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Contributed Paper

Effect of Suspension TiO_2 Nanoparticles on MinD Protein Dynamics

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ABSTRACT

On the basis of previous findings that nanoparticles (NPs) have profound effects on the environment and living cells, we applied a biophysical approach, spot tracking technique, to study NP effects on *Escherichia coli* with regard to MinD protein dynamics. We used TiO_2 NPs that were concentrated in the range of 5-35 mg/L. Investigations on size distribution showed that NP cluster formation was concentration-dependent. Above 25 mg/L, NPs were found to aggregate, forming large clusters of more than 100 nm in average diameter. In addition, no interference in MinD oscillation periods was found. Below 25 mg/L, we observed less particle aggregation, measuring a mean cluster size below 100 nm. In contrast to the higher concentrated and larger NP clusters, the lower concentrated NPs had a significant effect on the protein period and protein intensity. These results indicate that only the specific sized of the suspension particles had an effect on protein dynamic system. To the best of our knowledge, this is the first report on the NP effects observed on the Min protein oscillation system. Importantly, this study may increase the awareness of the effect of NPs on living cells, particularly in an aqueous environment.

Keywords: bacteria, Min protein dynamics, titanium dioxide, nanoparticles

1. INTRODUCTION

In *Escherichia coli* (*E. coli*), the best-prokaryotic model, Min proteins are a group of proteins arranging a mid-cell site for Z-ring formation in cell division process to prevent chromosome-less minicell of daughter cells [1-4]. Min proteins consist of MinC, MinD, and MinE. MinC inhibits every cell site for division and co-localizes with MinD, forming MinCD complexes [5-7]. MinD is a membrane-bound protein that can stop binding or rebinding depending on its interaction with MinE [8]. MinE regulates

MinC and MinD to enable mid-cell site for Z-ring formation [9]. Due to these regulations, Min proteins are crucial for cell survival. Thus, cells lacking these proteins result in a minicell phenotype, which can affect cell division and, subsequently, the cell growth rate [1, 10].

Thus far, there have been some studies of TiO_2 NPs (TNPs) effects in *E. coli* using various approaches to determine the affected cell number [11-14]. Evidently, these studies indicate strong efforts to demonstrate the effects of TNPs on the cell reproduction. However, there has been no effect study on Min proteins, which regulate the site for cell division. Thus, our research is the first study to investigate the TNP effects on Min protein dynamics. To achieve this purpose, we applied spot tracking technique (STT) to observe the cluster positions of Min proteins, which normally move pole-to-pole with the oscillation period at around one minute at room temperature [15, 16]. We investigated the effect of TNPs on MinD protein dynamics by measuring the oscillation period and monitoring the oscillation pattern of MinD as a representative of the Min protein system. We hypothesized that TNPs have effects on *E. coli* by the significant change in the period. In addition to the change, the relationship of the period and TNP size, TNP concentration, and protein intensity was also established.

2. MATERIAL AND METHODS

2.1 Preparation of TNP Suspension

All suspensions of TNP were performed in Luria Broth (LB) medium supplemented with 50 mg/L ampicillin and 0.25% glucose. The original powder form of P25 type, TNP was obtained from Evonik Digussa GmbH (Frankfurt, Germany). The primary size, surface area and composition were 25 nm, 50 ± 15 m^2/g, and 80:20 of anatase: rutile mixture, respectively. These particles were suspended in LB medium to obtain a designated concentration as following procedure. First, the powder was aliquoted into 15±5 mg in 1.5 mL microcentrifuge tube, sterilized for 15 minutes at 121°C, and dried at 100°C for 2 hours. Second, LB medium was added into the aliquot of the powder to obtain 5 mg/L suspension. The suspension was mixed for 10 seconds using a vortex mixer. Finally, the mixed suspension was serially diluted to achieve the designated concentration. This designated concentration of suspension particles was confirmed via optical density measurement at 490 nm using a spectrophotometer.

2.2 *E. coli* Cell Culture and TNP Exposure

E. coli cells, RC1/ PFX9 (Δ*min*/P_{lac}-*gfp*::*minD minE*) [17], called MinD cells, were used as a bacteria model to study the effect of NPs on the protein dynamics. First, the cells were grown overnight at 37°C, 200 rpm (Environmental Shaker-Incubator ES-20; Biosan, Latvia) in LB medium to obtain an optical density at 600 nm in the range of 0.3-0.5. Second, 25% of the cells from the first step were added into various concentrations of suspension TNP (0-35 mg/L), as previously obtained, supplemented with 10 µmol/L isopropyl-b-D-thiogalactopyranoside (IPTG) for protein induction and incubated at 37°C, 200 rpm for 2 hours prior to microscopic observation. The zero mg/L TNP suspension addition served as the control.

