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Single molecule force spectroscopy discovers mechanochemical switches in biology: The case of the disulfide bond

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Abstract

By studying with the single molecule force spectroscopy (SMFS) methodology the mechanical behaviour of single biomolecules, we are learning how mechanical forces like those present in the extracellular space modify the conformation of proteins, possibly leading to functional switches. We also understand that the functional efficiency of those mechanical switches can rely on their coupling to some independent biochemical control of the protein conformational changes. The disulfide bonds have been recently proposed to act as potential redox switches, even if their structural bases are unclear. Here we discuss, also on the basis of experimental evidences based on SMFS, the possibility that disulfide bond switching and mechanical deformation of extracellular proteins can be coupled, thus leading to an efficient and highly tuned switch for protein function. We propose this as one of the biological mechanisms that regulate extracellular protein functionality.

Keywords: Disulfide bonds; Protein; Mechanochemistry

1. Introduction

Disulfide bonds are commonly thought to have been selected by evolution to serve one main purpose: to sustain and protect the native conformation of a protein [1,2]. In fact a disulfide bond is rare in intracellular proteins but is a common feature of proteins that work in the extracellular space, i.e. a space that offers a particularly challenging environment for protein folding. The disulfide bonds reduce the conformational space accessible to the native folded structure of the proteins, by linking adjacent strands with a covalent bond (Fig. 1(A)). The disulfide bonds can even form a sort of ladder in a protein structure that must be maintained in a particularly harsh environment. That is for instance the case of laminin gamma-chain, a structural protein of the basement membrane [3] (Fig. 1(B)).

This point of view looks at the disulfide bond just as an inert structural feature. On the other hand the disulfide bond can be reversibly cleaved and reformed by various physiological agents. It is therefore natural to imagine that the disulfide bond can also act as a reversible switch able to be turned off and on. In fact, several protein functionalities have been already proposed to depend on switching mechanisms based on disulfide bonds (see Section 2).

Mechanical tensions are generated along the physical connections between the extracellular matrix and the adherent cells [4]. These forces play an important role in many processes in cells and tissues life cycle. Mechanical stresses have been proved to have regulatory effects on gene expression [5–8], on intracellular nuclear structure [8] and on extracellular matrix (ECM) remodelling [9]. Also pathological defects as muscle hypertrophy [7], atherosclerosis, hypertension [10], and various heart dysfunction [11] have been proved to result from mechanical stresses.

The determination of the mechanisms by which mechanical forces are transformed into biochemical signals that trigger biological responses is still a very open challenge. These transduction processes are expected to rely on the force induced deformation of the biomolecules under stress [4,12] (see Section 3).

We have recently obtained evidence that a redox and a mechanical mechanism of biological regulation can be hierarchically coupled to reach more complex and more precisely tuned protein functionalities (Grandi et al., to be published). In this paper, after a critical overview of the intrinsic efficiency of a redox disulfide bond-based switch and

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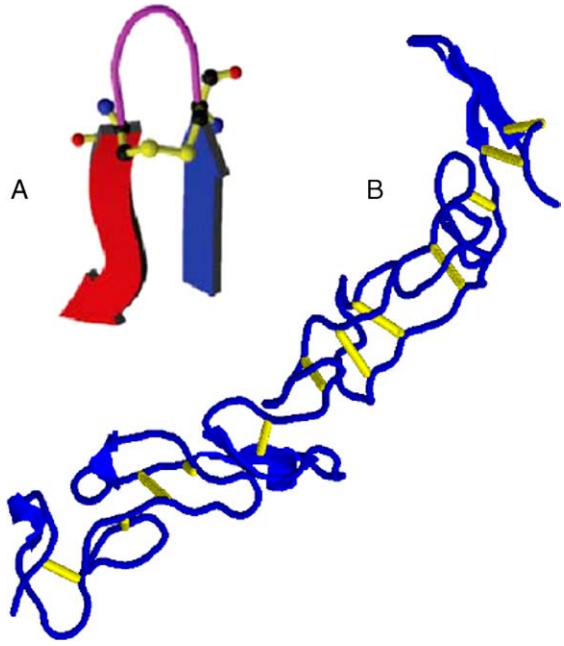

Fig. 1. Disulfide bonds protect the protein folding. (A) Cross-strand disulfides connecting a beta hairpin in the influenza virus B neuraminidase (from [30]). (B) The structure of the laminin gamma chain (1 KLO in the Protein Data Bank, [63]) is an example of a structure with a high concentration of disulfide bonds. The fully oxidized structure is a rigid rod kept together by a ladder of 12 disulfide bonds.

of a mechanical switch, the functional gain that can be ensured by their coupling is analyzed and discussed.

