FEBS Letters 355 (1994) 195–200

FEBS 14818

Distinct cadherin–catenin complexes in Ca^{2+}-dependent cell–cell adhesion

Stefan Butz, Rolf Kemler*

Max-Planck-Institut für Immunbiologie, D-79108 Freiburg, Germany

Received 22 September 1994

Abstract Catenins are peripheral cytoplasmic proteins originally identified in association with the mouse epithelial cell adhesion molecule E-cadherin. Molecular cloning and primary structure analysis demonstrated that α-catenin is homologous to vinculin and that β-catenin is homologous to human plakoglobin and the *Drosophila* gene product *armadillo*. With the use of peptide-specific anti plakoglobin antibodies we confirm here that plakoglobin is a component of the cadherin-catenin complex and that it is most likely identical to γ-catenin. We show that plakoglobin binds directly to E-cadherin. We consolidate the biochemical evidence for the existence of two distinct and separable E-cadherin-catenin complexes in the same cell. One complex is composed of E-cadherin, α- and β-catenin, the other of E-cadherin, α-catenin and plakoglobin. A similar distinct association with catenins is also found for other cadherins. Comparison of different cell lines revealed that the relative amounts of the two complexes vary depending on cell types.

Key words: Cell adhesion; Cadherin; Catenin; Plakoglobin

1. Introduction

Classical cadherins associate via their cytoplasmic domains with peripheral cytoplasmic proteins termed α-, β-, and γ-catenin, respectively 102, 88 and 80 kDa in size [1,2]. Several independent lines of evidence clearly point to the importance of catenins for cadherin-mediated cell adhesion (for review, see [3,4]). Molecular cloning and primary structure analysis of catenins revealed homologies to other peripheral cytoplasmic proteins. α-Catenin is homologous to vinculin [5,6], a protein localized in adherens junctions and in focal contacts. Biochemical analysis indicated that α-catenin does not bind directly to the cytoplasmic domain of cadherins [7,8], rather mediating the connection of the cadherin–catenin complex with actin filaments [9]. The importance of α-catenin for cadherin function has been demonstrated by transfection of the cDNA in α-catenin deficient cells, which restored the cadherin-mediated cell adhesion [10]. β-Catenin exhibits homology to human plakoglobin, a component of desmosomal plaques and adherens junctions [11,12], and to the product of the *Drosophila* segment polarity gene, *armadillo* [13,14]. Pulse–chase experiments and the analysis of different non-ionic detergent cell lysates indicated that β-catenin binds directly to E-cadherin [7]. The molecular identity of γ-catenin has remained less well understood. Recently it became evident that plakoglobin is a component of the cadherin–catenin complex and that γ-catenin is most likely identical to plakoglobin [15].

We have recently cloned the cDNAs encoding mouse β-catenin and plakoglobin and produced specific anti-peptide antibodies for each protein [16]. With the use of these antibodies we confirm that plakoglobin is a component of the cadherin–catenin complex and that it is most likely identical to γ-catenin. Plakoglobin, like β-catenin, binds directly to E-cadherin. Our results show that two separable E-cadherin–catenin complexes exist in the same cell, one composed of E-cadherin, α- and β-catenin, and the other of E-cadherin, α-catenin and plakoglobin. These results agree with recent data from another group [17,18] and provide further evidence for the existence of two distinct E-cadherin–catenin complexes in the same cell. We have also extended these studies to show variations in different cell types and implicate other cadherins.

2. Materials and methods

2.1. Cell culture

The following cell lines were used: A431, human epidermoid carcinoma cells (ATCC CRL 1555); Int407, human embryonic intestine cells (ATCC CCL 6); MDBK, bovine kidney cells (ATCC CCL 22); LLC-PK1, porcine kidney cells (ATCC CL 101); CMT, murine rectal carcinoma cells (ATCC CCL 223); CSG, salivary epithelial cells [19]; eEnd2, embryonic endothelioma cells [20] and PCC4aza1, embryonal carcinoma cells [21]. L1–1 cells are derived from Ltk^- cells transfected with E-cadherin cDNA [1]. All cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 15% FCS at 37°C in a 10% CO_2 atmosphere. For metabolic labelling experiments 2×10^7 cells were grown in methionine-free DMEM, supplemented with 10% dialysed FCS, for 2 h prior to the addition of 50 μCi/ml [^{35}S]methionine (Amersham) for overnight incubation.

