Interactions of the Neural Cell Adhesion Molecule and the Myelin-associated Glycoprotein with Collagen Type I: Involvement in Fibrillogenesis

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Abstract. To gain insights into the functional role of the molecular association between neural adhesion molecules and extracellular matrix constituents, soluble forms of the myelin-associated glycoprotein (MAG) and the neural cell adhesion molecule (N-CAM), representing most of the extracellular domains of the molecules, were investigated in their ability to modify fibrillogenesis of collagen type I. MAG and N-CAM retarded the rate of fibril formation, as measured by changes in turbidity, and increased the diameter of the fibrils formed, but did not change the banding pattern when compared to collagen type I in the absence of adhesion molecules. Scatchard plot analysis of the binding of MAG and N-CAM to the fibril-forming collagen types I, II, III, and V suggest one binding site for N-CAM and two binding sites for MAG. Binding of MAG, but not of N-CAM, to collagen type I was decreased during fibril formation, probably due to a reduced accessibility of one binding site for MAG during fibrillogenesis. These results indicate that the neural adhesion molecules can influence the configuration of extracellular matrix constituents, thus, implicating them in the modulation of cell–substrate interactions.

CELLS can adhere to each other or to the extracellular matrix (ECM)[1] they are in contact with. Cell surface molecules involved in such interactions are termed adhesion molecules and distinctions have been made between those mediating cell–cell interactions (cell adhesion molecules [CAMs]) and those involved in cell–substrate interaction (substrate adhesion molecules) (for reviews see Takeichi, 1988; Edelman, 1986). While it was originally thought that CAMs are exclusively membrane-associated glycoproteins, there is now increasing evidence for the appearance of soluble and ECM-associated forms of these molecules (for references see Lander, 1989). Two CAMs, the myelin-associated glycoprotein (MAG) and the neural cell adhesion molecule (N-CAM), were initially described as integral membrane glycoproteins but increasing evidence suggests that they may also be functional as part of the ECM.

MAG is a constituent of central and peripheral myelin (for review see Quarles, 1983) and is involved in neuron-oligodendrocyte and oligodendrocyte-oligodendrocyte adhesion (Poltorak et al., 1987; Sadoul et al., 1990). In addition to its cell surface localization, MAG has been found associated with Schwann cell basal laminae and interstitial collagens (Martini and Schachner, 1986, 1988). Specific interactions between MAG and the ECM constituents heparin and different collagen types could indeed be shown in binding studies (Fahrig et al., 1987). A soluble form of the molecule presumably containing most of its extracellular part is generated by an endogenous protease present in myelin (Sato et al., 1984). The physiological role of this protease has, however, remained obscure. N-CAM is expressed by a wide variety of different cell types predominantly of neural and mesenchymal origin (Thiery et al., 1982; Chuong and Edelman, 1984; Crossin et al., 1985; Keilhauer et al., 1985; Rieger et al., 1985; Covault and Sanes, 1986; Mirsky et al., 1986; Seilheimer and Schachner, 1987; Thor et al., 1987; Klein et al., 1988) and is involved in cell adhesion for which homophilic and heterophilic binding mechanisms have been suggested (for review see Rutishauser and Jessell, 1988). N-CAM from mouse is expressed in three major forms with apparent molecular masses of 180, 140, and 120kD (N-CAM 180, N-CAM 140, N-CAM 120; Gennarini et al., 1986), which are derived from four to five different mRNA species (Gennarini et al., 1986; Cunningham et al., 1987; Barbas et al., 1988). N-CAM 180 and N-CAM 140 are integral membrane glycoproteins (Gennarini et al., 1984; Murray et al., 1986), whereas N-CAM 120 is inserted into the plasma membrane via a phosphatidylinositol anchor (He et al., 1986; Sadoul et al., 1986). Soluble forms of N-CAM have

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1. *Abbreviations used in this paper*: CAM, cell adhesion molecule; ECM, extracellular matrix; MAG, myelin-associated glycoprotein; N-CAM, neural cell adhesion molecule.

