Evaluating Effect of Metallic Ions On Aggregation Behavior of Amyloid A β 42 By AFM Imaging and SERS

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Research Article

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Abstract

Background: Excessive aggregation of β -amyloid peptides ($A\beta$) is regarded as the hallmark of Alzheimer's disease. Exploring the underlying mechanism regulating $A\beta$ aggregation remains challenging and investigating aggregation events of $A\beta$ in the presence and absence of metal ions at molecular level would be meaningful in elucidating the role of metal cations on interactions between $A\beta$ molecules. In this study, chemical self-assembled monolayer (SAM) method was employed to fabricate monolayer of β -amyloid peptides $A\beta 42$ on gold substrate with a bolaamphiphile named 16-Mercaptohexadecanoic acid (MHA). Firstly, the samples of gold substrate (blank control), the MHA-modified substrate and the $A\beta 42$ -modified substrate were detected by X-ray photoelectron spectroscopy (XPS) to track the self-assembly process. Aggregation behaviors of $A\beta 42$ before and after metallic ions (Zn^{2+} , Ca^{2+} , Al^{3+}) treated were monitored by atomic force microscopy (AFM) and the interaction between $A\beta 42$ and metallic ions (Zn^{2+} , Ca^{2+} , Al^{3+}) was investigated by surface-enhanced Raman Scattering (SERS), respectively.

Results: The XPS spectra of binding energy of gold substrate (blank control), the MHA-modified substrate and the $A\beta 42$ -modified substrate are well fitted with the corresponding monolayer's composition, which indicates that $A\beta 42$ monolayer is well formed. The recorded surface morphology of different experimental groups obtained by AFM showed markedly different nanostructures, indicating occurrence of aggregation events between $A\beta 42$ molecules after adding metal ions to the solution. Compared to the control group, the presence of metal ions resulted in the increased size of surface structures on the observed 3D topography. Further study by SERS showed that the Raman strength of $A\beta 42$ changes significantly after the metal cation treatment. A considerable part of the amide bonds interacts with metal cations, leading to a structural change, which is characterized by the weakened β -fold Raman peak.

Conclusion: The AFM imaging results suggest that aggregation events occurred between $A\beta 42$ molecules with the addition of metal cations. Furthermore, the effect of metallic cations on the conformational change of $A\beta 42$ studied by SERS supported the results obtained by AFM imaging. Taken together, the results showed that the presence of substoichiometric metal cations promotes aggregation behavior between $A\beta 42$ molecules on the substrate at pH 7.4.

1. Background

In recent years, Alzheimer's disease (AD) is paid more and more attention for its terrible influence on the health of the elderly. Amyloid plaques with high density of β -amyloid peptides ($A\beta$) in the brain tissue of patients have been reported to be the major pathological feature of AD ¹. It was reported that the concentration of deposited $A\beta$ in the cerebral cortex correlates with the degree of dementia and synaptic loss ². Main components of the plaques were studied to be $A\beta 40$ and $A\beta 42$, which are two homologous isomers of $A\beta$ ³. While $A\beta 40$ is the most abundant homologous isomers ever discovered, $A\beta 42$ has been reported to be the most toxic ^{4,5}. Hence, the article focuses on the aggregation of $A\beta 42$, whose neurotoxicity has essential correlation with the pathology of AD ⁶. There remains a key question in the

pathology of AD: what are the risk factors that affect the aggregation of A β ? In recent years, several neurotoxic metal ions were proposed as affecting factors in the misfolding and aggregation processes of A β ⁷. It has been found through the autopsy of AD patients that abnormally high concentrations of Zn²⁺, Ca²⁺ are present along with A β in the senile plaques of AD ^{8,9}, where Al³⁺ is also detected ¹⁰.