2.2. Antibodies

Affinity-purified rabbit antibodies raised against the extracellular part of E-cadherin have been described [1]. Rabbit antisera against α-catenin, β-catenin and plakoglobin were produced against synthetic peptides. The peptides, the coupling by glutaraldehyde to keyhole limpet hemocyanin (Sigma) and the immunization procedure have been described for α-catenin [5] and β-catenin [16]. Anti-peptide antibodies recognizing the cognate sequence in plakoglobin were generated using resin-immobilized lysine-branched synthetic peptides. The peptide sequence DGLRPPYPTADHMLA (in plakoglobin this sequence is located at the carboxy-terminus) was synthesized on a polystyrene–polyoxyethylene graft co-polymer carrying a branched heptalysine [22]. All anti-peptide antibodies were affinity purified using the corresponding peptide–resin conjugates. Mouse monoclonal antibody 3.10 directed against desmoglein I was a kind gift of Dr. W.W. Franke (DKFZ, Heidelberg, Germany).

2.3. Preparation of lysates

Cells were washed three times with PBS at room temperature; all following steps were performed at 4°C. Cell extracts were obtained by applying cold lysis buffer (800 μl per 2×10^7 cells) for 10 min; lysis buffer: 20 mM imidazole, pH 6.8; 100 mM KCl; 2 mM $MgCl_2$; 10 mM EGTA; 300 mM sucrose; 1 mM Na-vanadate; 1 mM Na-molybdate; 1 mM Na-fluoride; 0.2% Triton X-100 and 1 mM PMSF. Lysates were centrifuged twice at $16,000 \times g$ for 5 min and then subjected to immunoprecipitation. In some experiments 0.2% Triton X-100 was

*Corresponding author. Fax: (49) 761-5108474

SSDI 0014-5793(94)01205-9

replaced by either 0.5% saponin, 0.5% digitonin, 0.02% lauryl dimethylamine oxide (LDAO), or 0.2% C_8E_4; or else 1% Triton X-100, 1 mM $CaCl_2$, 1 mM PMSF in PBS, pH 7.4, was used. In some experiments, α-catenin was removed from the cadherin–catenin complex by treating the lysate or the immunoprecipitate with 40 mM octylglucoside (OG) as described before [7]. To analyze detergent-insoluble material, pellets obtained from the first centrifugation were washed two times with lysis buffer, mixed with lysis buffer containing 10% betaine, and subsequently sonicated twice for 5 s each, at the highest level, with a probe sonicator (sonopuls, Bandelin, Berlin, Germany) with a probe of diameter 4 mm. After 20 min on ice, lysates were centrifuged at 16,000 × *g* for 5 min and supernatants were subjected to immunoprecipitations.

2.4. Immunoprecipitation and immunoblotting

Immunoprecipitations were carried out at 4°C. Cell lysates were precleared by incubation for 1 h with 200 μl/ml lysate of 10% (w/v) protein A-Sepharose beads (Pharmacia, Freiburg, Germany) preabsorbed with lysis buffer containing ovalbumin (1 mg/ml), then centrifuged at 1,000 × *g* for 5 min, followed by centrifugation of the supernatant at 16,000 × *g* for 5 min. Cell lysates equivalent to 4×10^6 cells were incubated with the appropriate antibody (5 μg anti-E-cadherin, 10 μg anti-α-catenin, 30 μg anti-β-catenin and 20 μg anti-plakoglobin) and 50 μl protein A-Sepharose for 1 h. Beads were washed 5 times with lysis buffer supplemented with ovalbumin. In sequential immunoprecipitations a further incubation with protein A-Sepharose was intercalated to remove traces of unbound antibodies before the next immunoprecipitation step was started. SDS-PAGE of the immunoprecipitates was performed under reducing conditions [23] and gels were fluorographed or immunoblotted. For fluorography, gels were fixed in 10% acetic acid, incubated with 1 M Na-salicylate and subsequently dried.

For immunodetection the proteins separated by SDS-PAGE were transferred electrophoretically to nitrocellulose. Filters were blocked with 5% powdered milk disolved in 10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% Tween 20, at 37°C for 1 h, and incubated with 2–5 μg/ml primary antibody and then with alkaline phosphatase-conjugated secondary antibody (Dianova, Hamburg, Germany). Bound antibodies were detected using Nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as substrates (NBT/BCIP) (Promega, Madison, USA).

