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Influence of DNA condensation state on transfection efficiency in DNA/polymer complexes: An AFM and DLS comparative study

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Abstract

Atomic force microscopy (AFM) is used to describe the formation process of polymer/DNA complexes. Two main objectives of this research are presented. The first one is to apply AFM as an effective tool to analyse DNA molecules and different polycation/DNA complexes in order to evaluate their degree of condensation (size and shape). The other one is to search for a relationship between the condensation state of DNA and its transfection efficiency. In this study, linear methacrylate based polymers and globular SuperFect polymers are used in order to induce DNA condensation. Ternary complexes, composed of methacrylate based polymers and polyethylene glycol (PEG)-based copolymers, are also investigated. AFM allows us to confirm good condensation conditions and relate them (or not) to transfection efficiencies. These AFM results (obtained after drying in air) are compared with measurements deduced from Dynamic Light Scattering (DLS) experiments performed in water. This comparison allowed us to identify the structural modifications resulting from deposition on the mica surface.

Keywords: Atomic force microscopy; DNA; Gene therapy vehicle; Dynamic Light Scattering

1. Introduction

The development of an efficient gene delivery vehicle is a major challenge for gene therapy. The DNA condensation process has thus drawn a large interest in biology the last decade. Two types of vectors of nucleic acids exist: viral agents (like recombinant retroviruses and adenoviruses (Ledley, 1996; Wilson, 1995)) and synthetic vectors (as cationic lipids and

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polymers). However, viral agents yet suffer from a limited success in delivering genes, partially due to the immune and toxic response they induce, but also because of biosafety problems (Miller, 2003; Maguire-Zeiss and Federoff, 2004; Feldman, 2003). To avoid these problems, non-viral carriers are presently investigated in many laboratories. For example, DNA condensation is achieved with proteins like protamine (Allen et al., 1997) or liposomes and lipid intermediates (Gao and Huang, 1996). Promising results are also obtained with polycationic polymers (van de Wetering et al., 1997, 1998), such as poly-L-lysine (Choi et al., 1999; Toncheva et al., 1998) or protonated amino-functionalised polymethacrylate (Pirotton et al., 2004). Such polymer/DNA complexes are formed through electrostatic interactions between the negatively charged phosphate groups of DNA and protonated amino groups of the polymer.

Although polycationic polymers have some advantages over viral vectors, their efficiency as a transfecting agent is still limited (Itaka et al., 2003). Indeed, several biophysical requirements on such polyelectrolyte particles are encountered. Apart from the polymer characteristics (charge density, molecular weight), which have been previously discussed (Pirotton et al., 2004), we wondered if the physico-chemical properties of the formed polymer/DNA complexes influence the transfection efficiency process. Indeed, a physical view of the condensates (including their dimensions) related to a biological view (including transfection efficiency) could facilitate improvement in this gene delivery technology (Dunlap et al., 1997). In this context, atomic force microscopy (AFM) has proven to be an excellent tool having the ability to image soft biological structures like cells, bacteria or proteins (Radmacher et al., 1992; Razatos et al., 1998; Reich et al., 2001). DNA molecules, such as plasmids in particular, have been intensively studied and detailed structures of individual molecules have yet been revealed (Hansma et al., 1992; Yang et al., 1992; Lyubchenko et al., 1993; Argaman et al., 1997). Moreover, AFM measurements have the advantage on DLS experiments that they can be performed after deposition on any surface and have an intrinsic better resolution.

Polycationic polymethacrylate derived chains are sufficient to condense DNA in order to produce binary complexes capable of transfecting cells in culture. However, these chains alone do not protect the particles from the environment. The interactions between the serum proteins and the complexes often impair the transfection in vitro. In vivo, these interactions lead to the rapid elimination of the complexes by phagocytosing macrophages (Howard et al., 2000). These problems can be avoided by the introduction of hydrophilic sequences of poly(ethylene glycol) (PEG) at the surface of the complexes. Previous results indeed highlighted that cell transfection with binary complexes is feasible exclusively in the absence of serum and that, on the contrary, the PEGylated complexes allowed transfection even in the presence of serum. The presence of the hydrophilic sequences of PEG at the complex surface also improved the hemocompatibility properties of the complexes (Pirotton et al., 2004). These kinds of complexes are also analysed by AFM.

Finally, globular dendrimeric polymers are also used to condense DNA. Dendrimers represent a class of polymers that exhibit a molecular architecture characterized by regular dendritic branching with a radial symmetry (Frechet, 1994). Their structural difference compared to linear polycationic polymers induces different condensed structures, which are also observed and discussed in detail.

