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Visualisation by electron microscopy of the unique part of the cytoplasmic domain of a desmoglein, a cadherin-like protein of the desmosome type of cell junction

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Abstract Part of the cytoplasmic domain of a human desmoglein, Dsg1, a cadherin-like protein found in desmosomes of epithelial cells, has been visualised by electron microscopy. The cloned fragment contains five repeats of a 29 ± 4 residue sequence unique to desmogleins, followed by a glycine-rich region. In rotary shadowed preparations the molecule consists of a globular head attached to a thin tail, the latter perhaps corresponding to the glycine-rich region. This portion of the molecule is thought to span the width of the inner dense plaque. The structure and dimensions concur well to the configuration deduced from the protein sequence.

Key words: Desmosome; Desmoglein; Electron microscopy; Rotary shadowing

1. Introduction

Desmosomes form a major class of adhesive junctions found especially in epithelial tissues, where adjacent cells are linked together by well-defined plaques situated on the plasma membrane [1,2]. Internally, the plaques are associated with the intermediate filament network extending from cell to cell. Molecules which might perform an adhesive role between desmosomes of neighbouring plasma membranes are the desmocollins (predicted M_r = 84,633 and 78,447 for the mature forms of human Dsc2) and desmogleins (predicted M_r = 107,578 for the mature human Dsg1), two transmembrane glycoproteins related to the calcium-binding family of cell adhesion molecules, the cadherins [3,4] (for nomenclature of desmosomal cadherins, see [5]). Major non-glycosylated proteins, the desmoplakins I and II (predicted M_r = 310,000 and 238,000, respectively for human DPI and DPII) [6,7], appear likely candidates forming a link between desmosomes and intermediate filaments [8].

Whilst some of the major proteins of the desmosome have been localised to particular regions of the plaque, no detailed model is yet available to describe the molecular structure of desmosomes. Some of the major desmosomal proteins such as the desmogleins and desmoplakins have been cloned and sequenced, or isolated in purified form from desmosome-rich tissues. These studies have provided a store of data from which secondary structure predictions have been made. In one case, that of the desmoplakins, the structural predictions [6] can be closely correlated with the appearance of the purified molecules seen by electron microscopy [9]. The availability of purified molecules could provide a means of studying the interactions between components of the desmosome as well as yielding information concerning the topology of individual domains.

In this paper we describe the first visualisation of a desmoglein, viz. part of the cytoplasmic domain of human desmoglein Dsg1 (predicted M_r = 29,367) [10,11] cloned and expressed in *E. coli*; this part of the protein contains the sequence unique to the desmogleins consisting of five repeats of a 29 ± 4 residue sequence with a core consensus of N-V-V/I-V-T-E-R/S-V-I/V, followed by a glycine-rich region. Our results show that the molecule is similar in shape and size to the configuration deduced from structural considerations.

2. Materials and methods

2.1. Cloning and expression in E. coli

A 1204 bp *Sty*I-*Xho*I fragment (2364 - 3567 bp in EMBL database sequence X56654; residues 714–1000 of the mature protein) [10,11] located at the 3′ end of the human DSG1 gene, starting with the amino acid sequence LGKES and ending in the 3′-untranslated region, was filled-in using the Klenow fragment of DNA polymerase and subcloned into the *Sma*I site of the pMal-c expression vector [12] (New England Biolabs), thus retaining the reading frame. This sequence starts just prior to the end of homology with the classical cadherins, and contains the remainder of the cytoplasmic domain including the region of desmoglein-unique repeats. It contains at its amino terminus part of a 19-amino acid sequence that is reported to be important for binding to another plaque protein, plakoglobin [13]. *E. coli* (strain TB1) carrying this construct were grown to mid-log phase, induced with 0.3 mM IPTG (isopropyl β-D-thiogalactopyranoside) for 2 h, and a soluble protein extract obtained by sonication and centrifugation.

