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Interaction between β-amyloid and lens αB-crystallin

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Abstract In Alzheimer's disease, β-amyloid peptides (βA_{1-40} and βA_{1-42}) are deposited on the brain cell surfaces as neurotoxic plaques. Some reports indicate that small heat shock proteins, Hsp27 and αB-crystallin, colocalize in the plaques, but their functions are not known. Interaction between βA and αB-crystallin must be determined in order to understand the role of αB-crystallin in βA fibril formation. We used a pyrene (Pyr)-labeled βA_{1-40} in a fluorescence energy transfer experiment. Upon incubation together at 37°C, energy transfer between Trp of αB-crystallin and Pyr of Pyr-labeled βA was observed, indicating that βA participated in subunit exchange of αB-crystallin, which promoted fibril formation.

Key words: β-Amyloid; αB-Crystallin; Fluorescence; Fluorescence energy transfer; Circular dichroism

1. Introduction

In Alzheimer's disease (AD), the plaque around the brain cell surface contains mainly aggregated β-amyloid (βA) and is responsible for neurotoxicity [1,2]. The βA peptides 1–40 and 1–42 sequences are the proteolytic degraded products of the amyloid precursor protein. Some reports indicate that the small heat shock proteins Hsp27 and αB-crystallin colocalize in the plaques [3–6]. αB-Crystallin is one of two components of lens α-crystallin, the other being αA-crystallin. While αA-crystallin is more or less lens specific, αB-crystallin is widely expressed in other tissues and its expression increases with neurodegenerative diseases [7,8]. The role of αB-crystallin in the development of these diseases is largely unknown but may be related to its functional role as a small heat shock protein [9,10]. Recently, α-crystallin was reported to act as a chaperone molecule capable of protecting other proteins from denaturation [11,12]. Whether this chaperone function is active in vivo either in the lens or in other tissues is unknown. To understand the effect of αB-crystallin on βA fibril formation, we need to determine whether they interact to each other. In the present study, we used fluorescence energy transfer for this purpose. A pyrene derivative dye was labeled to βA to serve as an acceptor, while Trp in αB-crystallin served as a donor. The results indicated that there was an interaction between βA and αB-crystallin when they were incubated together. A possible mechanism for this interaction and its implicated significance in vivo were discussed.

2. Materials and methods

2.1. Materials

Human lens recombinant αB-crystallin was prepared as described elsewhere [13]. βA peptides (βA_{1-40} and βA_{1-42}) were purchased from AnaSpec (San Jose, CA, USA). Fluorescence probes thioflavin T (ThT) and 1-pyrenebutanoic acid succinimidyl ester (Pyr) were obtained from Sigma (St Louis, MO, USA) and Molecular Probes (Eugene, OR, USA), respectively.

Monomer βA was prepared by dissolving βA in 50 mM phosphate buffer, pH 7.4. βA_{1-40} is soluble, and a fresh sample is in a monomer state; βA_{1-42} is apparently in an aggregate state. At the same concentrations and measuring conditions, ThT fluorescence is much greater for βA_{1-42} than for βA_{1-40} (Fig. 1). Mild sonication of βA solutions decreased ThT fluorescence for βA_{1-42} but not for βA_{1-40}. For convenience and easy handling, only βA_{1-40} was used in the present study. αB-Crystallin was also dissolved in the same phosphate buffer.

2.2. Fluorescence

Fluorescence was measured with an RF-5301PC Shimadzu spectrofluorometer (Shimadzu, Columbia, MD, USA). ThT ($\varepsilon = 26.62$ at 416 nm) fluorescence was scanned between 460 and 550 nm with excitation wavelength 450 nm [14,15]. Aliquots (20 μl) of ThT (30 μM) were added to 3 ml of protein samples and allowed to sit at room temperature for 15 min before measurement. The titration was continued until saturation was reached.