2.3 TNP Size Characterization

The designated concentration of TNP suspension which was similarly prepared as previously (see section 2.1); except that before TNP mixing in medium, the medium was filtered through a 0.2-µm filter to remove contaminants. The mixed suspension

was incubated at 37°C, 200 rpm for 2 hours in the presence of 10 µmol/L IPTG. Immediately after incubation, the hydrodynamic diameters of the suspension particles were measured using the Dynamics Light Scattering (DLS) technique on a Zetasizer NanoZS (Malvern Instruments, UK). The size was reported as the distribution of particle number.

2.4 MinD Oscillation Period and MinD Intensity Determinations

We followed similar procedures as described by previous reports [16] for microscopy, image acquisition and period determination. We performed experiment on totally 123 different cells for all treatment of TNP suspension. In details, we used 20, 18, 14, 15, 14, 13, 14, and 15 cells for the treatment at 0, 5,10,15,20, 25, 30, and 35 mg/L TNP suspension, respectively. Briefly, two-dimensional image sequences of the cells were obtained using a charge-coupled device (CCD) camera (monochrome QEI, Nikon). When applying STT, the images were converted into gray-scale intensity array. After noise reduction (by Gaussian filters), the highest intensity position was spotted and tracked across the image sequences [16]. The STT output consists of two-dimensional positions (x, y), where x is defined by the horizontal position along the polar axis and y by the vertical position. It has been demonstrated that only the values of x change periodically from pole-to-pole [16]. The procedure to obtain the MinD oscillation period from the obtained x data was performed as previously described, using the trajectory and velocity profile [16].

For MinD intensity determination, we used totally 84 cells which are the same cells as those from oscillation period analysis. In details, we used 19, 13, 13, 12, 13, and 14 cells for the treatment at 0, 15, 20, 25, 30, and 35 mg/L TNP suspension, respectively. We selected a circular region of interest (ROI) at polar zone, at the time which had the first maximum intensity and the first minimum intensity, providing the average intensity, I_{max} and the I_{min}, respectively. The determined MinD polar intensity was $I_p = \frac{I_{max} - I_{min}}{2}$. The ROI diameter was chosen to be approximately the same as diameter of the bacteria cells.

3. RESULTS AND DISCUSSION

3.1 TNP Size Characterization

Size characterization of suspension TNP between 15-35 mg/L was shown in Figure 1A. In the figure, the average hydrodynamic diameter measured by DLS technique of suspension TNP at 15 mg/L after a 2-hour incubation is around 47 nm, It was also found that increasing the concentration to 25, 30, and 35 mg/L, the particle diameter became larger. In Figure 1B, as we defined S-TNP is a suspension particle which has size approximately not greater than 100 nm, we found the number percentage S-TNPs became smaller with concentration. Increasing the concentration from 15 mg/L to 20 mg/L resulted in a one-third reduction in the number percentage of S-TNP. These increasing aggregation behaviors suggested that when the concentration was increased, the stability of a colloidal suspension was affected [18, 19]. However, no detailed mechanism discussion on suspension particle diameter and S-TNP concentration changes will be provided as we mainly focus on the change of the protein dynamics in these suspensions. Note that, the absence of S-TNP at 25 mg/L also indicated the limitation of detection using the DLS technique when the S-TNP concentration is extremely low.

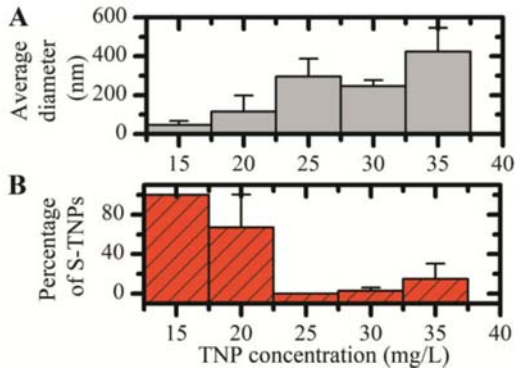

Figure 1. TNP hydrodynamic diameter (A) and S-TNP percentage (B) of prepared TNP suspension at various concentrations.

