POTENTIAL PROTECTIVE MECHANISM OF CROCIN AGAINST AMYLOID FIBRIL FORMATION OF E46K A-SYNUCLEIN IN PARKINSON DISEASE

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Abstract - The E46K mutation of the α-synuclein gene has been linked to autosomal dominant early-onset of PD. Crocin is a carotenoid chemical compound of saffron that has been shown antioxidant and neural protective activity. This study examined the effect of Crocin in preventing the amyloid fibril in the E46K α-synuclein, through in vitro studies and computational simulations. These results support that Crocin is a potent inhibitor of E46K α-synuclein fibrillization and it could be considered as a potential therapeutic agent in the treatment of Parkinson disease.

Index Terms - Crocin, E46K α-Synuclein, Amyloid, Protection.

I.INTRODUCTION

Parkinson's disease (PD) is a chronic and progressive of the nervous system disorder that affects movement. It is caused by a loss of nerve cells in the area of the brain called the substantia nigra [1]. The hallmark sign of Parkinson's disease is clumps of a protein α-synuclein, which is the main component of Lewy bodies. α-Synuclein is a protein that predominantly found in the human brain but smaller amounts are found in the heart, muscles, and other tissues [2]. Crocin is a water-soluble carotenoid pigment of saffron (Crocus sativus L.) that is responsible for its color. Saffron has been used as a spice for flavoring and coloring food preparations. Many studies have examined the pharmacological properties of Crocin and have shown that Crocin has memory improving properties [3]. Considering neuroprotective functions of Crocin, this study aimed to investigate the effectiveness, function and mechanism of Crocin in preventing amyloid fibril formation in E46K α-synuclein related with Parkinson disease.

II.MATERIAL AND METHODS

2.1 Fibril formation and kinetic evaluation

Amyloid fibril formation of E46K mutant forms of α-synuclein was performed in the presence and absence of crocin at different molar ratios (1:0, 1:0.25, 1:0.5) by sampling aliquots of misfolding reaction at different time point.

2.2 Light scattering measurements

A 10 µL aliquot of each sample contain α-synuclein alone and in the presence of crocin at 1:0.25, 1:0.5 molar ratio of α-synuclein: crocin was added to 0.99 ml of phosphate buffer (50 mM, pH 7.4). A 90° light cattering measurement of samples were taken in a quartz cuvette using a Varian spectrofluorimeter (Varian, USA) with both excitation and emission wavelengths set at 450 nm and a 2.5 nm slit width.

2.3 Fluorescence spectroscopy

To detect hydrophobicity changes in the α-synuclein structure, an ANS-binding assay of the α-synuclein (0.8 mg/mL) alone and in the presence of two different concentrations of crocin in molar ratios was done. Fluorescence titration experiments were performed on a Varian spectrofluorimeter (Varian, USA) at 37ºC. The experiment was done in a 50 mM phosphate buffer, pH 7.4.

A.Result

The ability of α-synuclein to form amyloid fibril and the chaperone ability of crocin to prevent amyloid fibril formation was investigated by ThT binding assay. Thioflavin T (ThT) is a dye fluorescence that binds selectively to β-sheet structure in amyloid fibril and has been chosen as a technique to monitor fibril formation and kinetic evaluation [Biancalana 2010].
ThT study of α-synuclein showed increase in fluorescence intensity during the exponential phase (figure 1). Addition of crocin at 1:0.25 molar ratio of α-synuclein: crocin decreased the ThT fluorescence intensity about 26.7 %. By increasing the concentration of crocin to 0.5 molar ratio, a further decrease in fluorescence intensity was observed (about 73.33%) which is indicative the efficient concentration-dependent inhibition of fibril formation (Figure 1A). This is also evident from the first order rate constant which is in Table 1

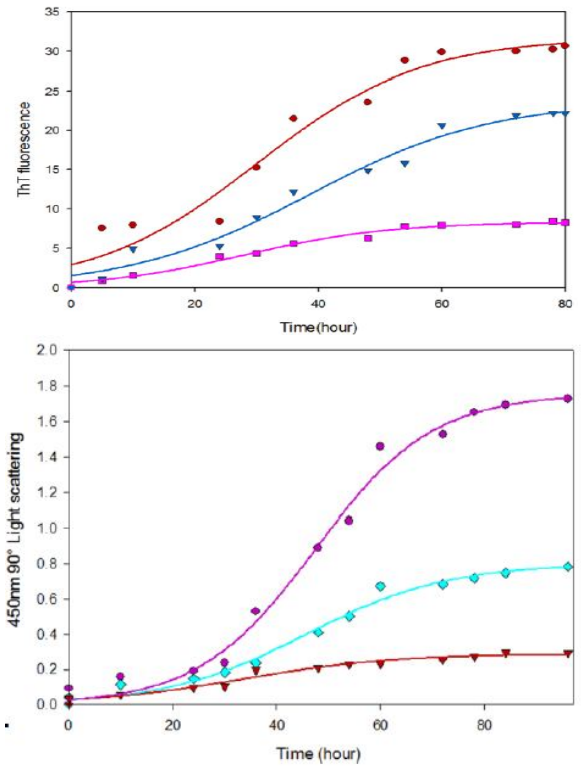

Figure 1: (a) Amyloid fibril formation of E46K α-synuclein (0.8 mg/mL) in the absence (●) and presence of two different concentration of crocin (1:0.5 ■, 1:0.25 ▼). (b) 90° static light scattering follows the increase in particle size of E46K α-synucleain (0.8 mg/mL) over time in the absence (●) and presence of two different concentration of crocin (1:0.5 ▼, 1:0.25 ♦). The protein was in a 50 mM phosphate buffer, 37 °C and pH 7.4. The ratios indicated are of E46K α-synuclein: crocin, in an (M : M) ratio.

