Zipper-like properties of [poly(L-lysine) + poly(L-glutamic acid)] β-pleated molecular self-assembly

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Received (in Cambridge, UK) 19th July 2005, Accepted 16th September 2005
First published as an Advance Article on the web 10th October 2005
DOI: 10.1039/b510150f

Through the application of Molecular Force Spectroscopy, here we demonstrate nanomechanical properties of a β-pleated, reversible, and pH-controllable molecular "zipper" formed by the spontaneous self-assembly of two sequenceless polypeptides.

Topologies, configurational variability, and dynamics of biomolecular systems have been inspiring novel strategies in engineering of molecular devices. Materials manufactured through spontaneous self-assembly of biopolymers may serve as a prominent example here.[1,2] For instance, the phenomenon of protein aggregation, which has been implicated in the etiology of several neurodegenerative disorders, such as Alzheimer Disease, was successfully employed in this field leading to some spectacular achievements, *e.g.* amyloid-based conducting nanowires.[3] Molecular Force Spectroscopy yields force *vs* distance dependencies of stretched molecules and is an insightful technique to explore the nanomechanics of polycarbohydrates,[4] DNA,[5,6] proteins[7,8] and their force-induced conformational transitions.

Through the application of molecular force spectroscopy, here we show that the spontaneous self-assembly of two sequenceless polypeptides—poly(L-lysine) and poly(L-glutamic acid) leads to the formation of β-sheet-rich aggregates, which display the properties of a reversible molecular zipper. Since the mutual compensation of positive charges of lysine residues and negative charges of glutamic acid side chains is the main driving force underlying the "zipping" event, the "velcro" function may be controlled through pH.

Fig. 1 shows infrared spectra of 1 wt.% aqueous solutions of poly(L-glutamic acid) and poly(L-lysine), both from Sigma, USA, molecular weights were 17.6 kD, and 66.7 kD, respectively. The spectra were collected at neutral pD (measurements were carried out in D_2O) before (A, B) and after the mixing of their equimolar solutions (C). Insoluble aggregates with the predominantly β-sheet structure[9,10] form immediately and precipitate, which is accompanied by the characteristic splitting of the amide I' band around 1645 cm^{-1} (A, B), assigned to a random coil, into two spectral components: minor at 1679 cm^{-1} and major at 1610 cm^{-1}, both of which are indicative of the antiparallel β-sheet.[10] The persisting minor intensity around 1645 cm^{-1} reflects the presence of some residual, loose chains. While the chains' extended conformation and the interchain hydrogen bonding patterns constitute a topological scaffold for the aggregate, it is the electrostatic attraction between lysine's and glutamic acid's side chains that acts as the main driving force of the self-assembly. Thus, at extreme values of pH (pD), wherein the side-chains are depleted of the charges, the two polypeptides do not form the mixed β-sheet structure.

In order to probe the nanomechanics of the self-assembled homopolypeptides and their interactions in thin layers, poly(L-glutamic acid) and poly(L-lysine) were covalently immobilized onto an AFM tip (Si_3N_4) and AFM substrate (mica), respectively. Covalent immobilization of multiple oligonucleotide chains was employed earlier in the pioneering force microscopy study on a DNA "molecular-velcro".[5] In our work, chemical functionalization has been enhanced by a brief (30 seconds) exposure of the tip