3. Results

3.1. Two E-cadherin–catenin complexes

Affinity-purified anti-peptide antibodies against mouse plakoglobin and β-catenin were tested in immunoblots against lysates of various cell lines from mouse, human and several other mammalian species. The antibodies reacted each with a single protein, one of 88 kDa for β-catenin and one of 80 kDa for plakoglobin (not shown). When E-cadherin–catenin complexes immunoprecipitated with anti-α-catenin antibodies from metabolically labeled cell lysates of human epithelial A431 cells (Fig. 1, lane 2) were probed with anti-plakoglobin antibodies, a protein of 80 kDa was detected, with an electrophoretic mobility identical to γ-catenin (Fig. 1, lane 3). Immunoprecipitates collected with anti-E-cadherin antibodies gave identical results when subsequently stained with anti-plakoglobin on immunoblots (not shown). These results confirm that plakoglobin is a component of the cadherin–catenin complex and that plakoglobin is most likely identical to γ-catenin. Attempts to distinguish between γ-catenin and plakoglobin led only to detection of the 84 kDa proteolytic fragment of E-cadherin, which frequently arises in preparative analysis (H. Hoschützky and A. Scherer, unpublished results). To further elucidate the relationship between plakoglobin and the cadherin–catenin complex, immunoprecipitation experiments on cell lysates of metabolically labeled cells were performed with either anti-β-catenin or anti-plakoglobin antibodies. The relative amounts of β-catenin and plakoglobin in the immunoprecipitated complexes varied depending on whether anti-β-catenin or anti-plakoglobin antibodies were used for immunoprecipitations (Fig. 1, compare lanes 4 and 5). These results were an indication for two different E-cadherin–catenin complexes in a given cell, one composed of E-cadherin, α- and β-catenin, the other of E-cadherin, α-catenin and plakoglobin. Treatment of cell lysates or purified cadherin–catenin complexes with octylglucoside (40 mM) specifically removes α-catenin from the complex [7]. Such experiments were carried out to investigate the possible interaction of plakoglobin with the other components of the complex. As expected, the amount of α-catenin was greatly reduced in the complex when cell lysates treated with octylglucoside were immunoprecipitated with anti-β-catenin antibodies (Fig. 1, lane 6). Most remarkably, in analogous experiments performed with anti-plakoglobin antibodies, the complex immunoprecipitated was largely composed of E-cadherin and plakoglobin (Fig. 1, lane 7). These results indicate that plakoglobin, like β-catenin, binds directly to the cytoplasmic domain of E-cadherin.

Additional evidence for two E-cadherin–catenin complexes in the same cell was obtained by sequential immunoprecipitations: cell lysates were first depleted of one of the two complexes with either anti-β-catenin or anti-plakoglobin antibodies, and the second complex was subsequently immunoprecipitated with the other antibody. In either order, the second complex was clearly detectable in supernatants after depletion of the first (Fig. 2A,B). These results show that neither β-catenin nor plakoglobin was reduced in the complex due

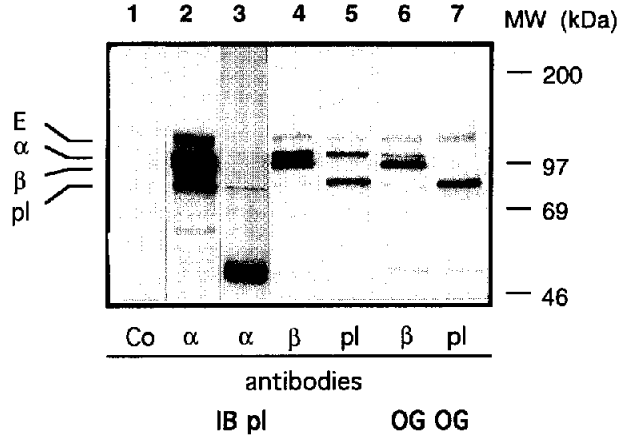

Fig. 1. Plakoglobin is a component of one of two distinct cadherin–catenin complexes and binds directly to E-cadherin. Cell lysates from metabolically labeled A431 cells solubilized in lysis buffer, 0.2% Triton X-100, were subjected to immunoprecipitation using affinity-purified antibodies specific for α- or β-catenin or plakoglobin. (Lane 1) Co, controls with no antibody. (Lane 2) Anti-α-catenin precipitates (like anti-E-cadherin, not shown) the previously known complex composed of E-cadherin and catenins. Blotting of anti-α-catenin immunoprecipitates and subsequent staining with anti-plakoglobin revealed that plakoglobin is a component of this complex (lane 3; IB pl, immunoblot with anti-plakoglobin antibodies). Immunoprecipitates collected with anti-β-catenin (lane 4) or anti-plakoglobin (lane 5) differed in the molecular composition of the cadherin–catenin complex in that either β-catenin or plakoglobin was greatly reduced or absent in the two alternative immunoprecipitates. Cell lysates treated with 40 mM octylglucoside (OG) and immunoprecipitated with anti-β-catenin (lane 6) and anti-plakoglobin (lane 7) had α-catenin specifically removed from the complex, thus demonstrating that plakoglobin binds directly to E-cadherin (lane 7). The positions of E-cadherin (E), α-catenin (α), β-catenin (β) and plakoglobin (pl) are indicated on the left.