More precisely, in this research, linear methacrylate based polycationic polymers (PDMAEMA), PEGylated linear methacrylate based polycationic polymers and globular SuperFect dendrimers are used in order to induce DNA condensation. AFM data obtained on PDMAEMA/DNA binary complexes are exposed and discussed as a function of the polymer/DNA weight ratio and transfection efficiency. Characteristics of ternary complexes are then compared to the binary complex properties. Measurements obtained on these two kinds of complexes are thus compared to Dynamic Light Scattering data, obtained in wet conditions. The globular SuperFect/DNA structures are finally exposed. In order to appreciate the architecture of polymer/DNA complexes, an image of uncondensed dsDNA has been provided for comparison.

2. Materials and methods

2.1. Complex formation

The pCMVβ plasmid, with the β-galactosidase gene of *Escherichia coli* under control of the CMV pro-

moter (Clontech, USA), was amplified and purified at large scale by Plasmid Factory (Germany). It is a plasmid of 7.2 kb (corresponding to a $M_w = 4.77 \times 10^6$ Da) with a high supercoiled content and low bacterial endotoxins (<0.1 E.U./μg DNA). It has been purchased in water (1 mg/ml). The poly(2-dimethylaminoethyl methacrylate) (PDMAEMA) homopolymers and P(DMAEMA-b-MAPEG) palm-tree like copolymers (where MAPEG stands for methacrylate end-functionalised polyethylene glycol macromonomer) are synthesized by atom transfer radical polymerisation (ATRP) as described elsewhere (Pantoustier et al., 2003; Pirotton et al., 2004). The average molar mass (M_n) and the polydispersity index (M_w/M_n) of the homopolymer are 49,100 and 1.59, respectively, as determined by size exclusion chromatography with reference to poly(methylmethacrylate) standards. For the palm-tree like copolymer, M_n is 24,100 and the polydispersity index is 1.45. The average mole number of tertiary amino groups per gram of polymer (N) is determined by titration: it is 6.32 and 4.22 mmol/g for the homopolymer and the copolymer, respectively.

Ternary complexes are prepared as follows: a small volume of concentrated PDMAEMA is mixed with the DNA and left to condense for 30 min at room temperature. Afterwards, the copolymer is added for a second incubation during 30 min. When PDMAEMA is tested alone, the solvent is added after the first 30 min incubation instead of the PEG-based copolymer. The final concentration of DNA is 20 μg/ml. Complexes are formed in water for the AFM and in a HEPES-buffered solution (HBS: 20 mM HEPES, 155 mM NaCl, pH 7.4) for the transfection experiments. The SuperFect/DNA complexes are prepared by immersion in water during 15 min at a 6/1 (w/w) ratio. SuperFect is provided by Qiagen, Germany.

2.2. Transfection

Cos-7 cells are cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with HEPES (20 mM), penicillin (100 U/ml), streptomycin (100 μg/ml) and Fœtal Bovine Serum (FBS) (10%). DMEM and FBS were obtained from Gibco Invitrogen Corporation (UK). Penicillin and streptomycin were purchased from Biowhittaker (Verviers, Belgium). The day before transfection, the cells are seeded in 12-well plates (2×10^4 cells/well) in complete culture medium. The cells are then incubated with the complexes, prepared as described above, in DMEM + 5% FBS under a humidified 5% CO_2 atmosphere, at 37 °C. After 3 h, the excess of complexes is removed, the cells are washed with DMEM and further cultured for 24 h in culture medium with 5% FBS. In order to evaluate the β-galactosidase expression, cells are washed twice with PBS and lysed with the mammalian protein extraction reagent (M-PER), obtained from Pierce (USA). Hundred microliter of β-galactosidase substrate (*o*-nitrophenyl β-D-galactopyranoside or ONPG, 4.4 mM) are mixed with 100 μl of cell lysate and incubated at 37 °C. Half an hour later, A_{405} (absorbance at 405 nm wavelength) is measured. For each sample, the protein concentration is determined according to the BIO-RAD Protein Assay protocol with bovine serum albumin (BSA) as standard. Results are expressed in arbitrary units (a.u.) per 100 μg of proteins, as the mean standard deviation of triplicate determinations.

For transfection experiments, DNA needs to be condensed by polycations. Complexes formed with two types of polycationic polymers, differing by their structure, have been analysed: on the one hand, SuperFect (SF) is a polyamidoamine dendrimer of the sixth generation and has a globular structure. On the other hand, PDMAEMA is a polymer with a linear structure. Both types of condensing agents have a similar molar mass (45,000–49,000).