Exploring the underlying mechanism regulating A β aggregation remains challenging and the mechanism of metal cations' effect on A β remains elusive. Until now, the lack of study on revealing molecular events of A β raised a controversy about whether metal cations are helpful to the aggregation of A β molecules. Therefore, a study on aggregation events of A β in the presence and absence of metal ions at molecular level would be of great significance in terms of pathology and methodology. In recent years, atomic force microscopy (AFM) has been widely used for studying the interaction between molecules, especially protein-protein interaction ^{11,12}. Since the turn of the century, new perspectives have been opened with the advent of AFM in the investigation of biomolecular interactions ¹³. Up to now, some research methods have been developed to study protein-protein interactions, such as surface plasmon resonance (SPR) ¹⁴, enzyme immunoassay (EIA) ¹⁵, surface force apparatus (SFA) ¹⁶, and atomic force microscopy (AFM) ¹⁷. Compared with other methods, AFM has the advantage of obtaining images with high spatial resolution and carrying out measurements under near physiological conditions ¹⁸. Consequently, AFM opens novel avenues for studying pathways of interactions between proteins and makes the study of physical behaviors of proteins achievable. In addition, along with the advantage of obtaining protein molecules' topographies on nanoscale, AFM enables researchers to monitor the aggregation behaviors between A β monomers. This study focuses on the effects of several metal ions (Zn²⁺, Ca²⁺, Al³⁺) on A β 42 aggregation. The three-dimensional morphology measured by AFM has high spatial resolution. All experiments were carried out under near-physiological conditions and a low ion concentration, closer to physiologically relevant values, is applied. By using AFM, it's achievable to concentrate on what we can find by "looking" at the protein molecules and analyze biological phenomena ¹⁹.

However, the main challenge with AFM testing is the sample preparation, especially the stabilization of protein molecules onto the gold substrate. In this study, the possibility of investigating molecular events in physiological solution is realized through a sample preparation method called Self-assembly monolayer (SAM), which has been developed over the last two decades and widely applied by biologists and chemists ^{20,21}. To achieve SAM chemically, a cleaned gold substrate should be immersed in a solution of thiols (16-Mercaptohexadecanoic acid (MHA)), which is followed by spontaneous reaction between the thiol and gold. Gradually, MHA monolayer on the gold surface with ordered and stable bonds (Au-S bond) is formed. Afterwards, the carboxyl terminus of MHA is activated by 1-ethyl-3-(dimethylaminopropyl) carbodi-imide hydrochloride (EDC) and N-Hydroxysulfosuccinimide (NHS) and then immersed into protein (A β 42) solution. Eventually, a stable and ordered monolayer of A β 42 on the thiols-modified gold surface is formed. The mechanism of mercaptan self-assembly method is depicted in Figure 1.

In order to track the formation of A β monolayer, X-ray photoelectron spectroscopy (XPS) were employed. The composition characteristics of the gold surface, the A β 42 monolayer, and the MHA film were studied. Next, we monitored the nanostructure changes of A β in the presence and absence of metallic ions (Zn²⁺ and Ca²⁺ and Al³⁺) by employing AFM imaging. The interaction between metallic ions and A β is furtherly probed and discussed by Surface-Enhanced Raman Scattering (SERS), which is an emerging sample surface analysis technique²². The changes of chemical bonds and groups in molecules lead to different molecular rotation or vibration states, which can be judged by the change of Raman scattering light frequency²³. For proteins, Raman scattering can obtain not only important information about amino acid composition, but also secondary structure information such as β - sheet and α -helix. It has been reported that the Raman cross sections of Au are enhanced to some extent when they adsorb different molecules²⁴. Because this effect occurs in the metal adsorbed molecular system, many important processes such as surface studies are related to it. The purpose of SERS study is to quantitatively characterize the effect of metal ions on the conformational transition of A β 42, and to probe the role of these metal ions in the process of abnormal aggregation of A β 42. Combined with the results of AFM study, the aggregation behavior of A β in the absence and presence of metallic ions was elucidated.

2. Results

2.1 Results of X-ray photoelectron spectroscopy (XPS) test

The surfaces of different samples (blank substrate, MHA films, A β 42 monolayer) were investigated by XPS and all measurements should be repeated 3 times for each sample. Full spectrum of elements obtained by XPS showed changed element binding energy of the bare gold-MHA-modified and the A β 42-modified gold sample. The full XPS spectra of the three surfaces are shown in Figure 2.