2. Is the disulfide bond cleavage a mechanism for protein function control?

Extracellular regulation by disulfide cleavage has been specifically demonstrated in secreted proteins like thrombospondin-1 [13], von Willebrand factor (vWF) [14] and plasmin [15], in cell-surface receptors including CD-4 T cell receptor [16], integrins [17] and the HIV gp120 [18]. The functional effects of this regulation are different and not always clear. Disulfide regulation directly affects the multimer size of vWF [14] and triggers the auto proteolysis process that leads to the production of angiostatin from plasmin [15]. In the case of the CD-4 receptor both oxidised and reduced forms of the molecule co-exist in equilibrium on the T-cell surface, and T-cell activation leads to a shift to the reduced form. This suggests a definite but still unknown functional role of the disulfide reduction in the CD-4 receptor [16] and indicate that the reversible cleavage of disulfide bonds in extracellular proteins may be an important tool for the regulation of their function. CD-4 disulfide reduction has been shown to block HIV infection [19].

In all these cases, except in that of plasmin, disulfide cleavage is triggered by the action of unspecific oxidoreductases of the protein disulfide isomerase (PDI) superfamily like thioredoxin or PDI itself. There is a general evidence that free thiols are exposed on the extracellular face of many plasma membrane proteins and that PDI controls the exofacial thiol/disulfide equilibrium [20]. Thioredoxin and PDI are known to be secreted outside the cytoplasm and to be present and active on the cell surface, despite the lack of obvious secretory signal sequences for both proteins [21–23]. It is still unclear if these proteins act as single turnover reductants or if they act in a catalytic-like manner: in the latter case a PDI- or TRX-reductase system fuelled by NAD(P)H, for example, must be present. Evidence for a working extracellular TRX/TRX-reductase system has been found [24].

The main plasmin reductase has been demonstrated instead, quite surprisingly, to be phosphoglycerate kinase (PGK), the sixth enzyme of the glycolytic pathway [25]. PGK appears to act with a still unknown disulfide independent mechanism unrelated to that of oxidoreductases of the PDI superfamily. Both alkaline hydrolysis [26] and acid-based assisted hydrolysis [27] mechanisms have been proposed. There is a definite functional difference between dithiol–disulfide redox exchange and thiol independent mechanism for disulfide reduction. The first class of proteins have broad specificity and require at least two additional factors to act catalitically: this means they are unlikely to act in a specific and efficient fashion. The second mechanism, although still unclear, can act both catalytically and with high specificity.

Disulfide bonds can also be enzymatically reformed in the extracellular matrix. Sulphydryl oxidases are disulfide bonding catalysts that seem to act in the extracellular matrix along with PDI [28].

The cleavage of a disulfide bond therefore seems a fast, specific and reversible switch for protein functions. However, in all the cases known in which a biochemical signal is triggered by disulfide bond cleavage, it is still unclear which is the structural mechanism that underlies this kind of regulation. The common explanation postulates that the cleavage of the disulfide bonds itself can trigger a conformational switch and therefore directly influence the protein function. This purely redox switching mechanism, although it can directly affect protein flexibility, is not expected to be very effective in altering the protein conformation, and it is unclear how widespread it may be [29].

In some cases disulfide bonds somehow ‘trap’ an otherwise frustrated fold, that relaxes in the minimal energy state only once the disulfide bond is broken. This can be the case of disulfide bonds that have high potential energy stored: examples are cross-strand disulfides [30] and vicinal disulfide turns [31]. However, the cleavage of most extracellular disulfide bonds is known to be reversible [29] and the existence of highly strained disulfide bonds is not expected to be general. After the cleavage of the disulfide bond, it should be difficult for a protein to ‘come back’ and reform spontaneously a disulfide bond that stores high potential energy. Even if there are rare cases of reversible conformational switches triggered by disulfide bonds [32], the general rule is that disulfide bonds do not drive the folding, but merely stabilize protein structures [33].

Therefore, after the cleavage of a disulfide bond it might be desirable to ‘help’ the system to drive the protein to the specific structural change that is required to activate a specific functionality. This help might have a mechanical origin.

3. The transduction of mechanical forces into biochemical signals

Both the extracellular and the intracellular spaces are environments where mechanical stresses are constantly developed. The continuous remodeling of ECM induces variations in its mechanical compliance and results in the application of mechanical tension on the adherent cells [34]. On the other hand, all living cells generate internal tension within contractile microfilaments in their cytoskeleton and they exert this tension on their surface membrane and consequently on the extracellular environment [35,36].

These mechanical forces are transmitted along the cytoskeleton–ECM physical connection mediated by focal adhesions [37] (Fig. 2) A few mechanisms by which mechanical forces are transduced into biochemical signals have been intensively studied in the last few years [4,12,38]. Most of these mechanisms are still poorly understood.

Force modulated ion channels are at the basis of force transduction in mechanosensory cells: tension transmitted via the linkers to the ion channel can modulate local ion transduction, which, in turn, may activate a variety of signal events [38,39]. Ionic movements are therefore at the basis of this transduction mechanism.

