Volume 247, number 1, 13–16 FEB 06984 April 1989

The assembly of the major desmosome glycoproteins of Madin-Darby canine kidney cells

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Received 3 February 1989

Madin-Darby canine kidney (MDCK) cells are unable to form desmosomes when cultured in low-calcium medium ($[Ca^{2+}] < 0.1$ meq./l), but can be induced to do so by raising the calcium to physiological concentrations (1–2 meq./l). We have previously demonstrated that this block correlated with increased desmosomal protein turnover. Here we have immunoprecipitated the major desmosome glycoproteins [DGI (150 kDa) and DGII/III (120/100 kDa)] from non-ionic detergent-soluble and -insoluble fractions prepared from metabolically labelled MDCK cells cultured in standard or low-calcium medium. Pulse-chase studies showed that both DGI and DGII/III became unextractable in non-ionic detergent before their arrival at the cell surface, whether cells were grown in standard or low-calcium medium. The non-ionic detergent insolubility of these membrane components is therefore a separate step which precedes the formation of morphologically recognisable desmosomes.

Assembly; Desmosome; Glycoprotein; Ca^{2+}; (MDCK cell)

1. INTRODUCTION

The biochemical composition of the desmosome junction of bovine muzzle epidermis is now well elucidated [1,2]. However, little is known of how this multisubunit complex assembles to confer strong, stable adhesion and detergent insolubility. Using a combination of metabolic labelling of Madin-Darby canine kidney (MDCK) cells in culture and immunoprecipitation with antisera to bovine epidermal desmosomal proteins, we have previously studied the biosynthesis, and co- and post-translational modifications of the major desmosome glycoproteins [3]. DGI (150 kDa) is synthesized with the co-translational addition of 2–4 high-mannose cores which are processed to complex oligosaccharide chains within 30 min, causing a decrease in mobility on SDS-PAGE. DGII/III (120/100 kDa), as well as similar carbohydrate addition and processing, has been shown to undergo proteolytic processing to lose 10 kDa from each polypeptide, both within 60 min. DGI and DGII/III were found to be expressed at the cell surface (as determined by their sensitivity to trypsin), by 60 min for DGI and slightly later for DGII/III.

All epithelial cell types so far examined fail to form desmosomes when cultured in low-calcium medium (LCM; <0.1 meq./l Ca^{2+}), but can be induced to do so by raising the calcium concentration of the medium to physiological levels (SCM; 1–2 meq./l Ca^{2+}) [4–7]. We have shown that this inability to form desmosomes could not be correlated with any changes in the synthesis or processing of the major DPs or DGs, but rather with a greatly accelerated degradation of the DGs [8]. Here we have examined the assembly of DGI and DGII/III in MDCK cells, as well as DPI (250 kDa) and DPIII (83 kDa), by extraction of labelled cells with a physiological buffer containing non-ionic detergent before immunoprecipitation, thus monitoring their transition from a soluble to an insoluble fraction. Such treatment has been shown to leave a framework of nuclei, insoluble cytoplasmic IF network and junctional complexes [9]. Indeed, we found that the initially soluble DGI and

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

DGII/III became insoluble under these extraction conditions. We also show that cells cultured in LCM were able to assemble their DGs as in SCM, and with similar kinetics. Thus, insolubilization of the DGs can be uncoupled from formation of morphologically recognizable desmosomes.

2. MATERIALS AND METHODS

Conditions for passaging and experimentation of MDCK cells in SCM or LCM have been described elsewhere as have the properties of antisera to DGI and DGII/III [3,8]. Confluent monolayers of cells in 50-mm plastic dishes were preincubated for 30 min in methionine-free SCM or LCM prior to labelling with 0.5 mCi/ml [^{35}S]methionine (spec. act. 800 Ci/mmol, Amersham) for 10 min. Isotope was removed by two rapid washes with SCM or LCM and incubation continued at 1 mM methionine for the chase. After two rinses, cells were harvested directly into 1 ml ice-cold PBS by scraping with a disposable plastic pipette tip. Cells were recovered by centrifugation at 11500 × g_{max} for 1 min at 4°C, extracted twice with 100 μl RIP buffer [20 mM Tris-HCl, 0.15 M NaCl, 1 mM EDTA, 1% (w/v) NP-40, pH 7.5] for 10 min at 4°C and recentrifuged for 5 min to yield a supernatant (RIP-soluble) and pellet (RIP-insoluble). The RIP-soluble fraction was precipitated with 9 vols acetone (−20°C) for 1 h, centrifuged at 4°C for 5 min at 11500 × g and the pellet dried in a Savant Speed Vac concentrator; the insoluble fraction was also washed with acetone and dried. Both were then solubilized in sample buffer and processed for immunoprecipitation and fluorography as described [3,8]. A preclearing step was included by incubation of samples for 1 h with 20 μl of a 1:1 suspension of Sepharose 4B. All experiments were performed at least 3 times with similar results.

