Structural transitions as a mode for expression and regulation of physiological functions of fibronectin

M. Y. Khan*

Department of Biotechnology, B. B. Ambedkar University, Lucknow 226 025, India

Protein conformations other than those representing their unique three dimensional native states are generally believed to be insignificant structural entities occurring only during their turn over. However, the conventional compact native conformation ('C' state) of fibronectin has been found to be functionally inactive/less active as compared to its non-compact, extended ('E' state) structure formed during expression of its functions. It has been proposed that an equilibrium, 'C ↔ E', between the two states is vital in explaining all the functional properties of fibronectin. The equilibrium is shifted towards right when fibronectin ought to express its activities and a 'go-slow' tag is imposed by shifting the equilibrium towards left should a need arise to that effect.

Keywords: Fibrillogenesis, fibronectin, regulation, structural transitions.

PROTEINS are polymers of amino acids linked in a distinctive sequential manner with a potential to acquire unique three-dimensional structures characterized by discrete biological function(s). Conformations other than the ones representing the 'unique three-dimensional native states' are generally regarded as inconsequential entities occurring during the genesis and degradation of the functional structures. The breakout of spongiform encephalopathy in England in the 1980s and its subsequent correlation with the 'misfolding of a normal form' of a protein to a deadly conformation[1], however, provided the impetus to look for possible roles for naturally occurring 'nonnative conformational states' of proteins. I review the subject with an aim to draw the attention to this vital biological phenomenon that has been, to a large extent, grossly ignored so far. Thus, owing to its occurrences in multiple tissues, vivid structures, diversity in functions and the availability of adequate literature, fibronectin (FN) makes an ideal choice for evaluation of the possible roles of structural transitions in generating functional versatility in proteins. As exhaustive and excellent accounts of the early research on FN are available elsewhere[2,3], I would restrict the number of citations of publications and focus more on the recent findings related to the central theme of this article.

*e-mail: profmykhan@rediffmail.com

Occurrences, isoforms and structural modules of fibronectin

Fibronectin is a large glycoprotein found in a soluble form in micromolar concentrations in the blood plasma and in an insoluble multimeric form in the extracellular matrix[2,3]. FN is composed of multiple homologous structural domains which are made up of three 'homology types' or motifs/modules termed types I, II and III. Each one of these structural motifs is repeated several times in an array analogous to a 'string of beads'[4–7] and is named as FN-I_1, FN-I_2, FN-II_1, FN-II_2, FN-III_1, FN-III_2 and so on (FN followed by the homology type with subscripts indicating their positional serial number, or sub-homology type, starting from the amino-terminal end of the protein). In addition, FNs may also contain a combination of two alternatively spliced type-III domains named as FN-III_{EA} (E for 'extra-domain'), FN-III_{EB} and one FN-III_V (v for variable, also called CS, connecting segment) domain[2,3,8] (Figure 1). Thus a total of 12 type-I modules, two type-II, 15–17 (depending on splicing) type-III and a variable (III_V) domain constitute the entire unit of the FN molecule. According to its domain composition, therefore, FN exists in various isoforms in a tissue and specific cell-type dependent manner.

Structural transitions in fibronectins

At physiological pH and ionic strength, plasma FN exists in a compact conformation[3,7,9–11]. Even minor alterations in the solvent conditions lead to significant tweaking in the overall organization of the domains of FN without necessarily causing major changes in its secondary structures[3,9,12,13]. Plasma FN secreted by hepatocytes in compact and soluble isoform is not active and is incapable of forming a three-dimensional matrix in the absence of cells that induce some structural alterations in the protein upon binding with it[14]. FN conformation presents characteristics of both fibrous and globular proteins. It has a large adaptability for association with other matrix proteins or other FN molecules. Elasticity of FN originates from its unfolding and refolding propensity in general and its FN-III modules in particular[15,16]. These structural