been detected in different body fluids (Ibsen et al., 1983; Bock, 1987) and in culture medium of neural and muscle cells in vitro (Cole et al., 1985; Cole and Glaser, 1986; He et al., 1987). Furthermore, N-CAM immunoreactivity has been observed in the ECM of peripheral nerves (Martini and Schachner 1986, 1988; Rieger et al., 1985, 1988) and surrounding blood vessels (Gulbenkian et al., 1989). Recently, an N-CAM–specific mRNA coding for a secreted form of N-CAM has been identified (Gower et al., 1988). First hints as to the functional relevance of soluble N-CAM have been obtained from binding studies demonstrating an association of N-CAM with glycosaminoglycans and different types of collagen (Cole and Glaser, 1986; Nybroe et al., 1989; Probstmeier et al., 1989).

Since structure and composition of the ECM changes during development and in regeneration (see, for example, Fleischmajer et al., 1983; Salonen et al., 1987) and cell behavior is influenced by the structural composition of the ECM (see, for example, Tucker and Erickson, 1984), we decided to obtain further insights into the functional roles of the soluble forms of MAG and N-CAM. We have isolated the NH_2-terminal proteolytic fragments of MAG and N-CAM with apparent molecular masses of 90 kD (MAG 90) and 110 kD (N-CAM 110), respectively, from adult mouse brain (Fahrig et al., 1987; Sadoul et al., 1988; Probstmeier et al., 1989) to investigate their influence on the formation of collagen type I fibrils in vitro.

Materials and Methods

Purification of Soluble Forms of N-CAM and MAG

Soluble forms of MAG and N-CAM were purified from adult mouse brains from several inbred strains as described previously (Fahrig et al., 1987; Sadoul et al., 1988; Probstmeier et al., 1989). These forms with apparent molecular masses of 90 kD for MAG (MAG 90) and 110 kD for N-CAM (N-CAM 110) are proteolytic degradation products of the membrane-bound forms since they are released by an incubation of a crude membrane fraction for 2 h at 37°C. For preparation, 50 g of brain tissue were homogenized in 400 ml 1 mM $NaHCO_3$, 0.2 mM $CaCl_2$, 0.2 mM $MgCl_2$, 1 mM spermidin, pH 7.9, at 4°C and centrifuged for 15 min at 60 *g*. The supernatant was then centrifuged for 45 min at 25,000 *g* and the resulting pellet homogenized in 250 ml RPMI containing 10 mM Hepes. The suspension was incubated for 2 h at 37°C and centrifuged for 1 h at 100,000 *g*. The supernatant was applied, in sequence, to Sepharose 4B columns to which mAbs to N-CAM (Hirn et al., 1983) and MAG (Poltorak et al., 1987) had been covalently coupled. Columns were then washed with 10-column volumes of 20 mM Tris-HCl, 400 mM NaCl, 1 mM EDTA, 1 mM EGTA, 0.5% Triton X-100, pH 7.4, followed by 30-column volumes of 20 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, pH 7.4. Antigens were eluted with 2-column volumes of 100 mM diethylamine, 1 mM EDTA, 1 mM EGTA, pH 11.5. Eluates were immediately neutralized, concentrated in Amicon chambers by pressure dialysis, dialyzed against PBS, pH 7.4, and stored at −70°C.

The NH_2-terminal amino acid sequences of MAG 90 and N-CAM 110 are identical to those of the membrane-bound forms previously published (Rougon and Marshak, 1986; Arquint et al., 1987; Barthels et al., 1987), as determined by amino acid sequence analysis. The COOH termini of MAG 90 and N-CAM 110 are located close to the membrane-spanning regions of the molecules: MAG 90 is recognized by an antiserum raised against a synthetic peptide corresponding to a sequence between the fourth and fifth Ig domain, N-CAM 110 is recognized by an antiserum to the fibronectin-like domains of the molecule.

