N-cadherin detected in the membrane fraction of lens fiber cells

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Summary. N-cadherin was identified as a glycoprotein present in the fiber cell membranes of frog, chick, bovine, rabbit and human lenses. The molecular size of N-cadherin varies with the species. Homogenization of the chick lens in the presence of Ca^{2+} resulted in a decrease in the concentration of N-cadherin. This suggests that the lens contains a Ca^{2+}-activated protease which can act on N-cadherin.
Key words. Lens; membranes, N-cadherin; glycoproteins.

N-cadherin is a Ca^{2+}-dependent cell adhesion molecule which appears to play an important role in the selective adhesion of cells at adherens junctions[1]. It has been identified in embryonic chick lenses[2] and is possibly identical to the adherens junction-specific cell adhesion molecule (A-CAM) which was detected in the plasma membranes of cultured lens epithelial cells and isolated fiber cell membranes[3]. In this study, we report on the identification of N-cadherin as a glycoprotein of lens membranes from different vertebrate species.

Materials and methods

Lenses were dissected from frog (*Rana pipiens*), chicken (*Gallus domesticus*), rabbit (*Oryctolagus cuniculus*) and bovine (*Bos taurus*) eyes immediately after death. Human lenses were obtained from the Michigan Eye Bank (Ann Arbor, MI). Membranes were prepared from fiber cells and solubilized in sodium dodecyl sulphate (SDS) as previously described[4]. Electrophoresis in polyacrylamide gels (PAGE) was done according to Laemmli[5]. After SDS-PAGE, protein was transferred to nitrocellulose paper, and glycoproteins detected with biotinylated concanavalin-A (Con-A)[4]. Immunological reactions were also developed on the nitrocellulose paper using rabbit polyclonal antibodies prepared in this laboratory against a chick lens 130 kDa glycoprotein[4], and polyclonal antibodies to chick neural retina N-cadherin[6] obtained from Dr J. Lilien (University of Wisconsin).

Results and discussion

Figure 1 shows the Coomassie Blue-stained gel pattern of the chicken, frog, rabbit, bovine and human lens fiber cell membranes (A–E) and the corresponding immunoblot (F–J) and glycoprotein staining pattern (K–O). A distinct single immunoreactive band was detected in each membrane preparation using the antiserum to the chick lens 130 kDa glycoprotein. Identical results were obtained with the antiserum to retina N-cadherin. Furthermore, the immunoreactive bands fitted precisely into the more complex pattern demonstrated by the Con-A reaction for each species. The position of the reactive bands varied slightly in molecular weight as judged by the migration in the gel. The chicken glycoprotein showed the lowest molecular weight (130 kDa), while the mammalian protein had the highest molecular size (140 kDa). The frog protein was of intermediate size. A comparison of the position of immunoreactive bands using the antiserum to the chick lens 130 kDa glycoprotein and the antiserum to chick neural retina N-cadherin is shown in figure 2. The bands are identical in position for each

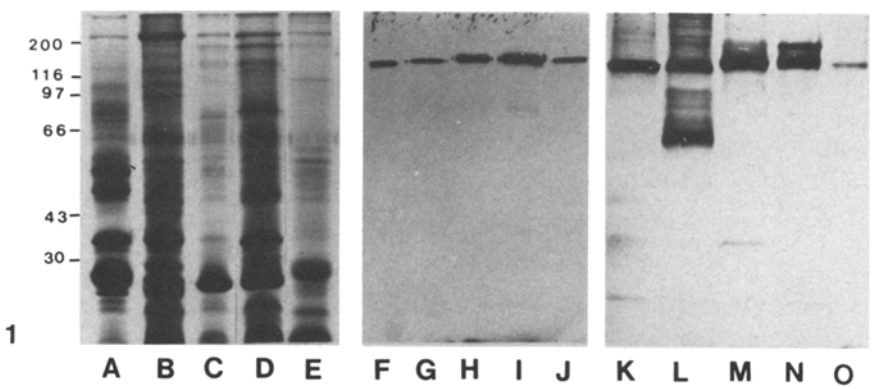

Figure 1. Coomassie Blue-stained SDS-PAGE pattern of chicken, frog, rabbit, bovine and human lens membranes respectively (A–E), the corresponding immunblot (F–J) and the Con-A pattern (K–O). The antiserum used was prepared in rabbits against the chick lens 130 kDa protein[4]. The numbers indicate molecular weight markers in kilodaltons.