2.2 Results of AFM imaging

Figure 4 shows the histogram of average diameter of the nanostructures on the images of the A β 42 monolayer in blank solution, 10 μ M Zn²⁺ solution, 10 μ M Ca²⁺ solution and 10 μ M Al³⁺ solutions, respectively. Firstly, a three-dimensional topography of A β 42-modified substrate was recorded in PBS by AFM as a comparison (Figure 3(A1)). The topography of A β 42 monolayer in the absence of metallic cations display homogeneous sharp granular-like structure after incubating for 24h or 48h (Figure 3(A1)). However, the topography of A β 42 monolayer in the presence of Zn²⁺, Ca²⁺ and Al³⁺ showed irregular smooth spheroid-like structures after 24h (Figure 3(B1)-(C1)-(D1)). With the increase of incubation time to 48 hours, globular features were still observed on the surface of A β 42 monolayer treated with metallic cations(Figure 3(B2)-(C2)-(D2)).

2.3 Results of SERS imaging

In general, the enhancement of the surface signal is 106 times, which is equivalent to the amplification of the surface monolayer to more than one million layers. Therefore, the advantage of SERS is that it can avoid the signal interference caused by the same substance in the solution, and obtain high-quality

surface molecular vibration and rotation signals, which is of great significance for a detailed understanding of the interaction mode between molecules (such as metal ions) and self-assembled monolayers and the structural changes of molecules. Since the discovery of SERS, it has been successfully applied in many fields such as chemistry, biology and so on.

Above all, stable SERS signal is of great significance for accurate analysis results. Hence, the SERS properties of the obtained A β 42 modified-substrates were evaluated in different metal ion environments. In order to investigate the stability of Raman measurement results, the changes of SERS spectra of A β 42 molecular layer were recorded under continuous laser irradiation. As shown in Figure 4, when different integral time was set, no obvious change in the peak shape of Raman spectrum curves for different samples was seen, which means the A β 42 monolayer in these three metal ion solutions has good stability under continuous laser irradiation.

In Figure 5, curve A stands for the Raman spectrum of the blank control group and curve B stands for the experimental group. The results indicated that β -folds (peak at 1669cm⁻¹) and Amide II (peak at 1375cm⁻¹) is the characteristic structure for the natural conformation of A β 42. With the addition of Zn²⁺, the peak intensity of the β -folded conformation at 1669cm⁻¹ and amide II band at 1375cm⁻¹ was weakened to a certain extent, respectively (Figure 5(1)). Moreover, in blank control group, the Raman peak signal at 1375cm⁻¹ was determined to be stronger than that at 1669cm⁻¹, which means the vibration attributed to N-H is greater than that attributed to β -fold. On the contrary, the signal intensity of Amide II (1375cm⁻¹) was greatly weakened after adding Zn²⁺, which is lower than that of β -fold (1669cm⁻¹). As shown in Figure 5(2), the addition of Ca²⁺ also caused a structural change of A β 42, which is characterized by the reduced Raman intensity of Amide II from 156.13 to 115.36, and the reduced Raman intensity of β -fold decreased from 144.669 to 117.76. It's worth noting that the Raman peak of Amide II at 1375cm⁻¹ was greatly weakened and almost disappear, and the intensity of the β -fold peak at 1669cm⁻¹ was also decreased compared with the blank control group, indicating that a considerable part of amide bonds in A β 42 molecule probably interacted with Al³⁺ which resulted in a conformational change (Figure 5(3)).

3. Discussion

3.1. Preparation of chemically immobilized A β 42 monolayers

It's of crucial importance to consider immobilization carefully for the sensitivity and reproducibility of bioassays. Self-assembly monolayer (SAM), which is reliable for protein immobilization, is a topic of current interest in biological studies. SAM method can be used for self-assembly of protein molecules without altering the stability and activity of protein²⁵. It has been studied that thiol concentration of 1 mM and immersion time of 24h are befitting for the formation of Mercaptan molecular film²⁶. Besides, MHA with a proper length of the chain serves as a spacer to minimize the interference from gold substrate²⁷.

