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Evaluation of the Photo-degradation of Alzheimer's Amyloid Fibrils with a label-free approach

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Abstract

Degradation of Amyloid- β (A β) aggregates has been considered as an attractive therapeutic and preventive strategy against Alzheimer's disease (AD). However, a *in-situ*, real-time, and label-free technique is still lacking to understand the degradation process of A β aggregates. In this work, we developed a novel method to quantitatively evaluate the degradation of A β fibrils by photoactive meso-tetra(4-sulfonatophenyl) porphyrin under UV irradiation with quartz crystal microbalance (QCM).

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Quartz crystal microbalance is a useful method to quantitatively evaluate the degradation of A β fibrils by porphyrin under UV irradiation.

Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder¹ and has become a very serious health and social problem due to the increasing life-expectancy. The accumulation of amyloid- β (A β) plaque could be an important factor in the pathogenesis of AD.² Therefore, there is considerable interest in understanding the process of A β aggregation and degradation, which is very helpful for new treatment development.³ A large number of studies have focused on the inhibition and degradation of A β aggregation.⁴ Porphyrin and its derivatives⁵ as one of the compelling therapeutic strategy have been widely applied to degrade amyloid fibrils by generating reactive oxidative stress (ROS).⁶ The accumulation of amyloid- β aggregates can be degraded in the brain by photoactivatable compounds by Kanai's group.⁷ To explore the degradation efficiency of AD's amyloid fibrils is a significant work for evaluating the therapeutic strategy based photo-degradation. However, some widely used characterization methods of amyloid aggregation and degradation still have drawbacks. In Thioflavin T (ThT) assay, it is difficult to real-time study amyloid aggregation or degradation process since small molecule may interfere with ThT test and the fluorescence would be decreased.⁸ Mass spectroscopy⁹ and western blot¹⁰

[†]Footnotes relating to the title and/or authors should appear here.

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have the similar weakness of denatured procedures. Therefore, it is necessary to develop a powerful method to study the degradation of protein aggregates in real-time and with a label-free approach to address the problem above. Quartz crystal microbalance (QCM) can kinetically detect tiny mass change during chemical transformations and degradation process.¹¹ Herein, we developed a QCM-based label-free method that can *in situ* quantitatively characterize the photo-degradation process of A β 42 in real-time induced by meso-tetra(4-sulfonatophenyl) porphyrins (named as the porphyrins in the following text) with the UV light irradiation (Fig. 1).¹² This work provided a feasible method to screen drug candidates to degrade amyloid fibrils *in vitro*, and evaluate the degradation efficacy of photo-active molecule and agent in photodynamic therapy not only on amyloid beta, also including tau,¹³ amylin,¹⁴ α -synuclein¹⁵ and mHtt (mutated form of the huntingtin protein)¹⁶ fibrils.

We studied the aggregation process of A β 42 by ThT assay. The s-shape growth curve suggested a typical A β 42 self-assembly (Fig. S1a). The short lag time could be ascribed to a quick nucleation of A β 42 at 37°C. ThT fluorescence intensity reached a plateau in 24 hours, suggesting the conformation of β -sheet stabilized. β -sheet conformation was confirmed by CD (circular dichroism) spectra with the negative peak (215 nm) and the positive peak (208 nm) (Fig. S1b).¹⁷ The fibrillar morphology of A β 42 aggregates were confirmed by atomic force microscopy (AFM) (Fig. 3a). Before the degradation of A β 42 fibrils, we examined the stability and signal-to-noise ratio of QCM in aqueous solution under UV light for 6 hours without and with porphyrin. Quartz crystal microbalance (QCM) can detect tiny mass change during the degradation process, that bases on the Sauerbrey equation to estimate the mass change of the sensor surface:

$$\Delta f = \frac{-2f_0^2 \Delta m}{A \sqrt{\mu_q \rho_q}} \quad (1)$$

where Δf is the shift of the frequency, Δm is the mass change. f_0 is the fundamental frequency of the QCM device, A the electrode area, μ_q is the shear modulus of quartz, and ρ_q the density of quartz. The Δf decreasing represents the increment of the mass of the sensor surface.¹⁸ As shown in Fig. S2a, the frequency baseline of QCM is stable, which represents the mass variation per unit area. UV irradiation can cause a constant quantitative frequency uprush and go back to the normal when light is off. The addition of porphyrin cannot cause the baseline with any frequency shift (Fig. S2b).

