Investigation of the Elasticity of Polymer Nanoparticle by Vibrating Scanning Polarization Force Microscopy

2006 Chinese Phys. Lett. 23 2315

(http://iopscience.iop.org/0256-307X/23/8/096)

Investigation of the Elasticity of Polymer Nanoparticle by Vibrating Scanning Polarization Force Microscopy *

ZHANG Li-Juan(张立娟)[1,2**], WANG Hua-Bin(王化斌)[1,2]

[1]*Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800*
[2]*Graduate School of the Chinese Academy of Sciences, Beijing 100049*

(Received 9 March 2006)

The elasticity of an individual polymer nanoparticle may be greatly different from that of the bulk one. Understanding the properties of individual particles such as elasticity and deformation under external forces is of great importance in controlling the final structures and functions of bulk materials. We study the compression properties of single polyethylenimine (PEI) particles using vibrating scanning polarization force microscopy. By controllably imaging PEI particles at different vibration amplitude set-point values, it is demonstrated that we can compress the single PEI nanoparticle with an atomic force microscopy tip in different loads. Based on the force–height and force-strain curves obtained, Young's moduli of PEI (5–160 MPa) in three force regions are estimated according to the Hertz model. The results indicate that PEI has excellent elasticity, which may contribute to its high efficiency as vectors in gene transfection.

PACS: 87. 64. Dz, 61. 41. +e, 62. 20. −x

Polymer microspheres are found ubiquitously in the food, pharmaceuticals, coating, and chemical industries. At the nanometre scale, polymer particles may have some properties different from those of the bulk ones. Understanding the properties of individual polymer nanoparticle such as elasticity and deformation under external forces is of great importance in controlling the final structures and functions of the bulk polymer–built materials.[1] Polyethylenimine (PEI) is a branched, water-soluble polymer, which is widely used in the paper industry, food, cosmetics, and pharmaceutical researches.[2] More importantly, PEI has been reported to promote transgene delivery to the nucleus in mammalian cells.[3] In comparison with viral vectors, the vectors formed by PEI-DNA complexes retain high attractiveness in gene therapy due to their excellent safety profile and PEI's high transfection efficiency.[4−6] However, the exact mechanism of PEI mediating transfer action remains to be elucidated. More frequently, researcher studied its transfection mechanisms from the view of chemical modifications, such as high-density amine groups, grafting, linear PEI.[7−9] Nevertheless, it is possible that its mechanical property (flexibility or elasticity) may be one of the main factors influencing the transfection processes (such as binding with DNA molecules, entering cell membrane and degrading in cell).[10] Thus it is imperative to know how 'soft' this polymer may be, particularly, at the nanometre scale.

At present, atomic force microscopy (AFM) perhaps is an unique technique that allows the measurement of both the force and the resulting deformation in the same time at the nanometre scale.[11] The AFM tip has been widely used to explore the local mechanical properties of materials at the nanometre scale.[12−21] For example, the deformability of some soft materials like carbon nanotubes[18] has been successfully studied using the tapping-mode AFM (TM-AFM) recurring to TM-AFM's virtues of eliminating the lateral forces and lowering the forces between the tip and the sample. However, the conventional tapping mode is in fact of inability to investigate very small deformations of soft materials because the force needed to maintain the stability of the tip is still too large to provide the detailed information of the small force region (< 0.5 nN).

Recently, we developed a method termed the vibrating scanning polarization force microscopy (VSPFM),[22−24] which is an imaging mode of combining non-contact mode and tapping mode under the participation of polarization force. The detailed mechanism of VSPFM is described in elsewhere.[23,25] Several typical advantages of VSPFM are as follows: (1) It is very easy to perform the transformation between the non-contact mode and the tapping mode by adjusting the height of the AFM tip. (2) It can image stably under a force (< 0.5 nN) that is much smaller than that of imaging in TM-AFM (1–10 nN).[26] (3) The polarization force is only used as a 'trace force' that can keep the imaging process very stable before the biased tip touches the sample surface, and as soon as the tip have touched the sample the repulsive force dominates the tip–sample interaction. Thus based on the above mentioned virtues, soft materials, such as biomolecules or polymers, can be imaged by VSPFM with smaller destroy than that in TM-AFM. Using this method, we have successfully measured the heights and radical compressive elasticity of DNA and protein deposited on mica surface.[23,24]

In this Letter, we investigate the elasticity of a

* Supported by the 100 Persons Project of the Chinese Academy of Sciences, the National Natural Science Foundation of China under Grant No 10474109, the Foundation of Ministry of Personnel of China.

