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Review article

Cryptic epitopes and functional diversity in extracellular proteins

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ABSTRACT

The functional diversity of proteins is a major factor determining the complexity of cells and tissues. Both translational and post-translational modifications contribute to this diversity. Recently, protein unfolding and refolding has been recognised as another mechanism for diversity by unmasking buried or cryptic sequences (epitopes) that possess physiological functions. In the current review, we focus on extracellular proteins where folding dynamics can be influenced by mechanical forces, protein-protein interactions and denaturation. Many cryptic epitopes in these proteins are exposed following proteolytic cleavage, but recent data indicate that unfolding/refolding play an important role in regulating the physiological behaviour of extracellular proteins. By understanding how and when hidden sequences are exposed, novel techniques for manipulating the function of these proteins may be uncovered.

1. Introduction

Proteins are essential to all aspects of cellular function. They catalyse enzymatic reactions, regulate gene expression and ion transport, communicate between the extracellular environment and the intracellular space, control the structure, shape and mobility of cells, and modulate the movement of cells within tissues. A single gene can encode a protein with diverse functions depending on various events such as alternative splicing, post-translational modifications and compartmentalisation. Some proteins perform multiple tasks that are mechanistically distinct (moonlighting). For example, pyruvate kinase carboxylates pyruvate but, in some species, it also acts as a transporter for other proteins (Ozimek et al., 2003). Similarly, cytochrome C participates in mitochondrial electron transport but, in the cytosol, it forms a complex with Apaf-1 to initiate apoptosis (Hao et al., 2005). There are numerous examples of these multi-purpose proteins (Huberts and van der Klei, 2010). The different translational and post-translational modifications that a protein can undergo gives rise to functional diversity that contributes to the biological complexity of cells and tissues. In recent years, subtle changes in protein structure due to induced unfolding and refolding has been recognised as a further contributor to this functional diversity (Uversky, 2015).

The vast array of functions that proteins perform is enabled by the precise folding of an amino acid sequence into a specific but dynamic three-dimensional conformation. Differences between structural elements distinguish one protein from another and represent the most fundamental property affecting the biological activity of proteins. Correct biological functionality requires a protein to exist in its native conformation. Misfolding can lead to proteopathy, diseases caused by protein aggregation, which include amyloidosis, Alzheimer's disease, amyotrophic lateral sclerosis, tauopathies, retinitis pigmentosa, Huntington's disease and Parkinson's disease.

Proteins are structurally dynamic with ordered and disordered regions that unfold and refold over time and in response to various stimuli (Jakob et al., 2014). It has been widely reported that changes in the folding state of a protein can expose functional domains normally hidden or shielded within the protein. This has been best illustrated by antibody recognition of various antigenic determinants (epitopes) in misfolded proteins (Sela-Culang et al., 2013). Epitopes can be specific sequences (linear peptides) or motifs comprised of several structural elements in close proximity. Upon unfolding of a protein, the antigenic site (cryptic epitope) is exposed and elicits an immunological reaction (Laver et al., 1990). This is the basis for many auto-immune diseases. For example, the plasma transport protein transthyretin is involved in several forms of amyloidosis. Following unfolding and aggregation of transthyretin (Goldsteins et al., 1999; Phay et al., 2014), a normally buried β-strand is exposed (Gustavsson et al., 1994). Moreover, antibodies to this β-strand are common in patients with transthyretin amyloidosis but not in controls. The influenza coat protein hemagglutinin is another well-characterised example of a molecule that partially

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unfolds in order to elicit a biological effect, in this case, fusion with the endosomal membrane during infection (Huang et al., 2003).

While our understanding of cryptic epitope recognition by antibodies is well established, recently evidence has begun to emerge that similar hidden sequences responsible for non-antigenic biological activities are present in some proteins. This is particularly evident in extracellular matrix proteins (Schenk and Quaranta, 2003) where exposure of these buried motifs, referred to as 'matricryptic' sites, involves conformational changes and/or proteolytic cleavage (Davis et al., 2000). Some of these are central to the primary role of the protein while others create novel functions that are not obvious unless the buried peptide sequence is exposed. These cryptic epitopes increase the functional diversity of many extracellular and intracellular proteins (Fig. 1). The difficulties in identifying functional cryptic epitopes are well reported but recent studies into the effects of protein binding to nanomaterials suggest a model for partially unfolding proteins that can be used to study their altered biological effects (Deng et al., 2011; Mortimer et al., 2014). The current review briefly summarises recent advances in our understanding of functional diversity focussing on key extracellular proteins.

2. Learning from extracellular matrix proteins: matricryptic sites

Extracellular matrix proteins commonly carry out multiple and diverse biological functions, many of which are the result of cryptic site exposure (Maquart et al., 2004). Although these masked sequences can be activated following either proteolytic cleavage or conformational modifications (Davis et al., 2000; Tran et al., 2005), discussion here will focus on the latter. Readers are directed to other reviews that cover examples of cryptic sites in extracellular matrix proteins revealed by enzymatic digestion (Davis et al., 2000; Schenk and Quaranta, 2003). Exposure of bioactive sites by conformational modifications can be induced by several different mechanisms including mechanical force (Hocking et al., 2008), heterotypic binding to other molecules (Dardik and Lahav, 1999), self-assembly reactions (Mao and Schwarzbauer, 2005; Wierzbicka-Patynowski and Schwarzbauer, 2003), and denaturation. The unmasking of cryptic epitopes by molecular stretching has been well characterised for intracellular proteins such as talin and alpha-catenin (del Rio et al., 2009; Yonemura et al., 2010).