3.2 MinD Protein Oscillation Behavior

The fluorescence signal of the MinD protein in Figure 2 showed that the dynamic behavior after incubation in TNP suspension in various concentrations did not change except for the oscillation frequency. In the control (panel A), the proteins formed horseshoe structures [20] on the cytoplasmic membrane at one pole and moved to another pole according to the proposed reaction-diffusion of the Min protein between the cytoplasm and membrane [21]. The time duration for the presence of horseshoe-shaped non-exposure TNP was 15 seconds (panel A, 20-35 seconds), but this time duration was shortened to 10 seconds for the 15 mg/L TNP exposure lot (panel C, 5-15 seconds). These results demonstrated that the oscillation frequency changed as the proteins detached and swept faster between the two poles.

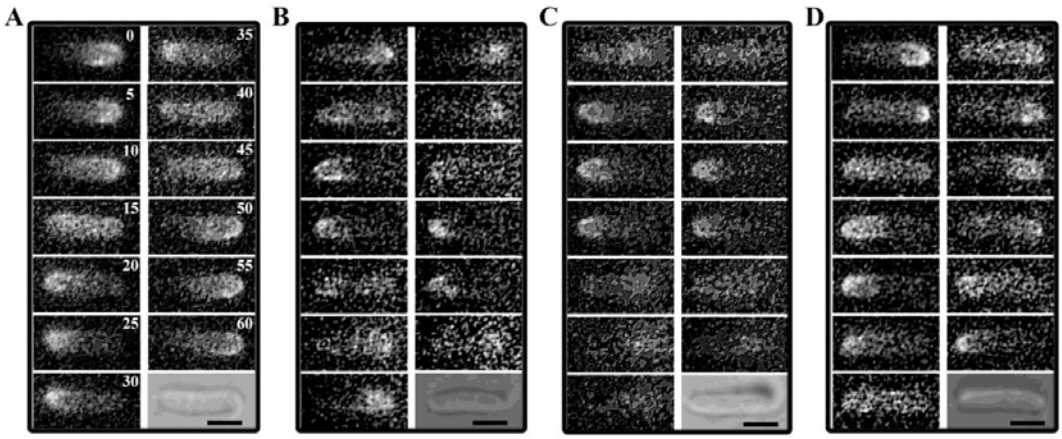

Figure 2. Oscillations of MinD proteins within *E. coli* are presented in the fluorescence image sequences for 0, 5, 15, and 30 mg/L TNP exposure in panels A, B, C, and D, respectively. The times (in seconds) are indicated in the top-right corner in the first panel and are respectively applied to the other panels. The dark solid bar is 2 μm long.

3.3 MinD Dynamic Alterations

As we observed oscillation frequency changed, we aims to quantitatively investigate the protein dynamical change in terms of oscillation periods .The protein periods in *E. coli* was measured after exposure at various concentrations of TNP suspension, in the range of 0-35 mg/L, with 5 mg/L interval. After TNP exposure, the periods are determined from spatial-temporal cluster position along polar axis or x axis as shown in Figure 3A. The period results in Figure 3B revealed that there was a group of experimental TNP concentrations (indicated in ellipse) that significantly affected the period (p-value < 0.01). The group contained

a small number of concentrations, consisting of 5-20 mg/L TNP. They fastened the MinD oscillation frequency by approximately one-fifth from the control lot. Previous studies revealed that the MinD oscillation period in wild-type *E. coli* was dependent on many factors, for example, the shape [22], strain/genotype [15, 23], temperature [15], number of MinD/MinE copy ratio in the cell [21, 24, 25] and cell length [16]. In this study, the shape and strain/genotypes were fixed. The temperature and cell length were controlled at 26±1°C and 5±1 μm, respectively.

