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Structural elucidation of full-length nidogen and the laminin–nidogen complex in solution

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ABSTRACT

Nidogen-1 is a key basement membrane protein that is required for many biological activities. It is one of the central elements in organizing basal laminae including those in the skin, muscle, and the nervous system. The self-assembling extracellular matrix that also incorporates fibulins, fibronectin and integrins is clamped together by networks formed between nidogen, perlecan, laminin and collagen IV. To date, the full-length version of nidogen-1 has not been studied in detail in terms of its solution conformation and shape because of its susceptibility to proteolysis. In the current study, we have expressed and purified full-length nidogen-1 and have investigated its solution behavior using size-exclusion chromatography (SEC), dynamic light scattering (DLS) and small angle X-ray scattering (SAXS). The *ab initio* shape reconstruction of the complex between nidogen-1 and the laminin γ-1 short arm confirms that the interaction is mediated solely by the C-terminal domains: the rest of the domains of both proteins do not participate in complex formation.

1. Introduction

The nidogen-1 protein is a key basement membrane molecule in animals that is produced primarily by mesenchymal cells. It mediates the attachment of epithelial cells (Alstadt et al., 1987; Tsao et al., 1990) by binding to $\alpha_v\beta_3$ and $\alpha_3\beta_1$ integrins (Dedhar et al., 1992; Dong et al., 1995), as well as playing a key role in the assembly of the basement membrane and the transfer of signals through integrins (Chung and Durkin, 1990; Pujuguet et al., 2000). In addition, it has been shown to affect neuromuscular organization and junction (Ackley et al., 2003).

Nidogen-1 consists of a single polypeptide chain with a molecular weight of approximately 139 kDa. Previous studies by Paulsson et al. (1986, 1987) that utilized denaturing conditions to purify nidogen-1 from EHS tumor indicated that it has two globular domains and a dumb-bell shape. However, studies with recombinantly expressed nidogen-1, have shown it to be composed of three globular domains G1, G2 and G3 (Fox et al., 1991). Domains G1 and G2 are connected by a 10 nm linker fragment, whereas domains G2 and G3 are separated by a 15 nm rod segment that primarily contains multiple EGF repeats (Fox et al., 1991) (see also Supplementary Fig. 1). A major function of nidogen-1 is the connection of the laminin —and collagen IV networks in basement membranes (Fox et al., 1991; Reinhardt et al., 1993; Poschl et al., 1994). These contacts are mediated by the globular domains G2 and G3 (Gerl et al., 1991; Mayer et al., 1993). Additional binding epitopes of nidogen for perlecan, fibulins and fibronectin have been mapped to either domain G2 or G3 (Hsieh et al., 1994; Sasaki et al., 1995a,b). Nidogen-1 and -2 are highly homologous proteins with common structural features such as the presence of three globular domains. However, nidogen-2 is much larger and is more extensively glycosylated (Kohfeldt et al., 1998). Nidogen-2 shares with nidogen-1 the ability to interact with laminin γ-1 chain but with lower affinity (Kohfeldt et al., 1998). The lack of expression of both nidogens has been proposed to be associated with gastrointestinal cancer (Ulazzi et al., 2007). Although nidogen-1 null mice presented no significant problems in terms of basement membrane formation and maintenance (Murshed et al., 2000), the expression of nidogen-2 was found to be up-regulated in the absence of nidogen-1, suggesting that nidogen-2 may compensate for the loss of activity. Subsequent studies on nidogen-2 null mice resulted in fertile and viable mice without any problems with the basement membrane formation (Schymeinsky et al., 2002). However, mice deficient in both nidogens died within 24 h due to complications with the lung and heart (Bader et al., 2005). The absence of both nidogens has also been linked to difficulties with limb development (Bose et al., 2006) and basement membrane assembly in skin-organotypic 3-dimentional co-cultures (Nischt et al., 2007). Although both nidogens are present in basement

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membranes and have overlapping binding partners (Ho et al., 2008), they seem to exhibit tissue specific roles: only nidogen-1 is recruited *via* laminin γ-1 chain to the basement membrane (Mokkapati et al., 2011).

Laminins are multifunctional heterotrimeric glycoproteins that play crucial roles not only in the formation and maintenance of basement membranes but also in early embryogenesis and muscle cell integrity (Tzu and Marinkovich, 2008). Whereas the α-chains participate in interactions with integrins that influence the processes of cell adhesion, migration and differentiation (Colognato and Yurchenco, 2000; Mercurio and Rabinovitz, 2001), the β- and γ-chains interact with various proteins found in the extracellular matrix. Of particular interest to this investigation is the high-affinity interaction between the C-terminal region of the laminin γ-1 short arm and nidogen-1. The nidogen-1 binding site on the laminin γ-1 short arm is located at the LE domain γ1 III4 and has been previously studied at atomic resolution (Baumgartner et al., 1996; Stetefeld et al., 1996). More recently, the specific domains of the laminin γ-1 short arm (17.5 kDa) and nidogen-1 (26 kDa) involved in complex formation were determined at atomic level (Takagi et al., 2003). However, there are no reported solution studies of complex formation between the complete laminin γ-1 short arm (109 kDa) and full-length nidogen-1 (139 kDa) that may provide detailed insight into any conformational changes. In this extension of a previous investigation of the solution behavior of the laminin γ-1 short arm that established its existence as an extended and curved multidomain assembly (Patel et al., 2010) we now report corresponding physicochemical studies on nidogen-1, which is also shown to exhibit an extended and curved nanostructure. Indeed, the degree of complementarity between the two structures suffices to allow postulation of a solution structure for the 1:1 complex between nidogen-1 and laminin γ-1.