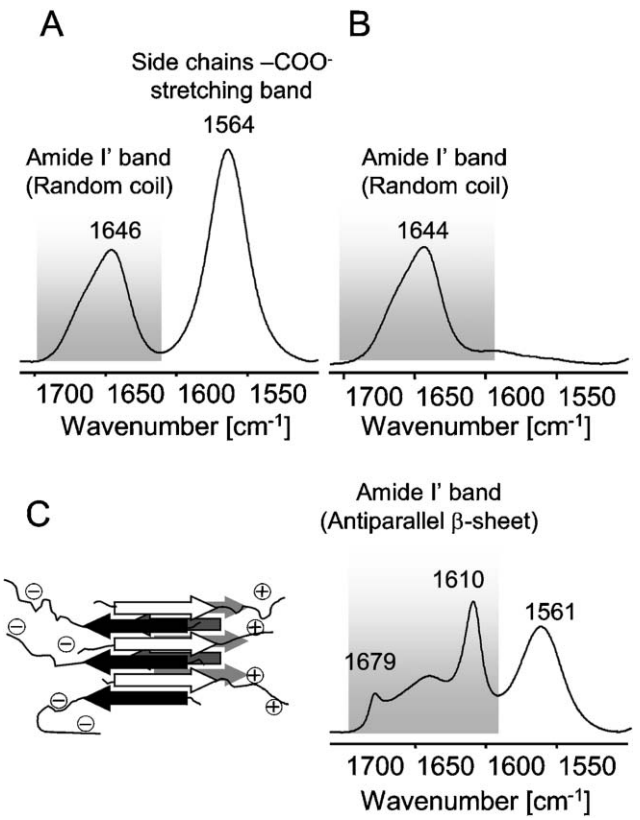

Fig. 1 FT-IR spectra of the amide I' band (shadowed) of 1 wt.% poly(L-glutamic acid), M.W. 17.6 kD (A), and poly(L-lysine), M.W. 66.7 kD (B) in D_2O, pD 7.0, and their mixture (C). The profound spectral changes in the amide I' band region reflect the conformational transition of solvated, randomly coiled polypeptides into the stacked antiparallel β-sheet network (A, B, C).

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and mica to $SiCl_4$ vapor at room temperature, followed by a 30 minute incubation in 4 wt.% (3-aminopropyl)-triethoxysilane (APTES) in isopropanol. All chemicals used for immobilization were from Sigma-Aldrich. After an excess of amorphous and partly hydrolyzed APTES was removed with 5 wt.% acetic acid (1 hour), the amino-functionalized tip and substrate were incubated for 30 minutes in a solution containing 4 wt.% *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC), and 3 wt.% *N*-hydroxysuccinimide (NHS) in 0.5 wt.% aqueous solution of terephthalic acid, pH 6.0. This has rendered the tip and mica surfaces covered with reactive EDC/NHS-modified carboxyl groups, which are competent to bind amine groups and form stable amide bonds. Immediately after this step, 1 wt.% polypeptide solutions in 50 mM HEPES buffer, pH 7.0 containing 4 wt.% EDC, and 3 wt.% NHS were dropped onto the carbodiimide-activated tip and mica surfaces and left for an overnight incubation. Finally, excess of the reactive EDC-activated carboxyl groups was blocked through a brief (10 minutes) incubation in 10 mM hydroxylamine at pH 8.2 and room temperature. This multi-step procedure allowed us to obtain a desirable surface density of the polypeptide chains, *i.e.* relatively abundant polylysine chains were coupled to mica through multiple ε-amine groups, while the fewer N-terminal amine groups of polyglutamic acid resulted in a limited functionalization of the AFM tip.

In the course of this study we have established that such conditions of covalent attachment of the polypeptides to tip and mica surfaces are critical for observation of unzipping events of single β-sheets. Namely, immobilization of larger quantities of the polypeptides, *e.g.* through abundant side chain carboxyl groups of poly(L-glutamic acid), is likely to result in large aggregates, wherein nanomechanical behavior of single β-strands is obscured by collective and simultaneous unzipping of multiple β-sheets. Force spectroscopy measurements were carried out on a home-made single-axis AFM instrument equipped with an AFM detector head from the MultiMode AFM (Veeco, USA) and a piezoelectric actuator with an integrated strain gauge for direct measurements of the travel of the piezo (open-loop travel resolution of 0.15 nm; Physik Instrumente, Germany). The instrument was controlled by a PC computer using a 16 bit A/D D/A interface and a program written in LabView (National Instruments). The spring constant of each cantilever (Veeco) was calibrated using the equipartition theorem as described earlier.[11] Force spectrograms were obtained by a procedure aimed at minimizing the nonspecific adhesive interaction between the tip and the substrate: the sample was brought into the cantilever proximity by moving the piezo in small steps, a few nanometer each without touching the substrate. At each step, the sample was withdrawn and the interaction forces between the tip and the substrate were monitored on retraction.

