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RESEARCH ARTICLE

Protein corona formation by over nano-gold colloids and reversible aggregation

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Abstract

The SARS-CoV-2 alpha spike protein adsorbed over nano-gold colloids, forming a protein corona, was investigated under the pH change between pH~3 and pH ~11. The acidic condition (i.e., pH~3) exhibited a sign of aggregation through unfolded conformation of RBD (Receptor Binding Domain) segment of a spike protein. As the pH was repeatedly altered between acidic (~pH 3) and basic (~pH 11), the aggregation was quasi-reversibly formed at ~pH3 and deformed at ~pH 4. However, a definite conformation and exact adsorption orientation was not assigned yet. The conformational change was examined and compared to the case by omicron-spike protein, and reversible conformational change was observed. The addition of ACE2 to the spike protein coated 50 nm gold colloid showed two stages of conformational change implying there was a sign of two different sets of conformational change before and after roughly 25 minutes once spike protein was exposed to pH 3 condition. This was followed by a conformational change forming an aggregate at basic condition. This work demonstrated that the aggregation process of nanoparticles with SARS-CoV-2 protein corona was controlled by an external pH change.

Keywords: *SARS-CoV-2 omicron, SARS-CoV-2 alpha, spike protein, gold nanoparticles, protein corona, ACE2, protein folding, reversible self-assembly, SPR band*

Introduction

The adsorption of proteins over the nano-particles has been categorized as protein corona. Recently, considerable attention has been drawn to protein corona formation due to its value in designing new bio-nano-materials [1-6], and there have been many important applications of protein corona in immunology [7-10]. The aggregation process through protein corona reveals mechanism of protein corona formation and role for protein-protein interaction. A layer of protein corona was divided into two regimes: hard and soft. The designation of hard or soft corona is dependent on the relative strength of the binding energy [11]. Our lab specifically focuses on amyloidogenic peptides and their adherence to the gold nanoparticles in order to identify the intermediates of fibrillogenesis of the amyloids. We prepared the amyloidogenic peptide coated gold nanoparticles and controlled the conformational change by externally alternating the pH, allowing peptides to unfold in acidic conditions and refold in basic conditions (Figure 1). Since the segments used for the adsorption over gold nanoparticles won't be critical for protein networking, the investigation of the aggregation process would focus on the segments responsible for protein-protein interaction. The protein-coated nanoparticle has revealed the features of conformation, which was formed due to interaction at specific nano-size restrictions. This was prepared with nano-spherical particles with core diameters ranging between 10 ~100 nm. While the overall observations were in-vitro conditions, the extract results reflected the crucial protein conformation responding to an external pH change under simplified condition to reveal an essential nano-size dependence.

Our group has established a methodology to characterize the surface property change in the aggregation of nano-gold colloidal particles by utilizing a spectroscopic approach. The SPR (Surface Plasmon Resonance) band associated with a collective motion of electrons located over the nano-gold colloidal surface was found to be very sensitive to the formation of aggregates. As shown in Figure 1, the SPR band of amyloid beta 1-40 ($A\beta_{1\text{-}40}$) coated gold-nanoparticles shifts to red as the pH was set at pH~4 with unfolded conformation of $A\beta_{1\text{-}40}$ from folded conformation at pH > 7. This was examined by the Mie scattering theory [12] and due to the formation of gold colloid aggregates as indicated in the sketches in (Figure 1). The spectrum corresponding to (1) shows a single absorption peak around the SPR (Surface Plasmon Resonance) band located around 525 nm representing an absorbance by a collective motion of the electrons (surface plasmons). As the sketch and TEM image by $A\beta_{1\text{-}40}$ a status of gold colloid in pH 7 or 11was disperse and no sign of aggregates was observed. On the other hand, at pH 4, the aggregates formed and the SPR band shifted drastically to the red as shown in the spectrum (2) and sketch/TEM image in (Figure 1).

