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Cadherin cell adhesion molecules with distinct binding specificities share a common structure

Yasuaki Shirayoshi, Kohei Hatta, Masaya Hosoda, Susumu Tsunasawa[1], Fumio Sakiyama[1] and Masatoshi Takeichi

Department of Biophysics, Faculty of Science, Kyoto University, Kyoto 606 and [1]Institute for Protein Research, Osaka University, Suita 565, Japan

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Ca^{2+}-dependent cell–cell adhesion molecules, termed cadherins, are divided into subclasses with distinct tissue distributions and distinct cell-binding specificities. To elucidate the biochemical relationship of these subclasses, we compared the pattern of tryptic cleavage and the partial amino acid sequence of mouse liver E-cadherin with those of chicken brain N-cadherin. Although these two cadherins are distinct in their cell-binding and immunological specificities, they showed an identical mol. wt and a similar tryptic cleavage pattern. We isolated tryptic fragments of E- and N-cadherin, and determined the sequences of nine amino acid residues of their amino terminus. The results showed that sequences of amino acids from the amino terminus to the 7th residues are identical in these two cadherins. We thus suggest that cadherins with distinct specificities have a common genic origin.

Key words: adhesive specificity/amino acid sequence/cadherins/cell–cell adhesion

Introduction

The mechanisms of selective cell adhesion are important for understanding the molecular basis of animal morphogenesis. Recent studies suggest that Ca^{2+}-dependent cell–cell adhesion molecules, termed cadherins, play a critical role in selective cell adhesion (Takeichi *et al.*, 1985b). Cadherin activities are detected in most kinds of mammalian and avian tissues, but they are divided into subclasses, such as E type (Yoshida-Noro *et al.*, 1984), N type (Hatta *et al.*, 1985; Hatta and Takeichi, 1986) and P type (A.Nose and M.Takeichi, in preparation), with distinct tissue distribution patterns. The E type of cadherin (E-cadherin) whose mol. wt is 124 kd, also called uvomorulin (Peyrieras *et al.*, 1983) or cell-CAM 120/80 (Damsky *et al.*, 1983), was originally identified in mouse teratocarcinoma cells (Yoshida-Noro *et al.*, 1984) and was found to be distributed exclusively in epithelial cells of various tissues such as liver (Ogou *et al.*, 1983; Hatta *et al.*, 1985). The chicken equivalent of E-cadherin was termed L-CAM (Gallin *et al.*, 1983). The N type of cadherin (N-cadherin) was originally identified in mouse and chicken brain (Hatta *et al.*, 1985; Hatta and Takeichi, 1986), and its mol. wt was found to be 127 kd. The tissue distribution of N-cadherin is distinct from that of E-cadherin; these two cadherins tend to show complementary, mutually exclusive distribution patterns in embryos (Hatta *et al.*, 1985; Hatta and Takeichi, 1986).

A most interesting property of cadherin subclasses is their specificities in cell–cell binding. For example, cells expressing E-cadherin do not cross-adhere with cells expressing N-cadherin in *in vitro* experiments (Takeichi *et al.*, 1985b). We also observed that cells expressing N-cadherin segregate from cells expressing E-cadherin during normal morphogenetic processes such as gastrulation and neural tube formation (Hatta and Takeichi, 1986). These observations prompted us to elucidate the biochemical basis of the binding specificity of heterotypic cadherins. In the present study, we isolated E-cadherin from mouse liver and N-cadherin from chicken brain, compared their patterns of trypsin sensitivity and determined their amino-terminal amino acid sequences. The results show that these two cadherins display a common tryptic cleavage pattern and possess an identical amino acid sequence extending from the amino terminus to the seventh amino acid residue. We thus suggest that cadherin subclasses with distinct specificities have a common genic background, constituting a 'cadherin family'.

