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Short communication

Glycerol facilitates the disaggregation of recombinant adeno-associated virus serotype 2 on mica surface

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Abstract

Preparation of distributed virus on a solid substrate is a prerequisite for investigation of the properties and individualism of virus, while many previous studies showed that virus has a tendency to aggregate on solid substrates. In this communication, we report a novel approach by which well-separated recombinant adeno-associated virus serotype 2 (rAAV2) could be prepared on bare mica surface. The key technique in this approach is the addition of less than 3% (v/v) glycerol into the virus solution and subsequently deposition onto mica surface for the sample preparation. The possible mechanisms are also briefly discussed.

Keywords: Recombinant adeno-associated virus 2 (rAAV2); Disaggregation; Glycerol; Atomic force microscope; Dynamic light scattering

1. Introduction

Virus is ubiquitous on the Earth and can greatly influence the life of humans as well as other organisms. For example, various diseases are caused by viruses which are including the fatal viruses such as Human Immunodeficiency Virus (HIV) and Ebola Virus. Recently, the study on single virus has attracted attentions. On the one hand, investigation of the properties of virus, particularly its individualism is of great biological and medical significance [1,2]. On the other hand, new emerging techniques make it possible to measure the mechanical properties of a single virus that may offer new information to understand different characteristics during the period of maturation [3].

In many cases, studies on single virus particle need the samples of separated individual viruses. However, a growing body of evidence indicates that viruses, like other nanoparticles, have an intrinsic tendency to form clumps, even at an extremely diluted solution [4]. Thus, how to prevent virus aggregation becomes an important scientific and technical issue. In pioneering work, the mechanisms and the kinetics of salts on viral disaggregation in suspension have been extensively investigated [5,6]. In some cases additives present in particle suspension may cause various effects on dispersing of the particles. Wadu-Mesthrige et al. have reported that bovine serum albumin could be used to disperse the tobacco mosaic virus both in suspension and on mica surface [7]. For the sake of further investigation on single virus particle, it is important to find suitable additives that can promote viruses well-separated on solid substrates.

In this paper, we propose to utilize glycerol to disperse recombinant adeno-associated virus serotype 2 (rAAV2) particles on mica. Glycerol is used as cosolvent that can be favor to prevent proteins aggregation [8]. Since the virus genome is usually packaged in a protein shell and a whole virion can be considered as a protein particle, and according to the report that glycerol could disperse the AAV2 particles in suspension [9], we speculate glycerol may possibly facilitate rAAV2 disaggregating on mica surface. Herein the atomic force microscope (AFM) and dynamic light scattering (DLS) were employed to reveal the aggregation information.

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AAV is a short-length (with a diameter about 20 nm of capsid) and single-stranded DNA virus of the *Dependovirus* genus that constitutes one of the four *Parvoviridae* family geniuses [10,11]. Due to their lack of pathogenicity, wide range of infectivity, and ability to establish long-term transgene expression, rAAV2 has been applied in clinical gene therapy. Therefore, it has been the subject of intense scientific scrutiny in past years for its great promise [12–14] and extensively investigated in preclinical studies for a wide variety of genetic diseases (for more detailed information in review [15]).

AFM has been widely used to study various materials such as DNA, proteins and virus since its invention in 1986 [16] for its high-resolution imaging ability. In comparison with other techniques such as scanning electron microscope and transmission electron microscope, the prominent advantages of AFM are its capability of imaging in ambient/liquid conditions and the requirement of relatively simple sample preparing procedures [17]. DLS technique is one of the most popular methods to determine the size of spherical particles in solutions, which is mainly applied to measure the sphere size distribution and give a description of the particle's motion in the medium [18].

2. Materials and methods

2.1. Sample preparation

Recombinant adeno-associate virus serotype 2 was purchased from Vector Gene Technology Company Limited (Beijing, PR China). Particle concentration was 6×10^{11} particles/mL. The original virus suspension (PBS) was diluted 10-fold into 0.5, 1, 2 and 3% glycerol aqueous solution, respectively. As a positive control, another stock of virus was diluted 10-fold into Milli Q water (18.2 MΩ). High ionic strength buffers were not used here to avoid the salt crystallization on mica surfaces. A 4 μL droplet of diluted virus suspension was deposited on bare mica for 2 min and the remained drop was gently blown off. Then the surface was rinsed by 15–25 μL Milli Q water, with excess wicked away, before sample was dried by flowing air. Finally, the mica was taped on a steel disk and observed by AFM in air. Unless otherwise noted, Milli Q water was used in the preparation of all aqueous solutions.

2.2. Atomic force microscope

The Multimode Nanoscope IIIa (Veeco/Digital Instruments, Santa Barbara, CA) was operated at tapping mode. Commercially available silicon cantilevers (NSC11, MikroMasch) with a nominal constant of 48 N/m and a typical resonant frequency of 330 kHz were used. All images were collected at 20–25 °C, relative humidity of 25–30%.

