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Neuronal tau induces DNA conformational changes observed by atomic force microscopy

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Neuronal tau associates with chromosome scaffold and localizes in the nuclear and the nucleolar organization regions in neuronal and some non-neuronal cells. Observing the interaction of neuronal tau with DNA under AFM shows that tau binds to DNA as a monomer, and tau–DNA complex forms a beads-on-a-string structure when the mass ratio is 1:10 (molar ratio of tau/DNA ~1:700 bp). A beads-on-a-coil structure, in which tau is as polymers, will appear when the mass ratio is up to 1:5 (molar ratio of tau/DNA ~1:350 bp). The present observation that neuronal tau bends the DNA double strands indicates that the appearance of the tau–DNA complex is dependent upon the mass (or molar) ratio of tau and DNA. *NeuroReport* 15:000–000 © 2004 Lippincott Williams & Wilkins.

Key words: Atomic force microscopy (AFM); Beads-on-a-coil; Beads-on-a-string; Neuronal tau; Nuclear tau; Tau–DNA complex

INTRODUCTION

Neuronal tau is one of the microtubule-associated proteins, promoting microtubule assembly and stabilizing microtubules both *in vitro* and *in vivo* [1–3]. This protein is most probably of central importance in the pathogenesis of neurodegenerative diseases, such as AD, PSP, and CBD [4]. Davis *et al.* [5] have showed that tau populations within apoptotic neuronal PC12 cells exhibit different phosphorylation. That is, tau may involve in the procession of apoptosis. Brandt *et al.* have found that tau acts as a mediator between microtubules and plasma membrane [6]. Furthermore, as reported by some investigators, tau may function in signal transduction pathway related to phospholipase C-γ (PLC-γ) [7] and src-family non-receptor tyrosine kinases, for instance fyn [8].

Identified by immunoflourescence and Western blot assays, tau exists in the nucleus of neuroblastoma cells [9,10]. Thurston and his colleagues have found that nuclear tau is located in the nucleolus of interphase cells and the nucleolus organization regions (NORs) of acrocentric chromosomes [11]. Further investigation shows that nuclear tau localizes in human non-neuronal cells and shares common structural and functional features with neuronal nuclear tau [12]. These observations highlight the association of tau with chromosome scaffold and suggest that tau is involved in nucleolus reformation and/or rRNA synthesis during cell division.

Our previous study showed that tau binds to double-stranded but not single-stranded DNA [13]. An increase in the melting temperature (Tm) reveals that tau protects the double helix of DNA from thermal denaturation [14,15]. The binding of tau to DNA is in protein conformation-dependent and phosphorylation-independent manner [16]. However, the detailed manner of tau interacting with DNA and conformational changes of the double-strands is worth investigating.

Atomic force microscopy (AFM) is commonly used to study the interaction of DNA with proteins [17,18]. It does not require the sample to be stained, shadowed or labeled, and provides a way to visualize protein, DNA, and protein-nucleic acid complexes at nano-scale [19]. Thus, we employ AFM to observe the interaction of neuronal tau with DNA at different mass ratios.

MATERIALS AND METHODS

The prokaryotic expression vector, Prk172, bearing tau40 (the largest isoform of human neuronal tau protein), was kindly provided by Dr. Goedert (Medical Research Council, Molecular Biology Unit, Cambridge, U.K.). Histone VII-S (lys-rich subgroup f2b) and bovine serum albumin (BSA) were from Sigma Inc. λ-DNA was from Sino-American Biotechnology Company. Sephadex G50, Q-Sepharose and SP-Sepharose were from Pharmacia. Monoclonal antibody Tau-1 (1:1000) was a kind gift from Dr Cheng-xin Gong (The State Institute of Disability and Ageing, Staten Island, New York, USA). The bicinchoninic acid (BCA) protein-assay kit came from Pierce Biotechnology Inc. [20]. The other reagents used were analytic grade without further purification.