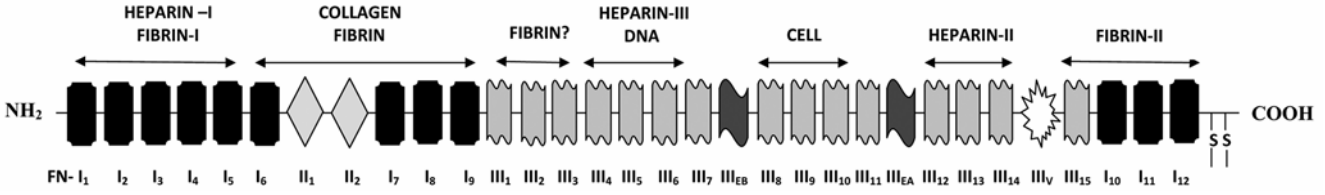

Figure 1. Diagrammatic illustration of fibronectin showing relative positions of its different structural modules in the intact molecule. The thin line connecting the sub-domains (shown by different shapes) of the different module types represents connecting primary structure segments of the protein. The amino- and carboxyl-terminal ends are marked with NH_2 and COOH respectively. Only one subunit has been depicted that is connected to the second unit (not shown) through its two disulphide bonds shown at the carboxyl terminal end of the chain. Ligand-binding positions are marked on the top of the schematic representation.

transitions in the FN molecule involve multiple conformational states and therefore offer an excellent tool for understanding the functional mechanism of the protein[17].

Role of structural transitions in FN-mediated processes

Fibronectin is involved in a number of biological activities which have been discussed elsewhere[2,3] (Figure 1). Instead of describing the mechanistic details of these FN activities/properties, the following account focusses on the structural transitions that are associated with the expression/regulation of some important biological activities of this vital protein.

Unfolding–refolding-assisted FN fibrillogenesis in the matrix assembly

Fibrillogenesis begins with the binding of FN to integrin and other cell receptors leading to its unfolding to an extended structure that exposes buried binding sites to promote the interaction of FN with other FN molecules and the matrix components[15–19]. Different degrees of FN unfolding can be observed along the newly formed FN fibrils. This variability in the degree of unfolding is due to different levels of tension that the cell cytoskeleton applies on individual FN fibrils. Treatment with cytochalasin D, which disrupts cytoskeleton tension, prevents fibril formation suggesting that FN modules refold when tension is released[18]. It is therefore conceivable that FN undergoes repeated cycles of important unfolding/refolding events during its assembly in the matrix[16].

Conformation-mediated role of FN in ageing and disease

The progression of several diseases including cancer and asthma has been linked with the changes in the remodelling and stiffening of the extracellular matrix[20,21]. FN is believed to be an active player in such stiffening processes that have been attributed to structural transitions in the protein. Zheng *et al.*[22] have recently shown that myofibroblast-driven unfolding of the secondary structure of matrix FN plays a role in the initiation and/or maintenance of chronic inflammation within the tumour microenvironment. Similarly, maturation of the matrix progresses during the course of ageing that in turn is associated with increased stretching of the FN fibrils. The stretching tendencies thus developed lead to partial unfolding of the secondary structures of the individual FN structural modules[23].

Increased levels of FN have been reported in patients suffering from arthritis[24,25] and the protein has been implicated in the aggravation of the disease through its structural domains. FN is readily degraded into fragments representing its various domains and their different combinations[25]. Once released, these fragments sometimes manifest properties which are not shared by their parent intact FN molecule. The heparin-binding amino- and carboxyl-terminal domains, for instance, have been found to play pathological roles in joint destruction by enhancing nitric acid (NO) production in the rheumatoid arthritis cartilage[26,27]. Intact FN, on the contrary, did not enhance NO production[27]. Although the exact mechanisms by which FN fragments contribute to the disease pathogenesis is still largely unclear[28], the observation that intact FN is not active but its separated domains are, suggests that some cryptic interacting sites are masked in the native structure of FN and they are exposed when these domains are separated and allowed to interact independently. There are two possible ways that could lead to this situation *in vivo*. One, FN undergoes reversible structural transition leading to separation of domains without any significant loss of its regular structures, a possibility I strongly believe in (see below). Alternatively, the active, disease causing/aggravating fragments are formed when bacterial and inflammatory proteases breakdown the matrix FN[28], a process that is likely to be accompanied by domain separation within the intact FN molecules as mentioned above.