2.3. Dynamic light scattering

Dynamic light scattering measurements were performed using an Autosizer 4700 light scattering spectrometer (Malvern Instruments Malvern, Worcestershire, UK) equipped with a 10 mW Ar^+ ion laser (488 nm). Measurement was performed at an angle of 90° and a temperature of 25 °C.

3. Results and discussion

As shown in Fig. 1a, it is obvious that the virus particles easily aggregated into clumps without glycerol. While the 0.5% (v/v) glycerol was added into virus solution, the clumps of virus were partial dispersion on the substrate (Fig. 1b). Subsequently, when 1–3% glycerol was blended, the virus particles could be well dispersed on bare mica (Fig. 1c, f and g).

Additionally, the diameter distribution of rAAV2 particles measured by AFM was shown in Table 1, whereas Fig. 1a and c were chosen to compare the difference between samples without and with 1% glycerol. The average apparent diameter of particles was determined from the height images using particle size analysis program bundled with the Nanoscope III software. In the process of data analysis, the particles with heights lower than 10 nm were overlooked for the presumption of impurities mixed in virus suspensions. Prior to measurements, flatten operation was applied to each image.

The standard deviation (S.D.) of diameters in Fig. 1a is larger than that in Fig. 1c (Table 1). Although the true diameters of viral particles is smaller than the data measured by AFM due to the tip convolution effect, the measured S.D. of particles' diameters would be proportional to that of true diameters. The larger S.D. of particles diameters (22.2 nm) indicates that more viral particles aggregated as clumps for the sample without glycerol.

It is well known that the rAAV2 has a limited solubility and can easily form aggregates in suspension at neutral pH values [19]. However, high concentrations and dispersion of pure AAV2 ($4.4–18 \times 10^{14}$ particles/mL) for crystallization could be obtained when 2.5–12.5% (w/v) glycerol was added [9], another report pointed that the glycerol could not disaggregate AAV particles if the concentration of virus was below $\sim 10^{14}$–10^{15} particles/mL [20]. While the concentrations of AAV we adopted here are far below those mentioned in the literatures, the results showed glycerol could also facilitate distribution of rAAV2 particles on bare mica.

Two possible factors would contribute to this discrepancy. First, glycerol can help to reduce the aggregation of proteins in suspension. When compared to the physiological conditions, isolated proteins are usually subjected to severe environmental conditions that might lead to unfolding and aggregation. Glycerol that has been proved to be an efficacious kind of stabilizer

Table 1
The average diameter of virus particles with/without adding glycerol in the sample preparation measured by AFM

	a (390)[a]		b (102)	
	0% (v/v)[b]		1% (v/v)[b]	
	Range	Mean	Range	Mean
Diameter (nm)	22.0–164.9	37.1 ± 22.2	22.0–54.0	33.0 ± 8.9

[a] The total viral particles number was counted from AFM data.
[b] Concentration of glycerol.