We monitored the self-assembled processes by X-ray photoelectron spectroscopy (XPS) to ensure that A β 42 was successfully modified on the surface of the gold substrate. Electron emission can be observed when the sample is exposed to electromagnetic waves with short enough wavelengths, i.e. high photon energy. This phenomenon is called photoelectric effect or photoionization because of the presence of observable photocurrents. In this process, the binding energy of material can be expressed by the following equation:

$$E_k = h\nu - E_b - \varphi_s$$

E_k -the kinetic energy of photoelectrons (in eV); $h\nu$ - the energy of photons in the X-ray source (in eV); E_b - the binding energy in the specific orbit of an atom (in eV); φ_s -the work function of the spectrometer (in eV).

Figure 2 shows that the elements on the surface of the three samples are identical to the modified molecules during self-assembly. The peak values of Au4f binding in the XPS spectra of blank substrate are 86.7 eV and 83.9 eV, respectively, which are consistent with the standard spectra. The peak values of Au4f binding shifted to 87.9eV and 90.8eV after the MHA molecule was modified on the surface of gold film. The formation of Au-S bond should be the cause of the binding-energy shift of Au4f. What's more, peak position of the S2p binding energy spectrum (Figure 2(d)) of the MHA film is lower than 164eV, indicating that there is no unbounded MHA molecule on the sample surface²⁸. There are two main peak in the binding energy spectrum of S2p. The peak at 161.2 eV may be due to the bond between MHA and Au, which reduces the binding energy of S2p²⁹. The peak at 161eV may be attributed to the C-S bond³⁰. The results suggest that the immobilization of A β 42 on the gold substrate is successful.

3.2. Topographic images of A β 42 monolayer imaged by AFM in solution

The sizes of the nanostructures on the surface of each sample were characterized by average diameter by using software analysis. The average size of the observed nanostructure in control group was calculated to be 43.12nm at the incubation time of 24h (Figure 6, blue bar) and 44.32nm at the incubation time of 48h (Figure 6, red bar), respectively. After treated with Zn²⁺, the average size of the surface nanostructures increased to 65.87nm at the incubation time of 24h and 76.13nm at the incubation time of 48h, respectively. Compared to blank group, the increased size of nanostructure in the presence of Zn²⁺ indicated that A β 42 aggregation behaviors were promoted by Zn²⁺. For A β 42 monolayer treated with Ca²⁺, the average size of the surface nanostructures was calculated to be 56.31nm at the incubation time of 24h and 65.34nm at the incubation time of 48h. It's worth noting that in the presence of Al³⁺, the average size of nanostructures observed on the image significantly increased to 72.67nm at the incubation time of 24h and 89.12nm at the incubation time of 48h. This observed size enlargement phenomenon indicated that A β 42 molecules react with neighboring A β 42 molecules and formed A β 42 aggregates.

By applying software analysis, it was found that the surface roughness of Aβ42 monolayer in different experimental groups varied significantly. The surface roughness of each topography is characterized by an average roughness (R_n) (shown in table 1). The change of surface particles can be indicated by R_n . The results showed that the roughness of control group (in physiological solution) was 1.958 at the incubation time of 24h and little change of R_n was observed at the incubation time of 48h. For Aβ42 monolayer incubated in 10 μM Zn^{2+} , Ca^{2+} and Al^{3+} solutions for 24 hours, the R_n was calculated to be 1.762, 1.82 and 1.672, respectively. For Aβ42 monolayer incubated in 10 μM in 10 μM Zn^{2+} , Ca^{2+} and Al^{3+} solutions for 48 hours, the R_n was calculated to be 1.54, 1.622 and 1.412, respectively. It can be found that with the addition of metallic ions, the decrease of R_n occurred and with the increase of incubation time, R_n of Aβ42 monolayer in the presence of metallic ions also decreased in varying degrees. We believe that the aggregation of Aβ42 particles leads to the collapse of molecular morphology, and more and more intermolecular gaps are covered, eventually resulting in the decrease of R_n .