2.3. AFM imaging

Prior to AFM measurements, the complex suspension is diluted 10-times with water and 2 μl of the resulting solution is then deposited onto a freshly cleaved mica substrate. Samples are imaged after water evaporation. Experiments are performed with a commercial STM/AFM (Nanoscope IIIa, Digital Instruments) operating in tapping mode (TM) AFM, using standard silicon cantilevers with ~42 N/m spring constant and 369.22 kHz working frequency (true resonance frequency = 369.32 kHz, $A_0 = 23.3$ nm and $A_{sp} = 5.5$ nm). All images are recorded in air at room temperature, at a scan speed of 2.5 Hz. The background slope is resolved using first or second orders polynomial functions. No further filtering is performed.

2.4. Dynamic Light Scattering

Particle size measurements are performed by Dynamic Light Scattering (DLS) analysis adopting a Brookhaven Instrument (BI 2030 AT linear digital correlator; 128 channels). The time-variations of the scattered light intensity (emitted by particles in solution) are analysed at 90°. Samples are incubated at 25 °C in water. The incident Ar laser wavelength is fixed at 488 nm (∼10 mW). The quality of the instrument is checked with a Beckman Coulter certified latex standard (PCS control L300). A minimum of three measurements is performed on each sample. The autocorrelation curves are deconvoluted with either Cumulant, Exponential Sampling or Contin methods.

3. Results and discussion

Results exposed in this paper highlight the formation of complexes from dsDNA in the presence of polymers. Previous studies using the AFM technique demonstrated the condensation of closed and linear dsDNA in the presence of different condensing agents (Dunlap et al., 1997; Rackstraw et al., 2001; Vijayanathan et al., 2004; Lin et al., 1998; Martin et al., 2000; Reschel et al., 2002; Hansma et al., 1998). Such agents produce different sizes of condensates and require different incubation times and concentrations. Concerning the polymers used in our experiment, optimal conditions (charge, polymer/DNA ratios, incubation times. . .) have been determined previously and applied in this paper (Pirotton et al., 2004).

3.1. Plasmid DNA

We performed AFM imaging in the tapping mode (TM-AFM). The results are stable and reproducible. Plasmid DNA molecules deposited onto freshly cleaved mica substrate are imaged after drying in air. The substrate appears to be homogeneously flat with double-stranded DNA (dsDNA) molecules on it. A typical TM-AFM high-resolution image obtained by scanning over a small area is presented in Fig. 1. The dsDNA molecules display a closed geometry, looking like relaxed circles, with little twisting of the strands. This structure is characteristic of uncondensed DNA morphology (Rackstraw et al., 2001). Almost

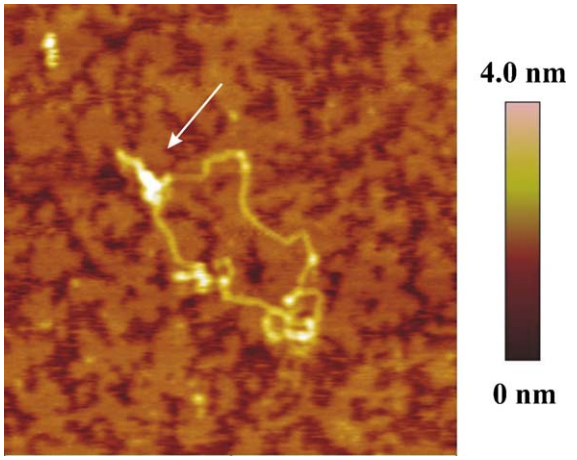

Fig. 1. High-resolution TM-AFM image (1 μm × 1 μm) of plasmid DNA observed on mica.

everywhere in the image, the average apparent height and width of DNA molecules correspond to 0.6 and 20.0 nm, respectively. The DNA strands are piled up at the upper left part of Fig. 1 (indicated by an arrow). At this point, the DNA strands have a height of 1.0 nm, about twice a helix apparent height.