Human neuronal tau40 was expressed and purified as described previously [13,14]. All purification steps were carried out at 4°C. The purified tau40 yielded a single

protein band in SDS-PAGE. The tau fraction was checked by westernblot using the monoclonal antibody Tau-1 (data not shown). After dialysis against 4 mM Hepes and 2 mM $MgCl_2$ (pH 6.8), tau was lyophilized and stored at −70°C until use. The concentration of tau was determined by BCA protein assay kit [20].

All of the solutions used were passed through a 0.22 μm filter for AFM sample preparation. For pure DNA or protein experiments, samples were diluted to desired concentration using phosphate buffer (25 mM Na_2HPO_4-NaH_2PO_4, pH 7.2, containing 2 mM $MgCl_2$, 5 mM NaCl). 2 mM $MgCl_2$ was added to enhance the adsorption of DNA to mica surface. For the binding experiment, tau protein (0.5, 1.0 and 2.5 μg/ml final concentrations, respectively) were mixed with λ-DNA (5 μg/ml final concentration). The corresponding molar ratios of tau/DNA in base pairs were 1:700, 1:350 and 1:140, respectively. The mixture was kept at room temperature for 10 min before adsorption. Mixtures of λ-DNA (5 μg/ml) with histone VII-S (2.5 μg/ml) and BSA (2.5 μg/ml) were used as positive and negative controls, respectively.

A 10 μl drop of sample was pipetted onto freshly cleaved mica, and was kept in air for 5 min at room temperature (25°C). The mica was washed with 200 μl aliquots of ultrapure water to remove unbound DNA or/and protein for five times. After drying the surface with a gentle stream of N_2 for 5 min, the sample was used for AFM imaging. A multimode SPM (Nanoscope IIIa, Digital Instruments, USA) operated in tapping mode was employed. Super sharp Si tips NSC12 (Silicon-MDT Ltd., Russian) were used. Once the tip was lowered on the surface of the sample, it was auto tuned, engaged, disengaged, and then re-auto tuned to ensure the appropriate drive amplitude. Once the tip was engaged, the setpoint value was adjusted to minimize the