The forward movement of the piezo was stopped at the onset of the retraction forces. Thus, force spectrograms were obtained at a minimal contact between the tip and the substrate with no indentation, which could damage both the substrate and the coating of the tip

The representative spectrograms collected for the poly(L-glutamic acid) + poly(L-lysine) aggregate formed between the tip, and the mica, are shown in Fig. 2. The data obtained at pH 7.0 reveals long, flat features expected for the unzipping of elongated and non-covalently bound structures. A series of three subsequent

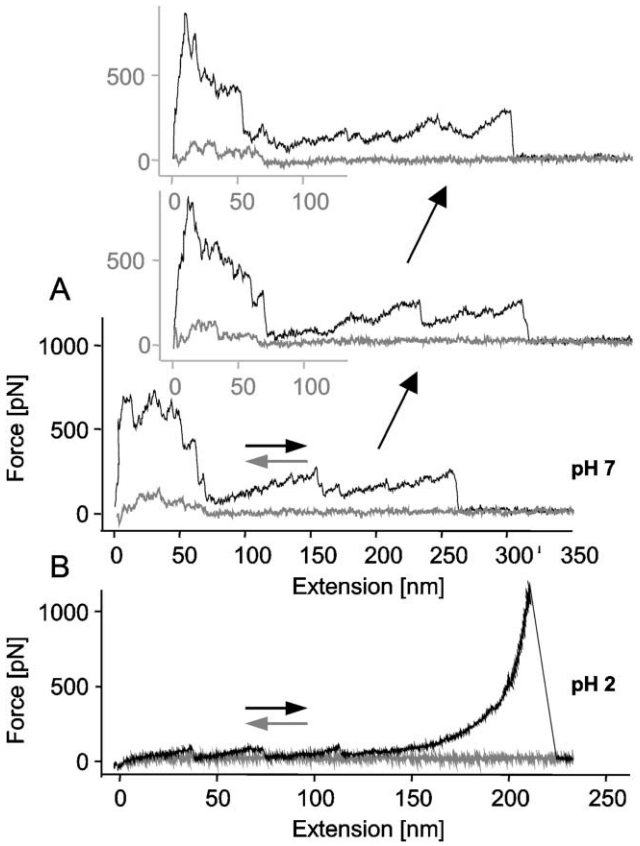

Fig. 2 Repetitive stretching of the molecular zipper formed spontaneously between poly(L-glutamic acid) immobilized on the AFM tip, and poly(L-lysine) immobilized on mica, at pH 7.0 (A) and pH 2.0 (B). The solution contained 100 mM NaCl in order to reduce the electrostatic adhesion. The initial effects, visible up to the distance of ~70 nm (A), are likely to originate from non-specific adhesive forces. The actual "velcro" effect corresponds to the long and flat part of the force–extension curve. The black lines denote unzipping events, while the gray lines correspond to the tip re-approaching the surface.

unzipping events at the same surface locus (tip departing from and re-approaching the mica surface) shown in Fig. 2A shows that the unzipping occurs in a reversible manner. The averaged stretching force is 150–200 pN, which is in a good agreement with a recent force microscopy study on unzipping β-sheet structure within Aβ1-40 fibrils.[8] We hypothesize that the unzipping of a single β-strand formed by the two polypeptides is accompanied by a continuous rupture—alongside the chain—of the Lys–Glu salt bridges, which contributes to the significant stability of velcro structure. It is clear, however, that the force spectrograms have a positive slope and the stretching force is gradually increasing until a major (or final) rupture event takes place (Fig. 2A). These slopes are likely to result from increasing entropic tension in the polymer system between the tip and the substrate (the WLC-type elasticity).[12] The entropic tension is modulated by small force peaks reflecting the breaking hydrogen bonds and untangling of polymer loops (major force peaks). The protonation of the glutamic residues at pH 2.0 disrupts the ionic association of the side-chains and the unzipping event is not observed under these conditions (Fig. 2B). The force spectrograms at such a low pH are reminiscent of the worm-like chain elasticity profiles obtained for