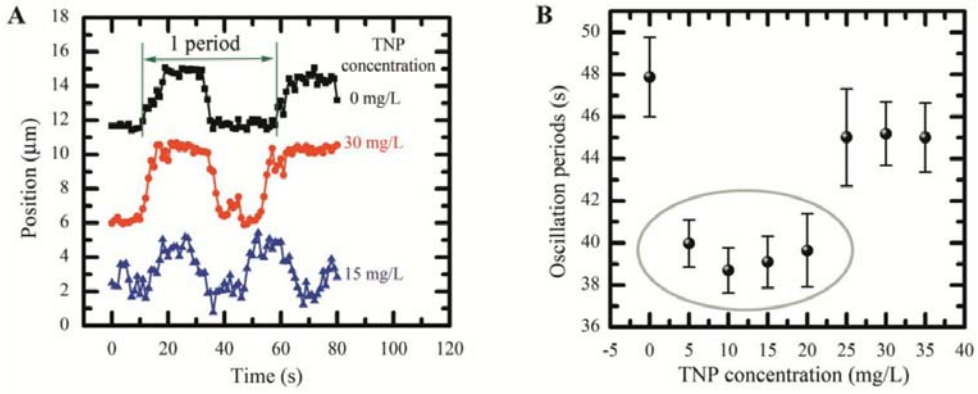

Figure 3. MinD protein oscillation period changes. After TNP exposure, the periods are determined from spatial -temporal cluster position along polar axis or *x* axis as shown in (A). The periods appear to be decreased with the group of low concentrations of TNP suspension as indicated in ellipse (B). Bar represents standard error.

Figure 3 revealed that the periods were changed according to the small number of TNP suspension concentrations. Thus, we focused on understanding this issue by plotting the period against average diameter as shown in Figure 4A and against S-TNP concentrations as shown in Figure 4B, where the S-TNP concentrations were correlated from the number percentage data at various prepared TNP concentration in Figure 1. In addition, the oscillation period of the control lot is provided as the indicated gray region. Qualitatively, Figure 4A shows that the periods are increased according to the increased average particle diameter. In addition, Figure 4B demonstrates the trend that the periods were rapidly reduced with increasing S-TNP concentration. Moreover, if we look more closely and quantitatively, as expected, the data can be classified into two groups (a classification criterion is in Table 1), i.e., L-TNP and S-TNP which are defined from a relatively low S-TNP concentration (large diameter) group and high S-TNP concentration (small diameter) group, respectively.

Table 1. Classification of the oscillation period according to the TNP concentration.

TNP concentration (mg/L)	*p*-value	Group of TNP concentration	
		S-TNPs (p-value ≤ 0.01)[a]	L-TNPs (p-value > 0.01)[a]
15	0.000	√	
20	0.000	√	
25	0.878		√
30	0.245		√
35	0.222		√

[a]Based on statistical *t*-test between the period of the control and various experimental lots.

Based on Figure 4B and Table 1, the S-TNP concentration group or small particle size group (inside ellipse) exhibited a high concentration of 13-15 mg/L S-TNPs. This group corresponded to the shorter period around 10% than those in the L-TNP concentration group which occupied S-TNPs ranging from 0-5 mg/L (outside ellipse). Furthermore, the average oscillation periods were significantly affected or changed after the threshold was reached (p-value < 0.01) i.e., S-TNP concentration approximately over 10 mg/L (Figure 4B-inside ellipse) or particle diameter approximately less than 120 nm (Figure 4A-inside ellipse). In other words, the periods were not significantly affected when the diameters were more than 240 nm (Figure 4A-outside ellipse) or when the concentrations were below 10 mg/L (Figure 4B-outside ellipse). To the best of our knowledge, this is the first study to quantitatively present the alterations of Min protein dynamics regarding to TNP size and S-TNP concentration. Thus, this study not only provides insight into the effects of TNP but it also reveals more profound findings, namely the conditions of TNP effects, i.e., the particle size should be less than approximately 100 nm and the S-TNP concentration should be sufficiently large, i.e., more than 10 mg/L in this system. Self-cleaning material manufacturer such as TiO_2 NP doped film manufacturer may use these conditions as guidelines for antibacterial application [26].

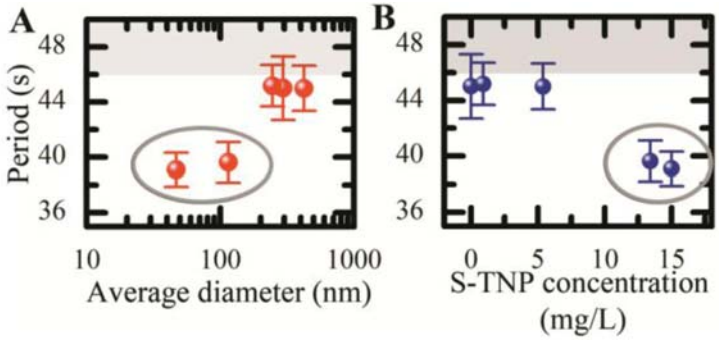

Figure 4. Relationship of TNP average diameter (A) and S-TNP concentration (B) on MinD oscillation periods, classified into two groups of TNP concentrations, i.e., S-TNP (inside ellipses) and L-TNP (outside ellipses). The shaded area represents the oscillation period of the control lot.