Table 1 Average roughness of Aβ42 monolayer in the absence and presence of metallic ions at different incubation time

sample	R_n	sample	R_n
control(24h)	1.958	control(48h)	1.954
Zn^{2+} (24h)	1.762	Zn^{2+} (48h)	1.54
Ca^{2+} (24h)	1.82	Ca^{2+} (48h)	1.622
Al^{3+} (24h)	1.672	Al^{3+} (48h)	1.412

On the basis of the above results, it can be suggested that in the presence of metallic ions, the aggregation behavior of Aβ42 was induced to varying degrees. In other words, the presence of metal ions leads to disorder and clumping on the surface of Aβ42 monolayer. What’s more, stable aggregation state was observed during the experiment with the extending of incubation time. The results indicated that the addition of Zn^{2+} , Ca^{2+} and Al^{3+} drastically destabilized Aβ42 and stabilize aggregation of Aβ42. The result is consistent with the mode proposed in Alies' paper ³¹. Besides, the aggregation state of Aβ42 in 10 μM Zn^{2+} , Ca^{2+} and Al^{3+} solution differed in size and morphology of nanoparticles imaged by AFM. In conclusion, AFM imaging results showed large amounts of heterogeneous and conglobate-shaped aggregates were produced in the presence of metallic ions. The influence of different kinds of metal ions on the process of Aβ42 shows some differences, which may be attributed to the following reasons: 1) different charge amount, different molar ratio of Aβ42 to metal ions; 2) action modes and action sites on the Aβ42 peptide chain are different in the presence of different metal cations.

3.3. SERS analysis

Furthermore, the change of molecular conformation was evaluated by the Raman intensity ratio (I_{1375} / I_{1669}) (displayed in Figure 7). In comparison, I_{1375} / I_{1669} of Aβ42 monolayer in the presence of Al^{3+} is the

lowest, which indicates that Al^{3+} make the greatest effect on the conformation change of $\text{A}\beta_{42}$. The result is consistent with Banks's study, found that Al^{3+} can stabilize the aggregation structure of $\text{A}\beta$ to a greater extent ³².

Combined with Figure 3, the nanostructures with aggregation state on the surface in the presence of metallic ions is consistent the results obtained by SERS. Based on these results, it can be inferred the presence of metallic ions plays a vital role in the occurrence of the abnormal aggregation events of $\text{A}\beta_{42}$. The implications of this phenomenon are as follows: misfolding of $\text{A}\beta$ conformation at the early stage leads to a destabilization and the interaction between metal cations and $\text{A}\beta$ results in a conformational change of $\text{A}\beta$, which promotes formation of aggregates.

4. Conclusion

A study of aggregation events between $\text{A}\beta_{42}$ immobilized on MHA monolayer in the absence and presence of metallic ions by AFM imaging and SERS analysis is presented in this paper. The topographic images of $\text{A}\beta_{42}$ monolayer either in the absence of metallic ions or in the presence of metallic ions (Zn^{2+} , Ca^{2+} and Al^{3+}) show significantly different surface structures. The obtained three-dimensional surface topography of $\text{A}\beta_{42}$ monolayer show more pronounced state of aggregation in physiological solutions with added metal ions, which is characterized by the enlargement of nanoparticle sizes on the surface. The imaging results suggest that aggregation events occurred between $\text{A}\beta_{42}$ molecules with the addition of metal cations. Furthermore, the effect of metal cations on the conformational change of $\text{A}\beta_{42}$ was studied by SERS, which furtherly supported the results obtained by AFM imaging. These results, therefore, suggest that the presence of metallic ions plays a crucial role in the accelerated aggregation behavior of $\text{A}\beta_{42}$. It is rational to assume that to block the effects from metal cations or obstruct the interaction between $\text{A}\beta$ and metal cations may have great potential in new drug design for AD.