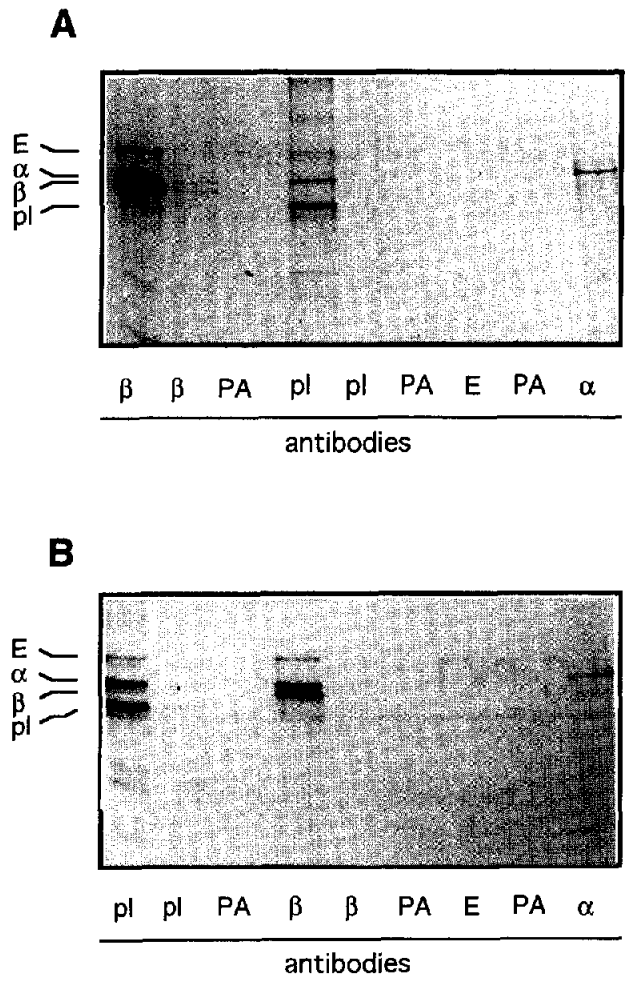

Fig. 2. Two distinct E-cadherin–catenin complexes in the same cell are separable by sequential immunoprecipitations. These were performed with anti-β-catenin and anti-plakoglobin antibodies on cell lysates of metabolically labeled A431 cells. In panel A, cell lysates were first depleted with anti-β-catenin (β) and unbound material was subsequently immunoprecipitated with anti-plakoglobin (pl); in panel B the order was reversed: first depletion with anti-plakoglobin (pl) followed by anti-β-catenin (β). An intermediate step with protein A-Sepharose (PA) was intercalated to remove residual antibodies. After depletion of cell lysates with both anti-β-catenin and anti-plakoglobin, no E-cadherin (E) was detected in the remaining supernatant by immunoprecipitation, but free α-catenin (α) was present.

to interference by the bound anti-plakoglobin or anti-β-catenin antibodies. Additional immunoprecipitations with anti-E-cadherin antibodies revealed no free E-cadherin, but from the depleted supernatants free α-catenin was detectable with anti-α-catenin antibodies (Fig. 2A,B). This finding verifies earlier observations pointing to a pool of free α-catenin in a cell.

The two distinct cadherin–catenin complexes were found reproducibly, independent of cell culture or cell lysis conditions, including the following experimental parameters: (i) growth support (polystyrene cell culture dishes or porous polycarbonate membrane inserts); (ii) confluency of cell cultures (semiconfluent, 2 days confluent, or 5 days confluent); (iii) lysis buffer (see section 2); (iv) detergent used for lysis (see section 2); and (v) time period of lysis (between 5 and 30 min) (data not shown).

3.2. The relative amounts of the two cadherin–catenin complexes vary depending on the cell type

Human Int 407, porcine LLC-PK1, and murine CSG and PCC4aza1 cells were metabolically labeled with [^{35}S]methionine, and cell lysates were immunoprecipitated with anti-α-, anti-β-catenin or anti-plakoglobin antibodies to investigate whether the results obtained with A431, human epidermoid carcinoma cells, could be extended to other cell types (Fig. 3). Two E-cadherin–catenin complexes were also clearly detected in PCC4aza1 embryonal carcinoma cells when anti-β-catenin or anti-plakoglobin antibodies were used for immunoprecipitations (Fig. 3, PCC4; β-catenin migrates as a doublet in these cells, [24]). Similar results were obtained with the other cell lines; in LLC-PK1 cells (or eEnd2 cells, not shown), more than one cadherin is expressed (Fig. 3, LLC-PK1). Also an additional cadherin, with an electrophoretic mobility distinct from E-cadherin, is complexed either with α- and β-catenin or with α-catenin and plakoglobin. The identity of the cadherins of Int 407 and LLC-PK1 cells were not further determined, but the protein bands were immunoreactive with an anti-pan-cadherin antibody in immunoblots (not shown) and therefore marked with 'Cad' for not determined cadherin.