An important point for consideration regarding cryptic sites in extracellular matrix proteins is the gain in functionality. This is highlighted in work linking matricryptic site exposure and cell responses following tissue injury (Davis, 2010). Matricryptic sites can also be similar to those of pattern recognition receptors. For instance, DMBT1 (a basement membrane matrix protein also referred to as hesin) binds polyanionic ligands including LPS in a similar manner as scavenger receptors (End et al., 2009). It has been proposed that unmasking of these site that engage pattern recognition receptors helps regulate cell responses during tissue injury (Davis, 2010).

2.1. Collagen

Collagens are the most abundant proteins in the extracellular space involved in the organisation of the structural support to surrounding cells throughout the body (Di Lullo et al., 2002). Collagen has multiple diverse biological effects such as inhibition of angiotensin 1-converting enzyme (ACE), anti-angiogenesis activity, tumor growth inhibition, chemotaxis and oxidative stress relief (Banerjee et al., 2015). Cryptic binding sites contribute substantially to this functional diversity. The majority of these sites require proteolytic activation (Marneros and Olsen, 2001; Sasaki et al., 2002). However, conformational changes to collagen can also expose hidden binding domains. For instance, the pro-angiogenic HUIV26 cryptic epitope within the triple helix of collagen IV can be exposed by temperature-induced unfolding (Hangai et al., 2002; Xu et al., 2001) as well as ionizing radiation (Brooks et al., 2002). This motif has been linked to several biological effects of collagen, including cell migration, adhesion and angiogenesis (Favreau et al., 2014; Xu et al., 2001). Despite attempts to identify the HUIV26 epitope, the amino acid sequence remains unknown suggesting it may represent a non-contiguous binding domain. Importantly, this epitope has been shown to have a role in tumor metastasis and may be a novel target for drug development (Roth et al., 2006).

Mechanical forces transmitted across the collagen molecule can lead to localized unfolding (micro-unfolding) (Bourne et al., 2014). In one domain of the protein, located in the N-terminus of the alpha1 chain, mechanical force exposes a chymotrypsin cleavage site. Mutations in this region, as seen in Ehlers-Danlos syndrome and osteogenesis imperfecta, significantly alter the unfolding kinetics of the collagen molecule (Makareeva et al., 2006). Tissue injury and remodelling of extracellular proteins can also result in conformational changes in this region of the collagen protein (Leikina et al., 2002; Persikov and Brodsky, 2002).

Collagen is known to bind to cellular integrins via a cryptic epitope. This tripeptide sequence (arginine-glycine-aspartate or RGD) is exposed when collagen unfolds or is denatured (Engvall et al., 1978; Ingham et al., 1985). The RGD epitope plays a central role in the function of collagen as a cell signalling molecule and regulates cell proliferation and differentiation (Taubenberger et al., 2010). In MC3T3-E1 cells, partially denatured collagen engages $\alpha_5\beta_1$- and α_v-integrins to promote phosphorylation of focal adhesion kinase (FAK), which eventually stimulates cell differentiation. Unfolding has also been shown to transform collagen type I from a $\beta1$ integrin-associated ligand to an $\alpha v\beta3$-dependent ligand due to exposure of an RGD epitope (Davis, 1992; Montgomery et al., 1994). A similar pathway has been identified for collagen-stimulated endothelial cell proliferation in angiogenesis (Ames et al., 2015).

Clearly, the collagens are multi-function proteins that play an important role in the remodelling of the extracellular matrix. Much of the diversity seen with the protein originates from cryptic epitopes that bind to a variety of proteins and receptors once the collagen molecule is partially unfolded.

2.2. Fibronectin

Two forms of fibronectin mediate a diverse array of biological effects. While the plasma form is involved in coagulation, most fibronectin functions are attributed to the less-soluble cellular form that interacts as part of the extracellular matrix. These functions include cell adhesion, growth, migration and differentiation as well as tissue responses to wound healing, malignant transformation, inflammation and haemostasis. There are various matricryptic sites within fibronectin, the majority of which are exposed following proteolysis (see Table 2 in Schenk et al.) (Schenk and Quaranta, 2003). However, there is evidence for unmasking of cryptic epitopes by partial unfolding. For example, incorporation of fibronectin in the extracellular matrix occurs by fibrillogensis, which involves self-association (polymerisation) of fibronectin into fibrils. Some of the sites mediating self-association are cryptic or partially cryptic and require conformational changes (such as mechanical stretching) to enable accessibility. Cell-driven increase in tension leads to unfolding and extension of the Fn III modules of the protein exposing cryptic polymerisation sites (Hao et al., 2005). Controlled strain experiments on fibronectin fibers show that stretch-induced exposure of cryptic sites increases gradually over the full range of fiber extension. This is accompanied by the onset of Fn III module unfolding (Klotzsch et al., 2009). The ability of fibronectin to unfold and