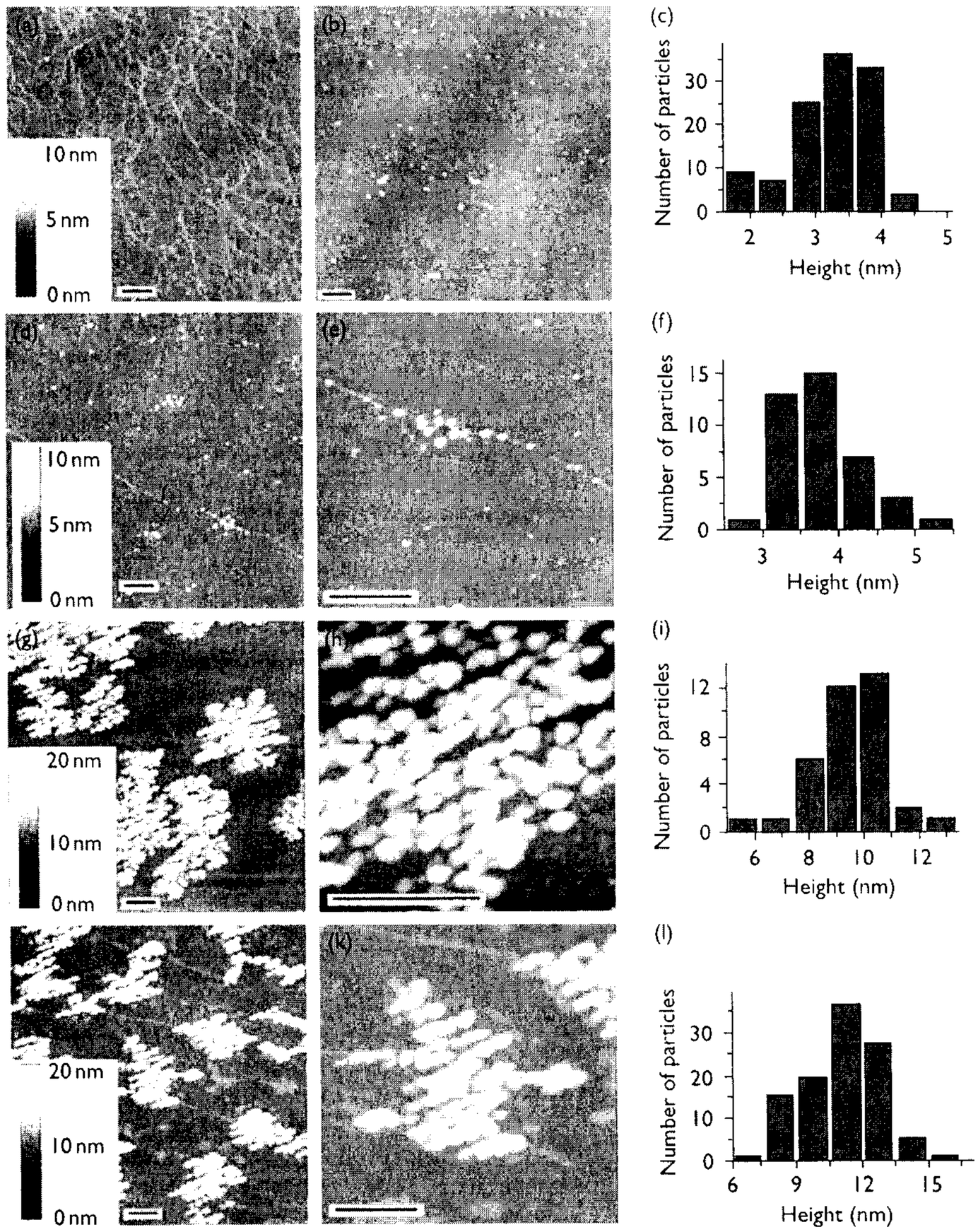

Fig. 1. AFM images of tau–DNA complexes. λ-DNA (5 μg/ml final concentration) as incubated with neuronal tau40 at different concentrations. (**a**) AFM image of λ-DNA alone (5 μg/ml, 10 μl); (**b**) AFM image of tau40 (0.5 μg/ml, 10 μl) alone; (**d–f**) λ-DNA (5 μg/ml, 10 μl) in the presence of 0.5 μg/ml tau40; (**g–i**) λ-DNA (5 μg/ml, 10 μl) in the presence of 1.0 μg/ml tau40; (**j–k**) λ-DNA (5 μg/ml, 10 μl) in the presence of 2.5 μg/ml tau40. Bar=0.5 μm. Panels c,f,i and l show the size of particles in samples b,e,h and j, respectively.

Table 1. The sizes of particles of tau- and histone-DNA complexes.

Samples	Tau/DNA			Histone/DNA		
Mass ratio	0	1:10	1:5	1:2	0	1:2
Count	115	40	37	94	23	99
Means ± s.d. (nm)	3.24 ± 0.62	3.74 ± 0.54	9.47 ± 1.37	11.05 ± 1.70	3.48 ± 0.61	13.17 ± 1.35

The average size means the average height of the particles under AFM.

force exerted on the sample while maintaining the sharpness of the image. The AFM images were initially scanned at a 5 μm scan size with a scan rate of 1–2 Hz and height of 5 nm or 30 nm and then zoomed in as necessary. The captured images were analyzed using NanoScope version 5.0 Software. The center of the image was zoomed in a 1–2 μm size. The height of the particles bound to DNA was measured using cross section analysis. For each assay, particles were analyzed and the heights were averaged and given a calculated standard deviation.