In Figure 3B, after *E. coli* are exposed to TNP, whether the MinD oscillation periods will change is critically dependent on the TNP suspension concentrations. Specifically, from our estimation and hypothesis, the change in period is exclusively dependent on the proportion of the specific sized particles, referred to as S-TNPs (see Figure 4B). Generally, in any suspension of TNP, the suspension consists of both S-TNPs and the larger size TNP or L-TNPs. Thus, it is observed in Figure 4B that the relatively high concentrations of S-TNPs result in a reduction in the oscillation periods. To determine the cause of the reduction, we further analyze the MinD protein fluorescence intensity at cell poles. We found that the protein intensity (Figure 5B, spheres) at the polar zone is significantly decreased (p-value < 0.05) in the presence of S-TNP, both low and high S-TNP. If we assumed the linear relation between the measured intensity and MinD protein number involved in the oscillation on inner membrane, the candidate for the reduced period could either be a change in the reaction or a change in the diffusion process associating to the reduced MinD number appearing on membrane [21]. Previously studies showed that the main cause of the reduced period is a change in the reaction process [15, 27]. The reaction rate, called hydrolysis rate, are the rate at which MinE hydrolyzes MinD on the membrane to the cytoplasm could affect the period *in silico* [15, 27]. Besides the rate, a reduction of the MinD/MinE number ratio also results in a shorter period and inter-division time [21, 24, 25]. In our study, the decreased intensity of MinD in the polar zone indicates a high possibility of a reduction in the number of MinD participating in the oscillation. Consequently, the ratio is reduced and the MinD oscillation period is reduced accordingly. In addition, by this mechanism, the MinD polar zone intensity is also significant reduced (p-value < 0.05) at the low S-TNP of around 0-5 mg/L (Figure 5B), implying that MinD number at this interval is probably affected but is not relevant to the shorter periods. Reduction of MinD number though may not only factor for oscillation period reduction.

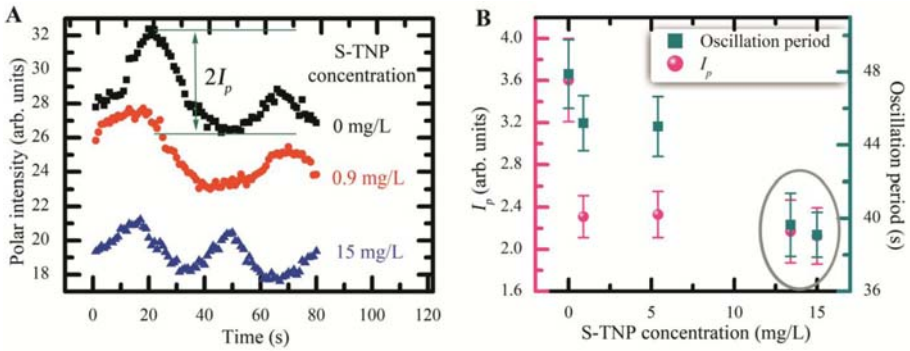

Figure 5. Polar intensities (I_p) are changed according to S-TNP concentration. I_p is determined as shown in (A). The zero S-TNP concentration served as the control. Remarkably, high reduction of polar intensities and high reduction of oscillation periods relates to the presence of high S-TNP (ellipse in B). The intensity and period data are denoted by spheres and squares, respectively.

4. CONCLUSIONS

We investigated the dynamic alterations of MinD proteins after TNP suspension exposure in the range of 5-35 mg/L TNP suspension. MinD oscillation period significantly changed in 5-20 mg/L TNP suspension. Compared to the control lot, the alterations in dynamics are revealed by twenty percent reduction of oscillation periods. Moreover, we quantified the particle size and percentage of TNPs in various concentrations of TNP suspension. We found that a TNP group correlates with the reduction in oscillation periods. Particles with a size approximately below 100 nm, referred to as S-TNPs, play a considerably critical role in the alteration of Min protein dynamics. Furthermore, the presence of a high S-TNPs concentration is related to the reduction in the oscillation period. On the basis of the reaction-diffusion theory of Min proteins and the MinD intensity change evidence, it may indicate that the reduced periods upon increasing S-TNP concentration partly results from reduced MinD numbers involved in the oscillation. The potential benefit of this study is its implication for the health awareness of suspension nanoparticles, particularly with sizes that are less than 100 nm in an aqueous environment.

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