The relative abundance of the two complexes seems to vary depending on the cell type. While in A431 cells (see Figs. 1 and 2) the two complexes appeared in roughly similar amounts, only a small amount of the complex was immunoprecipitated with anti-β-catenin antibodies in Int 407 cells (Fig. 3, Int 407) or with anti-plakoglobin antibodies in CSG and LLC-PK1 cells (Fig. 3, CSG, LLC-PK1). The plakoglobin-containing complex was even less prevalent in CMT murine rectal carcinoma cells and existed only in trace amounts in L1-1 cells (data not shown). In all these experiments a constant amount of cell lysate was immunoprecipitated with an excess of affinity-purified antibodies; thus these results clearly indicate a different relative distribution of the two complexes in different cell types.

3.3. Analysis of cadherin–catenin complexes derived from the Triton X-100-insoluble fraction

Cadherin–catenin complexes partition variably into the detergent-soluble and -insoluble cytolskeletal fractions, depending on the cell type [25]. Therefore, we compared the complexes immunoprecipitated from the detergent-soluble and -insoluble fraction by anti-β-catenin and anti-plakoglobin antibodies (Fig. 4). To make the Triton X-100-insoluble fraction accessible to immunoprecipitation, we sonified this material in the presence of lysis buffer containing 10% (w/v) betaine to release cadherin–catenin complexes without disrupting them. Hereby we avoided steps, like cross-linking of proteins prior to cell lysis, which might possibly alter the affiliation of the complexes to defined fractions. The β-catenin and plakoglobin containing complexes immunoprecipitated in the detergent-soluble and -insoluble fractions with the respective antibodies are shown in Fig. 4 (lanes 1–4). On long-time exposed fluorographs, residual β-catenin or plakoglobin was detected in the reciprocal complexes, so the separation of β-catenin and plakoglobin in two distinct complexes was apparently not complete. This phenomenon was more pronounced in immunoprecipitations from the detergent-insoluble fraction (Fig. 4, lanes 1–4). These findings most likely reflect co-immunoprecipitation of a minor subpopulation of 'higher order complexes' including both cadherin–catenin complexes.