3.2. Methacrylate based complexes

3.2.1. PDMAEMA/DNA binary complexes

The DNA used to prepare the complexes is the pCMVβ plasmid, encoding the β-galactosidase of *Escherichia coli*. The transfection is defined as the introduction of exogenous genetic material, its subsequent internalization into a host cell (the transfected cell) and the subsequent expression of the encoded protein. In our case, the transfection efficiency is determined by measuring the β-galactosidase activity (β-gal) in transfected cells. Fig. 2 exhibits results of transfection experiments (representing the β-gal activity as a function of the polymer/DNA weight ratio) carried out at a fixed plasmid concentration and a varying PDMAEMA concentration. Cationic polymers thus electrostatically bind to plasmid molecules (presenting a net negative charge), leading to polymer–DNA complexes. It seems evident that PDMAEMA promotes the cellular uptake and its subsequent expression. Moreover, the transfection efficiency is dramatically affected by the weight ratio and presents a maximum. The maximal β-gal activity is observed when complexes are

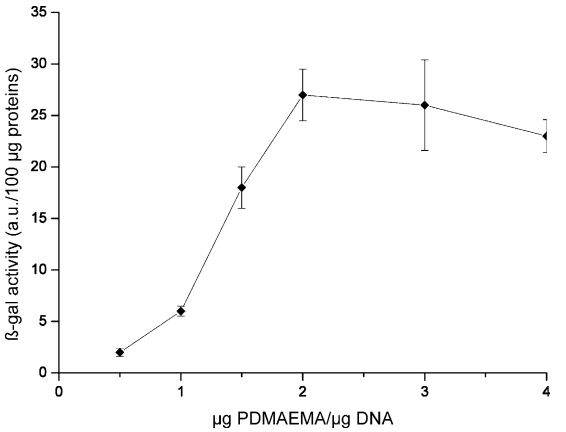

Fig. 2. Transfection efficiency of the PDMAEMA/DNA complexes, followed by measuring the β-galactosidase activity (β-gal) in transfected cells, as a function of the PDMAEMA/DNA weight ratios used.

prepared with 2 µg of polymer/µg (or more) of DNA. Lower PDMAEMA/DNA weight ratios lead to a lower transfection efficiency. In order to determine whether the structure (shape) and size differences could explain the β-gal expression evolution, complexes giving maximal and submaximal β-gal activity were analysed. The effect of the PDMAEMA/DNA weight ratio on the size of the formed complexes is thus exposed below.

Complexes obtained with a 1/1 polymer/DNA weight ratio produce poorly condensed structures, as observed in Fig. 3A. Large flat aggregates (composed of linear strands and loop-like structures) are visible on the top of this image. These aggregates are about 25 nm wide, 50 nm long and 1.2 nm high. This apparent height is twice as big as measured on bare DNA. In the centre of Fig. 3A (arrow), we observe a structure similar to a bare DNA molecule, as already depicted in Fig. 1. Toroidal structures were also observed but in a limited number (data not shown). From Fig. 3A, it is obvious that the optimal condensation conditions (in size and shape) are not reached. In this case, the poor transfection efficiency observed in Fig. 2 (for a 1/1 polymer/DNA weight ratio) could be related to the poorly condensed polymer/DNA aggregates. As already previously observed, at such lower weight ratios (<1.5), rather large particles are formed (Cherng et al., 1996). It was therefore postulated that PDMAEMA molecules probably act as a linker for dsDNA molecules, resulting in the formation of large aggregates. These particles are

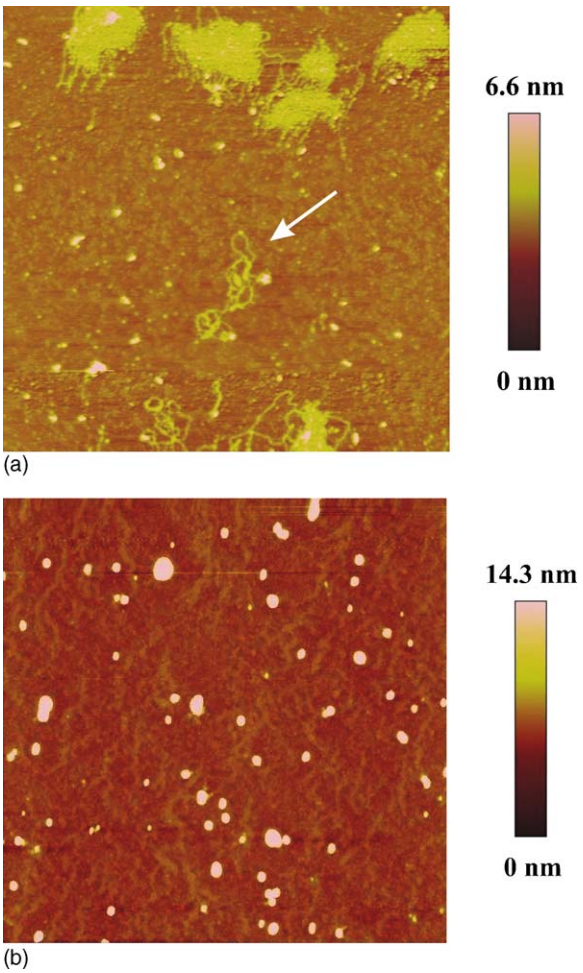

Fig. 3. (a) TM-AFM image (2 µm × 2 µm) of PDMAEMA/DNA complexes (1/1, w/w), at ambient air. (b) TM-AFM image (3.5 µm × 3.5 µm) of PDMAEMA/DNA complexes (2/1, w/w), at ambient air.