2.2. Dsg1 purification and characterisation

The maltose binding protein-Dsg1 fusion protein present in the sonicate was purified by affinity chromatography on an amylose resin (manufacturer's standard protocol). Dsg1 was cleaved from the resin-bound maltose binding protein carrier by digestion with Factor Xa (1 μg enzyme/100 μg fusion protein) for 4 h at 22°C. This Dsg1 preparation (in 20 mM Tris-HCl, 100 mM NaCl) was concentrated to 0.1 mg/ml by centrifugal ultrafiltration (Centricon). Purity and identity of the preparation were checked by analysis of silver-stained 10% SDS-PAGE gels, and N-terminal amino-acid microsequencing respectively.

2.3. Electron microscopy

Samples were first examined by negative staining on thin carbon films prepared on freshly cleaved mica, using 1–4% sodium silicotungstate (pH 7.0) or 1% ammonium molybdate, pH 7.2. For rotary shadowing, aliquots (0.1 mg/ml.) were diluted with Tris-buffer (to 0.01 M) and glycerol (to 30%) and then sprayed onto the surface of a piece of freshly cleaved mica [14]. Samples were rotary shadowed with tungsten at an angle of 5° in a Leybold-Heraeus EPA100 vacuum coating unit equipped with a turbo-molecular pump. Following tungsten evaporation a thin carbon film was deposited onto the mica at an angle of 90°.

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The thickness of tungsten and carbon was measured with a Nanotech film thickness monitor (Nanotech). The mica sheet was scored into small squares before the replicas were floated onto the surface of distilled water and subsequently picked up onto 400 mesh copper/rhodium grids. Specimens were examined at 80 kV in a JEOL 1200EX electron microscope. The magnification was calibrated with catalase crystals.

3. Results

3.1. Characterisation of the Dsg1 cytoplasmic domain

SDS-PAGE analysis of the purified and cleaved Dsg1 cytoplasmic domain preparation revealed that greater than 95% of the sample consisted of a single M_r = 30,000 protein whose identity with Dsg1 was confirmed by N- terminal amino acid sequencing.

3.2. Electron microscopy

Rotary shadowing proved to be the most suitable method for examining the cytoplasmic domain of Dsg1. The basic shape and predominant form of the molecule was that of a globular head of diameter 9.2 ± 0.89 nm (mean ± S.D., n = 42) attached to a thin tail 23.2 ± 1.8 nm (n = 42) in length (Fig. 1A). Also present, but less frequently (perhaps amounting to 10% of the molecules observed), were tail–tail associations (Fig. 1B–D) as well as long tails (~130 nm in length) associated with only one head (Fig. 1E) as well as many heads bearing no obvious tail (Fig. 1A,B). Very long molecules were omitted from the estimation of mean dimensions. Unidirectional shadowing from an angle of 5° showed also that the head was approximately 10nm in diameter and of roughly spherical shape. The dimensions given also contain a contribution derived from tungsten/carbon evaporation and glycerol spraying and form a significant addition to the size of such a small structure. The amount of metal deposited was estimated to be about 2 nm, as judged from the use of a film thickness monitor. The dimensions given above for the molecule are therefore likely to be overestimates, the true average sizes being about 4 nm diameter for the head and 19 nm in length × 1 nm in width for the tail.