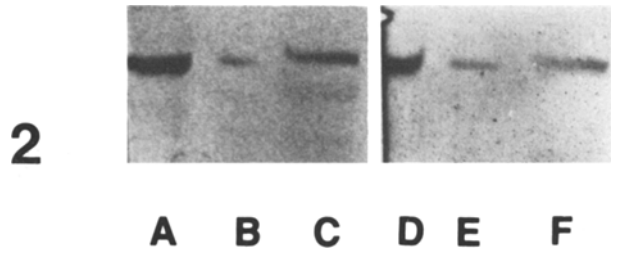

Figure 2. Immunoblot reactions with the antiserum to lens 130 kDa protein (A–C) and with the antiserum to chick neural retina N-cadherin (D–F). Lanes A and D contained lens fiber cell membrane protein from chicken; lanes B and E from human, and lanes C and F from bovine.

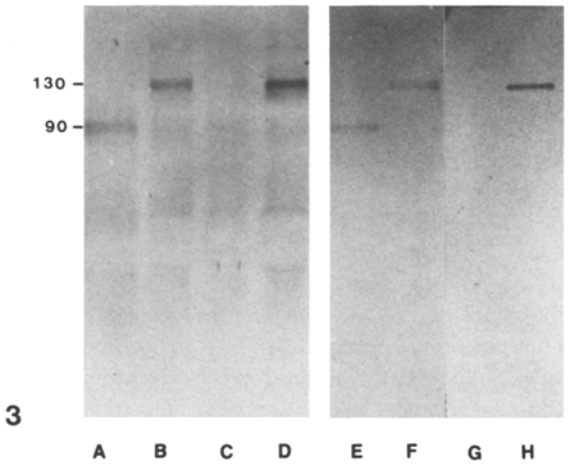

Figure 3. Con-A reaction (A–D) and corresponding immunoblot (E–H) of chick lens membranes. The antiserum against the chick lens 130 kDa protein was used for the immunoblot.
Lanes A and E: membranes prepared in the presence of Ca^{2+} and then treated with trypsin.
Lanes B and F: membranes prepared in Ca^{2+}.
Lanes C and G: membranes prepared in the presence of EGTA and then treated with trypsin.
Lanes D and H: membranes prepared in EGTA.

antiserum. Additionally, the difference in electrophoretic mobility between the chick, human and bovine proteins is more clearly evident. The apparent molecular weights were calculated as 130 kDa for the chicken, 135 kDa for the human and 140 kDa for the bovine protein. The identical results obtained with the two antisera indicated that the lens antiserum was also specific for N-cadherin. This was confirmed by the fact that the lens antiserum reacted specifically with chick neural retina N-cadherin after two-dimensional electrophoresis (Dr. Lilien, personal communication).

The effect of Ca^{2+} on lens N-cadherin was tested by homogenization of chick lenses in 2 mM Tris-HCl buffer pH 7.4, containing 2 mM Ca^{2+} or 2 mM EGTA. After isolation of the crude membrane by centrifugation at 37,000 × g for 20 min, the insoluble membrane-rich material was resuspended in the original buffer. An aliquot of each was treated with trypsin (100 µg/ml) and incubated for 10 min at 37 °C. Samples were then subjected to SDS-PAGE, and the protein transferred to nitrocellulose for Con-A and immunological analyses. Figure 3 demonstrates that homogenization of the lens in Ca^{2+} alone resulted in a decrease in the concentration of the 130 kDa protein as reflected by the intensity of the Con-A staining and immunoreactivity, when compared with the EGTA treated lens membranes. Two faint immunoreactive bands were observed at the 130 kDa position in the Ca^{2+} treated membranes (lane F). The band of slightly lower molecular weight probably represents a partially degraded form of the native 130 kDa protein. The addition of trypsin at the concentration used resulted in the complete digestion of the 130 kDa glycoprotein in the EGTA treated membranes. No degradation products of the 130 kDa protein were detected either by the antibody or by Con-A. However, Ca^{2+} plus trypsin treatment yielded a major fragment of about 90 kDa.

The results of this study show that N-cadherin is present in the membranes of frog, chick, bovine, rabbit and human fiber cell membranes. Furthermore, this molecule varies somewhat in molecular size in different species. The reason for the difference in molecular size, whether due to the amount of carbohydrate or to a true difference in protein size remains to be determined. The broad species cross-reactivity of the antisera provides a useful tool to study regional and age-related changes in the membrane composition of lens cells that normally differentiate into fiber cells and are retained throughout life. Such membranes are known to contain adherens junctions [7]. The antisera can also be used to investigate the effects of induced cataractogenesis on lens cell membranes in animal models, and of the age-related cataracts in man.

Although it is known that Ca^{2+} protects N-cadherin against degradation when trypsin is added to cells in culture [2], this was not the case when lens cells were homogenized in the presence of Ca^{2+}. Only partial protection was afforded under these conditions. Homogenization in Ca^{2+} alone resulted in a decrease in concentration of the glycoprotein when compared to membranes prepared in the presence of EGTA. This suggests that a Ca^{2+}-activated protease endogenously present in the lens acts on the 130 kDa glycoprotein. The addition of trypsin probably degrades this enzyme so that N-cadherin is not further broken down beyond a 90 kDa peptide.

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