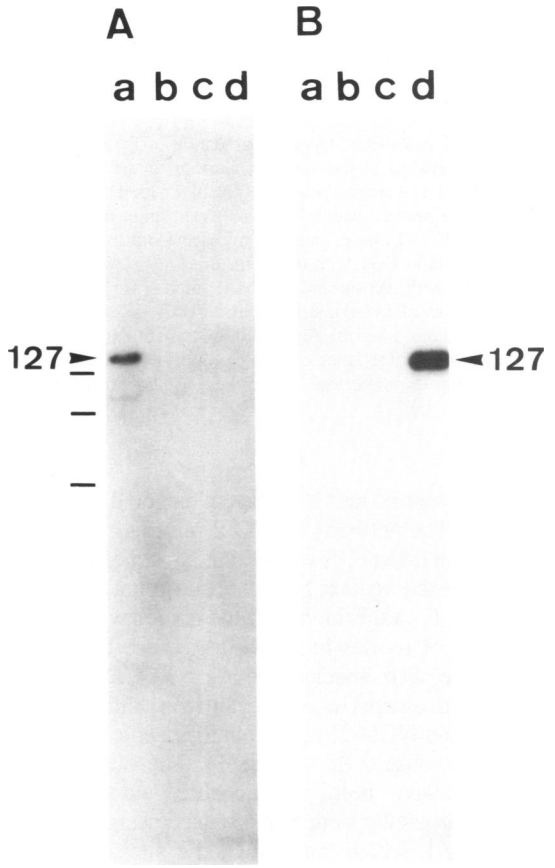

Fig. 1. Immunoblot analysis of the cross-reactivity of the antibody ECCD-2 to mouse E-cadherin and the antibody NCD-2 to chicken N-cadherin. **(A)** Reaction with ECCD-2. **(B)** Reaction with NCD-2. **Lane a**, mouse liver; **lane b**, mouse brain; **lane c**, chicken liver; **lane d**, chicken brain. These tissues were directly dissolved in SDS sample buffer as antigen sources. Positions of three mol. wt markers, β-galactosidase (mol. wt 116 kd), phosphorylase b (mol. wt 94 kd) and bovine serum albumin (mol. wt 68 kd), are shown by bars.

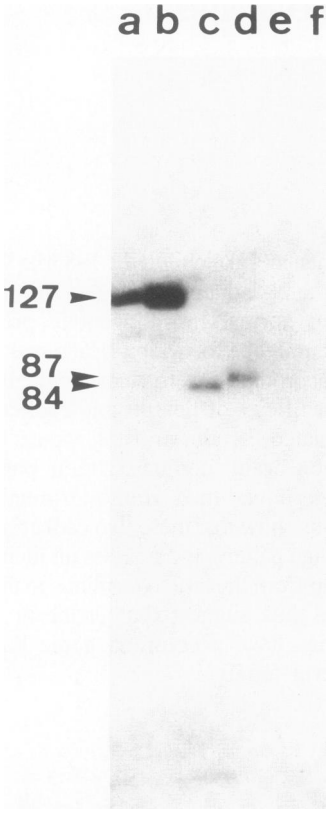

Fig. 2. Immunoblot analysis of tryptic degradation of E- and N-cadherins. **Lane a**, intact E-cadherin of mouse liver. **Lane b**, intact N-cadherin of chicken brain. **Lane c**, material released from liver membranes treated with trypsin and Ca^{2+}. **Lane d**, material released from brain membranes treated with trypsin and Ca^{2+}. **Lane e**, material released from liver membranes treated with trypsin and EGTA. **Lane f**, material released from brain membranes treated with trypsin and EGTA. Samples were prepared as follows: Intact tissues of liver or brain were directly dissolved in SDS sample buffer for lanes a and b. An aliquot of trypsin extracts was mixed with SDS sample buffer for lanes c – f. The mol. wts of bands were determined by using the same mol. wt markers as shown in Figure 1.

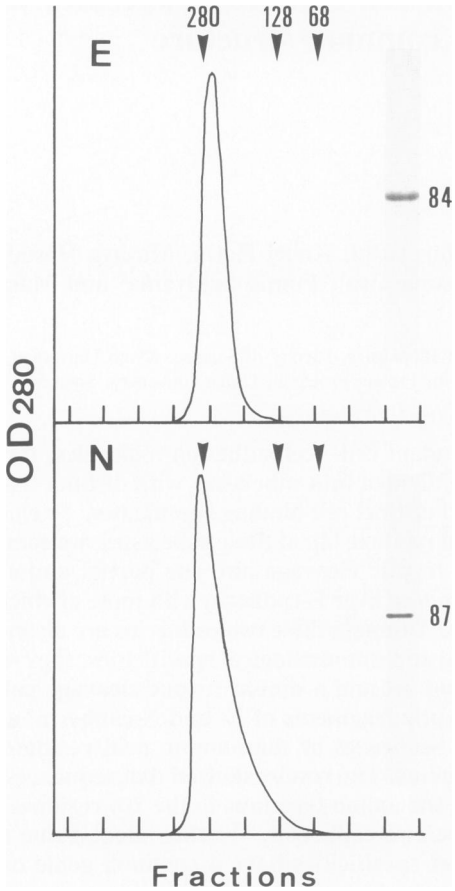

Fig. 3. The patterns of gel filtration of the 84 kd E-cadherin fragment **(a)** and the 87 kd N-cadherin fragment **(b)**. Samples which had been purified by a TSK G300SW gel filtration were again fractionated by the same gel system. Inserts are the Coomassie blue stain of gels after SDS – PAGE of the peak fractions. Mol. wt markers were glutamate dehydrogenase, 280 kd; glucose-6-phosphate dehydrogenase, 128 kd; and serum albumin, 68 kd.