Proposed mechanism of structure-mediated action of FN

The exposure of FN to moderate alkaline or acidic pH, high ionic strength, or to its natural ligands like heparin and collagen leads to the separation of its structural

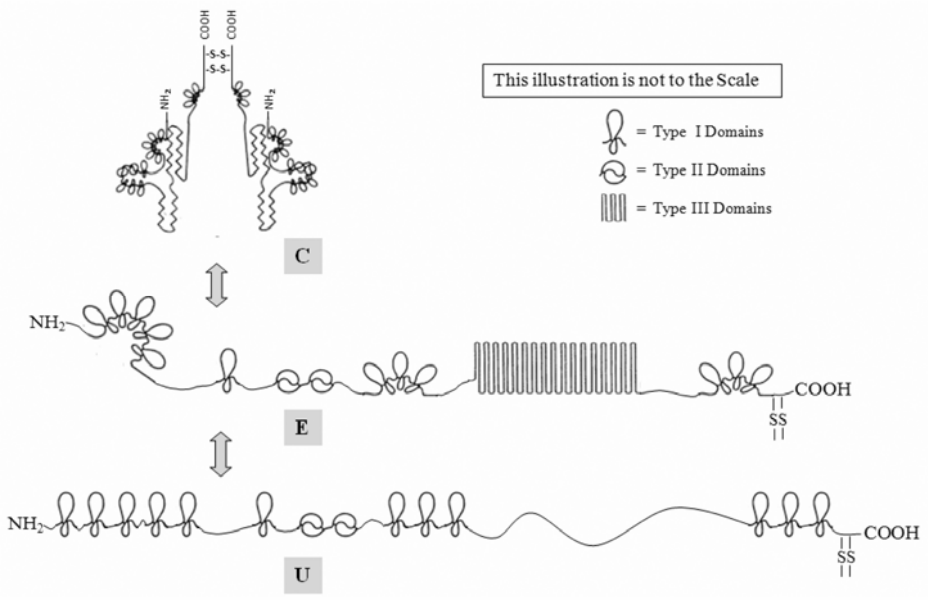

Figure 2. Schematic depiction (modified after Khan *et al.*[9]) of structural transitions required for the functional manifestation of fibronectin. The *C* state represents the compact conformational state of fibronectin found in the soluble form in the plasma. This state is stabilized essentially by inter-domain ionic interactions[3]. The *E* state is the extended conformational state of fibronectin which is formed due to separation of its domains from each other without essentially losing its regular secondary structures. Depending on the conditions (see text), the *E* state may vary in size (due to difference in the extent of domain separation) and shape (depending on the location(s) and the type(s) of the structural module(s) undergoing severance). The *E* state shown may therefore be envisioned only as a representative state of several such states that are possible under the actual physiological conditions. The *U* state is the fully unfolded state that has lost all its secondary structures. The *U* state retains its disulphide bonds and is generally a non-functional entity[3,9,17].

domains from each other[3,7,9,12,13,29,30]. The salient features of these structural alterations are illustrated in Figure 2. I had earlier proposed that FN, in its soluble form, exists in a compact *C* state which is converted to an extended state *E*, under the above-mentioned conditions[9]. The *E* state, as proposed in the model (Figure 2), represents just one of the numerous possible *E* states that might be formed during the expression of the FN function. Although the extent of severance of the constituent domains may vary according to the environmental conditions FN is exposed to, the nature of all these structural transformations is essentially similar as it does not involve any major loss of its conventional secondary structures. Under harsher conditions like, for instance, during *in vitro* denaturation studies[9,17], the *E* state is converted into the unfolded state *U*. Thus, equilibrium, $C \leftrightarrow E \leftrightarrow U$, is proposed to describe all conceivable structural transformations in FN (Figure 2), wherein *E* may be envisaged as the characteristic expanded state occurring under a given condition as mentioned above. I further suggest that analogous conformational transitions between the *E* and *C* states of FN might be a 'natural' phenomenon required for expression of the biological activities of the protein. The *E* state, which is more relaxed and flexible than the *C* state, would be potentially more active during the physiological functioning of FN[7]. Collagen binding, for instance, might be sterically hindered by the folding pattern in the *C* state and hence it would tend to change the *C* state to the *E* state. Likewise, the *E* state is expected to be advantageous during FN fibrillogenesis since the inter-domain interactions operating within the FN molecules could be replaced by intermolecular interactions involving the *E* state. As mentioned above, the *E* state obtained under different conditions/formed by different means[31] need not necessarily be the same and depending on the extent of its 'expansion', it may have different size and structural characteristics. Indeed, by exposing FN to a stretching force, Li *et al.*[32] have recently shown that the sub-domains of FN-III_{10} separate along two pathways, each involving the separation of different segments of the native secondary structures.