Labeling of Pyr to βA was performed by a method described in the supplier's production information. To label mainly the terminal amine, a buffer close to neutral pH was used. In brief, 2 mg βA was dissolved in 1 ml of 0.1 M sodium bicarbonate, pH 7.2; 100 μl of Pyr solution (0.5 mg in 0.5 ml DMSO) was added and incubated at room temperature for 1 h. The reaction was stopped by adding 100 μl of fresh 1.5 M hydroxylamine. The conjugated βA was separated from unconjugated βA in a column of Sephadex G-10. The ratio of labeling was estimated to be approximately 1 (molar ratio).

Upon excitation at 340 nm, an emission spectrum with three emission peaks at 370, 390, and 420 nm were observed for Pyr [16]. In the fluorescence resonance energy transfer experiment, the Trp in the unlabeled αB-crystallin served as a donor and Pyr in the labeled βA as an acceptor.

2.3. Far-UV circular dichroism (CD)

CD spectra were measured with an Aviv Circular Dichroism Spectrometer (model 60 DS; AVIV, Lakewood, NJ, USA). Five scans were averaged and smoothed by a polynomial-fitting program. The CD intensity was expressed as mdeg.

3. Results

3.1. ThT fluorescence

ThT is widely used in the detection of fibril formation of βA, and ThT fluorescence intensity is correlated with the aggregated state of the protein rich in β-sheet conformation

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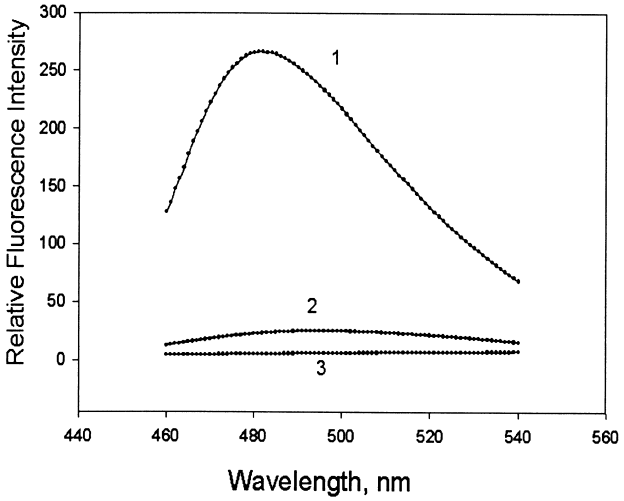

Fig. 1. ThT fluorescence of freshly prepared βA_{1-42} and βA_{1-40} peptides: 1, βA_{1-42}; 2, sonicated βA_{1-42} and 3, fresh βA_{1-40}. Concentrations of βA and ThT are 2 and 5 μM, respectively. Excitation wavelength is 450 nm.

[14,15]. Fig. 2A showed the increased ThT fluorescence intensity of βA by the presence of αB-crystallin after incubation for 24 h at 37°C. The control was the mixture of βA and αB-crystallin incubated separately.

The increased ThT fluorescence intensity was dependent on the amount of αB-crystallin in the incubation mixtures (Fig. 2B), in which the concentration of αB-crystallin increased while the amount of βA was fixed. The increase was insignificant with αB-crystallin incubated alone.

3.2. Far-UV CD

The far-UV CD for the individual βA_{1-40} and αB-crystallin is shown in Fig. 3A. αB-Crystallin displayed a far-UV CD characteristic of β-sheet conformation [13]. Non-aggregated βA was reported to exist mostly in random coil [17,18]. Our CD shows that the fresh βA_{1-40} samples existed in random coil conformation and old samples in β-sheet.

The corresponding CD for the mixture of βA_{1-40} and αB-crystallin is shown in Fig. 3B. Similar to ThT fluorescence, CD intensity was greater for the mixture of βA_{1-40} and αB-crystallin incubated together than for mixed substance incubated separately. Fibril formation of βA was accompanied by conversion of random coil to β-sheet.