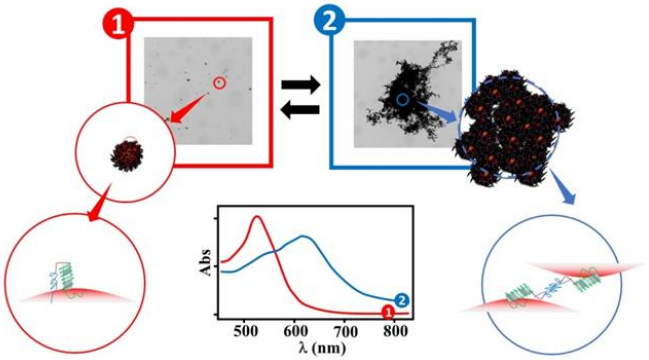

Figure 1: The absorption spectrum of SPR band, the TEM images corresponding to the situation at pH ~10 (marked as No. 1) and pH ~4 (marked as No. 2) for the case of $A\beta_{1\text{-}40}$.

Experimental

The preparation of gold colloid coated with alpha and omicron spike protein (S protein) was described previously [13,14]. The ACE2 (angiotensin-converting enzyme 2) receptor protein was purchased from ACRO Biosystems (Newark, Delaware, USA). The alpha and omicron SARS-CoV-2 strains have substantially identical molecular weight (138.2 kDa), and the fixed concentration of omicron S protein (~100 pico mols) was mixed with gold nanoparticles in the ratio of [S protein] / [Gold Colloid] ranging between ~5 and ~3400 [15]. Under the temperature of 24.5 ± 0.4°C, the pH was alternated between acidic at pH 3.5 ± 0.6 and basic condition around pH 10.5 ± 0.5 by inserting pre-tested volumes of hydrochloric acid (HCl) and sodium hydroxide (NaOH) to maintain pH ~3 and pH~11, respectively. All spectra were processed with a component of the band expressed by a Gaussian profile by Peak Fit function of OriginPro 2018b (Origin Lab), and the spectrum area weighted average peak position in the region between 450 nm and 850 nm was extracted [15].

Results

As the pH alternates between pH ~3 and pH 11 (pH hopping scheme), the undulation of peak shift was observed for the protein gold colloid of d ≥ 30 nm. While a routine pH hopping experiment was managed with three components of peaks of spr ranging between 400 nm and 900 nm, here $\bar{\lambda}_{peak}$ in figures 2–4 were extracted from only two components of spr presented between 400 nm and 700 nm. The third component resides around 725 nm did not move the peak position and gaining the intensity from the other components peaking around 1000 nm and diluting the peak shift feature of two SPR components in shorter wavelength. Here, in figure 2, the examples for core gold diameter (d), d = 10 nm and d = 50 nm are shown. The pH value at the n (operation number) = 1 is an initial pH and it was around pH ~ 7. Then, the pH at an even operation number maintains approximately pH ~ 3, and approximately pH ~ 11 for any odd operation numbers ≥ 3. The analytical formula was applied to explain this behavior and concluded that there was a reversible aggregation/disaggregation of gold nano-colloid mediated by alpha s protein at the interface. The discontinuity of the undulation feature (amplitude change) was observed at the operation number at n = 9 and n = 10 for s protein coated 50 nm gold colloid. This feature was confirmed to be reproducible as far as the operation time spent for each process was estimated between experiments. This was consistently featured at n = 8~9 for d = 40 and 60 nm as well [14]. The reversible process was interpreted to showcase the phenomenon of gold nano-particle dispersal and aggregation. The aggregation of the gold colloid was presumed to be due to the mediation of the s protein over the gold colloid surface. We have conducted the pH hopping scheme for Au 50 nm coated with gold colloid nanoparticles with the presence of ACE2. Quite surprisingly, the different binodal feature was observed by pivoting around n = 8. The amplitude of the reversibility were enhanced for ACE2 insertion up to n = 8, then it drastically enhanced at n ≥ 9 and gradually reduced its amplitude up to n = 20.

Opposed to what were observed in SARS-CoV-2 alpha S protein possessing bi-structural undulation (i.e., two different amplitude sections) we observed one single undulation feature in sars-cov-2 omicron s protein as shown in (Figure 4). A bi-structural undulation was explained as a type of kinetic factor that took place around ~25 minutes plausibly a rather drastic conformational change. This bi-structural feature was consistently observed for d = 40, 50, and 60 nm.