therefore too voluminous to be internalized by cells via endocytosis (Jones et al., 2000), as seems confirmed by the low transfection efficiency observed.

Complexes are also prepared with a 2/1 polymer/DNA weight ratio, which is the ratio corresponding to a higher transfection efficiency. Round-shaped and compact complexes are observed, as presented in Fig. 3B. Bare DNA molecules are never observed, which lead us to conclude that most of plasmid molecules are complexed with the polymer. The apparent diameter and height distributions are shown on Fig. 4A and B, respectively. The curves are obtained

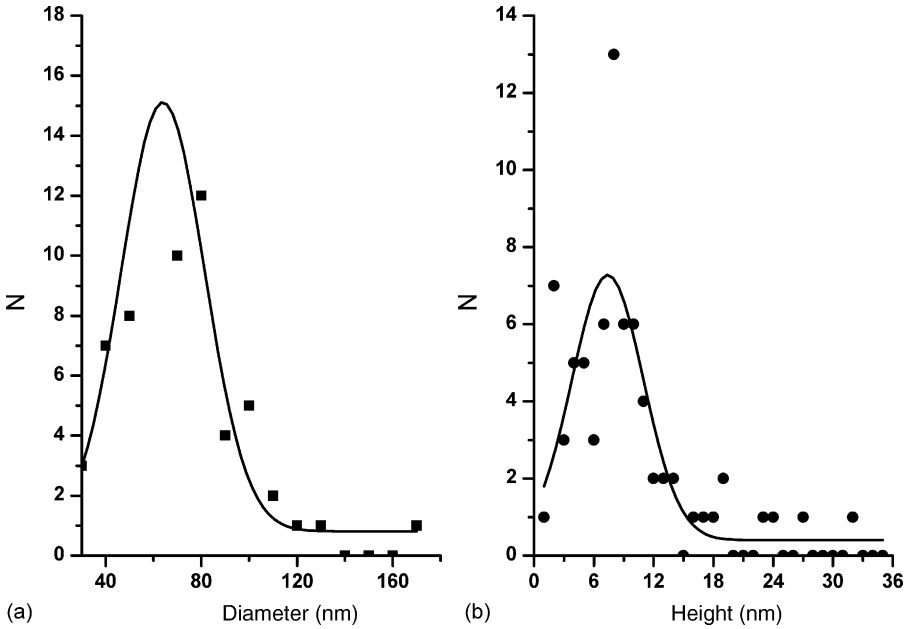

Fig. 4. (a) Diameter distribution of PDMAEMA/DNA complexes (2/1, w/w), calculated from TM-AFM images on about 75 complexes. The curve is a Gaussian fit of the data. (b) Height distribution of PDMAEMA/DNA complexes (2/1, w/w), calculated from TM-AFM image on about 75 complexes. The curve is a Gaussian fit of the data.

with a Gaussian fit to the experimental data. The distributions are calculated from about 75 complexes. The average diameter and height distribution are 63.9 ± 2.5 and 7.4 ± 0.5 nm, respectively. Approximately 5% of the particles have a diameter larger than 100 nm and 50% of the complexes a diameter larger than 60 nm. In any case, these binary complexes are well-condensed structures, which tend to confirm the close link between condensation and transfection efficiency. The diameters deduced from AFM data are compared to DLS results obtained in solution (Fig. 5). The DLS average diameter is 130.7 nm ($D_{\text{dls bin}}$), which is much larger than the value extracted from Fig. 4A.

Complexes obtained in a 1/1 PDMAEMA/DNA weight ratio produce poorly condensed structures (Fig. 3A). The observed aggregates seem to be intermediate between uncondensed DNA of Fig. 1 and condensed structures of Fig. 3B, as developed by Vijayanathan et al. (2004). In this case, the poor transfection efficiency observed in Fig. 2 could be related to the poorly condensed polymer/DNA aggregates. These results on binary condensates are comparable to those reported by other research groups using these techniques, although different DNA samples and condensing agents are used. Similar condensed structures are reported for complexes prepared with other polycations such as poly-L-lysine (Toncheva et al., 1998), poly(amino ester) or *n*-PAE (Lim et al., 2002) or other methacrylate polymers (Wolfert et al., 1999).