** To whom correspondence should be addressed. Email: lijuanzhang187@163.com

single PEI particle with VSPFM and find that PEI has excellent elasticity. The results provide some useful information for mechanical properties of PEI and present one of the possible reasons for its higher transfection efficiency.

The main principle of VSPFM is the utilization of polarization force during imaging in a dynamic vibrating mode, i.e. the conductive tip of an AFM is biased by an ac voltage and is polarized, to produce a local electric field. The schematic graph of VSPFM is shown in Fig. 1(a). The sample is mounted onto the piezoelectric tube scanner and the deflection of the cantilever under the influence of tip/surface forces is measured by the light beam reflection technique using a segmented photodiode detector. An ac voltage from a function generator (C & C Instrument Co., LTD, Korea) with a range of ± 10 V and frequency of 300 kHz–1 MHz is applied to the tip and the substrate to induce the polarization force. A conductive (metal coating probe of consists of a 10-nm Pt layer on a 20-nm Ti sublayer) rectangular cantilever (NSC18/TiPt, MikroMasch Inc.) with a spring constant of about 3.5 N/m and a resonant frequency of around 75 kHz was used here. All images were collected on a NanoScope IIIa SPM system (Digital Instruments/Veeco, Inc., Santa Barbara, CA) at a temperature of $25 \pm 2^{\circ}$C and a relative humidity of $30 \pm 3\%$.

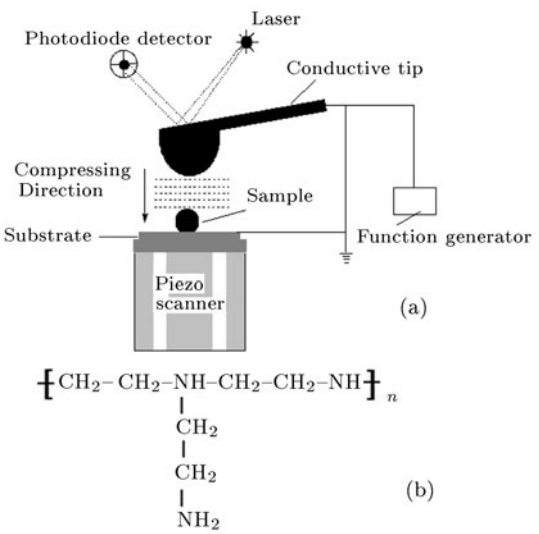

Fig. 1. (a) Schematic drawing describing the principle of VSPFM. (b) The structure of PEI.

PEI (see Fig. 1(b)) provided by Yao *et al.* in our institute was diluted to the concentration of 5 ng/ml with Millipore water (18.2 MΩ·cm) and then deposited onto the freshly cleaved mica.

We observed that PEI exists in the form of nanoparticles on freshly cleaved mica under our experimental conditions. The typical topographical image of PEI under the smallest force that can maintain the tip stable during imaging in TM-AFM is shown in Fig. 2. The average apparent height and width of these nanoparticles are about 8.3 ± 0.5 nm and 94 ± 3 nm, respectively. These values are reasonable for nanoparticles, taking the tip convolution effect into consideration. It is found that PEI particles uniformly adsorbed on mica, indicating their good dispersing property in water. Their sizes are also roughly the same.

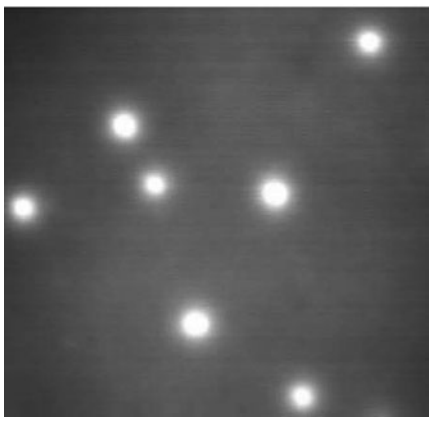

Fig. 2. The height image of PEI deposited on mica surface. Scan area: $1.5\ \mu m \times 1.5\ \mu m$. Data scale: 10 nm.