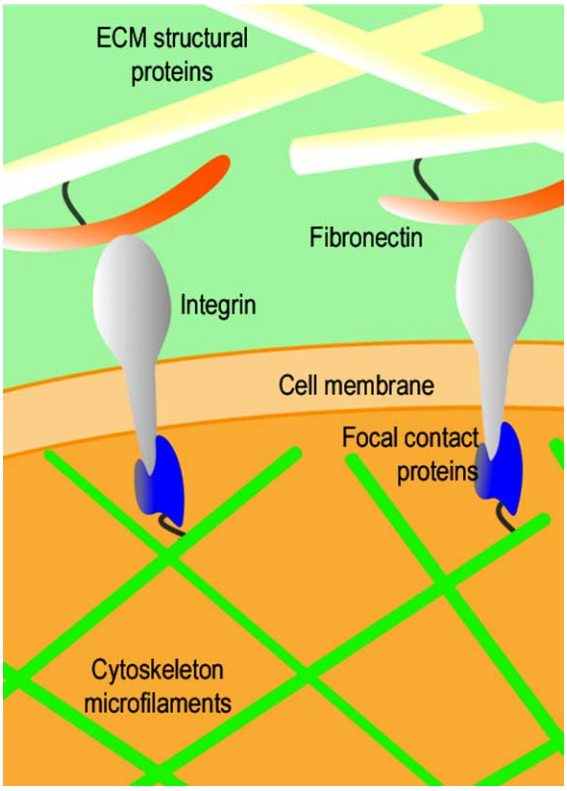

Fig. 2. Mechanical crosstalk between the outside and the inside of the cell. Focal adhesion complexes constitute the physical connection between the structural proteins composing the Extra-Cellular Matrix (ECM) and the intracellular cytoskeleton fibers network. This mechanical connection is based on the capability of transmembrane integrins to bind both the ECM protein fibronectin and the cytoskeleton microfilaments (via the focal contact proteins) [69]. Along this physical pathway, the mechanical tensions due to the contractions of the cytoskeleton microfilaments are transmitted from inside to outside the cell, whereas those due to ECM continuous remodelling are transmitted from outside to inside. Transmembrane focal adhesion complexes provide therefore dynamic, bi-directional, mechanical links between the inside and the outside of the cell [37].

It has been recently demonstrated that also the mechanical stretching of cell structures can activate signalling pathways. For example cytoskeleton stretching induces GDP–GTP exchange in the Rap1 G-protein, eventually leading to a tyrosine-kinase signalling cascade that activates regulators of cell division [40,41]. In this force induced signalling reaction no ionic movements are involved. This different mechanism of mechanochemical transduction is very likely based on the force induced alteration of the three-dimensional position and/or conformation of specific molecules, along the cytoskeleton–ECM physical connection [4,12].

The cellular mechanochemical transduction processes therefore rely primarily on the force induced deformation of the biomolecules therein involved. The force spectroscopy methodology has allowed so far to study at the single molecule level the response to mechanical stress of various biomolecules, evidencing that the application of a force at the ends of a protein chain can modify its native folding [42].

In the case of multimodular proteins, their single modules unfold sequentially and each unfolding is marked by a force peak in the force–extension curve. The forced unfolding of each single domain is an all or none process initiated by the catastrophic rupture of a specific structure element (most commonly a β-sheet), which leads abruptly to the unfolding of the whole domain structure into a random coil chain [12]. Once a module has been unfolded, it behaves as an entropic chain [43] (i.e. shows a worm-like chain force–extension profile) and is unravelled almost completely before another module unfolds (Fig. 3) [44].

When a force is applied to a protein domain, its effect is to lower the free energy barrier along the unfolding pathway thus strongly increasing the unfolding probability (Fig. 4(A)). The effect of the applied force is therefore only to facilitate the unfolding event, which remains itself a thermally activated process [12,45].

The mechanically forced transition from the folded to the unfolded state can occur along a complex free energy profile with multiple energetic barriers [46,47] (Fig. 4(B)), driving the protein domain to intermediate conformations. These mechanical deformations of the protein fold can trigger new functionalities.

Three basic mechanisms have been proposed, by which stretch induced deformations in a multimodular protein conformation can alter its functional state [48]. It might take place by the exposure of cryptic sites, through the change of the relative distance of synergic binding sites, or through the deformation of the structure of a binding site.

The function and/or regulation of many proteins of the extracellular matrix have been shown to depend from cryptic sites [49]. Normally, it is assumed that cryptic sites are exposed mostly by limited proteolysis of the target protein by enzymes like matrix metalloproteinases (MMPs). In some cases, the force applied on a protein module can stretch it into