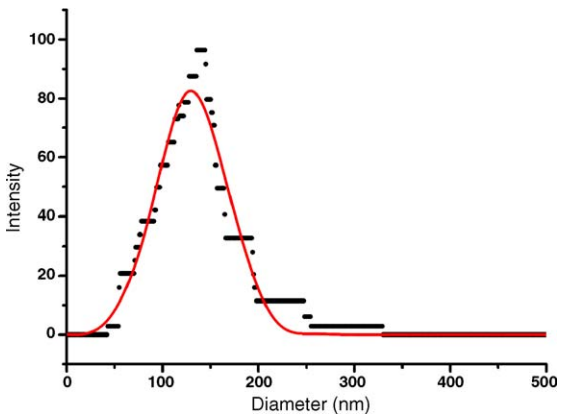

Fig. 5. Diameter distribution of PDMAEMA/DNA complexes (2/1, w/w), calculated from Dynamic Light Scattering data.

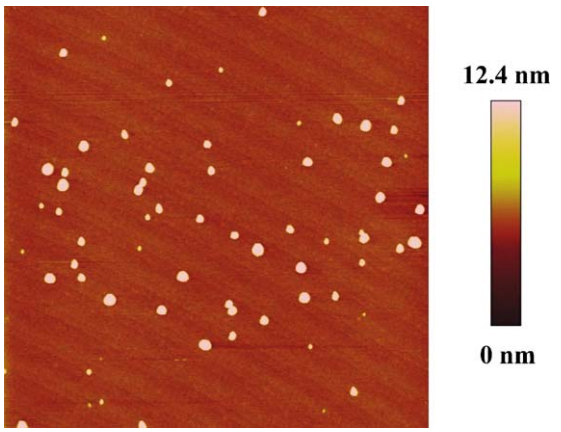

Fig. 6. TM-AFM image (4 μm × 4 μm) of copolymer/polymer/DNA ternary complexes (5/2/1, w/w/w), at ambient air.

3.2.2. Ternary complexes

A typical image of ternary complexes is presented in Fig. 6. The complexes are prepared with 2 μg of PDMAEMA and 5 μg of the copolymer. This is a polymer/DNA ratio giving maximal transfection activity (data not shown). The ternary complexes form very compact structures, similar to the PDMAEMA/DNA complexes. No tubular structure in or around the condensates is visible. Apparent diameter and height distributions are shown on Fig. 7A and B, respectively. These distributions are calculated from about 100 complexes. The apparent diameter and height mean values are 102.6 ± 2.0 and 9.7 ± 0.1 nm, respectively. These dimensions are about twice larger than those measured for the binary complexes. DLS experiments revealed the diameter distribution of wet complexes, as shown in Fig. 8. The average diameter is 329.6 nm ($D_{\text{dls tern}}$), which is about three times larger than the one calculated from Fig. 7A (obtained after drying in air).

The AFM image of Fig. 6 highlights the presence of dense structures, indicating the formation of ternary complexes. Therefore, the two-steps preparation method of those complexes prevents the interfering of the PEG during the condensation of the DNA with the PDMAEMA, as previously observed (Rackstraw et al., 2001). Nevertheless, it was not demonstrated that PEG copolymer does not substitute the PDMAEMA. However, it is unlikely because of the transfection efficacy of the ternary complexes. Indeed, if substitution occurred, there would be copolymer/DNA complexes in the population and previous studies have clearly