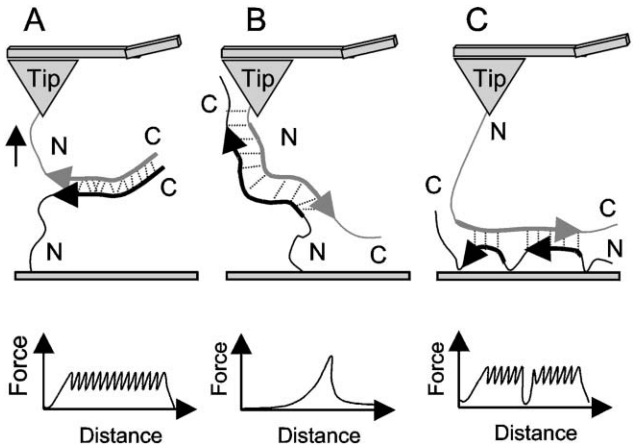

Fig. 3 Different orientations of the β-sheet structures formed by poly(L-glutamic acid) and poly(L-lysine) result in different stretching profiles, as seen in force spectrograms. A step-by-step unzipping of a loose parallel β-strand (A); cooperative breakage of hydrogen bonds within a loose antiparallel β-sheet structure (B); surface immobilization of the antiparallel β-sheet achieved in this study results in the step-by-step unzipping profile.

individual polymer chains.[12] Another difference between the spectrograms recorded at the low and neutral pH concerns vanishing of the initial effects up to the distance of ~70 nm (Fig. 2A) upon acidification (Fig. 2B). We propose that these effects are likely to originate from electrostatic attraction of the two oppositely charged polymers, which is associated with formation of highly distorted and coiled rather than regular β-sheet-rich structure.

The pH-controllable transition between different stretching profiles of the poly(L-glutamic acid) + poly(L-lysine) aggregates (Figs. 2A and 2B) is reversible and the "velcro"-like effect is restored when the pH is elevated back to the neutral level.

In Fig. 3 possible attachment and pulling configurations are schematically discussed for the poly(L-glutamic acid) + poly(L-lysine) aggregates composed of two β-strands oriented in a parallel (Fig. 3A) or antiparallel (Figs. 3B, C) fashion. For the sake of clarity, this has been handled in a simplified manner without indication of possible complications stemming from the previously discussed entropic tension.[12] The covalent immobilization procedure employed in this study attaches the poly(L-glutamic) acid to the AFM tip only by its N-terminus. On the other hand, poly(L-lysine) chains are more likely to bind the substrate at multiple sites through ε-amine groups of their side-chains. Thus, we expect that Fig. 3C represents the most likely pulling configuration between the AFM tip and the mica. The hypothetical force spectrogram predicted for such a configuration is shown in the lower panel of Fig. 3C. The sets of small force peaks, in this figure, corresponding to the unzipping of the hydrogen bonds are occasionally separated by events, which relax the stretching force, whenever an extra length of the polypeptide chain, as from a loop, is added to the stretched polymer (Fig. 3C). This hypothetical configuration is consistent with the experimental force spectrograms shown in Fig. 2A.

In conclusion, we have demonstrated that self-assembled poly(L-lysine)–poly(L-glutamic) acid aggregates have the properties of a pH-controllable molecular zipper. While this system constitutes an exciting model for studies on nano-dynamics of aggregating peptide conformations, it may also inspire new strategies for renewable immobilization of proteins. Using sequenceless polymerized amino acids may prove beneficial in terms of high stability against thermal denaturation and cleavage by proteases. On the other hand, a "velcro" system employing two oppositely charged homopolymers is highly sensitive to pH, and therefore easily controlled by the environment.

WD acknowledges the financial support from the Ministry of Scientific Research and Information Technology, No.77/E-72/SPB/COST/P-04/DWM38/2005–2007. PEM acknowledges the financial support from the National Science Foundation (USA)

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