3.3. Interaction between βA and αB-crystallin

Fig. 4A shows the Trp fluorescence spectrum for αB-crystallin (λ_{ex} at 295 nm) and the Pyr fluorescence spectrum for labeled βA (λ_{ex} at 340 nm). Because of the overlap between Trp emission and Pyr excitation, energy transfer may occur between these two probes if there is interaction between αB-crystallin and βA. Fig. 4B shows the decreased Trp fluorescence intensity and increased Pyr fluorescence intensity after they were incubated up to 48 h at 37°C. The changes were time-dependent, but intensity increase was complicated by aggregation-related scattering for samples incubated longer than 24 h. Samples incubated at room temperature or 4°C did not exhibit energy transfer.

4. Discussion

The increased ThT fluorescence in βA fibril formation was reported to be due to a conversion of β-sheet conformation [14]. Our results of increased ThT fluorescence clearly indicated that αB-crystallin promoted the formation of β-sheet in βA. The conversion of the helical and random structure of βA to β-sheet structure was also evident by the increased far-UV CD. A similar study of increased ThT fluorescence has been reported by Stege et al. [19]; they also show that αB-crystallin increases the βA toxicity in the cultured neurons. They suggest that intermediates during βA fibrillogenesis, called protofibril, may associate with α-crystallin. The mechanisms of the association, however, are unknown.

One report indicates that Hsp25 and Hsp27, but not α-crystallin, inhibit in vitro βA_{1-42} fibril formation [20]. A recent report, however, shows that both Hsp25 and α-crystallin promote βA_{1-40} fibril formation [19]. The latter report also