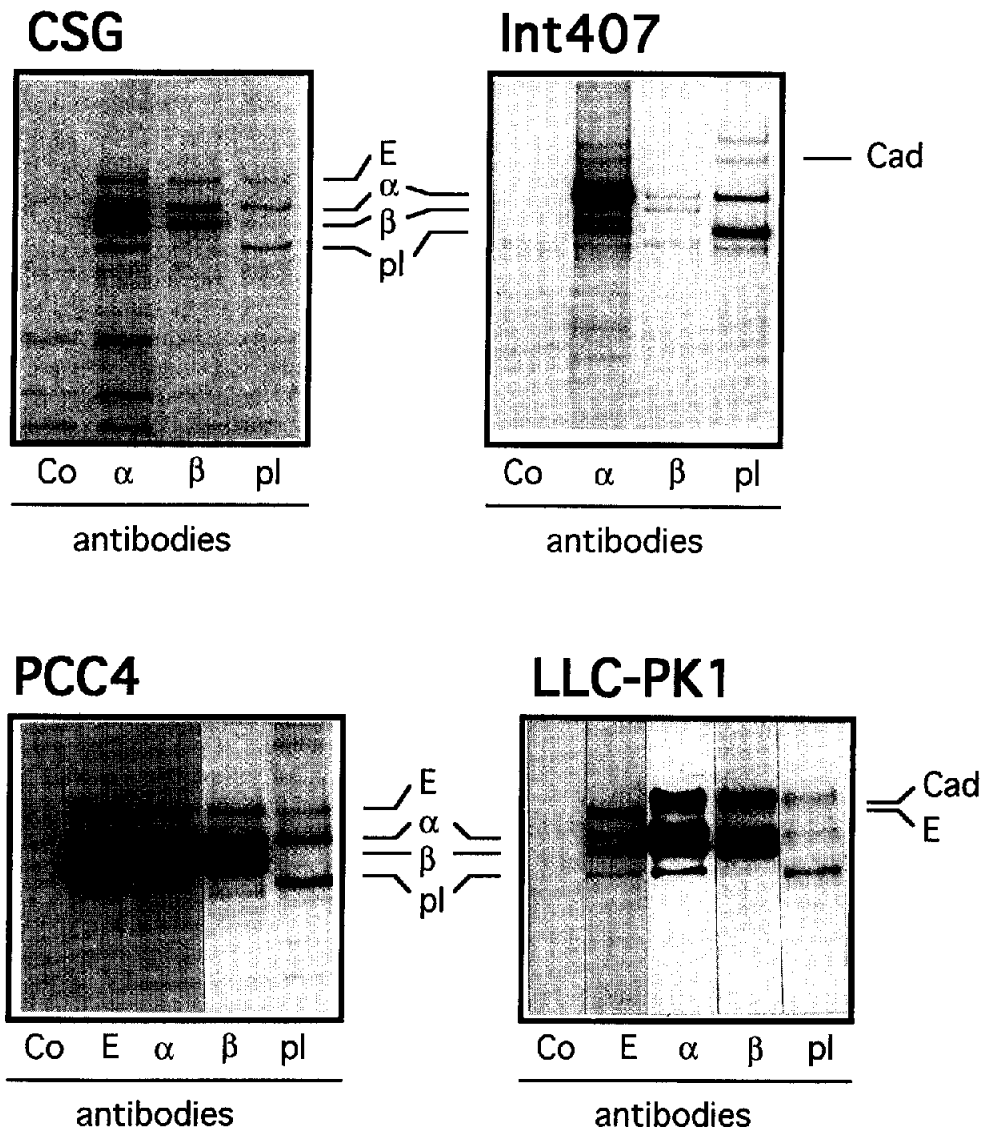

Fig. 3. Two complexes distinguished by antibodies against β-catenin and plakoglobin were detected in different cell lines. Cell lysates of metabolically labeled CSG, Int 407, PCC4aza1 and LLC-PK1 cells were immunoprecipitated with antibodies as indicated. The relative amounts of the two complexes vary depending on cell lines (compare PCC4, CSG and Int 407). In LLC-PK1 cells another cadherin was detected. Cad, 'not further determined' cadherin; E, E-cadherin; α,β,pl, α- and β-catenin, and plakoglobin; Co, controls omitting antibodies. The Cad protein bands were immunoreactive with an anti-pan-cadherin antibody in immunoblots (not shown).

Complexes immunoprecipitated by anti-plakoglobin antibodies contained additional proteins, including the cadherin desmoglein I. When the anti-plakoglobin immunoprecipitates shown in Fig. 4, lanes 2 and 4, were blotted and stained with anti-desmoglein I antibodies, desmoglein I was particularly enriched in the immunoprecipitates from the Triton X100-insoluble, cytoskeletal fraction (Fig. 4, lanes 5 and 6). Association of desmoglein I with plakoglobin was also seen in immunoprecipitates from cell lysates of MDBK cells with anti-desmoglein I antibodies (Fig. 4, lane 7), confirming earlier observations [14,26]. These data indicate that the anti-plakoglobin immunoprecipitates derived from the detergent-soluble or -insoluble fraction are composed of two distinct cadherin–catenin complexes, with plakoglobin associated either with E-cadherin or with desmoglein I. Other co-immunoprecipitated proteins have not yet been identified. A promising candidate for the protein band at 45 kDa in the immunoprecipitates of the detergent-insoluble fraction (Fig. 4, lanes 3 and 4), is actin, but this band also appeared in control experiments without antibodies (not shown).

4. Discussion

Catenins were originally identified complexed to E-cadherin in immunoprecipitations with anti-E-cadherin antibodies [1,2]. More recently the primary structures of α- and β-catenin have been determined [5,6,16,27]. We confirm here that plakoglobin is most likely identical to γ-catenin. In our earlier work the position of γ-catenin in the complex was less clear, since the relative amount in the complex varied from cell type to cell type and since γ-catenin was not consistently detected in all experiments [28]. Mainly for these reasons γ-catenin was placed at the