3. RESULTS

Detergent extraction of [^{35}S]methionine-labelled cells followed by immunoprecipitation was used to monitor the transition of newly synthesized DGs from soluble to insoluble form. Our antisera were found to recognize the DGs less efficiently after boiling in SDS as compared to solubilization in an NP-40-containing buffer (not shown) hence the RIP-soluble and RIP-insoluble fractions were treated in order to compensate for this (see section 2). However, even after taking this precaution, we found that the recovery of the two pools was not equivalent and therefore in figs 1 and 2 they could not be related quantitatively. We also cannot rule

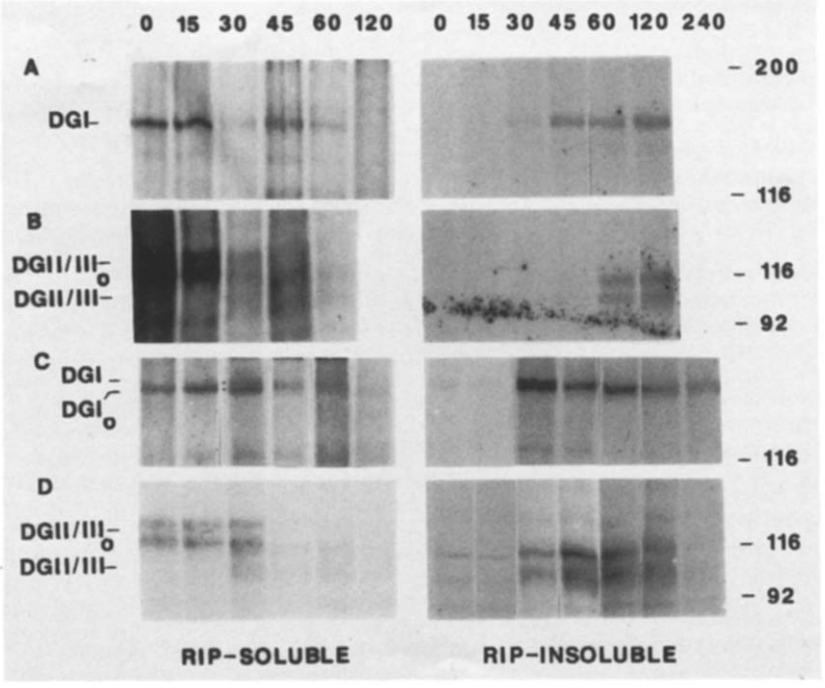

Fig.1. Assembly of DGI and DGII/III by MDCK cells grown in SCM (A,B) and LCM (C,D). MDCK cells were labelled for 10 min then chased for the times indicated (in min). RIP-soluble and -insoluble fractions were prepared and immunoprecipitated with anti-DGI (A,C) or anti-DGII/III (B,D) polyclonal antisera. The position of the markers was determined by staining the gel with Coomassie blue after fluorography. The subscript 'o' denotes the newly synthesized form of each DG.

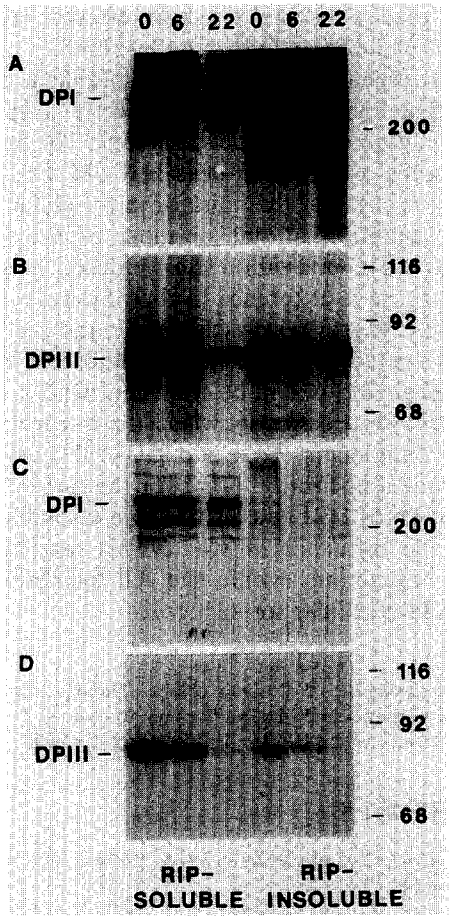

Fig.2. Assembly of DPI and DPIII by MDCK cells grown in SCM (A,B) and LCM (C,D). MDCK cells were pulse labelled for 10 min then chased for the times indicated (in h). RIP-soluble and -insoluble fractions were prepared and immunoprecipitated with anti-DPI (A,C) or anti-DPIII (B,D) polyclonal antisera.

out the unlikely possibility that endocytosed desmosomal remnants, residual structures from previous cell-cell contact in SCM, might persist and influence the assembly of DGs synthesized de novo in LCM.

