against *S. aureus* and *E. coli*. The α-Syn protein of indicated concentrations was incubated with *S. aureus* and *E. coli* cells and spotted onto Mueller Hinton agar plates. 25 mM Hepes buffer (pH 7.2) was used as a control (c). The colony survival of bacterial cells was then analyzed after 24 h incubation at 37 °C. The data are representative of five experiments, all of which gave similar results.

activity may explain the link between amyloid-β and the innate immune system in the brain.

As α-Syn and amyloid-β share many characteristics, including membrane binding and the ability to induce an innate immune response, we sought to determine if the human α-Syn protein is also an AMP. For the first time, we demonstrate that α-Syn does indeed have antifungal and antibacterial properties. Based on our findings, we propose that the AMP properties of α-Syn may contribute to the emerging field of innate immunity in the human brain.

2. Materials and methods

2.1. Materials

Carboxytetramethylrhodamine succinimidyl ester and SYTOX-green was obtained from Molecular Probes (Eugene, OR). All other reagents were of analytical grade.

2.2. Cloning of the a-Syn gene and the expression of the protein in E. coli

The full-length gene of the protein was isolated from a human cDNA library using PCR and then ligated into the pET28a vector (Novagen). The His-tag fusion system was used to generate the recombinant protein in *Escherichia coli* strain BL21 (pLysS). The α-Syn protein was purified with a Ni-NTA agarose affinity gel. The eluted protein was dialyzed with 25 mM Hepes (pH 7.2).

2.3. Assay for antibacterial activity

The antibacterial activity of α-Syn toward *Staphylococcus aureus* (American Type Culture Collection; ATCC 25923), *E. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 15692) and *Staphylococcus epidermidis* (ATCC 12228) was assessed using a microdilution assay performed in a plate according the method previously described [21].

2.4. Assay for antifungal activity

We used a radial growth inhibition assay to analyze the antifungal activity of the protein. We also carried out a microdilution assay in a plate in order to evaluate the effective concentration, as described previously [22]. The following fungal strains were used: *Aspergillus flavus* (KCTC 6905), *Aspergillus fumigatus* (KCTC 6145), *Aspergillus parasiticus* (KCTC 6598), *Candida albicans* (KCTC 7270), *Candida tropicalis* (KCTC 7221), *Filobasidiella neoformans* (KCTC 7003) and *Trichoderma harzianum* (KCTC 6043) were obtained from Korea Collection for Type Cultures, and *Rhizoctonia solani* (KACC 40138) were obtained from Korea Agricultural Culture Collection.

2.5. Confocal laser scanning microscopy

We used confocal laser scanning microscopy to analyze the cellular distribution of the α-Syn protein in *E. coli* and *Candida albicans*. Cell suspensions (10^4 conidia/ml) were put on poly-L-lysine-coated glass slides and the slides were incubated at RT for 45 min for cell adhesion to the slides. After PBS washing, rhodamine-labeled α-Syn was added to the cells. The slides were rinsed several times with PBS and then examined using a Zeiss (Gottingen, Germany) laser scanning microscope (LSM 510META).

2.6. SYTOX-green uptake ability of α-Syn in E. coli cells

E. coli cells grown to mid-logarithmic phase at 37 °C were suspended (2×10^7 cells/ml) in 10 mM sodium phosphate buffer (pH 7.2). The cells were then incubated with 1 μM SYTOX green for 15 min in the dark. After the addition of α-Syn protein with serial diluted concentrations, the time-dependent increases in fluorescence caused by the binding of the cationic dye to intracellular DNA were monitored (excitation wavelength, 485 nm; emission wavelength, 520 nm).

3. Results and discussion

α-Syn closely resembles amyloid-β in many features, including membrane binding and the formation of uncharacterized pore in β-

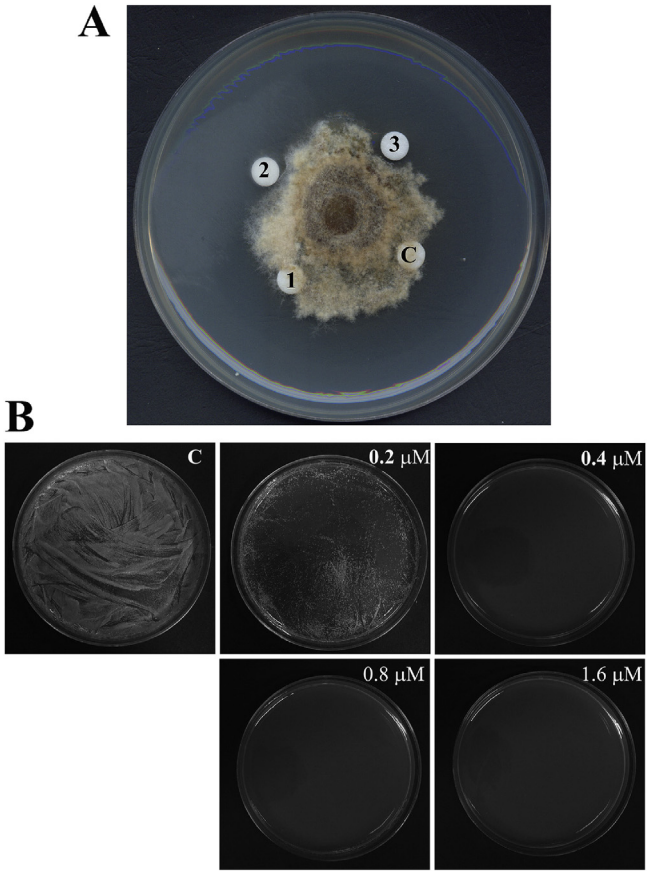

Fig. 2. Antifungal activity of α-Syn against fungal cells. Purified α-Syn protein was subjected to radial growth inhibition tests with *R. solani* (A), and *C. albicans* (B). (A) Paper disks were loaded with buffer alone as negative control (disk C; 25 mM Hepes buffer, pH 7.2) or with 1.6 μM (disk 1), 3.2 μM (disk 2), or 6.4 μM (disk 3) of purified α-Syn protein. (B) The *C. albicans* colony was then analyzed after 24 h incubation at 28 °C in the absence (c, 25 mM Hepes buffer, pH 7.2) or presence of α-Syn protein.