2. Results

Analysis of the recombinant full-length nidogen-1 preparation (M_r = 139 kDa) by SDS-PAGE has yielded an electrophoretic pattern that is consistent with the calculated molecular mass (Fig. 1A, Lane A). Furthermore, the observation of an essentially identical pattern for nidogen-1 subjected to Pngase F digestion (Fig. 1A, Lanes E and F) seemingly precludes extensive post-translational modification of the two reported N-linked glycosylation sites (Fujiwara et al., 1993). This purified protein is deemed suitable for physicochemical examination of the solution behavior of nidogen-1, the chosen conditions (50 mM Tris/HCl, pH 7.5, supplemented with 200 mM NaCl) being similar to those used previously for delineating the solution nanostructure of the laminin γ-1 short arm (Patel et al., 2010).

2.1. Size-exclusion chromatography

Preliminary information on the sizes of nidogen-1 as well as the laminin γ-1 short arm and their complex was obtained from SEC on the Superdex 200 column (Fig. 1B). The monomeric fractions of individual proteins were mixed together to allow formation of the complex formation, which was then purified by SEC. The shoulder observed in the elution profile of the laminin–nidogen complex is attributed to inexact matching of reactant concentrations in its preparation. The SEC purified

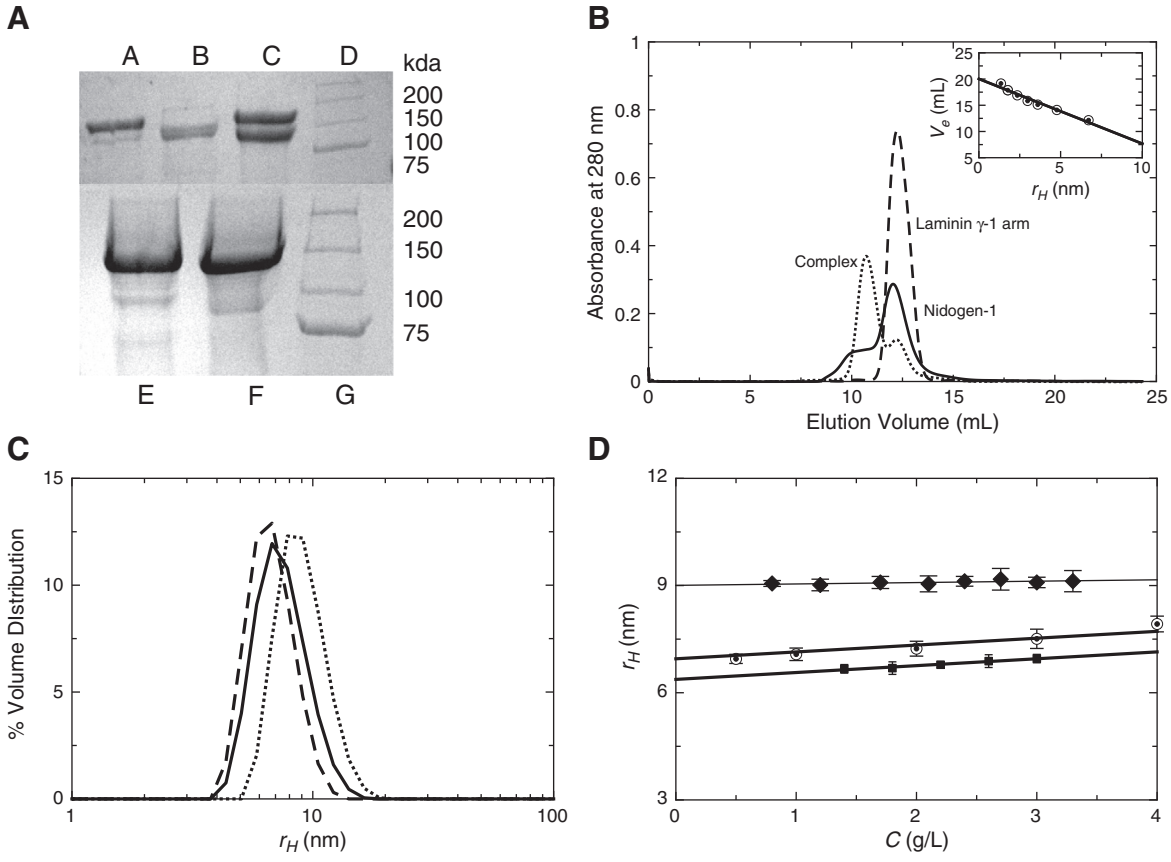

Fig. 1. Preparation and analysis of nidogen-1, the laminin γ-1 short arm and their complex by SDS-PAGE, SEC and DLS. (A) SDS-PAGE analysis of proteins where nidogen-1 (lane A); the laminin γ-1 short arm (lane B); complex of nidogen-1and laminin γ-1 (lane C); molecular weight markers (lanes D, G) as well as nidogen-1 before and after Pngase F digestion (lane E) and (lane F) respectively are presented. (B) Size-exclusion chromatography of nidogen-1, the laminin γ-1 short arm and the complex between the two proteins on a Superdex 200 column pre-equilibrated with TBS buffer (pH 7.5, I = 0.19 M). Also shown (inset) is a calibration plot of elution volume in terms of Stokes radius. (C) DLS profiles for nidogen-1 (—), laminin γ-1 short arm (– – –) and their complex (……) in TBS buffer (pH 7.5, I 0.19) presented at 3.0 mg/mL. (D) Concentration dependence of the mean hydrated radius (r_H) for nidogen-1 (○), laminin γ-1 short arm (■) and the complex (♦).

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