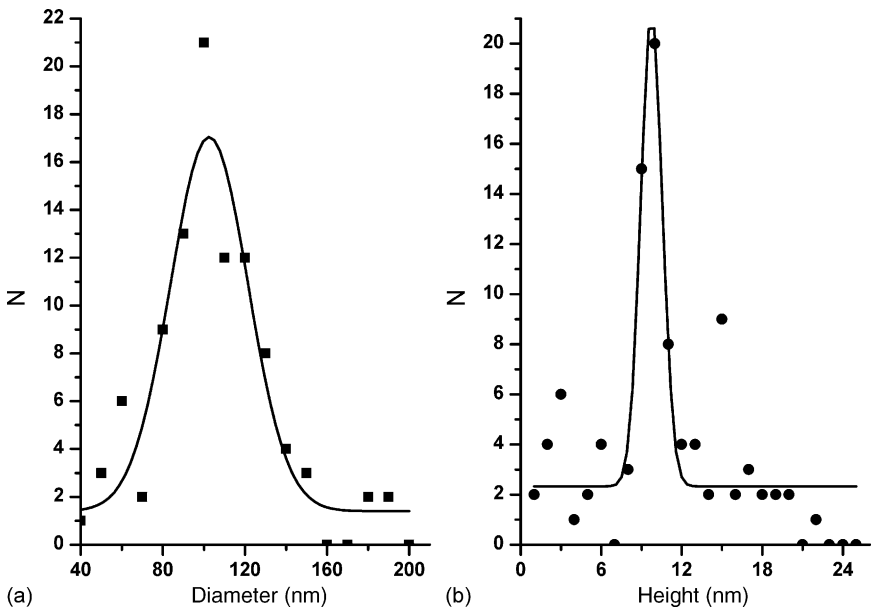

Fig. 7. (a) Diameter distribution of copolymer/polymer/DNA ternary complexes, (5/2/1, w/w/w), calculated from TM-AFM images on about 100 complexes. The curve is a Gaussian fit of the data. (b) Height distribution of copolymer/polymer/DNA ternary complexes (5/2/1, w/w/w), calculated from TM-AFM images on about 100 complexes. The curve is a Gaussian fit of the data.

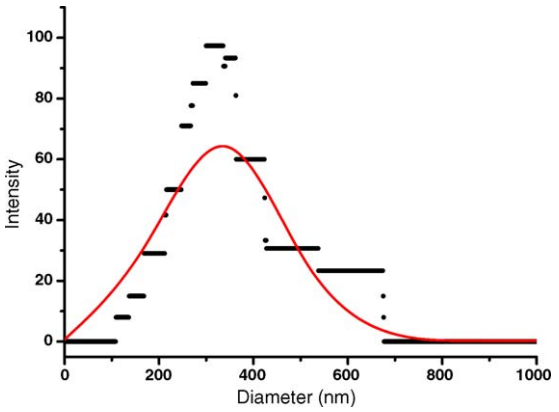

Fig. 8. Diameter distribution of copolymer/polymer/DNA ternary complexes (5/2/1, w/w/w), calculated from Dynamic Light Scattering data.

shown that this type of complexes are unable to transfect the cells (Pirotton et al., 2004). With a mixed population of complexes, a decrease in transfection efficacy rather than the reported increase should be observed.

The AFM and DLS diameters of ternary complexes are larger than those obtained on binary ones. Meanwhile, the size of the ternary complexes is much larger than those measured on binary complexes, whatever the experimental probe used. This is in agreement with the association of block copolymer with PDMAEMA/DNA binary complexes previously formed. However, transfection efficiencies observed with these two types of complexes are similar (Martin et al., 2000). These observations suggest that the optimal size of polyplexes can vary with the composition of the polyplexes and clearly differs from binary and ternary complexes, as previously observed by other research groups (van de Wetering et al., 1997; Congiu et al., 2004).

The apparent AFM sizes of binary and ternary complexes indicate that the condensates present a compressed-sphere shape. Such shapes are certainly related to AFM technique limitations. As a matter of fact, this phenomenon (compressed structures on the substrate) is the same as observed for bare dsDNA molecules. Indeed, the measured height and width of a single dsDNA molecule in Fig. 1 are, respectively, much lower and much larger than the DNA helix theoretical diameter of about 2.0 nm. These differences have been discussed in several recent publications. Other research groups have also observed and reported on this effect (Hansma et al., 1996; Maeda et al., 1999; Van Noort et al., 1997; Wyman et al., 1995; Yang et al., 1996). They commonly attributed it to the indentation of dsDNA. Moreno-Herrero et al. supposed that the compression of the molecule due to electrostatic attractive forces between the sample and the substrate contributes to the reduction of the measured heights (Moreno-Herrero et al., 2003). However, using non-contact AFM, correct heights measurements have been carried out by Anselmetti et al. (1994).

The very large measured width of dsDNA molecules (~20 nm instead of the common 2–3 nm width) only reflects the tip radius effect as developed by Lyubchenko et al. (1993). The DNA molecules and complexes imaged are indeed dilated due to the finite size of the tip, leading to overestimate sizes. In order to get more accurate diameters of the complexes, the AFM tip apex radius of curvature (R_t) have to be determined, using the apparent diameter measured for the DNA double helix ($W = 20$ nm). Considering objects with a circular cross-section, this apparent width is given by $W = 4\sqrt{R_0 R_t}$, with R_0 the radius of the object (~1 nm for the DNA molecule). The AFM tip apex radius of curvature is therefore estimated to be 25 nm. By posing the complexes have a spherical shape on the mica substrate and a R_t of 25 nm, the measured diameters can be corrected using the above-mentioned equation. The averaged diameters for the binary and ternary complexes are now calculated as 20.4 nm ($D_{0\,bin}$) and 52.6 nm ($D_{0\,tern}$), respectively.