A series of studies with FN fragments representing different domains of FN have shown that the separated domains were potentially more active than the intact FN[33–35], suggesting that the compact *C* conformation envisioned above suppresses the biological activity of the protein in its native form. The question as to what might be the biological role of the compact native conformation of FN, therefore, becomes more relevant than ever before. I propose that the existence of equilibrium between the *C* and *E* states of FN is paramount in explaining all the properties/functions of the protein. FN-mediated processes might utilize either of the two states of the protein, wherein the *C* state is primarily required for curbing the undesired manifestations of its functions. Accordingly, the 'go-slow' tag imposed by the *C* state is removed by shifting the $C \leftrightarrow E$ equilibrium towards the right should a situation arise wherein FN needs to express its activities.

The *in situ* cellular environmental factors such as ionic composition, fluidity and physical steric effects are the likely parameters that might control the above equilibrium and eventually the activities of FN.

1. Horwich, A. L. and Weissman, J. S., Deadly conformations: protein misfolding in prion disease. *Cell*, 1997, **89**, 499–510.
2. Mosher, D. F., *Fibronectin*, Academic Press, San Diego, California, 1989.
3. Hynes, R. O., *Fibronectins*, Springer-Verlag, New York, 1990.
4. Johnson, K. J., Sage, H., Briscoe, G. and Erickson, H. P., The compact conformation of fibronectin is determined by intramolecular ionic interactions. *J. Biol. Chem.*, 1999, **274**, 15473–15479.
5. Singh, P., Carraher, C. and Schwarzbauer, J. E., Assembly of fibronectin extracellular matrix. *Annu. Rev. Cell Dev. Biol.*, 2010, **26**, 397–419.
6. Rocco, M., Carson, N., Hantgan, R., McDonagh, J. and Hermans, J., Dependence of the shape of the plasma fibronectin molecule on solvent composition, ionic strength and glycerol content. *J. Biol. Chem.*, 1983, **258**, 14545–14555.
7. Khan, M. Y., Structure and properties of fibronectin. *Indian J. Biochem. Biophys.*, 1990, **27**, 63–68.
8. Schwarzbauer, J. E., Spencer, C. S. and Wilson, C. L., Selective secretion of alternatively spliced fibronectin variants. *J. Cell Biol.*, 1989, **109**, 3445–3453.
9. Khan, M. Y., Medow, M. S. and Newman, S. A., Unfolding transitions of fibronectin and its domains. Stabilization and structural alteration on *N*-terminal domain by heparin. *Biochem. J.*, 1990, **270**, 33–38.
10. Williams, E. C., Janmey, P. A., Ferry, J. D. and Mosher, D. F., *J. Biol. Chem.*, 1982, **257**, 14973–14978.
11. Erickson, H. P. and Carrell, N. A., Fibronectin in extended and compact conformations. Electron microscopy and sedimentation analysis. *J. Biol. Chem.*, 1983, **258**, 14539–14544.
12. Markovic, Z., Lustig, A., Engel, J., Richter, H. and Hormann, H., Shape and stability of fibronectin in solutions of different pH and ionic strength. *Hoppe-Seyler's Z. Physiol. Chem.*, 1983, **364**, 1795–1804.
13. Lai, C. S., Tooney, N. M. and Ankel, E. G., Structure and flexibility of plasma fibronectin in solution: electron spin resonance spin-label, circular dichroism, and sedimentation studies. *Biochemistry*, 1984, **23**, 6393–6397.
14. Mao, Y. and Schwarzbauer, J. E., Stimulatory effects of a three-dimensional microenvironment on cell-mediated fibronectin fibrillogenesis. *J. Cell Sci.*, 2005, **118**, 4427–4436.
15. Baneyx, G., Baugh, L. and Vogel, V., Coexisting conformations of fibronectin in cell culture imaged using fluorescence resonance energy transfer. *Proc. Natl. Acad. Sci. USA*, 2001, **98**, 14464–14468.
16. To, W. S. and Midwood, K. S., Plasma and cellular fibronectin: distinct and independent functions during tissue repair. *Fibrogenesis Tissue Repair*, 2011, **4**, 1–17.
17. Patel, S., Chaffotte, A. F., Batt-Amana, A., Goubard, F. and Pauthe, E., *In vitro* denaturation–renaturation of fibronectin. Formation of multimers disulfide-linked and shuffling of intramolecular disulfide bonds. *Int. J. Biochem. Cell Biol.*, 2006, **38**, 1547–1560.