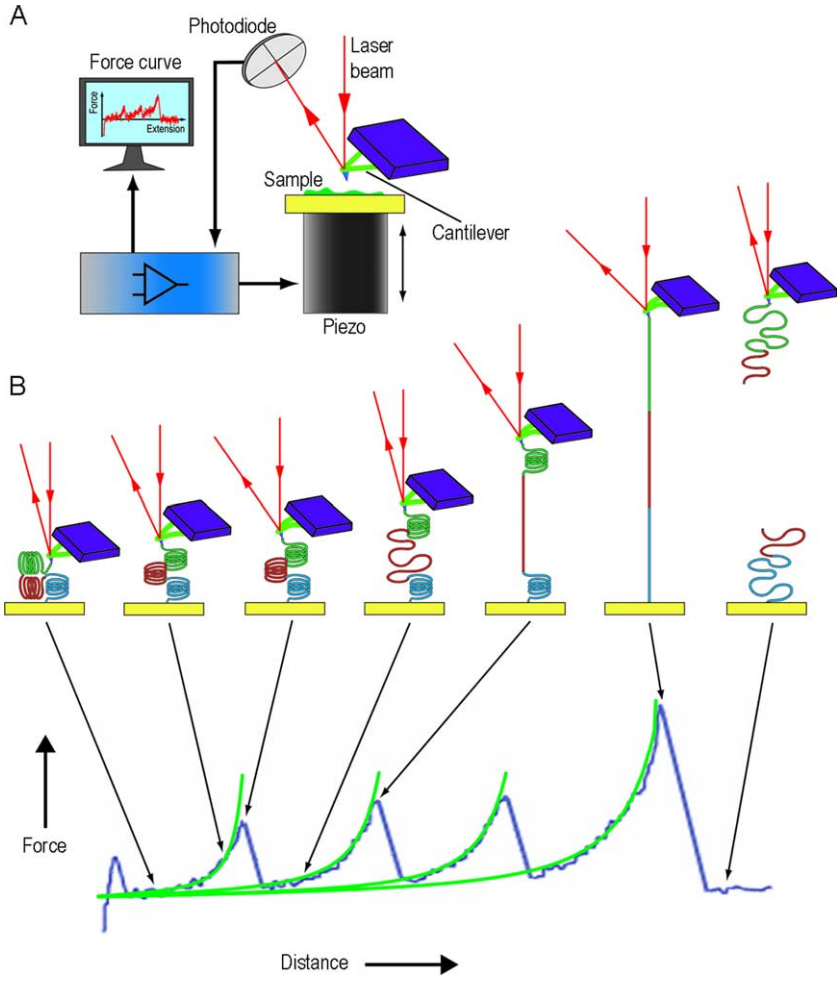

Fig. 3. Unfolding multimodular proteins by AFM single molecule force spectroscopy. In a typical AFM force spectroscopy experiment the cantilever tip approaches the surface, pushes on it and then retracts. During this approach-retraction cycle the force acting on the molecule is measured and plotted as a function of the tip-surface distance: the so-called 'force curve' is thus obtained [42]. The typical force curve corresponding to a multimodular protein unfolding experiment shows a saw tooth profile, where each dominant force peak represents one domain rupture. Immediately after the rupture of each domain, the tension is released and the force drops down. As the extension increases again, the ruptured module begins to unravel as a random coil chain and the force starts to rise up again. The increased tension applied on the remaining modules leads suddenly to another domain rupture. The rising of the force after each domain unfolding is due to the entropic elasticity of the unravelling chain. In fact, when a stretching force is applied at the ends of a polymer chain, its conformational space is shrinked and its entropy decreases, generating an opposing force. The dependence of this opposing force on the chain extension can be described by the worm like chain (WLC) model. The green curves reported on the saw tooth force profile represent the WLC fits.

a conformation in which a binding site, normally buried in the folded equilibrium conformation, is exposed, becoming able to bind a receptor (Fig. 5(A)). The effect of the force is therefore to turn on such a biochemical switch.

An applied force can also change the relative distance of two binding sites that bind the same receptor molecule. This effect can either destroy an existent synergy in the equilibrium conformation or bring the two sites to the correct distance for a synergic binding to the receptor molecule (Fig. 5(B)). In the latter case the applied force would result in an increased affinity of the protein–receptor binding.

A third mechanism to switch on biochemical signals is based on the deformation of a binding site conformation that makes the ligand fit or unfit the same site, thereby altering the receptor–ligand affinity (Fig. 5(C)).

This scheme has been demonstrated to work in fibronectin, a multimodular cell adhesion protein [48]. Cell-derived mechanical stretching has been found to be essential for fibronectin fibrillogenesis [36]. Fibronectin fibrils have been observed to be highly stretched in living culture (up to 4-fold their relaxed length) [35]. Fibronectin fibrillogenesis has been recently proposed to be initiated by the mechanically induced transition of FNIII1 module to a mechanically stable intermediate, that is about four times longer than the native folded state [50]. This elongated domain conformation exposes crucial nucleation sites for the assembly of fibronectin into its fibrillar form, that are buried in the FNIII1 native conformation, according to the scheme of Fig. 5(A).

The binding affinity of the fibronectin FNIII10 for integrins has been found to be force regulated. The RGD loop of the FNIII10 is the site by which fibronectin binds to integrins on cell membrane. In fibronectin native conformation, cell binding to the RGD loop of the FNIII10 domain is enhanced 40-fold in the presence of a synergy site in its neighbour