The A β fibrils were added into the aqueous solution, and gradually adsorbed onto the gold chip as published by us. The adsorption of A β 42 fibrils caused the frequency decreasing, and the frequency baseline was stable after 5-hours incubation until the adsorption is stabilized (phase I of Fig. 1, Fig. 2a). The addition of porphyrin didn't interfere the frequency baseline after the adsorption of A β 42 fibrils was stable (Figs. 2a and S2), suggesting porphyrin cannot degrade A β 42 fibrils without UV irradiation. The adsorption of A β 42 fibrils caused the frequency decreasing, termed as Δf_1 (Fig. 2b). Three individual experiments were performed, and Δf_1 is calculated to be ~ 100 Hz.

In the presence of porphyrin, the irradiation of UV can lead to the photo-degradation of A β 42 fibrils (phase II of Fig. 1, Figs. 2b, 2c and 2d). The baseline can went back to normal when the UV light is off, exhibited the frequency increasing (phase III of Fig. 1, Fig. 2b). The increment of frequency was termed as Δf_2 , which is 72 Hz, 86 Hz, and 88 Hz in three independent experiments. Importantly, this novel method can quantitatively determine the degradation efficiency and rate by porphyrin. We defined the degrading efficiency of amyloid fibrils as:

$$\eta = \Delta f_2 / \Delta f_1 \times 100\% \quad (2)$$

Where Δf_1 is the frequency shifts caused by A β 42 fibrils adsorption, and Δf_2 is the increment of frequency, as measured by QCM.

The degradation rate is obtained via the derivation of the curve equation of the degradation process as:

$$\Delta = dy/dt \quad (3)$$

Where y is the real-time frequency of the degradation process, and t is the real time of the degradation process.

With the different concentration of porphyrin (1 μ M, 2.5 μ M and 5 μ M), the efficiency of degradation is 72%, 77% to 90%, respectively (Figs. 2b to 2e), which is obviously different from the sample without the treatment of photoactive porphyrin. Interestingly, both the degradation process and the degradation rate of amyloid fibril conformed to the exponential function model by the nonlinear fitting, which presents an exponential variation (insert of Figure 2b, and Figure 2f). For instance, the porphyrin with the concentration of 5 μ M presented the better degradation efficiency of A β 42 fibrils, therefore, the degradation rate was explored further. In the real-time observation, the rate of degradation is not constant, the rate was high in the beginning, and gradually decreased exponentially over time. In general, the concentration effect of porphyrin on the degradation of amyloid fibrils displayed the difference.

QCM can provide a way to evaluate the degradation of amyloid fibrils with a label-free method based the systematic analysis above. To validate the accuracy of degradation level by QCM, we *in-situ* observe the morphology of A β 42 fibrils mediated by photo-degradation on the gold chip by AFM. Compared to A β 42 fibrils alone, obvious morphological changes of A β 42 aggregates were observed in the presence of porphyrin after UV irradiation (Fig. 3). The heights of A β 42 fibrils photodegraded by porphyrin with different concentrations were measured, and the average heights of A β 42 fibrils alone is 6 nm (Fig. 3a), and decreased to 5.4 nm, 4.4 nm, and 3.6 nm, respectively with the concentrations 1 μ M, 2.5 μ M and 5 μ M of porphyrin (Figs. 3b—e). Furthermore, in the presence of 2.5 μ M and 5 μ M of porphyrin, A β 42 fibrils were photodegraded into A β 42 particles. Strikingly, the amount of A β 42 fibrils represented by the coverage on the gold chip dramatically decreased from 14.5% (A β 42 fibrils alone) to 8.5%, 5% or lower photogradated by porphyrin with different

concentrations (Figs. 3a—d, Fig. 3f). The heights of A β 42 fibrils is positively correlated with the concentrations of porphyrin in the photodegradation, whereas the heights of A β 42 particles is negatively correlated. The data of *in-situ* observation on QCM gold chips by AFM validates the QCM results, suggesting that the degradation effect of porphyrin is correlated with its concentrations. Importantly, QCM can be applied to interpret the kinetic of photodegradation of protein aggregates, including degradation efficiency and rate. AFM can complementarily support the mechanism that A β 42 fibrils were degraded into particles in the end stage. Therefore, QCM is a feasible, reliable and informative method to study the photodegradation of protein aggregates.