18. Baneyx, G., Baugh, L. and Vogel, V., Fibronectin extension and unfolding within cell matrix fibrils controlled by cytoskeletal tension. *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 5139–5143.
19. Smith, M. L., Gourdon, D., Little, W. C., Kubow, K. E., Eguiluz, R. A., Luna-Morris, S. and Vogel, V., Force-induced unfolding of fibronectin in the extracellular matrix of living cells. *PLoS Biol.*, 2007, **5**, e268.
20. Liu, F., Mih, J. D., Shea, B. S., Kho, A. T., Sharif, A. S., Tager, A. M. and Tschumperlin, D. J., Feedback amplifications of fibrosis through matrix stiffening and COX-2 suppression. *J. Cell Biol.*, 2010, **190**, 693–706.
21. Levental, K. R. *et al.*, Matrix crosslinking forces tumour progression by enhancing integrin signaling. *Cell*, 2009, **139**, 891–906.
22. Zheng, M., Jones, D. M., Horzempa, C., Prasad, A. and McKeown-Longo, P. J., The first type III domain of fibronectin is associated with the expression of cytokines within the lung tumour microenvironment. *J. Cancer*, 2011, **2**, 478–483.
23. Antia, M., Baneyx, G., Kubow, K. E. and Vogel, V., Fibronectin in aging extracellular matrix fibrils is progressively unfolded by cells and elicits an enhanced rigidity response. *Faraday Discuss.*, 2008, **139**, 229–249.
24. Miller, D. R., Mankin, H. J., Shoji, H. D. and Ambrosia, R. D., Identification of fibronectin in preparations of osteoarthritic human cartilage. *Connect. Tissue Res.*, 1984, **12**, 267–275.
25. Homandberg, G. A., Wen, C. and Hui, F., Cartilage damaging activities of fibronectin fragments in human osteoarthritis and disease free articular cartilage. *Osteoarthritis Cartilage*, 1998, **6**, 231–244.
26. Gamba, T., Valbracht, J., Alsalameh, S. and Lotz, M., Focal adhesion kinase and mitogen-activated protein kinases are involved in chondrocyte activation by 29-kDa amino-13 terminal fibronectin fragment. *J. Biol. Chem.*, 2002, **277**, 907–911.
27. Yasuda, T., Kakinuma, T., Julovi, S. M., Yoshida, M., Hiramitsu, T., Akiyoshi, M. and Makamura, T., COOH-terminal heparin binding fibronectin fragment induces nitric oxide production in rheumatoid cartilage through CD44. *Rheumatology*, 2004, **43**, 1116–1120.
28. Ghosh, A., Park, J. Y., Fenno, C. and Kapila, Y. L., *Porphyromonas gingivalis*, gamma interferon, and a proapoptotic fibronectin matrix from a synergestic trio that induces c-Jun *N*-terminal kinase 1-mediated nitric oxide generation and cell death. *Infect. Immunol.*, 2008, **76**, 5514–5523.
29. Khan, M. Y., Jaikaria, N. S., Frenz, D. A., Villanueva, G. and Newman, S. A., Structural changes in the NH_2-terminal domain of fibronectin upon interaction with heparin. *J. Biol. Chem.*, 1988, **263**, 11314–11318.
30. Jaikaria, N. S., Rosenfeld, L., Khan, M. Y., Danishefsky, I. and Newman, S. A., Interaction of fibronectin with heparin in model extracellular matrices: role of arginine residues and sulfate groups. *Biochemistry*, 1991, **30**, 1538–1544.
31. Khan, M. Y. and Newman, S. A., The salting-out behavior of human plasma fibronectin and its possible correlation with heparin-induced cryoprecipitation of the protein. *Biochem. Int.*, 1991, **23**, 1–7.
32. Li, L., Huang, H. H., Badilla, C. L. and Fernandez, J. M., Mechanical unfolding intermediates observed by single-molecule force spectroscopy in a fibronectin type III module. *J. Mol. Biol.*, 2005, **28**, 817–826.
33. Hayashi, M. and Yamada, K. M., Domain structure of the carboxy-terminal half of human plasma fibronectin. *J. Biol. Chem.*, 1983, **258**, 3332–3340.
34. Rocco, M., Aresu, O. and Zardi, L., Conformational state of circulating human plasma fibronectin. *FEBS Lett.*, 1984, **178**, 327–330.
35. Akiyama, S. K., Hasegawa, E., Hasegawa, T. and Yamada, K. M., The interaction of fibronectin fragments with fibroblastic cells. *J. Biol. Chem.*, 1985, **260**, 13256–13260.

Received 27 January 2012; revised accepted 4 October 2012