RESULTS AND DISCUSSION

As shown in Fig. 1a, λ-DNA double-strands exhibit in strings on the mica surface detected by AFM. On the other hand, native tau as control (Fig. 1b) appears in globe-like structures under the same conditions, although tau contains a minimal content of ordered secondary structures in solution [21]. The average size (particle height, except where stated otherwise) of tau particles is about 3.24 ± 0.62 nm (n=115; Fig. 1c, Table 1). According to Shiomi *et al.* [22], a globular protein with molecular mass of 50 kDa should measure ~3.2 × 3 nm. The molecular mass of tau40 is 45.85 kDa, suggesting that tau is as a monomer adsorbed onto the mica surface. However, when tau is incubated with DNA with a mass ratio (tau/DNA) about 1:10 (equivalent to the molar ratio of tau with DNA base pair, e.g. one tau molecule to 700 bp), the resultant complex has a beads-on-a-string appearance (Fig. 1d,e). Its uniform appearance and the clarity of the surrounding mica surface indicate that the bound molecule is a protein rather than background, compared with λ-DNA and neuronal tau alone (Fig. 1a,b). The DNA-bound tau maintains a globular-like appearance and its average size is ~3.74 ± 0.54 nm (n=40) under AFM (Fig. 1f, Table 1). The results suggest that tau associates with the DNA double strands as monomer at this mass ratio. Although our previous results observed under electronic microscope also reveal a beads-on-a-string structure for neuronal tau40 binding to plasmid DNA under the same condition [13], the size of the bound tau cannot be precisely determined because metal Pt is sprayed to shadow the protein on the grid. The sprayed-metal enlarges the apparent size of protein tau under the electronic microscope.

As the protein concentration increasing (the mass ratio of tau/DNA, 1:5; equivalent to the molar ratio of tau/base pairs, 1:350), the association of tau with DNA is markedly enhanced. The average size of the bound tau becomes ~9.47 ± 1.37 nm (n=37; Fig. 1g–i, Table 1), suggesting that tau could be as polymers binding to the double-strands at high tau/DNA mass ratio. Simultaneously, the association of tau markedly bends the DNA double strands to form super-coiled structure (beads-on-a-coil). The average size of the DNA bound tau increases to 11.04 ± 1.70 nm (n=94) when the mass ratio (tau/DNA) increase to 1:2 (molar ratio of tau/base pairs, 1:140). No obvious changes in the appearance of tau–DNA complexes and their average size can be observed when tau concentration further increases. That is, the appearance of the complex is dependent upon the mass ratio of the two molecules (tau/DNA) when the ratio is <1:2.

As reported previously [13], histone VII-S (histone 2b) can replace the bound tau from DNA. Histone VI-S and VIII-S, however, are weaker in replacement of tau from DNA. Thus, we use histone VII-S as positive control for protein-DNA interaction in the experiments. Interestingly, under the same conditions, histone VII-S binds to DNA and induces the double-strands bend distinguishably (Fig. 2). Similar to tau as mentioned above, histone VII-S is a globe-like protein on the mica surface. The average size of histone VII-S alone is ~3.49 ± 0.61 nm (n=23), and it becomes larger (13.17 ± 1.35 nm, n=99) when histone binds to DNA at the mass ratio of 1:2. This suggests that histone VII-S also associates with DNA in polymer.

Bovine serum albumin (BSA) is commonly used as a negative control in protein-DNA binding assay [13]. Figure 3 shows that BSA does not affect the appearance of DNA. Compared to Fig. 1a, when BSA is added under the same conditions, no detectable changes of DNA occur and BSA simply overlaps DNA molecules on the mica surface. That is to say, BSA does not bind to DNA double-strands.

We suppose that tau binds to DNA in a histone-like manner on the basis of the observations as the following: (1) both histone and tau appear as beads-on-a-string in their complexes with DNA at a low mass ratio [23] (protein/DNA), but as beads-on-a-coil at a high mass ratio; (2) like histones (2a and 2b) [24], neuronal tau has the capability of increasing the melting temperature and preventing DNA from thermal denaturation [13]; (3) also similar to histones [25], a high ionic strength buffer such as high NaCl concentration (1 mol/l) can dissociate tau from tau–DNA complexes; (4) Histone 2b and neuronal tau can replace each other from the binding DNA [13]; tau and histone are alkaline proteins and finally tau and histones bind to DNA in a non-sequence-specific manner. It suggests that neuronal tau binds and bends the double-stranded DNA in a histone-like manner.