From fig.1A it can be seen that all the DGI synthesized in a 10 min pulse was RIP-soluble in cells cultured in SCM. Only by 30 min was any DGI observed in the RIP-insoluble form. This time corresponded to a decreased level of DGI in the RIP-soluble form and after the 2 h chase period no RIP-soluble DGI was detected. Similarly, DGII/III synthesized in a 10 min pulse (fig.1B) was all RIP-soluble and was only just detectable in the RIP-insoluble fraction after a 45 min chase period. After a 1 h chase little RIP-soluble DGII/III remained. These data suggested the complete conversion of both DG families from a newly synthesized RIP-soluble state to an RIP-insoluble form; however, as the two pools could not be related to each other quantitatively we could not be sure that some loss of the RIP-soluble pool was not due to degradation.

The timing of the RIP-soluble to -insoluble transition suggests that the assembly of the DGs occurred after the carbohydrate/proteolytic processing (i.e. after movement through the Golgi apparatus) yet before arrival at the cell surface [3]. In agreement with this the newly synthesized form of DGII/III was found only in the RIP-soluble fraction whilst that in the RIP-insoluble form had all undergone proteolytic processing (fig.1B).

Similar assembly and timings were observed for cells cultured in LCM (fig.1C,D). However, while both DGI and DGII/III appeared in the RIP-insoluble fraction at 30 and 30–45 min respectively, the level declined over the 4 h chase period in contrast to the RIP-insoluble DGs synthesized in SCM which were stable (fig.1A,B). No doubt this was due to the rapid turnover exhibited by DGI and DGII/III in LCM ($t_{1/2}$ = 2.7 and 1.7 h, respectively) [8]. Fig.1C also showed that the carbohydrate processing of DGI was occurring on the RIP-soluble form as both newly synthesized and processed polypeptides were resolved in the 30 min lane. From these data, for MDCK cells cultured in SCM or LCM, we would predict that little of the DGs would exist in an RIP-soluble form at steady state.

The situation with DPI and DPIII was different; for MDCK cells grown in SCM (fig.2A,B), DPI and III, whether synthesized in a pulse or after 22 h chase time, were found in both fractions and the proportion was similar with increasing chase times, though unassembled DPIII appeared largely degraded after a 22 h chase period. As discussed above, it was not possible to determine accurately the relative amounts in each pool. For cells grown in LCM, DPIII (fig.2D) behaved similarly though the turnover appeared to be accelerated (cf. fig.2B and D). However, DPI synthesized by cells in LCM (fig.2C) appeared to be predominantly RIP-

soluble in a pulse label and after a 22 h chase period.

4. DISCUSSION

The present data show that DGI and DGII/III became non-ionic detergent-insoluble with different timings (30 and 45 min, respectively) in agreement with their known different rates of carbohydrate processing and transport to the cell surface. We cannot say whether the RIP-insoluble form represents a heteromeric complex of DGI and DGII/III together with other components. As the time between insolubilisation and appearance at the cell surface [3] was found to be similar for both families of DG this is possible. The proteolytic processing step [3] might be required to allow interaction of DGII/III with DGI. Both families of DG are still transported to the cell surface when cells are cultured in LCM [8,10]. Here we found that they are also insoluble in non-ionic detergent in contrast to the report by Mattey and Garrod [7] who found DGII/III to be extractable on the basis of immunofluorescence staining only.

Our data on DPI agree with those of Pasdar and Nelson [11], the only other biochemical study to date on desmosome assembly. They did not address the problem of antisera recognizing antigens differently after denaturation and estimated that 25–40% of DPI/II is soluble in SCM-grown cells whereas 70% is soluble in LCM-grown cells. We found only soluble DPI in cells grown in LCM, but the exact proportions may vary according to the precise extraction conditions used or the degree of confluency of the cells and time in culture under various conditions (SCM or LCM). The significance of the soluble pool of DPIII is not clear especially as DPIII is also a component of non-desmosome plaques [12]. However, the proportion did not appear to vary significantly according to whether cells were grown in LCM or SCM.

Morphological studies on cells grown in LCM have revealed punctate structures containing desmosomal antigens and associated with intermediate filaments. These have been interpreted as desmosome precursors moving to the cell surface as a package [5,13] or as remnants of old desmosomes [6,7,14]. Our data suggest that both major families of DG were capable of assembling into a 'predesmosome' structure whether cultured in SCM or LCM as judged by insolubility in non-ionic detergent. Clearly, the insolubility of the membrane components is not directly coupled to the formation of stable cell junctions. The block in desmosome assembly in cells in LCM would appear to be either at the point of intercellular adhesion or addition of one or more of the plaque proteins at DG nucleation sites. Indeed, there does appear to be a difference in the physical state of DPI in LCM grown cells (this paper and [11]). The data presented here together with those of Pasdar and Nelson [11] would be consistent with a model for desmosome assembly where the integral membrane components (DGI and DGII/III) assemble to provide a nucleation point for the fast effective association of DPI and DPIII from a soluble pool under the influence of Ca^{2+}. This may require lateral interactions within the cell membrane, as well as intercellular interactions.

Acknowledgements: We thank Claire Thomas for expert technical assistance and Marilyn Brennan for typing. This work was supported by the UK Medical Research Council.

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