To begin the compression (operate the AFM in VSPFM), we selected a PEI particle as the target. First, the voltage applied to the tip and the substrate was increased gradually until the contrast of PEI particles disappeared in the image. In this case, the tip did not touch the top of the particle. With the decreasing amplitude at set point (A), the tip was then located to scan the top of the molecule at a larger A and further lowered step-by-step until a conventional TM-AFM image appeared, as shown in Fig. 3. The key steps are that the tip is first adjusted to slightly touch the top of PEI particle (defined as the top image) and then gradually lowers until the tip touches the substrate surface (defined as the bottom image), as illustrated in Refs. [23,24,27]. The corresponding A was termed as $A_{\rm top}$ and $A_{\rm bottom}$, respectively. A series of images could be collected during the approaching process. It is worth noting that the position of the tip can be precisely controlled with the accuracy about 0.1 nm and images are taken at each position by finely tuning the A of our VSPFM. The height H_j of PEI nanoparticle after compressing jth step is calculated by the following equations:

$$H_j = H_{j\text{-app}} + \alpha(A_j - A_{\rm bottom}), \quad (1)$$

$$H_T = H_{\text{top-app}} + \alpha(A_{\rm top} - A_{\rm bottom}), \quad (2)$$

$$\Delta H_j = H_T - H_j, \quad (3)$$

where A_j is the vibration amplitude; $H_{j\text{-app}}$ and $H_{\text{top-app}}$ denote the apparent heights of the PEI nanoparticle after compressing jth step and when the tip just contacts the top surface of the nanoparticle, respectively. These values can be determined from the offline treatment. H_T is the total height of the PEI nanoparticle, ΔH_j is the change of height of PEI after compressing jth step; α is a conversion coefficient, $\alpha = \Delta Z/\Delta A$. In a zero-scale scan on mica surface, when we change the set-point value A stepwise, a

given change of ΔA will make a corresponding change of ΔZ in the tip–sample distance, which can be directly measured from the offline topographic image. More detailed information can be found in Ref. [23].

Figure 4 shows a series of images that illustrate the deformation of a single PEI particle on mica at different A values. Figures 4(a)–4(c) are obtained with the VSPFM operated at sequentially decreasing A values (1.335 V, 1.290 V, 1.205 V). Figures 4(d) and 4(e) are obtained by returning A to 1.290 V and 1.335 V. These images show clearly the large deformation of the PEI particle under small forces and the deformation is remarkably reversible. The deformation of the particle was calculated in terms of Eqs. (1)–(3) according to the corresponding A. The force loading on the nanoparticle is calibrated by a soft cantilever with known spring constant.[27,28]

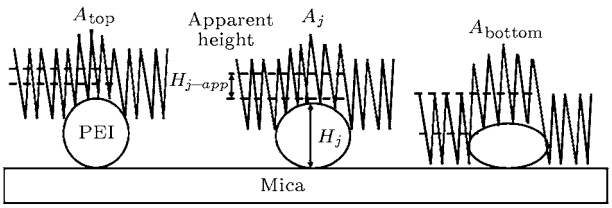

Fig. 3. Sketch of a PEI nanoparticle deposited on a mica surface and an AFM tip at three different set point values ($A_{top} > A_j > A_{bottom}$). The scanning tip only images the PEI particle and does not image the mica surface (A_{top} and A_j).

