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LETTER

Comment on the mechanical properties of the amyloid fibre, poly(ValGlyGlyLeuGly), obtained by a novel AFM methodology

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Poly(ValGlyGlyLeuGly) is an elastin-like polypeptide belonging to the glycine-rich sequences of elastin [1]. When suspended in water and deposited on silicon wafers, it self-assembles in amyloid-like fibres. These fibres, and those from similar sequences show a remarkable resistance to bending, tending to remain straight across large fields of view, even at bridging points between fibres [2, 3]. We have now confirmed this apparent axial stiffness by measurement of mechanical properties using atomic force microscopy (AFM).

Materials and methods

The synthesis of poly(ValGlyGlyLeuGly) was performed as previously described [4]. AFM force experiments were carried out with a NanoScope III Multimode microscope (DI, CA, USA) and with ultrasharp NSCS12/C cantilevers (NT-MDT, Russia) with a specified force constant of 4.5 N m^{-1} and a resonant frequency of about 160 kHz. PMMA substrates were prepared by spin-coating on silicon wafers from a solution of 0.3% PMMA in $CHCl_3$. Microindentation experiments were performed with a MINILOAD hardness tester from Leica.

A problem in the direct determination of mechanical properties by AFM is the determination of cantilever spring constant, given that the spring constant specified by the manufacturer can differ from the actual value as much as 100% [5]. The available methods have been evaluated and compared in a recent paper by Clifford and Seah [6]. However, since the contact area between tip and surface is crucial in determination of the applied stress, the tip shape must be accurately evaluated, leading to a further series of problems.

In our laboratory, we have circumvented these problems by means of an indirect method in which the mechanical properties of poly(ValGlyGlyLeuGly) fibres were compared with the known mechanical properties of polymethylmethacrylate (PMMA). Many studies [7] have demonstrated that techniques normally used for the determination of mechanical properties of hard surfaces can be successfully used also in the case of elastic surfaces, provided that the elastic recovery is accurately taken into account. In advance of making such a detailed study, we have created a calibration curve to record the equivalent 'Vickers Hardness' of the amyloid fibres and thus made an initial comparison of axial and radial moduli. To do this we first demonstrated a linear correlation between the slope of the force–distance curve, for given AFM cantilevers measured in compression mode against flat surfaces of PMMA prepared by spin-coating onto silicon and the Vickers Hardness parameter for the material. A range of different hardness values was obtained by ageing PMMA for different times in an oven. The surfaces were then tested with a microindenter and their Vickers parameter was obtained. As shown in Fig. 1, this parameter is linearly correlated with the force–distance slope measured by AFM. Whilst the values obtained for the hardness are greatly influenced by the underlying substrate, the plot demonstrates that the spring constant of the AFM lever is in the required range for the required comparison of PMMA and poly(ValGlyGlyLeuGly) fibres.

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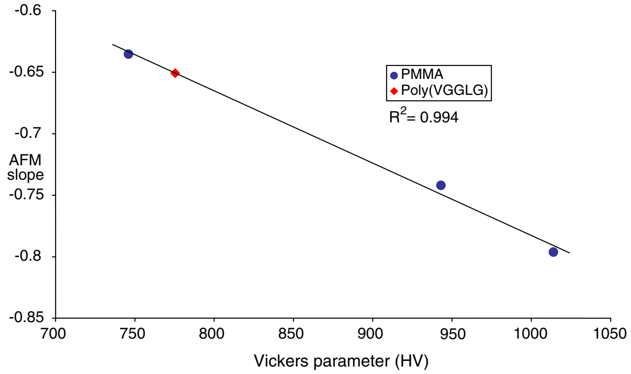

Fig. 1 The relationship between a composite ‘Vickers’ parameter and the slope of AFM force versus distance curves obtained from PMMA thin films supported on silicon 100 surfaces

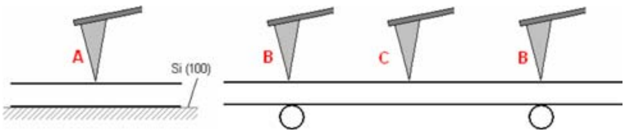

Fig. 2 Measurement points for force versus distance curves

Large numbers of force–distance curves were measured on the polymer sample, on many fibres and at different positions along the fibre axis. In particular, three specific points were examined, as schematically shown in Fig. 2:

- Point A: the fibre is lying on the silicon substrate;
- Point B: crossing point between two fibres;
- Point C: the fibre is suspended as a bridge between other two fibres. This condition was verified by very small angle 3D visualization (Fig. 3).