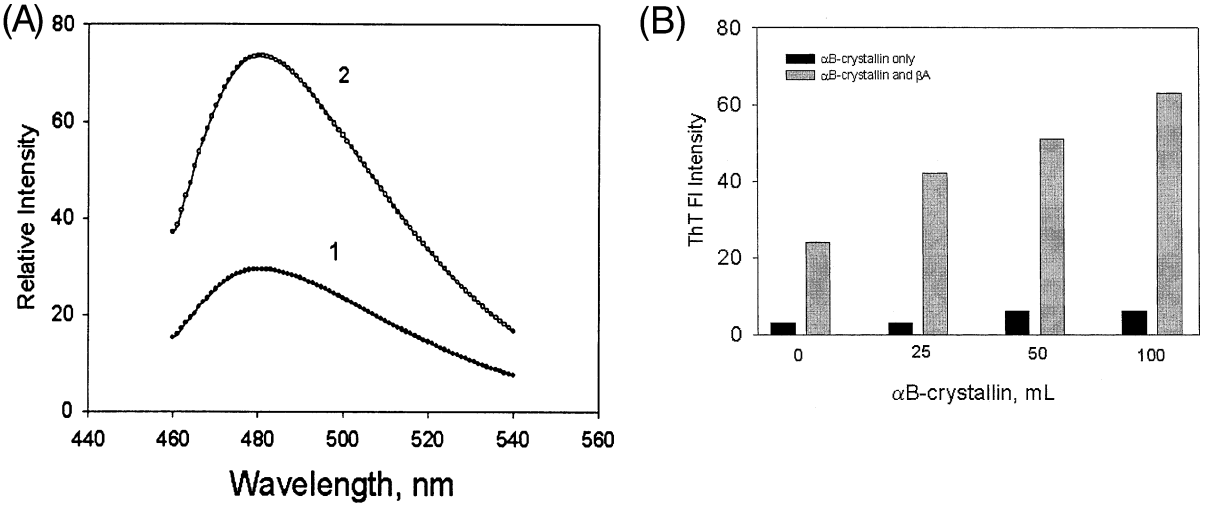

Fig. 2. A: ThT fluorescence of the mixture of βA_{1-40} and αB-crystallin. Spectrum 1 is the mixture of separately incubated βA_{1-40} and αB-crystallin (1:1 ratio) and spectrum 2 is the mixture of βA_{1-40} and αB-crystallin incubated together at 37°C for 24 h. Concentrations of βA_{1-40} and αB-crystallin are 8 μM and 0.08 mg/ml, respectively. ThT concentration is 2 μM. B: Effect of αB-crystallin on the ThT fluorescence intensity of βA_{1-40} after incubation at 37°C for 24 h. Increasing amount of αB-crystallin solution (0.08 mg/ml) was added to 1 ml βA solution (8 μM). The data were an average of two independent experiments and the difference between them did not exceed 5%.

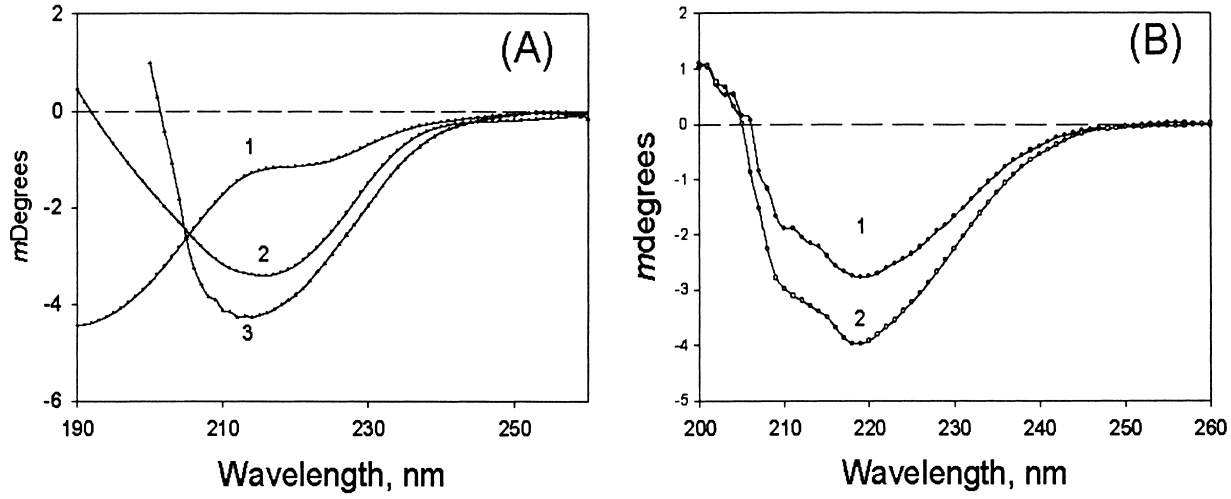

Fig. 3. Far-UV CD of βA_{1-40} and αB-crystallin. A: CD of individual components: 1 and 2, βA_{1-40} (46 μM, fresh and old samples); 3, αB-crystallin (0.17 mg/ml). Cell pathlength is 1 mm. B: CD of mixture of βA_{1-40} (4 μM) and αB-crystallin (0.04 mg/ml) with 5 mm cell, before and after incubation (curves 1 and 2) at 37°C for 24 h.

shows that, rather than fibrillar, it is non-fibrillar aggregates that are responsible for the neurotoxicity. Since we do not have EM data, it is not known whether αB-crystallin promoted βA fibril formation in our study. However, increased ThT fluorescence and far-UV CD clearly indicated increased β-sheet conformation (Figs. 2 and 3), which in a protein generally facilitates aggregation and insolubilization. In our study of human lens HMW α-crystallin aggregates, greater β-sheet conformation was observed than with LMW α-crystallin [21]. We believe that mechanisms similar to α-crystallin HMW aggregation may occur between βA and αB-crystallin.