CONCLUSION

We have used the atomic force microscope to visualize the interaction of tau with the double-stranded DNA. Neuronal tau is absorbed onto the mica surface in a globular appearance, similar to histone 2b. The complex of tau/λDNA appears as beads-on-a-string at a low mass ratio and as beads-on-a-coil at a high mass ratio. Tau bends DNA into a super-coiled conformation, which may gives us a

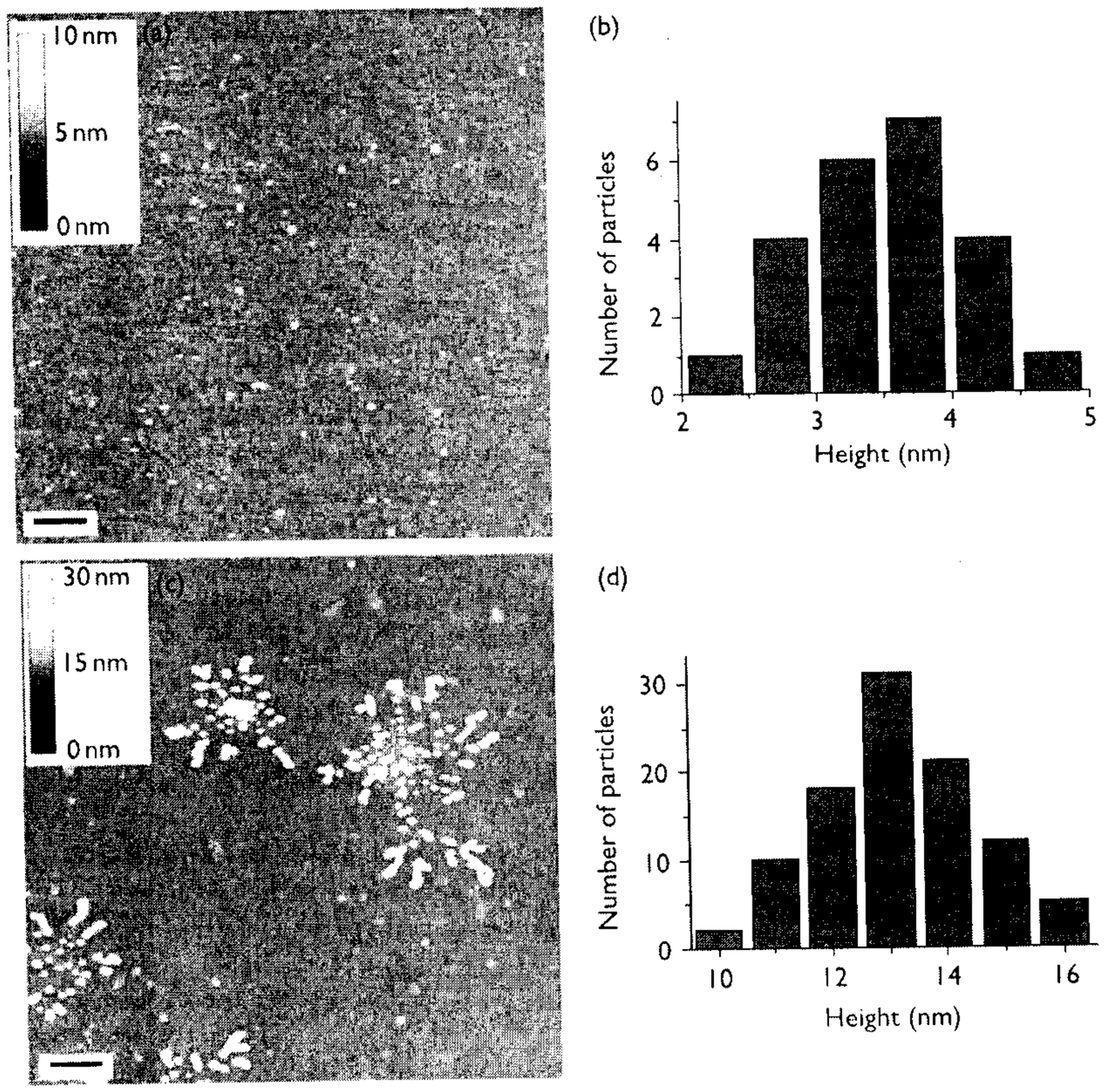

Fig. 2. AFM images of histone and histone-DNA complexes. Conditions were as in Fig. 1, except that histone was employed to incubate with DNA. (**a**) 2.5 