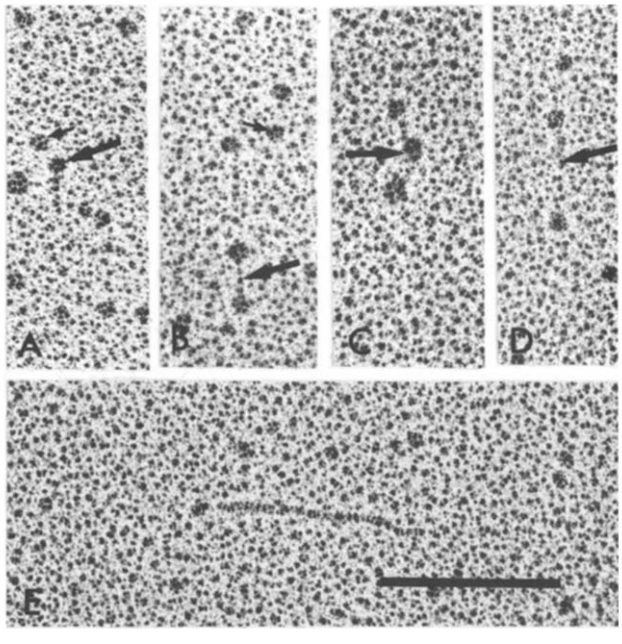

Fig. 1. Electron micrographs of rotary shadowed preparations of the unique part of the cytoplasmic domain of the desmoglein Dsg1, showing (A) a 'monomer' (large arrow) consisting of a globular head and thin tail and possible dimers (B,D, large arrows) and oligomers (C,E). Apparently free heads are shown (small arrows, A,B). Bar = 100 nm.

The shape of the molecule was similar after negative staining but the tail portion was not easy to discern (data not shown). None of these structures were visible when the pMal-c expression vector alone, without the Dsg1 insert, was induced.

4. Discussion

We have shown that the purified cytoplasmic domain of the desmosomal glycoprotein Dsg1 consists of a head portion of about 4 nm diameter and a thin tail some 19 × 1 nm. The presence of tail–tail structures (Fig. 1) suggests that the molecule may form dimers or possibly oligomers in solution.

Assuming that the head is spherical in shape, that the tail is a narrow cylinder, and that the protein has a partial specific volume of 0.73, a value characteristic of many proteins [15], the dimensions given would yield an approximate molecular weight of 39,000. Of this 39,000, some 27,000 would be contributed by the globular head and about 12,000 from the tail. This calculated approximate molecular weight is not grossly different from that predicted from the protein sequence, namely 29,367, and estimated by gel filtration. From the sequence data, the head would account for approximately 20,000 and the tail 10,000.

Although we have no data concerning secondary structure of the molecule, the shape and dimensions given (Fig. 1) are in good accord with the model described by Nilles et al. from structural considerations [16]. From Chou and Fasman analysis it is predicted that the unique part of the desmoglein molecule (residues 777–907, region CIII), consisting of five repeats of a 29 ± 4 residue sequence with a core consensus of N-V-V/I-V-T-E-R/S-V-I/V, would form an anti-parallel β-sheet [10,16]. In the model of Nilles et al. [16], this would form a head, shown to be approximately cylindrical and 8 nm in length and composed of four turns of beta-folded sheets. The tail is assumed to be about 16–19 nm in length and corresponds to the serine-glycine-rich portion of the sequence. We have no evidence for the presence of a washer-like structure in region CIVB (the final 35 residues of Dsg1). It is most unlikely that such a structure would be resolved by metal-shadowing. The dimensions reported by Nilles et al. [16] were reported to fit the dimensions of the plaque as measured in thin-section micrographs, with the head occupying the ~8 nm space between the outer and inner dense plaques.

Two other desmosomal proteins (isolated from bovine tissues) possess a globular head attached to a tail-like segment: desmoyokin (M_r = 640,000) [17] and desmoplakin I and II (predicted M_r = 310,000 and 238,000, respectively) [9] and ranging in length from about 70 to 200 nm. Another protein from the same source, desmocalmin (M_r = 240,000), consists of two polypeptide chains about 100 nm long lying together [18]. Whether these features of the molecules can be correlated with the laminated appearance of desmosomes in thin sections is not clear. It is, however, tempting to speculate whether the more electron-dense bands visible in sections might not correspond to the close-packing of the globular heads of the constituent protein molecules, resulting in an increase in the relative mass thickness (mass/unit area). The tail portion of Dsg1, which might be flexible in character, contains basic residues and might be

favourably located to bind to intermediate filaments [16]. Future studies may show the possible significance of dimers and oligomers in forming such associations. Although desmosomal plaques are regarded as insoluble, perhaps rigid structures, a limited degree of flexibility may exist within different regions of the plaque.

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