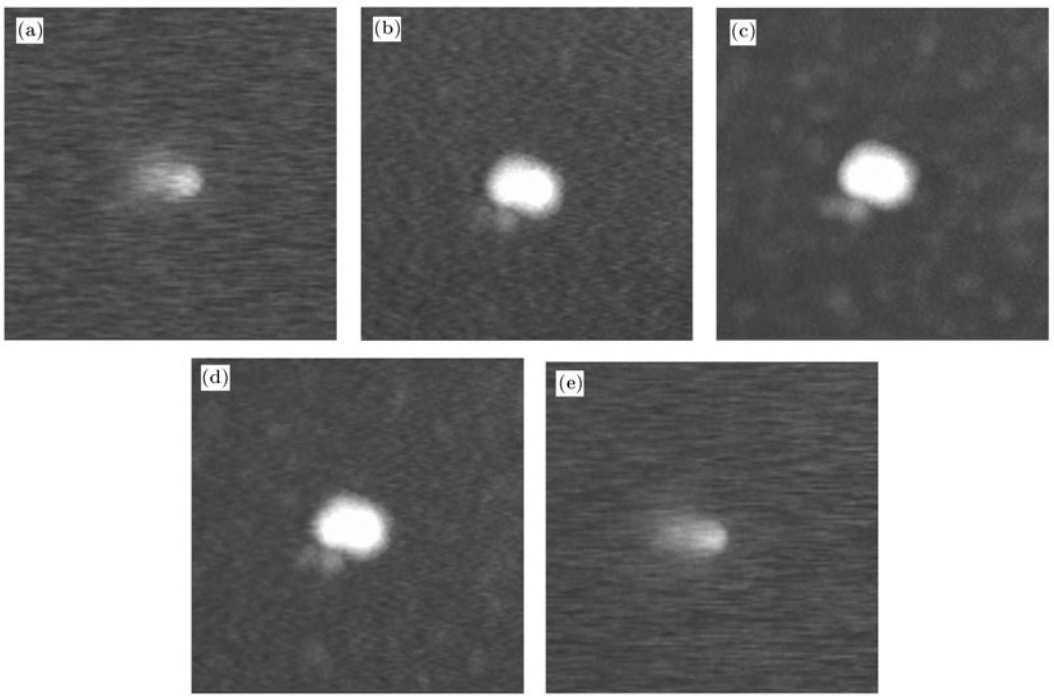

Fig. 4. The images obtained during the tip approaching to and retracting from the particle on a mica surface in VSPFM, for topographic images taken at A = 1.335 V (a), 1.290 V (b), 1.205 V (c), 1.290 V (d), and 1.335 V (e). Scan size: 250 nm×50 nm. Data scale: 15 nm.

Figure 5 shows typical height–force curves of the PEI particle when the tip approaching to and retracting from the particle. From these curves, we can find that the compression of a PEI nanoparticle takes over the three stages. In the initial compressing stage (< 0.5 nN, I), the curve is approximately linear with a large slope of about 1.67, and the height decreases rapidly with the applied force, suggesting that PEI nanoparticle is very 'soft' and is susceptible up to even small forces. It is possible that the forces maintaining polymer structures in this deformation regime are mainly from the hydrogen bonding and van der Waals interactions. When the average load is between 0.5 nN and 0.9 nN (Ⅱ), the height–force curve of the PEI nanoparticle becomes flatter (the slope of curve is about 0.5). At this stage, the drop of the height of the PEI nanoparticle with the applied force is slower than that in the small force range. The change of strain in this period could be derived from the change of configuration of polymer. Above 0.9 nN (Ⅲ), the height of the PEI nanoparticle decreases slowly with the applied force (the slope of curve is about 0.29). We speculate that the space between backbones is now greatly reduced and the external force is mainly exerted on the much stiffer hydrocarbon backbones. Compared to the result in our previous paper, the well known 'hard' gold nanoparticles exhibit a fairly flat curve of height versus force, which means that its height does not significantly change with the increase of applied forces.[24]

The strain–force curves for the approaching and retracting processes are illustrated in Fig. 6. From these

curves, it can be concluded that the deformation of the single PEI nanoparticle also is carried out in three steps: i.e., quick compression in a small force region (I), slight slowness in the middle force region (II), and large slowness in relatively large force region (III). Under the experimental forces, the PEI nanoparticle exhibits remarkable reversibility of the deformation according to the force-strain curves of the approaching and retracting processes, even the PEI nanoparticle is compressed to 70% of its height. In the small force region ($F < 0.5$ nN), the PEI nanoparticle is very soft, which makes its structure change freely and might help it to well bind with DNA molecules during the process of transfection.

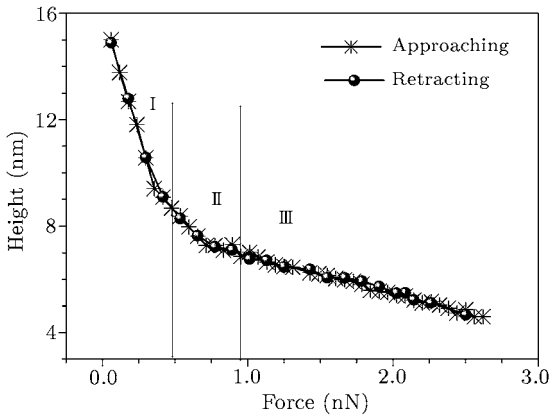

Fig. 5. Height–force curves obtained during the tip approaching to and retracting from the particle.

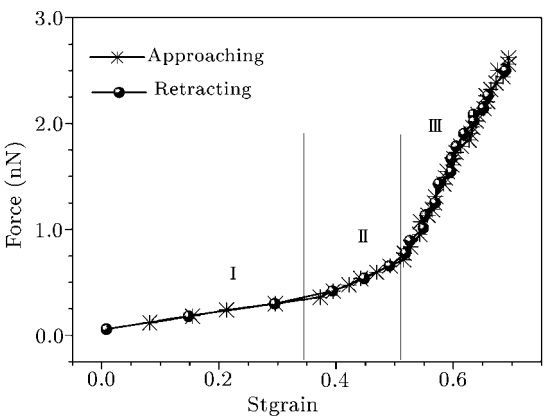

Fig. 6. Force–strain curves of the PEI particle.