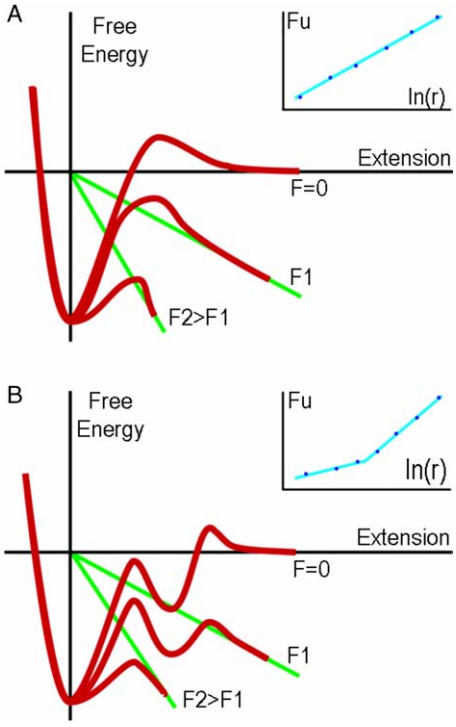

Fig. 4. Unfolding energy landscapes under an external applied force. (A) Two state transition of a protein domain from the folded to the unfolded state. An externally applied force tilts the free energy landscape along the mechanical reaction coordinate by an amount equal to the work done on the system by the force itself [12]. The most typical AFM force spectroscopy experiment is not performed at constant force but at constant velocity of tip retraction. As shown in the inset, the most probable force *Fu* at which the domain unfolding takes place depends linearly on the natural logarithm of the loading rate *r* (i.e. the variation of force with time). From this linear dependence is possible to estimate the barrier position and the lifetime at zero force. (B) Three state transition of a protein domain from the folded state to an intermediate and then to the unfolded state. The tilting effect of the force on the energy profile subverts the height of the different energy barriers [12]. In fact an applied force reduces to a greater extent the energy barriers located further away from the folded state than those located at closer positions. At low forces the transition can be still determined by the dominant barrier at zero force, whereas at sufficiently high forces the unfolding transition can be dominated by the intermediate barrier. This is normally evidenced in the force versus loading rate plot (in the inset), showing two regimes characterized by two different slopes.

domain FNIII9 [48]. An applied force drives the structure into an intermediate conformation where the distance between the two sites is increased and the synergy effect is destroyed [51]. Further, the stretching of the FNIII10 domain drastically changes the RGD loop geometry, strongly reducing its binding to integrins [52]. These two processes are in accord with switching off mechanisms based on the schemes of Fig. 5(B) and (C).

4. Coupling the redox equilibrium of a disulfide bond with an external mechanical force

An effective functional switch should be fast, specific, and reliable. A mechanical switch or a redox switch based on a disulfide bond, taken alone, fulfil only partially these requirements.

We have seen that just the opening of a disulfide bond can hardly lead to the relevant conformational changes that are expected to be required to activate specific functionalities. We have seen also that mechanical processes are able, instead, to deeply affect the structure and, therefore, the functionality of an extracellular protein. What these latter processes lack is a reliable control of the induced molecular extension.

Evidence was recently obtained by us that mechanical and disulfide regulations can have evolved as to complement each other (Grandi et al., to be published).

4.1. The disulfide bonds can control the extent of a protein domain that is accessible to mechanical unfolding

Disulfide bonds create topological loops that drastically affect the mechanical behaviour of a protein molecule under external force, by covalently connecting cysteines that are distant in the protein primary sequence. Being covalent, the disulfide bonds can withstand forces up to a few nanonewton [53]. The stretching forces normally generated in vivo have been estimated to range from one to a few tens of piconewton [54,55]. These forces would not be enough to break a disulfide bond. The action of the force will therefore ‘bypass’ the loop enclosed by the disulfide bond (Fig. 6): the contour length measured in a single molecule force spectrum thus results apparently shorter than that expected simply on the basis of the total number of its aminoacid residues.

An experimental evidence of this effect has been given by the human angiostatin K1-5 molecule [56]. Angiostatin is a protein composed of up to five very similar modules, called kringle domains. Each kringle domain has a globular structure defined by three internal disulfide bonds, forming a characteristic triple-loop topology [57] (Fig. 7(A)). The most external bond of each domain encloses practically all the module.

In the fully oxidized configuration angiostatin behaves as a short, inextensible molecule with an apparent contour length of a few nanometers, contrary to a contour length of the order of 150 nm, in the fully reduced form. By chemically reducing the disulfide bonds the internal topological loops of the kringle domains become accessible to the action of the force and can mechanically unfold. The more the reduction proceeds, the larger is the portion of each module that can unfold under force (Fig. 7(B and C)). The presence or the absence of disulfide bonds therefore modulates the mechanical properties of the polymer among states with different extensibility. In the case of angiostatin, the triple loop topology of kringle domains makes three different mechanical states to be accessible in each domain.

An analogous but simpler system has been described for the Ig-like domains of melanoma cell-adhesion molecule (Mel-CAM)[58].