Results

Figure 1 shows that E- and N-cadherin can be distinguished immunologically. The antibody ECCD-2 raised against E-cadherin did not react with brain cells expressing N-cadherin, and the antibody NCD-2 raised against N-cadherin did not react with liver cells expressing E-cadherin. The same results were obtained using other pairs of tissues in a species. These two monoclonal antibodies were also species specific; ECCD-2, which was originally raised against a mouse antigen, did not react with chicken cells, and NCD-2, raised against a chicken antigen, did not react with mouse cells (Figure 1). These results indicated that cadherins have both type-specific and species-specific epitopes. Similar results were also obtained using the monoclonal antibody ECCD-1, which can block active sites of mouse E-cadherin (Yoshida-Noro *et al.*, 1984), and the monoclonal antibody NCD-1 against mouse N-cadherin (Hatta *et al.*, 1985).

The immunoblot analysis also showed that E-cadherin of mouse liver migrates on SDS gels to the same position as N-cadherin of chicken brain whose mol. wt is 127 kd (see Figure 2). This suggests that the liver E-cadherin is slightly larger than the teratocarcinoma E-cadherin whose mol. wt was determined to be 124 kd. This was confirmed by comparing the mol. wt of E-cadherin obtained from these two sources on a single gel (data not shown).

E- and N-cadherin were similar not only in molecular size but also in their pattern of trypsin sensitivity. Previous studies (Yoshida and Takeichi, 1982; Hatta *et al.*, 1985) showed that both of these cadherins can be degraded by treatment of cells with trypsin in the absence of Ca^{2+}, but that Ca^{2+} protected them from proteolysis so that they remain in an intact form on the cell surface after trypsin treatment in the presence of Ca^{2+}.

A different pattern of degradation was observed when membrane fractions of cells were subjected to trypsin treatment. When a membrane fraction from liver was treated with trypsin in the presence of Ca^{2+}, E-cadherin was released from the membranes as a fragment of mol. wt 84 kd as determined by immunoblot analysis (Figure 2). This result was consistent with those obtained by Hyafil *et al.* (1980), Damsky *et al.* (1983), and Cunningham *et al.* (1984). When the membrane fraction was treated with trypsin in the absence of Ca^{2+}, the 84 kd fragment was further degraded. We found a strikingly similar degradation pattern with N-cadherin. When a membrane fraction from chicken brain was treated with trypsin in the presence of Ca^{2+}, a fragment of this molecule of mol. wt 87 kd was produced (Figure 2). This fragment was further degraded by treatment with trypsin in the absence of Ca^{2+}. Thus, these two cadherins appear to have very

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E-cadherin:   N-Asp Trp Val Ile Pro Pro Ile Val Val
N-cadherin:   N-Asp Trp Val Ile Pro Pro Ile Asn Leu
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Fig. 4. Amino acid sequences of the amino terminus of the 84 kd E-cadherin and the 87 kd N-cadherin fragments.

similar but not identical structures in terms of their patterns of susceptibility to trypsin and Ca^{2+}.

We purified the 84 kd fragment of E-cadherin and the 87 kd fragment of N-cadherin. Interestingly, the estimated mol. wt of each of these cadherin fragments, if globular, was found to be about three times higher (~280 kd) by gel filtration analysis than by SDS–PAGE (Figure 3), suggesting either that cadherin fragments exist in polymeric form, possibly as a trimer, or that they are highly asymmetric in their native stage. This result again suggested that E- and N-cadherins have similar structures.

We then determined the sequence of nine amino acid residues from the amino terminus of these fragments of E- and N-cadherins. Figure 4 shows that primary sequences from the amino terminus to the 7th residue were completely identical in these two cadherins. These sequences were also identical at the three positions determined at the amino terminus of chicken L-CAM reported by Cunningham *et al.* (1984).

Discussion

These results provided the first evidence supporting the hypothesis that cadherin subclasses with distinct tissue distribution and distinct binding specificities may arise from a common origin (Takeichi *et al.*, 1985a); the different cadherins may be encoded by a family of related genes. E-cadherin and N-cadherin resemble each other not only in mol. wt and pattern of tryptic sensitivity but also in amino acid sequence. This common amino acid sequence should have a key function in the cadherins, since it is conserved in cadherin subclasses, even those derived from different animal species. The present result also supports our previous assumption that L-CAM is the chicken equivalent of E-cadherin.