The compression elasticity of PEI nanoparticles can be extracted from the slope of the height–force curve with the Hertz model, which is widely used to estimate the elastic modulus of a single nanoparticle in AFM experiments. We note that the elastic moduli of the tip and the substrate (mica) are significantly higher than that of the PEI nanoparticles, thus the deformation of the tip and substrate can be ignored. According to this, the Hertz model is simplified to the following equation:[29]

$$d^3 = 9(1 - v)F_0^2/(16RE^2), \qquad (4)$$

where v, R, F_0^2, d, and E represent the Poisson ratio, tip radial, applied load, indentation, and Young's modulus, respectively. The tip radial R is about 20 nm, as provided by the manufacturer. By assuming a typical Poisson ratio of 1/3 for soft materials and fitting the functional dependence of Eq. (4) to several height–force curves, we obtain the effective elastic modulus of PEI nanoparticles. Their effective elastic modulus is 5–10 MPa in the small force region I (see Fig. 5), which is smaller than the DNA values of 20–70 MPa ($F < 0.4$ nN).[24] The elastic moduli in the regions II and III are 10–80 MPa and 80–160 MPa, respectively. The calculation of other six PEI nanoparticles agreed well with these values within a factor of 2–3.

The height of the PEI nanoparticles is obtained by VSPFM to be approximately 14.8 nm, which is much larger than that in TM-AFM (about 8.3 nm). The difference suggests that the tip pressure is one of the key factors that result in polymer deformation. Therefore, it is necessary to consider the effect of tip's pressure on the soft molecules in the AFM imaging process. For example, a recent AFM study on immunoglobulin (IgG) indicated that its severe deformation is induced by AFM tip's pressure.[26] In contrast to the conventional TM-AFM, our VSPFM-based method can study molecules when the tip–molecule forces almost approach zero, which offers new opportunities for investigation the properties of soft materials, such as polymers and biomolecules.

We thank Hu Jun, Fang Hai-Ping, and Fan Chun-Hai for helpful discussions.

References

[1] Zhang W K and Zhang X 2003 *Prog. Polym. Sci.* **28** 1271
[2] Nichol C A et al 1999 *Drug Delivery* **6** 187
[3] Pollard H et al 1998 *J. Biol. Chem.* **273** 7507
[4] Abdallah B et al 1995 *Biol. Cell* **85** 1
[5] Boussif O et al 1995 *Proc. Natl. Acad. Sci.* USA **92** 7297
[6] Pun S H et al 2004 *Bioconjugate Chem.* **15** 831
[7] Gosselin M A et al 2001 *Bioconjugate Chem.* **12** 989
[8] Tseng W C and Jong C M 2003 *Biomacromolecules* **4** 1277
[9] Furgeson D Y et al 2003 *Bioconjugate Chem.* **14** 840
[10] Brissault B et al 2003 *Bioconjugate Chem.* **14** 581
[11] Binnig G and Quate C F 1986 *Phys. Rev. Lett.* **56** 930
[12] Tan S et al 2004 *Langmuir* **20** 7015
[13] Shulha H et al 2003 *Macromolecules* **36** 2825
[14] Cappella B and Dietler G 1999 *Surf. Sci. Rep.* **34** 1
[15] Zhang Q et al 1993 *Surf. Sci. Lett.* **290** L688
[16] Tsukruk V V et al 2003 *Appl. Phys. Lett.* **82** 907
[17] Shen W D et al 2000 *Phys. Rev. Lett.* **84** 3634
[18] Yu M F et al 2000 *Phys. Rev. Lett.* **85** 1456
[19] Fang T H et al 2004 *Chin. Phys. Lett.* **21** 1117
[20] Sui L et al 2005 *Chin. Phys. Lett.* **22** 1010
[21] Yue Y et al 2005 *Chin. Phys. Lett.* **22** 1837
[22] Hu J et al 1995 *Science* **268** 267
[23] Li X J et al 2003 *J. Val. Sci. Tech.* B **21** 1070
[24] Zhou X F et al 2005 *Phys. Rev.* E **71** 0629011
[25] Salmeron M et al 1997 *MRS Bulletin* **22** 36
[26] García R and Pérez R 2002 *Surf. Sci. Rep.* **47** 197
[27] Zhou X F et al 2005 *Chem. Lett.* **34** 1488
[28] Su C et al 2004 *Ultramicroscopy* **100** 233
[29] Radmacher M et al 1994 *Langmuir* **10** 3809