Finally, spectroscopy including the ThT assay and CD spectra were applied for validation. Under the same conditions, porphyrin with different concentrations was added into A β 42 fibrils for 6-hours UV irradiation (Fig. 4a). A gradual decrement of ThT fluorescence intensity confirmed that porphyrin can strongly degrade A β 42 fibrils in a dose-dependent manner (Figs. 4a-b). Since the thioflavin T molecules binds to beta-sheet-rich structures,¹⁹ the fluorescence intensity represents the level of β -sheet secondary structures of protein aggregates. With the photodegradation, the β -sheet secondary structure as the major conformation of A β 42 aggregates was also disrupted (Figs. 4a-b). CD spectra was applied further confirming the β -sheet secondary structure transformation of A β 42 fibrils after the photodegradation (Fig. 4c). We observed that a gradual decrement of the CD spectra peak at 198 nm with the increasing concentrations of porphyrin (Figs. 4c-d), suggesting the photodegradation by porphyrin can lead to the decreasing of β -sheet secondary structure of A β 42 fibrils. Therefore, spectroscopy (ThT and CD) and AFM all support the data of QCM as published.²⁰

The degradation efficiency of amyloid fibrils by photo-active porphyrin under UV irradiation is proportional to the concentration of porphyrin with the other parameters constant. QCM is an easy way to evaluate the degrading efficiency of amyloid fibrils with photoactive porphyrin by QCM *in-situ* under UV irradiation, and also could become a novel strategy to analyze and assess the amyloid aggregate degradation.

In conclusion, we developed a novel label-free method that can kinetically and *in-situ* evaluate the degradation effect of photo-active porphyrin on A β 42 fibrils under UV irradiation. This QCM method can provide quantitative analysis of the degradation efficiency and rate of A β 42 fibrils combined with AFM, ThT assay and CD spectra, which makes up for the shortcomings of traditional methods. which provide may open up a route for assessing amyloid degradation and provides a new way for the investigation of various amyloid-targeted drugs aiming to eliminate the toxic amyloid aggregates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Schematic representation of QCM was used to quantitatively evaluate the photo-degradation efficiency of porphyrin on the amyloid fibrils with the light irradiation. phase I: the gradually stabilization of adsorption process of A β 42 fibrils on the gold chips represented by the sequency baseline. phase II: UV light on. phase III: UV light off, and frequency difference represents the degradation level. f_1 , decreased frequency caused by the adsorption of A β 42 fibrils. f_2 , increased frequency resulting from the photo-degradation of A β 42 fibrils adsorbed on the gold chip.

Fig. 2.

The degradation of Aβ42 aggregates under UV irradiation with different concentration of porphyrin monitored by QCM. (a) Porphyrin was injected onto the QCM crystal after an addition of amyloid fibrils without UV for 6 hours; b-d Porphyrin was introduced with different concentrations (b: 1 μM, c: 2.5 μM, d: 5 μM) after an addition of Aβ42 fibrils under UV irradiation for 6 hours, The curve equation of the degradation process can be obtained by nonlinear fitting, The black line represents the degradation curve between 1 and 2. The red line is the nonlinear fitting curve of the black line (b). when the porphyrin concentration is 1 μM, The curve equation is $y = -58.67 \cdot \exp(-x/126.49) - 21.71$ (The X-axis is time/minute and the Y-axis is f /Hertz, R-Square² 0.99). (e) The degradation efficiency of Aβ42 fibrils with different concentrations of photoactive porphyrin. (f) The degradation rate is obtained via the derivation of the curve equation of the degradation process (based on the curve of d, which is displayed in the inserted part with nonlinear fitting). The real-time degradation rate decreases exponentially with time lapses, and it conforms to the exponential model. The QCM signal can be considered to reach the equilibrium when the shift frequency is less than one Hertz within thirty minutes. The green line represents degradation to equilibrium.

Fig. 3. **Morphology changes of Aβ42 aggregates photodegraded by porphyrin with AFM.** (a—d) AFM images and height analysis of amyloid aggregates photodegraded by the porphyrin with different concentrations (a: 0 μM, b: 1 μM, c: 2.5 μM, d: 5 μM) under UV irradiation for 6 hours on the QCM gold chip. The percentage distributions of the height of Aβ42 aggregates photo-degraded by porphyrin (Lower panels). (e) The height analysis of amyloid fibrils and particles. With the increasing concentration of porphyrin, Aβ42 fibrils tended to decrease, whereas the amount of particles increases. (f) The analyses for the coverage of Aβ42 fibrils. The coverage of Aβ42 fibrils changed in the different concentrations of porphyrin (0 μM, 1 μM, 2.5 μM, 5 μM).

Fig. 4.

The secondary structure of Aβ42 aggregates change in the photo-degradation. (a-b) ThT assay of Aβ42 mature fibrils and Aβ42 fibrils in the photo-degradation with different porphyrin concentration (0 μM, 1 μM, 2.5 μM, 5 μM); (c-d) CD spectra of peptide in Aβ42 fibrils in the photo-degradation process with different concentration of porphyrin (0 μM, 1 μM, 2.5 μM, 5 μM).