The choice of points A, B and C was aimed to determine both radial and axial mechanical properties of the fibres, by separately testing their compression and bending modes (Fig. 4a–c).

The force versus distance curves [8] (Fig. 5) show the same slope regardless of the position along the fibre axis and regardless of the specific points A, B or C analyzed. This finding very clearly suggests that the fibre’s axial stiffness does not allow the fibre to bend, but fibres preferably buckle (Fig. 4c). The work confirms our conclusion, based on simple visual inspection, in previous studies of the homologous sequence poly(ValGlyGlyValGly) that amyloid-like fibres (i.e. having a helical structure [2]) have a high axial stiffness. The failure of the helical structure to deform by bending implies strong bonding between the turns of the helix so that the amyloid behaves as a cylindrical tube in its mechanical properties. The tendency to buckle or indent under a locally applied load is a well recognised property of thin walled tubes but is much more difficult to model for the present case and it is for this reason that the comparative method has been used. The value of the slope is indicated on Fig. 1 and shows that the radial stiffness is only slightly greater than that of cast PMMA (typically having a Vickers Hardness value equivalent to 218 MPa [9]). It is clear from the equivalence of points A, B and C that a bending mode is inappropriate. However, if the measured slope at point C is interpreted according to the standard theory for bending of a cylindrical beam [10] (using a fibre of diameter 90 nm and wall thickness 9 nm, suspended between points separated by 0.75 μm), then the axial modulus would appear to be in the range of tens of GPa whereas a simple interpretation of a buckle-type compression of the tube gives a value close to that obtained by reference to PMMA. The inordinately large value for axial modulus merely shows that the

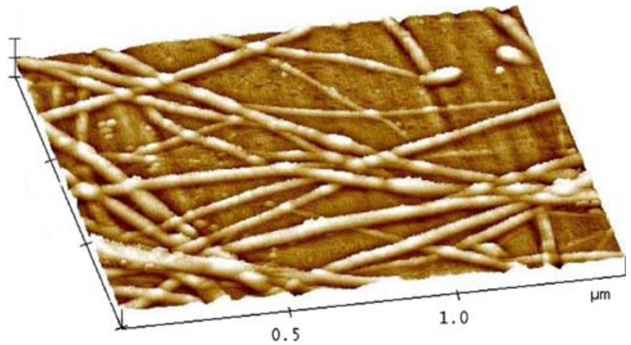

Fig. 3 Low-angle view of poly(ValGlyGlyLeuGly) amyloid-like fibres

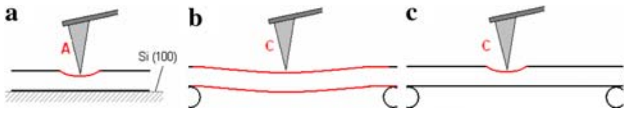

Fig. 4 Experimental setup for the independent determination of (**a**) radial and (**b**) axial moduli. Diagram (**c**) shows the case for radial modulus ≪ axial modulus

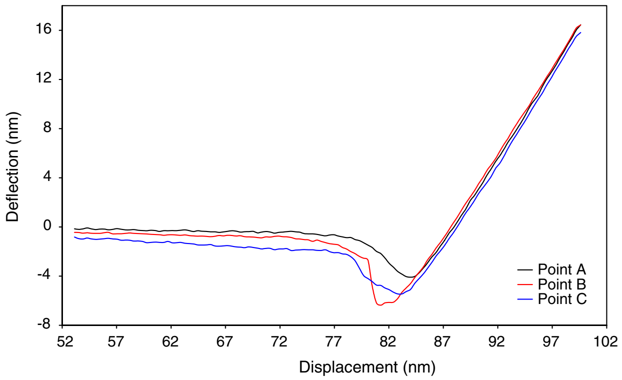

Fig. 5 Example of three force–distance curves taken from points A, B and C along the fibres. The complete curve for the approach of the tip to the sample surface is shown. After contact the cantilever bends with a positive deflection and a slope that depends on the surface hardness

bending model is inappropriate. However the fact the axial stiffness is so much greater than radial stiffness supports our view that bonding occurs between the helices whilst the large anisotropy between the axial and radial moduli probably stems from the properties of the ribbon from which the helix is wound.

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