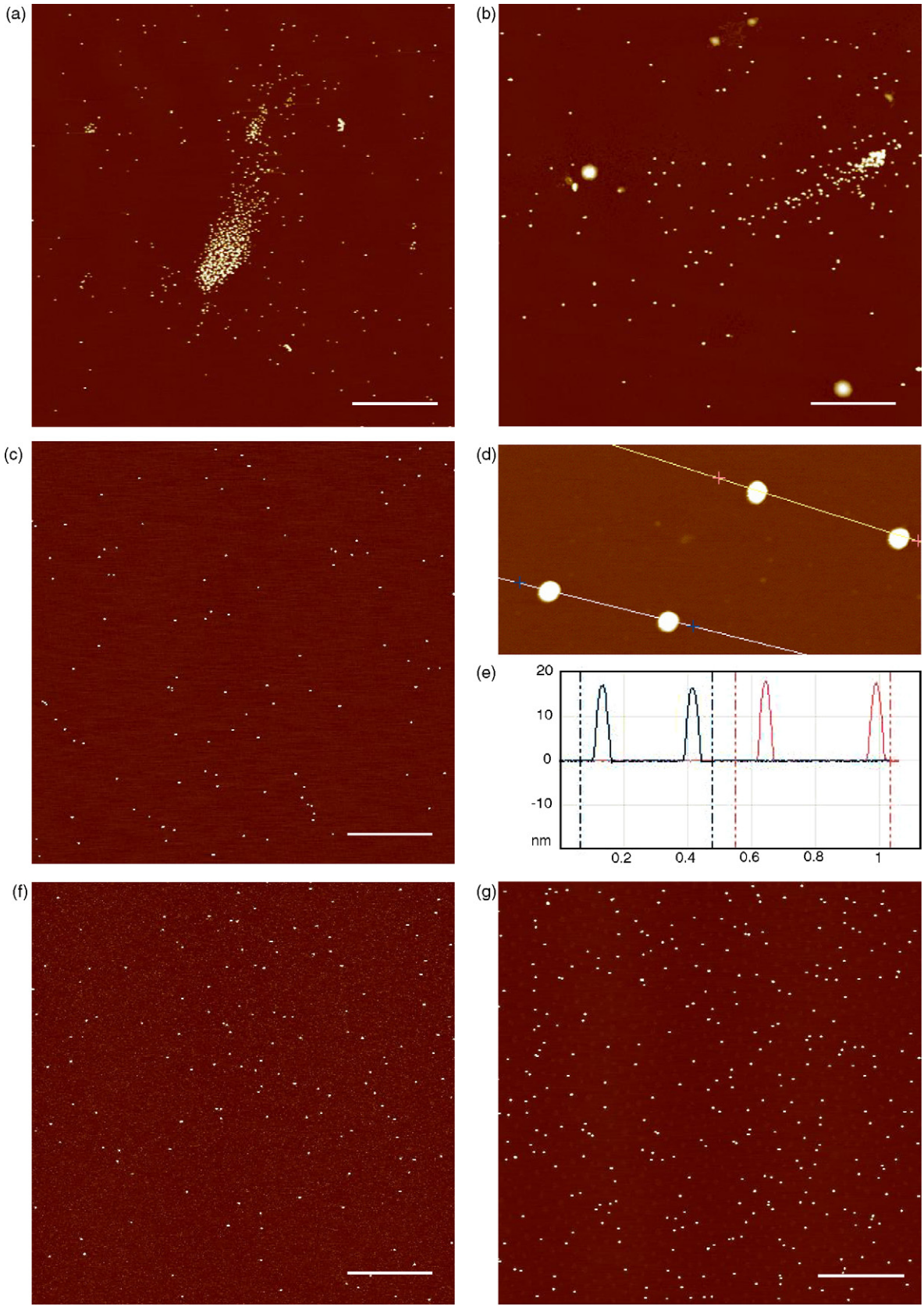

Fig. 1. AFM images of AAV samples treated without/with glycerol. (a) Virus suspension without glycerol; (b, c, f and g) the glycerol concentration is 0.5, 1, 2 and 3%, respectively. The bar scale each image is 2 μm and the height scale is 0–20 nm; (d) a zoom scan area, 1% glycerol, 1 μm × 500 nm, and the height scale is 0–40 nm; (e) the section analysis of (d).

Table 2
Average diameters screening by dynamic light scattering

Concentration of glycerol, % (v/v)	Average diameter (nm)
0	362.1
1	92.7

is widely used in the preservation of proteins [21]. According to the explanation of Gekko and Timasheff, the glycerol molecules are preferentially depleted from the proteins surface layers when the glycerol was added into solution [22]. That process is in favor of dissociating aggregated proteins [23]. Our DLS data provide another evidence to prove the dissociated effect of glycerol, in which average diameter (92.7 nm) of virus particles in 1% glycerol suspension is lower than that (362.1 nm) of particles in aqueous suspension (Table 2), though the average diameter of particles in glycerol suspension was still three-fold larger than the real ones.

Second, the influence of the substrate on the dispersion of particles would be considered. Xu and Salmeron found that a thickness of 1.5 nm inner glycerol layers strongly adhered to the mica through the spreading of droplet of water–glycerol deposited on bare mica [24]. The adhered glycerol layer may increase local concentration of glycerol, which may enhance the depletion effect of glycerol. The viscidity of adhered glycerol layer may help to capture particles in suspension, so the particle concentration at the surface was higher than that in suspension. Therefore, the dispersion of virus particles on mica was increased locally compared with that in suspension. In addition, we speculate that the layers maybe contribute to virus particles both attachment and dispersing on mica. When the precursor monolayer slides on the surface of substrate, the virus particles may be similar to the polymers that could diffuse following it [25–27]. The incompletely disaggregated particles stuck on mica could possibly be further pulled separately through the spreading of glycerol film. Under the cooperation of these effects, we got well-dispersed samples even at extremely low glycerol and particle concentration (as shown in Fig. 1c), which showed consistency with previous works.

To prove this, the disaggregated effect of glycerol concentrations below 0.5% or above 3% was also investigated. When the concentration of glycerol was lower than 0.5%, the virus particles were still easily aggregated to clumps. Although separated single particles on mica could also be obtained by added more than 3% concentration of glycerol, the higher concentration of glycerol sometimes smeared mica badly and more water was needed to rinse the surface, consequently, most virus particles were often unexpectedly washed away due to large volume of water flow. The 1% glycerol was found as a proper threshold concentration for the dispersion of virus particles on mica surface.