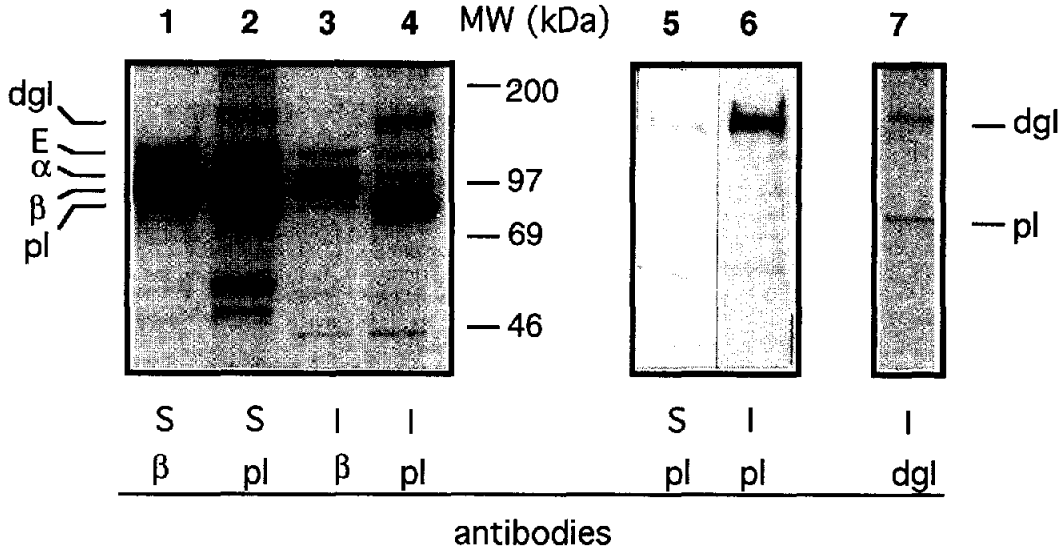

Fig. 4. Partitioning of the two cadherin–catenin complexes in the Triton X-100-soluble and -insoluble fractions. The detergent-insoluble fraction of metabolically labeled A431 cells was partially solubilized by sonicating the pellet in lysis buffer containing 10% (w/v) betaine, and subsequent immunoprecipitations were compared with those obtained from the detergent-soluble fractions (lanes 1–4). Complexes detected with anti-β-catenin or anti-plakoglobin antibodies separated into the detergent-soluble (S) and -insoluble (I) fractions. Anti-plakoglobin immunoprecipitates included additional proteins, suggesting a variety of protein associations, one of which is an association of plakoglobin with desmoglein I, as indicated by immunoblots of the immunoprecipitates shown in lanes 2 and 4 with anti-desmoglein I antibodies (lanes 5 and 6). An association of plakoglobin with desmoglein I was also found in immunoprecipitates from cell lysates of metabolically labeled MDBK cells with monoclonal antibodies to anti-desmoglein I (lane 7). S,I, Triton X-100-soluble and -insoluble fractions; dgl, desmoglein I; E, E-cadherin; α,β,pl, α-, β-catenin, and plakoglobin.

periphery of the cadherin–catenin complex. With the use of specific anti-plakoglobin antibodies we add important new information to our knowledge about the cadherin–catenin complex. Our results indicate that plakoglobin (γ-catenin) binds, as does β-catenin, directly to the cytoplasmic domain of E-cadherin. Because of the high degree of homology between β-catenin and plakoglobin in the central part of the proteins, this interaction might be mediated by a motif common to both proteins.

We report here that two different E-cadherin–catenin complexes co-exist in the same cell, one composed of E-cadherin, α- and β-catenin, and the other of E-cadherin, α-catenin and plakoglobin. This finding is in full agreement with results recently published by W.J. Nelson's group [17,18]. More importantly, we provide additional evidence for the co-existence of two distinct cadherin–catenin complexes in the same cell. Sequential immunoprecipitations demonstrated that the two complexes are not obtained artifactually by affecting the molecular composition of a supposed single cadherin–catenin complex due to interference by the bound anti-β-catenin or anti-plakoglobin antibodies. We have used different cell lysis conditions, e.g. several detergents known to exhibit different solubilization properties, but have always obtained two distinct and separate complexes. This, and our observations that the relative amounts of the two complexes vary from cell type to cell type, very likely indicate that the solubilization procedure does not account for the results obtained. We therefore favor the possibility that the two cadherin–catenin complexes reflect a differential association in which either β-catenin or plakoglobin is bound to the cytoplasmic domain of a given cadherin in a mutually exclusive fashion. The variation of the relative amounts of the two complexes in different cell types may well turn out to have an important biological function. It remains to be investigated whether cadherin-mediated cell–cell adhesion is indeed achieved independently of the type of complex that predominates in a cell. Besides E-cadherin, other cadherins, most likely N- or P-cadherin [15], can be also found in the two complexes. In the immunoprecipitations with anti-plakoglobin antibodies additional independent complexes are apparently detected, representing interaction of plakoglobin with desmoglein I and/or other as yet unknown proteins. Recently, other proteins have been found in association with catenins. The product of a tumor suppressor gene, adenomatous polyposis coli (APC), forms a complex with α-catenin and β-catenin [29,30]. The epidermal growth factor (EGF) receptor associates with the cadherin–catenin complex after EGF stimulation of cells, and this interaction appears to be mediated by β-catenin [31]. Possible consequences of such a differential association of catenins with cadherins or other proteins in the same cell might be that these proteins can participate differentially in the construction of sub-cellular compartments, be differentially connected to the cytoskeletal network, and/or be differentially involved in signal transduction pathways.