4.2. The redox modulation of the protein mechanics can switch new protein functionalities

The three mechanisms proposed in Section 3 and in Fig. 5, by which a mechanical stress can be transduced into

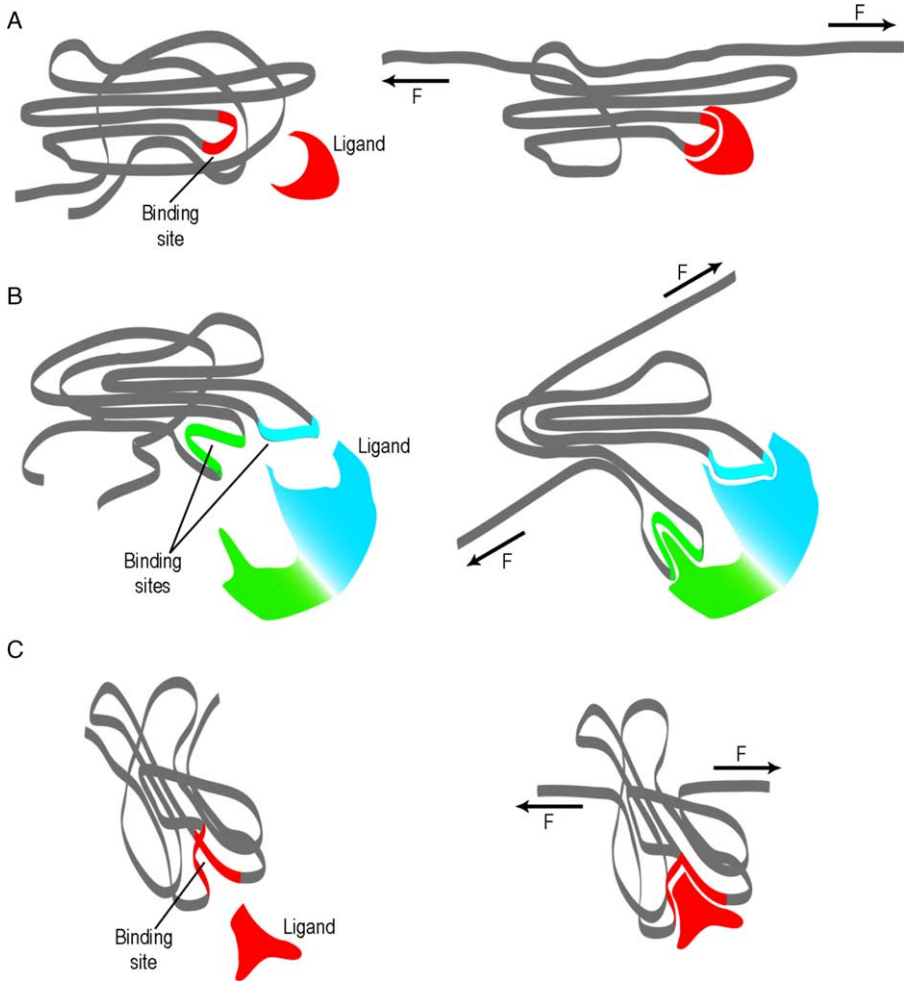

Fig. 5. Transduction of a mechanical tensions into biochemical signals by force induced conformational transitions. (A) Exposing cryptic sites. The binding site for the red coloured ligand is buried in the native domain structure (left side). In the force induced conformation the cryptic site is exposed and the binding can now take place (right side). (B) Changing the distance of two potentially synergic binding sites. The distance between the two binding sites in the native structure of the domain does not fit that of the relative ligand sites (left side). The applied force can modify their distance, making a synergic double binding possible (right side). (C) Changing the geometry of the binding site. The ligand binding site shape does not fit the tiny binding pocket of a protein domain (left side). The applied force deforms the pocket as to favour its binding (right side).

a biochemical signal, can meet a finer tuning whenever they are coupled to a redox equilibrium based on disulfide bonds. In fact, in the absence of disulfide bonds, potential cryptic sites are exposed each time there is a tensile stress on the protein. On the other hand, if the cryptic site is enclosed in a loop defined by a disulfide bond, the site will be hidden by default, and it would be exposed to a mechanical stress only after having unlocked the disulfide bond.

A signalling pathway on the cell surface can be therefore based on the overexpression and/or activation of extracellular disulfide reductases, that can finely control the exposure of cryptic sites in mechanically stressed proteins (Fig. 8).

The very first example of this kind of mechanochemical regulation has been found by our group during the study of the mechanical unfolding of angiostatin (Grandi et al., to be published). In this case the rupture of the most external disulfide bond of angiostatin K4 domain allows the formation of a mechanically unfolded intermediate that ensures an improved accessibility of a pair of domain segments that were previously demonstrated to be the most active at the anti-migratory level [59] (according to the scheme in Fig. 5(A)). The same redox and mechanical coupling also alters the distance between the binding sites of K2 and K3 domains to two subunits of endothelial plasma membrane F1-ATPase (a suspected angiostatin receptor [60]), making it possible for the angiostatin molecule to bind cooperatively both subunits (according to the scheme in Fig. 5(B)). We have also proved by mean of Single Molecule Force Spectroscopy that human thioredoxin is able to manage this kind of redox control. The same thioredoxin is in fact over-expressed on the surface of tumour cells [61,62]. The mechanical component of the overall mechanochemical control mechanism is induced by the proliferation of endothelial cells. The results have indicated that angiostatin activity therefore relies on the presence of both components, the redox and the mechanical one.