5. Methods

5.1 Materials

Metal salts used in this work are chlorides (ZnCl_2 , CaCl_2 and $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$), manufactured by Sigma Aldrich Chemical Co.. All chemicals were used as received. Phosphate buffered saline (PBS, pH 7.4) and absolute ethyl alcohol (guaranteed grade) were produced by Merck Co.. Ultra-pure water was made by Millipore purification system. 1-ethyl-3-(dimethylaminopropyl) carbodi-imide hydrochloride (EDC), N-Hydroxysulfosuccinimide (NHS), 16-Mercapto hexadecanoic acid (MHA) are made by Sigma Aldrich Chemical Co.. The amyloid peptides used in this work ($\text{A}\beta_{42}$) was obtained from AnaSpec. (USA).

5.2 Formation of protein films

5.2.1 Preperation of gold film

Besides, vapor deposition method was applied to obtain the film of gold particles. Gold particle spray film deposited on the mica plate in high vacuum by applying turbo evaporator (at $\sim 10^{-7}$ Torr), Using a radiator heater, mica plates were heated to 325°C for 2 h prior to deposition. The velocity of evaporation is limited within the range of 0.1-0.3 nm/s. The thickness of gold granular film is about 200 nm. In addition, a chromium film was deposited between the gold and the surface of mica to increase the adherence. Finally, the obtained gold film was annealed in H₂ flame for one minute.

Before use, the prepared gold plaque was immersed in piranha solution (v/v H₂SO₄:H₂O₂=3:1) for 30 min to remove organic pollutants on the surface. Then the gold surface was washed three times by absolute ethyl alcohol and ultrapure water in turn. Next, it was dried in nitrogen to avoid any pollution. The surface of gold substrate was characterized by XPS.

5.2.2 Formation of MHA film

MHA was dissolved in an ethanol solution at a concentration of 1 mM. After that, the gold substrate was immersed into MHA solution to form MHA membrane spontaneously. Ultrasonic cleaning was applied to remove unbound MHA molecules. Then the SAM film was irrigated by absolute ethyl alcohol and ultrapure water successively. Finally, it's necessary to dry the MHA film in nitrogen immediately. The sample of MHA monolayer was characterized by XPS.

5.2.3 A β 42 immobilization onto the MHA film

The carboxyl groups at the end of the MHA film reacts with the amino groups of lysine in A β molecule^{33, 34}. Taking advantage of this principle, steady A β 42 monolayer can be acquired. The A β protein was dissolved in a physiological solution (PBS), which was freshly prepared according to standard method. It should be noted that MHA film were activated for 1 h at normal temperature using the method mentioned above. Afterwards, the activated MHA film was rinsed as mentioned above and then immersed in 10 μ M A β 42 solution and stored in refrigerator at 4°C for 12 h. At last, the A β 42 film was prepared. Besides, it should be pointed out that the A β 42-modified substrates should be rinsed three times by ultrapure water and dried in nitrogen before testing. This step is to remove free protein molecules from the surface of the substrate. The surface of A β 42 monolayer was characterized by XPS.

5.3 AFM imaging

The powder of the three metal salts was dissolved in PBS respectively and finally diluted to 10 μ M before use. For the comparison of A β 42 aggregation events in the absence and presence of metal cations, the A β 42 monolayers were incubated in blank solution (PBS) and metal ionic solutions and placed in 37°C incubator for 24h and 48h respectively. Three-dimensional images of the incubated A β 42 monolayers were achieved at a resolution of 512×512 and a rate of 1 Hz. Surface topography of all samples were scanned by atomic force microscopy (JPK Nanowizard®II, Germany). Three-dimensional topography of blank group and experimental groups recorded in different conditions were analyzed by imaging processing software which is offered by the company.

5.4 SERS determination

A β 42 monolayer was prepared by the method mentioned above. 10 μ M concentration of metal salts was dissolved in PBS solution. The prepared A β 42 monolayer modified substrate was placed in a clean liquid pool, 2 mL of blank solution (PBS) and metal ion solution (Zn²⁺–Ca²⁺–Al³⁺) was added respectively, and immersed in an incubator (37°C) for 24 h.