Although E- and N-cadherins thus seem to have similar structures, they were distinguished by immunological and binding specificities. Northern blot analysis (Gallin *et al.*, 1985) showed that a cDNA clone complementary to L-CAM mRNA hybridizes only with mRNA obtained from cells expressing this adhesion molecule. Therefore, each type of cadherin must have regions of unique amino acid sequence relevant to its specificity. Active sites of cadherins probably also have such type-specific sequences, since the antibody ECCD-1, which can block the action of E-cadherin and recognizes a peptide moiety (Shirayoshi *et al.*, 1986) selectively reacts with E-cadherin but not other cadherins. Vestweber and Kemler (1985) recently identified a binding domain of uvomorulin/E-cadherin, facilitating further analyses of the structure of the active sites of this molecule.

Taken together, these data indicate that cadherins may be a family of molecules provided with both common and heterologous structures. We suppose that other unidentified types of cadherins are present in animal tissues, since the monoclonal antibodies to cadherins available at present do not react with all cells with cadherin activity. Further analysis of the structures of these cadherin subclasses should lead to an understanding of the molecular origin of the specificity of cell–cell adhesion, which is probably the most important regulatory factor for animal morphogenesis.

Materials and methods

Monoclonal antibodies and tissues

The rat monoclonal antibody ECCD-2 against E-cadherin of mouse liver was obtained as described elsewhere (Shirayoshi *et al.*, 1986). The rat monoclonal antibody NCD-2 against N-cadherin of chicken brain has been described previously (Hatta and Takeichi, 1986).

Liver was obtained from newborn ICR mice, and brain was obtained from newly hatched White Leghorn chick.

Trypsin treatment of membrane fractions

Mouse liver or chick brain were homogenized in 10 mM Tris–HCl buffer (pH 8.0) containing 2 mM $CaCl_2$ and protease inhibitors (1 μg/ml leupeptin, 1 μg/ml pepstatin A, 1 μg/ml antipain, 2 mM phenylmethylsulfonylfluoride) by a motor-driven Potter homogenizer in an ice bath. The homogenates were centrifuged at 16 000 *g* for 60 min. The resultant pellets, defined as the membrane fraction, were re-suspended and washed twice in 10 mM Hepes-buffered (pH 7.4) saline containing 2 mM $CaCl_2$ (HBS). For trypsin treatment in the presence of Ca^{2+}, the pellets were incubated with 0.01% trypsin in HBS for 60 min at 37°C. After centrifugation at 16 000 *g* for 60 min, the supernatant solution was passed through a benzamidine–Sepharose 6B column (Pharmacia) to remove trypsin. For trypsin treatment in the absence of Ca^{2+}, the samples were prepared by the same procedure as above, except that $CaCl_2$ in the trypsin solution was replaced by 1 mM EGTA. For electrophoretical analysis of these trypsin extracts, an aliquot was mixed with 2% SDS sample buffer (Yoshida and Takeichi, 1982) and subjected to SDS–PAGE.

Purification of tryptic fragments of cadherins

Membrane fractions of liver or brain were treated with trypsin in the presence of Ca^{2+} as described above. The trypsin extract of liver membranes was loaded onto an affinity column (Affigel 10, Bio-Rad) conjugated with ECCD-2 antibodies. After extensive washing with HBS supplemented with 1 M NaCl, bound materials were eluted from the column with 50 mM diethylamine (pH 11.5) containing 1 mM $CaCl_2$, and concentrated by an Amicon YM10 membrane filter. The sample was then chromatographed on a TSK G300SW gel filtration column using a Gilson h.p.l.c. system, and the peak containing the E-cadherin fragments was identified by immunoblot analysis using ECCD-2. When necessary, these steps were repeated to remove contaminating materials. The trypsin extract of brain membranes was processed in the same way as the liver extract, except that an affinity column conjugated with NCD-2 antibodies was used, and the peak containing the 87 kd fragment of N-cadherin was determined using NCD-2. The peak fractions contained pure 84 or 87 kd fragments of each cadherin, as judged by SDS–polyacrylamide gel electrophoresis (see Figure 3). Generally 1 μg of pure cadherin fragments were obtained from ~2 g of liver or 3 g of brain.

Amino acid sequencing

Cadherin fragments purified were dialyzed against 0.01% SDS in 5 mM NH_4HCO_3 and subjected to analysis by a gas-phase amino acid sequencer (Applied Biosystems, model 470A) as described (Hirado *et al.*, 1985). Identical sequences were obtained in duplicate sequencing runs.

Electrophoresis and immunoblot analysis

The SDS–PAGE was performed using 7.5% polyacrylamide as described (Yoshida-Noro *et al.*, 1984). All samples were dissolved in 2% SDS sample buffer and reduced by boiling with 5% 2-mercaptoethanol before electrophoresis. The immunoblot analysis was carried out as described previously (Hatta and Takeichi, 1986), except that nitrocellulose sheets with protein blots were pre-coated with 5% skim milk (Difco) instead of serum albumin to minimize non-specific antibody binding.

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