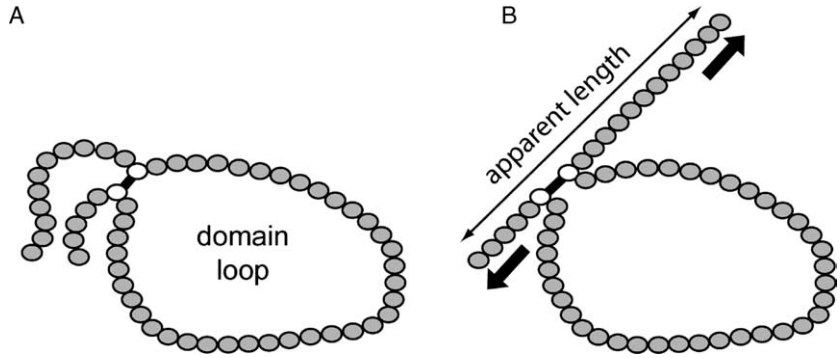

Fig. 6. Disulfide bonds hides portions of a protein domain from an external force. (A) Two dimensional sketch of a protein domain containing an internal disulfide bond. If the protein is made up of 56 residues, its contour length L should be of 19.6 nm (taking 0.35 nm as length/residue). The disulfide bond encloses a loop of 38 residues, corresponding to a loop length L_{app} of 13.3 nm. (B) The disulfide bond act as a barrier to mechanical unfolding. The apparent contour length L_{loop} resulting from the application of a force to the same protein sketched in A, is therefore given by $L_{app}=L-L_{loop}+SS_{bond}$, where L_{app} is the length of the disulfide bond itself (that can be safely neglected). In the case of this figure, the chain portion that is exposed to the external force, being composed of 18 residues, corresponds to a L_{app} of 6.3 nm only.

4.3. Candidate proteins for disulfide-controlled mechanical regulation

The angiostatin example is probably just a 'tip of the iceberg'. As discussed in the previous sections, the coexistence of mechanical tensions and active disulfide reductases is common in the extracellular environment. We can therefore speculate that all the extracellular structural proteins that contains disulfide bonds are potential candidates for disulfide-controlled regulation mechanisms similar to that supra described for angiostatin.

The structure of laminin, for example, strongly suggests the possibility that such a regulation mechanism might act in the basal membrane of vascolar endothelium. The basal membrane is the specialized extracellular matrix that sustains the growth and the survival of vascular endothelium [3] and it is normally subjected to the mechanical forces generated by migrating endothelial cells. Laminin is a trimeric protein and is one of the main structural components of the basal membrane. The structure of laminin shows a striking 'ladder' of disulfide bonds in the

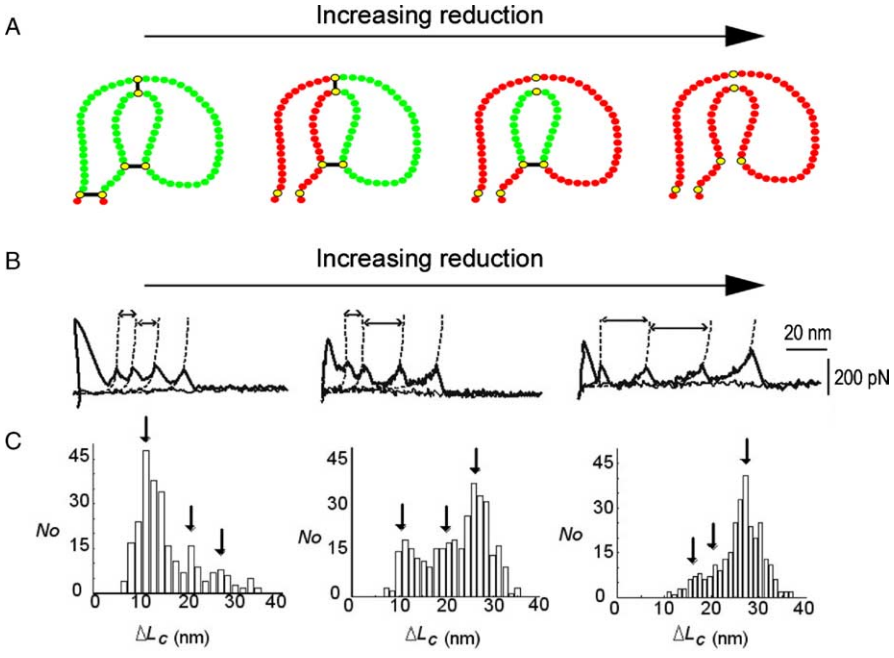

Fig. 7. Unfolding angiostatin modules at different reduction levels of their three internal disulfide bonds. (A) Two dimensional sketches of a kringle structure at different reduction states. The portion of the module that can unfold under force (red coloured in the figure) increases in length in response to an increased reduction. The topological loops accessible to mechanical unfolding are, respectively, 14 nm (40 residues) after the reduction of the most external disulfide bond, 20 nm (57 residues) after the reduction of the two most external disulfide bonds, and 28 nm (80 residues, correspondent to the whole chain) after the reduction of three disulfide bonds. (B) Experimental force curves. The apparent contour length, i.e. the distance between subsequent force peaks, that corresponds to the portion of the domain that has been unfolded, increases with the extent of reduction [56]. (C) Length increments distribution (i.e. distance between subsequent peaks) from angiostatin unfolding, at different DTT concentration. Distances are distributed around the three expected values (14, 20, 28 nm). The most populated peak shifts from the 14 nm one at low DTT concentration to the 28 nm one at high DTT concentration. On this basis the reduction state of each unfolded domain can be assigned [56].