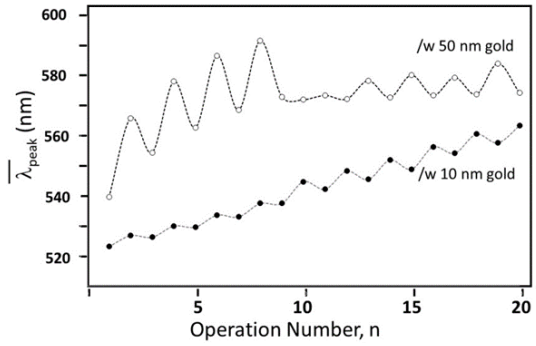

Figure 2: The comparison of the pH hop experiment ($\bar{\lambda}_{peak}$, average absorption peak shift) as an alternation of pH's between ~3 and ~11 for alpha S protein for 10 nm (black closed circles) and 50 nm (black open circles). The absolute absorbance was shifted in order not to show with a clear separation.

Discussions

The binding of the ACE2 to RBD is expected to cause a cleavage of the RBD followed by the formation [16] of pre-hairpin by an extension of protomers of fusion proteins [17-23], while the ACE2 has no effect if the RBD side was used for the adsorption contact site. Surprisingly, the pH hopping feature exhibited a drastic change at n =9 for both with and without ACE2.Thus, it may imply that ACE2 interacted with the S1 side at the time corresponding to the operation number, n, is at 9.

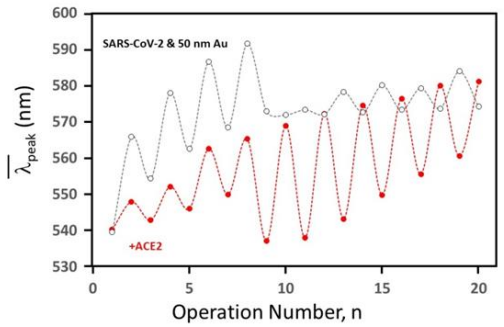

Figure 2: The pH hopping experiment for alpha S protein coated gold (black open circles) and with that after addition of hACE2 added (red closed circles). Here $\bar{\lambda}_{peak}$ was extracted from only two components of SPR presented between 400 nm and 700 nm.

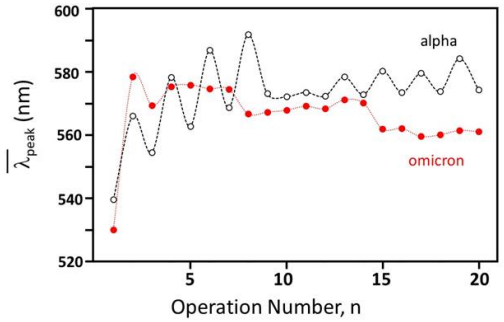

Figure 4: The sketch of pH hopping experiment for alpha S protein coated 50 nm gold (black open circles) and omicron S protein (red closed circles).

Omicron expressed a significant reduction in reversibility of the aggregation. It indicates that omicron S was more sensitive to the acidic condition and denatured more easily. Among all the mutations, the crucial mutations in the RBD of the Omicron variant are considered to be T478K, E484A, Q493R, and N501Y [24], since these mutations are considered to increase the affinity to the ACE2 receptor binding [25]. These mutations are, therefore, also considered to be highly responsible for denaturing the protein at the acidic condition.

In continuation to further analyze a sub-micro scale local conformational change of the form of adsorbed proteins, networking proteins or segments will be probed by SERS (Surface Enhanced Raman Scattering) imaging technique under various pH with/without ACE2 as a function of the nano-size to investigate the general role of fusion process involving S protein adsorbed over the gold-nanoparticles. As an example of ongoing investigation of more detailed gold-protein interaction, the amyloid existent in brain cell of a mouse was detected and the further detailed protein-gold surface is being examined [26].

Conclusions

The property and conformational change utilized for protein-protein interaction of S protein were investigated over the nano-gold colloidal surface. It revealed the response to an external pH change was quasi-reversible. The ACE2 was found to trigger a drastic conformational change, which may resemble the S1 shedding expected in the process of SARS-CoV-2 infecting a human cell. The current work demonstrated that protein corona formed by the S protein was investigated by isolating the conformational change, which would be significant for initiating the protein-protein interaction. While there was a great limit in identifying the kinetic process involved in a conformational change, it provided so many useful suggestions to move the direction of the study.

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