Table 1- Summary of rate constants for α-synuclein in Thioflavin-T binding assays

Sample	Rate Constant × 10^{-2} (min^{-1})
α-syn	7.1089 ±0.0004
α-syn: crocin (1:0.25)	4.65643±0.0001
α-syn: crocin (1:0.5)	2.07114±0.0001

. To confirm the thioflavin T binding result, static light scattering (SLS) was done to monitor the effect of crocin on E46K α-synuclein aggregation. As shown in Fig 1B, light scattering of E46K α-synuclein decreased in the presence of crocin in a dose-dependent manner indicating decrease in the aggregation of protein as compared to the control. These data are consistent with those from the ThT binding assay, and demonstrate that crocin inhibited E46K α-synuclein aggregation.

III. DISCUSSION

There is no drug or other types of treatment to effectively treat Parkinson's disease. All currently used therapeutic medications are able to control only disease symptoms and have many side effects. Recently, much attention has been focused on medicinal plants in preventing neurological diseases, due to their lower side effects (Ghahghaei, et al., 2013; Jiang, et al., 2010; Moshiri et al., 2006). In the present study, the effectiveness, function, and mechanism of Crocin against the amyloid fibril formation of E46K α-synuclein have been studied using both experimental and computational simulation analysis.

In this study, amyloid fibril formation of E46K α-synuclein and its interaction with Crocin has been investigated using Thioflavin T-binding assay. By incubation of E46K α-synuclein at a temperature of 37 ºC, a time dependent increase in the fluorescence intensity were observed indicating formation and accumulation of amyloid fibrils (Greenbaum, et al., 2005; Jiang, et al., 2010; Rekas et al., 2004; Waudby et al., 2010). Crocin dose-dependently inhibited amyloid formation by incubated E46K α-synuclein as proved by the reduction in Thioflavin-T fluorescence intensity. Static light scattering, also showed that Crocin caused a dramatic reduction in E46K α-synuclein aggregation.

α-Synuclein is a natively unfolded protein with little or no ordered structure (Uversky, Li, & Fink, 2001; Weinreb, Zhen, Poon, Conway, & Lansbury, 1996). It is believed that the core hydrophobic region of α□synuclein (NAC) (residue 66-74) and the negative charges at the C-terminal region, is responsible for fibrillation and cytotoxicity of α-synuclein and formation of cross β□structures (Rajagopalan and Andersen, 2001; Rodriguez, et al., 2015). The formation of α-synuclein amyloid fibrils occurs in a nucleation-dependent manner, so that its early stage is formation of partially folded intermediate known as pre-molten globule-like. Such intermediates are in rapid equilibrium with its monomeric form that is critical for α-synuclein fibrillation (Uversky, et al., 2001). It has been shown that a decrease in pH or an increase in temperature enforced α-synuclein to gain some ordered structure.

In this condition, conversion of partially folded intermediate to the amyloidogenic nucleus and subsequent propagation into fibrils has been occurred (Uversky and Eliezer, 2009). It has been suggested that this partially folded intermediate have significant nonpolar patches on their surface, which leads to hydrophobic interactions between the molecules and, as a result, leads to protein aggregation (Fink, 2006).

Therefore, the protective ability of Crocin may be due to its interaction with the hydrophobic surface area. Most likely Crocin interacts with the hydrophobic surface area of α-synuclein on the fibrillation pathway, and blocking its conversion into fibrils. This hypothesis is supported by the

observations that the ThT intensity decrease in the presence of increasing amounts of Crocin, indicating fewer fibrils formed in the presence of Crocin. In addition, TEM images show that the presence of Crocin completely eliminates the amyloid fibril and leads to increasing amounts of amorphous aggregate. There are many factor that prevents amyloid fibril formation in α-synuclein by stabilizing the monomer or oligomeric form (Fink, 2006; Goedert, 1999; Uversky, 2007). As the hydrophobic areas are absent in the natively unfolded monomeric α-synuclein, so it is likely that Crocin interact with a oligomeric precursor specious which has hydrophobic cluster area (Lashuel, Overk, Oueslati, & Masliah, 2013).

CONCLUSION

In conclusion, the present study examined the effect of Crocin on the fibril formation of E46K α-synuclein in vitro via both experimental and molecular docking. Our experiments results showed that Crocin had a potentially inhibitory effect against amyloid fibril formation of E46K α-synuclein in a concentration-dependent manner. Crocin applies its chaperone activity by binding to a partially exposed aggregation-prone region of E46K α-synuclein and inhibiting the structural change, thereby protecting the protein from amyloid fibril nucleation. The results of docking simulations also confirm the interaction of Crocin with the NAC and the C-terminal region of the E46K α-synuclein which supports the central role of the Crocin in recognizing and preventing interaction of the E46K α-synuclein with oligomeric precursor specious and subsequently fibril. The result from this study indicates that Crocin should be considered as a possible drug to prevent or delay protein aggregation and thus amyloid formation in Parkinson disease.

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