Acknowledgements: We are grateful to Dr. Heinz Hoschützky for his valuable advice on how to make the Triton X-100-insoluble fraction accessible to immunoprecipitation experiments. We thank Lore Lay for preparing the photographs, Rosemary Schneider for typing, Dr. Otmar Huber, Dr. Lionel Larue, Dr. Heinz Hoschützky and Dr. Randy Cassada for critically reading the manuscript.

References

[1] Ozawa, M., Baribault, H. and Kemler, R. (1989) EMBO J. 8, 1711–1717.
[2] Nagafuchi, A. and Takeichi, M. (1989) Cell Regul. 1, 37–44.
[3] Takeichi, M. (1991) Science 251, 1451–1455.
[4] Kemler, R. (1993) Trends Genet. 9, 317–321.

[5] Herrenknecht, K., Ozawa, M., Eckerskorn, C., Lottspeich, F., Lenter, M. and Kemler, R. (1991) Proc. Natl. Acad. Sci. USA 88, 9156–9160.
[6] Nagafuchi, A., Takeichi, M. and Tsukita, S. (1991) Cell 65, 849–857.
[7] Ozawa, M. and Kemler, R. (1992) J. Cell Biol. 116, 989–996.
[8] Aberle, H., Butz, S., Stappert, J., Weissig, H., Kemler, R. and Hoschützky, H., in press.
[9] Ozawa, M., Ringwald, M. and Kemler, R. (1990) Proc. Natl. Acad. Sci. USA 87, 4246–4250.
[10] Hirano, S., Kimoto, N., Shimoyama, Y., Hirohashi, S. and Takeichi, M. (1992) Cell 70, 293–301.
[11] Cowin, P., Kapprell, H.-P., Franke, W.W., Tamkun, J. and Hynes, R.O. (1986) Cell 46, 1063–1073.
[12] Franke, W.W., Goldschmidt, M.D., Zimbelmann, R., Mueller, H.M., Sehiller, D.L. and Cowin, P. (1989) Biochemistry 86, 4027–4031.
[13] Riggleman, B., Schedl, P. and Wieschaus, E. (1990) Cell 63, 549–560.
[14] Peifer, M., McCrea, P.D., Green, K.J., Wieschaus, E. and Gumbiner, B.M. (1992) J. Cell Biol. 118, 681–691.
[15] Knudsen, K.A. and Wheelock, M.J. (1992) J. Cell Biol. 118, 671–679.
[16] Butz, S., Stappert, J., Weissig, H. and Kemler, R. (1992) Science 257, 1142–1144.
[17] Hinck, L., Näthke, I.S., Papkoff, J. and Nelson, W.J. (1994) J. Cell Biol. 125, 1327–1340.
[18] Näthke, I.S., Hinck, L., Swedlow, J.R., Papkoff, J. and Nelson, W.J. (1994) J. Cell Biol. 125, 1341–1352.
[19] Knowles, M.A. and Franks, L.M. (1977) Cancer Res. 37, 3917–3927.
[20] Williams, R.L., Courtneidge, S.A. and Wagner, E.F. (1988) Cell 52, 121–131.
[21] Nicolas, J.F., Avner, P., Gaillard, J., Guenet, J.L., Jakob, H. and Jacob, F. (1976) Cancer Res. 36, 4224–4231.
[22] Butz, S., Rawer, S., Rapp, W. and Birsner, U. (1994) Pept. Res. 7, 20–23.
[23] Laemmli, U.K. (1970) Nature 227, 680–685.
[24] Peyrieras, N., Louvard, D. and Jacob, F. (1985) Proc. Natl. Acad. Sci. USA 82, 8067–8071.
[25] Shore, E.M. and Nelson, W.J. (1991) J. Biol. Chem. 266, 19672–19680.
[26] Korman, N.J., Russell, W.E., Eyre, M.D., Klaus-Kovtun, V. and Stanley, J.R. (1989) N. Eng. J. Med. 321, 631–635.
[27] McCrea, P.D., Turck, C.W. and Gumbiner, B. (1991) Science 254, 1359–1361.
[28] Ozawa, M. and Kemler, R. (1990) J . Cell Biol. 111, 1645–1650.
[29] Rubinfeld, B., Souza, B., Albert, I., Müller, O., Chamberlain, S.H., Masiarz, F.R., Munemitsu, S. and Polakis, P. (1993) Science 1731–1734.
[30] Su, L-K., Vogelstein, B. and Kinzler, K.W. (1993) Science 1734–1737.
[31] Hoschützky, H., Aberle, H. and Kemler, R., in press.