Our fluorescence energy transfer study indicated that there was an interaction between βA and αB-crystallin. The nature of the interaction is probably that βA participates in the subunit exchange in the αB-crystallin. The dynamic structure of α-crystallin was reported first by van den Oetelaar et al. [22] and later by many other investigators [23–25]. We have reported subunit exchange in the α-crystallin by fluorescence energy transfer using a dye-labeled αA-crystallin mutant W9F as an acceptor and Trp in the wild-type αA-crystallin as a donor [25]. The results indicate that the dye-labeled subunits are in the structure of the wild-type αA-crystallin; the dye probes in the W9F mutant are sufficiently close to Trp residues in the wild-type αA-crystallin to produce an energy transfer. The present observation of energy transfer between the dye-labeled βA and αB-crystallin clearly indicates that βA participated in subunit exchange. The exchange occurs when βA aggregates to sufficiently large size structures, possibly like reported intermediate protofibrils [26].

Although it is not clear how the cytoplasmic αB-crystallin is expressed or migrated to cell surface and colocalizes with βA in AD plaques, one study has shown that βA is present in the human lens and may be involved in cataract formation by facilitating HMW aggregation of α-crystallin [27].

AD is one of the protein condensation diseases that share the common phenomenon or etiology of protein aggregation as a stimulating factor [28]. To understand the mechanisms that promote or inhibit aggregation is very important in the

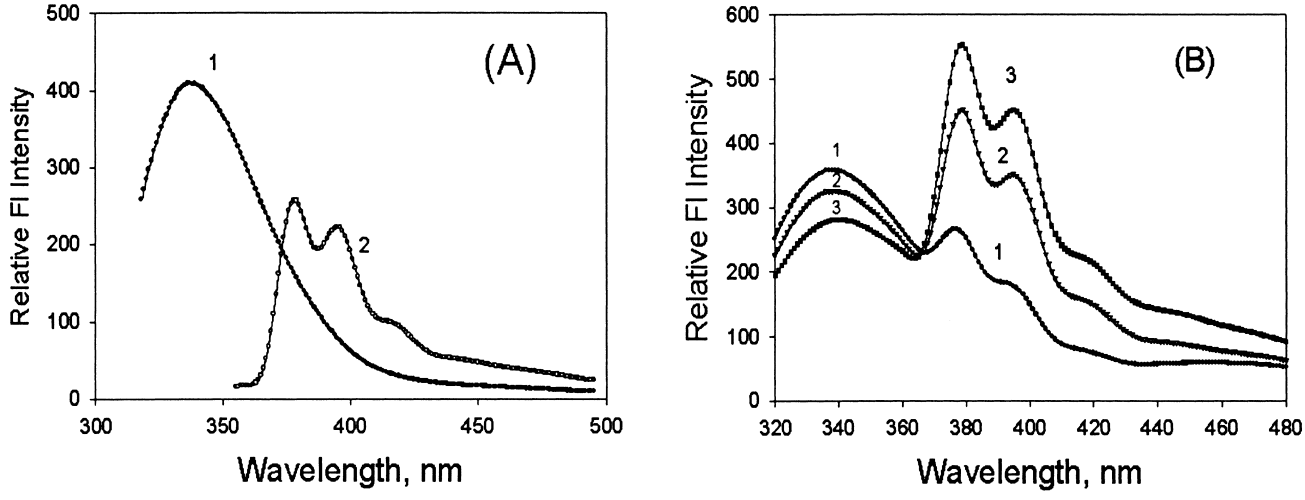

Fig. 4. A: Trp fluorescence of αB-crystallin (λ_{ex} at 295 nm, 0.11 mg/ml) and Pyr fluorescence of labeled βA_{1-40} (λ_{ex} at 340 nm, 4.5 μM). B: Fluorescence of the mixture of βA_{1-40} and αB-crystallin (1:1 mixture, λ_{ex} at 295 nm) before (curve 1) and after incubation at 37°C for 24 and 48 h (curves 2 and 3).

study of these diseases. Our study may help in the understanding of the role of αB-crystallin in AD and other neurodegenerative diseases.

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