4. Conclusions

In summary, by adding a proper amount of glycerol into rAAV2 solution, well-separated virus particles could be obtained on bare mica, which is vital for further single rAAV2 studies. The concentration of glycerol has an obvious influence on the dispersion of virus particles and the optimized concentrations range from 1 to 3% (v/v). In addition, the glycerol layers may help the attachment and dispersion of virus particles on mica.

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References

[1] Y.G. Kuznetsov, J.G. Victoria, W.E. Robinson Jr., A. McPherson, J. Virol. 77 (2003) 11896–11909.
[2] J.P. Michel, I.L. Ivanovska, M.M. Gibbons, W.S. Klug, C.M. Knobler, G.J.L. Wuite, C.F. Schmidt, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 6184–6189.
[3] N. Kol, M. Gladnikoff, D. Barlam, R.Z. Shneck, A. Rein, I. Rousso, Biophys. J. 91 (2006) 767–774.
[4] R. Floyd, D.G. Sharp, Appl. Environ. Microb. 33 (1977) 159–167.
[5] S.A. Overman, D.M. Kristensen, P. Bondre, B. Hewitt, G.J. Thomas Jr., Biochemistry 43 (2004) 13129–13136.
[6] B.J. Mccreedy Jr., K.P. Mckinnon, D.S. Lyles, J. Virol. 64 (1990) 902–906.
[7] K. Wadu-Mesthrige, B. Pati, W.M. McClain, G.Y. Liu, Langmuir 12 (1996) 3511–3515.
[8] S.E. Bondos, A. Bicknell, Anal. Biochem. 316 (2003) 223–231.
[9] Q. Xie, J. Hare, J. Turnigan, M.S. Chapman, J. Virol. Methods 122 (2004) 17–27.
[10] G.P. Gao, M.R. Alvira, L.L. Wang, R. Calcedo, J. Johnston, J.M. Wilson, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 11854–11859.
[11] Q. Xie, W.S. Bu, S. Bhatia, J. Hare, T. Somasundaram, A. Azzi, M. Chapman, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 10405–10410.
[12] G. Seisenberger, M.U. Ried, T. Endreβ, H. Büning, M. Hallek, C. Bräuchle, Science 294 (2001) 1929–1932.
[13] H.G. Zhang, Y.M. Wang, J.F. Xie, X. Liang, H.C. Hsu, X. Zhang, J. Douglas, D.T. Curiel, J.D. Mountz, Gene. Ther. 8 (2001) 704–712.
[14] A. Negishi, J.H. Chen, D.M. McCarty, R.J. Samulski, J. Liu, R. Superfine, Glycobiology 14 (2004) 969–977.
[15] Z.J. Wu, A. Asokan, R.J. Samulski, Mol. Ther. 14 (2006) 316–327.
[16] G. Binnig, C.F. Quate, Ch. Gerber, Phys. Rev. Lett. 56 (1986) 930.
[17] P. Wagner, FEBS Lett. 430 (1998) 112–115.
[18] F. Andreasi Bassi, G. Arcovito, M. De Spirito, A. Mordente, G.E. Martorana, Biophys. J. 69 (1995) 2720–2727.
[19] F.B. Johnson, A.S. Bodily, Soc. Exp. Biol. Med. 150 (1975) 585–590.
[20] J.F. Wright, T. Le, J. Prado, J. Bahr-Davidson, P.H. Smith, Z. Zhen, J.M. Sommer, G.F. Pierce, G. Qu, Mol. Ther. 12 (2005) 171–178.
[21] K.D. Miller, R. Barnette, R.W. Light, Chest 126 (2004) 1933–1937.
[22] K. Gekko, S.N. Timasheff, Biochemistry 20 (1981) 4667–4676.
[23] F.G. Meng, Y.K. Hong, H.W. He, A.E. Lyubarev, B.I. Kurganov, Y.B. Yan, H.M. Zhou, Biophys. J. 87 (2004) 2247–2254.
[24] L. Xu, M. Salmeron, J. Phys. Chem. B 102 (1998) 7210–7215.
[25] H. Xu, D. Shirvanyants, K. Beers, K. Matyjaszewski, M. Rubinstein, S.S. Sheiko, Phys. Rev. Lett. 93 (2004) 206103.
[26] H. Xu, D. Shirvanyants, K. Beers, K. Matyjaszewski, A.V. Dobrynin, M. Rubinstein, S.S. Sheiko, Phys. Rev. Lett. 94 (2005) 237801.
[27] H. Xu, S.S. Sheiko, D. Shirvanyants, M. Rubinstein, K.L. Beers, K. Matyjaszewski, Langmuir 22 (2006) 1254.