By the way, those newly calculated values do not agree with DLS measured diameters. This discrepancy may be attributed to differences in experimental procedures. Indeed, DLS experiments are performed under aqueous solution while AFM results were obtained after drying in air. So the dehydration of the complexes could be responsible of it. However, the ratio between ternary and binary complexes diameters calculated from AFM data ($D_{0\,tern}/D_{0\,bin}$) is about the same as the ratio deduced from DLS diameters ($D_{dls\,tern}/D_{dls\,bin}$), which are about 2.5. This ratio seems to confirm the validity of our AFM measurements. As a consequence, the ratio between DLS and AFM diameters of binary complexes ($D_{dls\,bin}/D_{0\,bin}$) is also similar to this ratio applied to ternary complexes ($D_{dls\,tern}/D_{0\,tern}$), which is around 6.2. This seems to set a relation between wet and dried complexes diameters, with this last ratio as a pro-

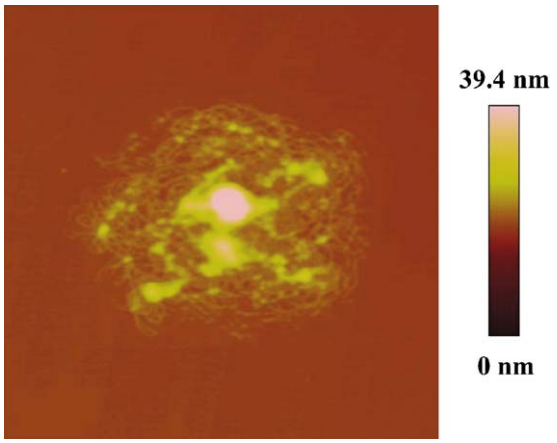

Fig. 9. TM-AFM image (2 μm × 2 μm) of SF/DNA complexes (6/1, w/w), at ambient air.

portionality factor. Thanks to AFM, it could therefore be possible, in further similar experiments, to relate AFM measurements (obtained on dried samples) to diameters of wet samples (on which experiments are more difficult to perform).

3.3. SF/DNA complexes

SF/DNA complexes are also prepared in the best conditions for transfection experiments, i.e. with 6 μg SF/μg DNA (data not shown). The complex structures are shown in Fig. 9. They appear as a protruding central dot surrounded by a spaghetti-like structure, i.e. a flat disk consisting of entangled filaments. The average disk diameter is about 850 nm while the central dot is about 18 nm high and 290 nm wide. The filaments have diameters ranging from 12 to 24 nm, and a height between 0.4 and 0.9 nm. These dimensions are similar to those measured on bare DNA in Fig. 1.

These results on SF/DNA complexes highlight the significance of the polymer architecture to the morphologies observed. The structure visible in the AFM image of Fig. 9 on such DNA complexes indicates that complexes do not form a compact structure. This structure can be explained by suggesting that the central dots are well-condensed SF/DNA complexes, surrounded by poorly condensed ones or by bare DNA. Those results are opposite to the compact structures observed by other research groups (Maksimenko et al., 2003). Whatever the reason, it corresponds to a good transfection activity (similar to one observed with binary complexes), forcing us to conclude that, taken separately, the polymer architecture is not, to a certain degree, a critical point in the transfection process.

4. Conclusions

Atomic force microscopy imaging of DNA and polymer/DNA complexes has been successfully achieved. We deduced apparent and "true" size and shape of the molecules and the condensates. Our results were compared to the dimensions deduced from Dynamic Light Scattering experiments, performed in wet conditions. These experiments demonstrated an original approach, based on direct observations, which reveal the effect of structural factors of complexes on the transfection efficiency. We showed that the AFM technique could give informations on the crucial factors (shape and size), we have to take into account in the elaboration of new transfection vectors. In particular, we deduced that the size of the complexes and the polymer architecture is not, to a certain degree, a critical point in the transfection process. Moreover, we put forward the effect of the surrounding environment on the complexes. We found a proportionality factor (of about 6.2) between the diameters observed in solution (obtained by DLS) and those observed after deposition on the mica substrate (observed by AFM).

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