μg/ml histone alone; (**c**) 2.5 μg/ml histone incubated with 5 μg/μl λ-DNA. Bar=0.5 μm. (**b**,**d**) show the size range of particles in samples of (a) and (c) detected by AFM, respectively.

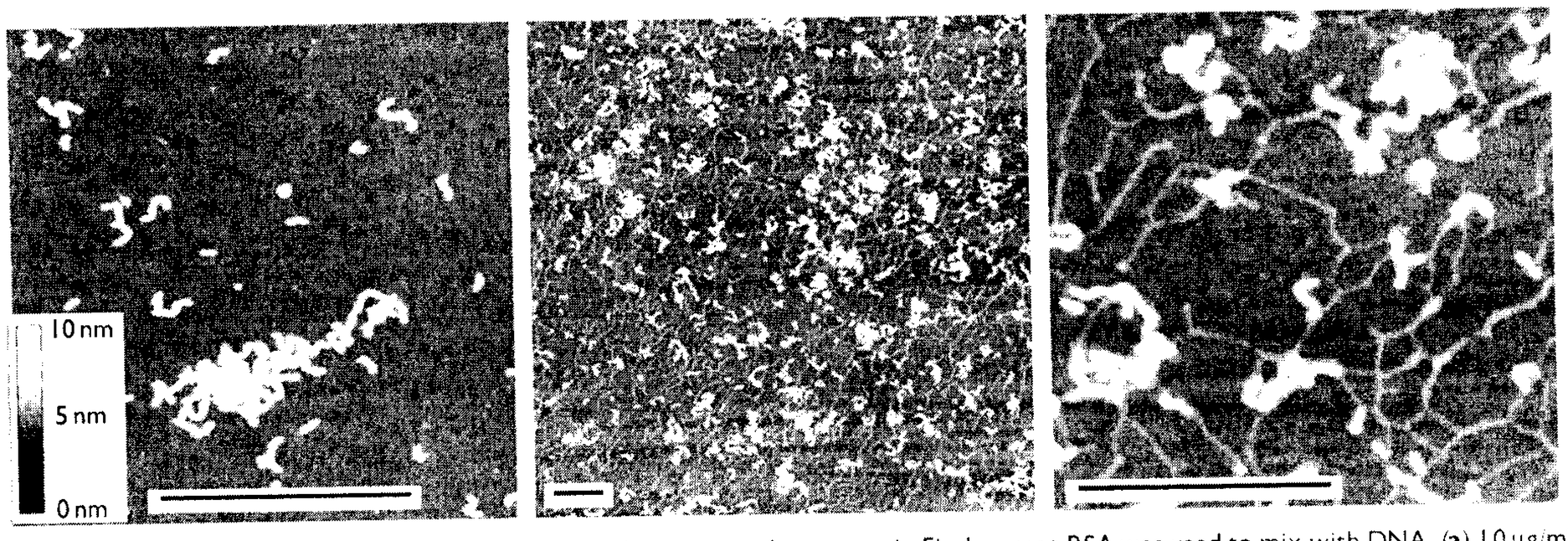

Fig. 3. AFM images of BSA and BSA mixed with DNA. Conditions were the same as in Fig. 1, except BSA was used to mix with DNA. (**a**) 1.0 μg/ml BSA alone; (**b**,**c**) AFM image of λ-DNA in the presence of 1.0 μg/ml BSA. Bar=0.5 μm.

structural explanation for tau to protect DNA and stabilize the double-strands.

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