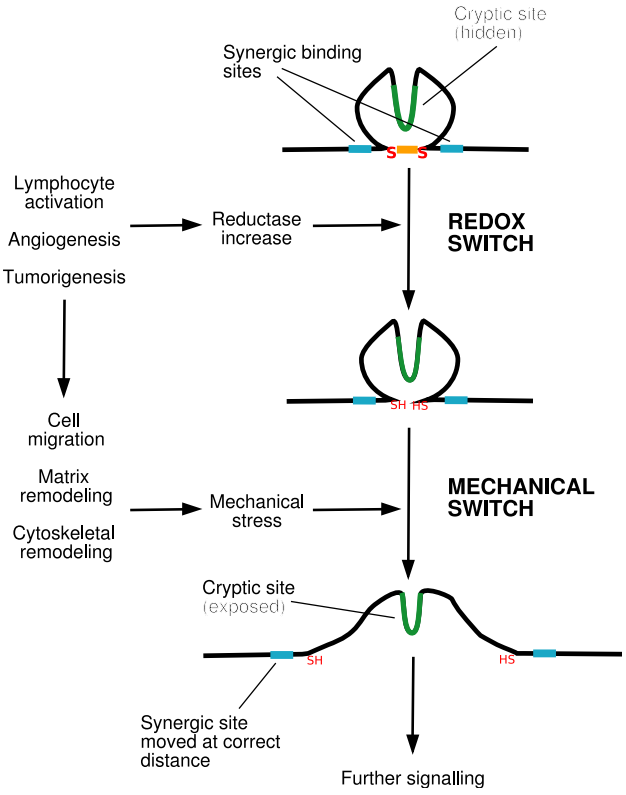

Fig. 8. A hypothetical scheme of how a disulfide-coupled mechanical signalling might work. An hypothetical extracellular protein module might contain a cryptic site (green) in a loop enclosed by a disulfide bond. It might even contain in addition two potentially synergic binding sites (blue) outside the loop. Processes like lymphocyte activation, angiogenesis and tumorigenesis have been shown to locally increase the expression and secretion of reductases [61,70,71], thereby leading to conditions in which the disulfide bond can be cleaved. The same processes are normally coupled with cell migration, and cytoskeletal/ECM remodelling [72–74], that generate mechanical stress on the extracellular proteins. Under these forces, whenever the disulfide bond has already been reduced, the module can partially unfold, both exposing the cryptic site and even repositioning the two synergic binding sites, thus switching on and off a biochemical signal. This type of pathway can be fully reversible: by relaxing the forces, the module can refold and by restoring the original redox potential the disulfide bond can be locked again.

gamma chain [63], and also three exposed disulfide bonds in the alpha-2 chain [64] (see Fig. 1(B)).

Thioredoxin has been shown to be able to reduce laminin disulfide bonds and this reduction seems to alter the growth of endothelial cells [65]. Moreover, the binding sites for nidogen (another essential component of the basement membrane) located on the 1III4 domain of the laminin gamma chain geometrically match with disulfide-connected loops in laminin. These binding sites are at least partially less active when reduced [66], suggesting a mechanism in which binding affinity could be locally regulated by a redox equilibrium.

Cell adhesion molecules and transmembrane molecules are normally subjected to the mechanical stresses generated along the ECM–cytoskeleton pathway (see Section 3). Many of these molecules also present disulfide bonds in their structure and constitute therefore other candidates for a disulfide-controlled regulation mechanism.

It has been proved that the CD4 receptor activity is regulated by disulfide reduction [16] (see Section 2), and that the mechanical properties of a cell adhesion molecule (CAM) can be modulated by mean of its disulfide bonds [58]. The vascular cell adhesion molecules (VCAMs) and CD2 [67] are two other examples of the many cell adhesion molecules that contain disulfide bonds in their structures.

Integrins are the transmembrane molecules that mediate cell adhesion. Also these molecules contain disulfide-bonded modules and their reduction has been shown to lead to their activation [17].

5. Conclusions

The majority of structural proteins present in the various kinds of ECM contain disulfide bonds. In ECM, as any other polymer gel, the degree of crosslinking strongly influences the gel mechanical behaviour. There is evidence that at least some ECM components are crosslinked by means of disulfide bonds [68]. Given also the presence of reductases and disulfide isomerases in the extracellular space we must expect that a disulfide bond mediated crosslinking can be a quite diffused phenomenon.

On the other hand, whereas a control mechanism based on the hierarchical coupling of a redox and a mechanical switch has been proved at a molecular level, the same mechanism at the inter-molecular level has not been evidenced so far. The Single Molecule Force Spectroscopy has already been demonstrated to be the technique of choice to study how mechanics and chemistry combine at the single molecule level to obtain complex and precise controls of biological functions. We expect that the discovery of the same type of controls acting in complex supramolecular systems like the ECM will be successfully pursued with the same technique.

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