A confocal Raman spectrometer of Horiba company was used to measure the Raman spectrum of A β 42 molecular film. The laser was a He Ne laser with excitation wavelength of 633nm, laser power of 6.0mw, resolution of 1cm^{−1}, and the measurement range was 800 ~ 2800cm^{−1}. With PBS solution as blank control, the conformational changes of A β 42 with the addition of metal cations were determined by Raman spectroscopy. For each experimental group, the Raman experiment was repeated at least three times.

In addition, we used Levenbery-Marquardt algorithm for peak split and fitting of the Raman spectral bands (corresponding to the β -folded conformation) (about 1600 ~ 1700cm^{−1}) and the characteristic peaks corresponding to amide bond at about 1400cm^{−1}. During the SERS testing, the same sample parameters were set. In order to get accurate results, each sample was repeated at least three times. The integration time of Raman data was initially set as 10s, and then the integration time was gradually increased to 60s.

Abbreviations

AFM: atomic force microscope; SAM: self-assembled monolayer; XPS: X-ray photoelectron spectroscopy; SERS: surface-enhanced Raman Scattering; AD: Alzheimer's disease; A β : β -amyloid peptides; MHA: 16-Mercaptohexadecanoic acid; EDC: 1-ethyl-3-(dimethylaminopropyl) carbodi-imide hydrochloride; NHS: N-Hydroxysulfosuccinimide; PBS: Phosphate buffered saline

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Experimental design and manuscript writing: Yang Xie. Data collection and analysis: Lin Yu, Yuna Fu and Heng Sun. Providing overall thinking: Jianhua Wang. All authors reviewed and approved the final version of the manuscript.

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Figures

Figure 1

A schematic diagram of mercaptan self-assembly method, which shows covalent attachment of Aβ42 on the carboxyl terminal of thiol-modified gold substrate

Figure 2

(a) Binding energy spectra of bare gold; (b) Binding energy spectra of MHA; (c) Binding energy spectra of Aβ42 monolayer; (d) Binding energy spectra of S 2p after MHA modification. The spectra were measured at 25 °C.

Figure 3

(A1) Topography of A β 42 monolayer imaged by AFM in PBS at the incubation time of 24h; (A2) Topography of A β 42 monolayer imaged by AFM in PBS at the incubation time of 48h; (B1) Topography of A β 42 monolayer in the presence of Zn $^{2+}$ at the incubation time of 24h; (B2) Topography of A β 42 monolayer in the presence of Zn $^{2+}$ at the incubation time of 48h; (C1) Topography of A β 42 monolayer in the presence of Ca $^{2+}$ at the incubation time of 24h; (C2) Topography of A β 42 monolayer in the presence

of Ca^{2+} at the incubation time of 48h; (D1) Topography of $\text{A}\beta_{42}$ monolayer in the presence of Al^{3+} at the incubation time of 24h; (D2) Topography of $\text{A}\beta_{42}$ monolayer in the presence of Al^{3+} at the incubation time of 48h. The concentration of metal ions is $10\mu\text{M}$. (The scanning range is $1000\text{ nm} \times 1000\text{ nm}$) (pH7.4)

Figure 4

Raman spectrum curves for $\text{A}\beta_{42}$ monolayer in the presence of Zn^{2+} (A) Ca^{2+} (B) Al^{3+} (C)

Figure 5

Raman Spectra about various metallic ions effects on $\text{A}\beta_{42}$ (integration time for 10s), with line A as control group; (1) in the presence of Zn^{2+} (2) in the presence of Ca^{2+} (3) in the presence of Al^{3+}

Figure 6

Average particle size of Aβ42 monolayer in the absence and presence of metallic ions at different incubation time

Figure 7

Intensity ratio I_{1375}/I_{1669} of Aβ42 molecule in the absence and presence of metal cations (integration time for 10s)