Soluble MAG and N-CAM preparations were free of contaminating glycosaminoglycans or proteoglycans as measured by the Alcian blue (Krueger and Schwartz, 1983) and Safranin O staining methods (Lammi and Tammi, 1988), or by ELISA and Western blots using a commercially available antibody directed against chondroitin sulfate (code No. C8035; Sigma Chemical Co., St. Louis, MO).

ECM Components

Collagen type I (collagen R) was obtained from Serva (Heidelberg). Collagen type II was prepared from chick cartilage (von der Mark et al., 1982). Collagen type III was prepared from fetal calf skin (Timpl et al., 1981). Collagen type IV with intact NC1 domain was extracted from the EHS mouse tumor (Kleinman et al., 1982). Collagen type V was purified from human placenta (Bentz et al., 1978). Chondroitin-4-sulfate was obtained from Serva and heparin (sodium salt, grade I; from porcine intestinal mucosa, code No. H 3125) from Sigma Chemical Co.

Analytical Procedures

For quantitation of collagen concentrations, a modified microbiuret method (Goa, 1953) was applied using collagen type I (collagen R from Serva, Heidelberg, FRG) as a standard. All other protein determinations were carried out according to Bradford (1976). SDS-PAGE was carried out according to Laemmli (1970) using 7% slab gels. Protein bands were visualized by the silver method of Merril et al. (1982).

Fibrillogenesis Assay and Determination of Fibril Diameter

Fibrillogenesis of collagen type I was measured turbidimetrically by monitoring the increase in absorbance of the collagen solution at 315 nm (Gross and Kirk, 1958). For the assay, 75 μl collagen solution (2.0 mg/ml in 0.1% acetic acid) were mixed with 10 μl ten times concentrated PBS and 15 μl of normal PBS. This solution was added to 900 μl PBS (with or without additives), mixed again, and immediately poured into a cuvette placed in a jacketed flow cell maintained at 30°C in a spectrophotometer. The pH of the final solution was in the range of 7.0. Before mixing, all solutions used were warmed up to 30°C. The absorbance was monitored every 2.5 min. To study the influence of MAG 90, heparin, chondroitin sulfate, or BSA on fibrillogenesis, different amounts of each molecule were diluted with PBS shortly before mixing with the collagen solution. N-CAM 110 was not included since it could not be obtained in amounts large enough for this assay.

To investigate the effects of MAG 90 or N-CAM 110 on fibril structure, 15 μg of collagen type I were mixed with 9 μg MAG 90 or 11 μg N-CAM 110 in a total volume of 100 μl PBS. This mixture was incubated for 2–3 h at 30°C, a time period sufficient for completion of fibril formation. A drop of each sample was then placed on a carbon film supported on a 300-mesh copper grid for 2 min. The grid was drained slowly with filter paper and floated inverted on a large volume of a freshly prepared aqueous solution of 1% sodium phosphotungstate, pH 7.4, for 5 min. The grid was then drained and air-dried. The preparation was examined with a Philips 400 electron microscope at 80 kV and micrographs were taken at a nominal magnification of 12,500. Usually, two identically prepared grids were examined and fibril-diameter determined from electron micrographs.

Radioiodination of MAG and N-CAM

Immunoaffinity-purified MAG 90 and N-CAM 110 were iodinated using Bolton-Hunter reagent (from Amersham; Bolton and Hunter, 1973) as described (Fahrig et al., 1987; Probstmeier et al., 1989). Briefly, proteins were dialyzed against PBS, pH 8.2, concentrated to a final volume of 200 μl, added to dried Bolton-Hunter reagent, and incubated for 30 min at room temperature. 50 μl 1 M glycine, pH 8.2, were added and incubated for 10 min at room temperature. The iodinated protein was extensively dialyzed against PBS. Specific activities were determined by TCA precipitation and ranged between 1×10^6 and 5×10^6 cpm/μg protein.

Binding Assays Utilizing Radioiodinated Proteins

Solid phase radioligand binding assay was carried out as described (Fahrig et al., 1987; Probstmeier et al., 1989). Briefly, individual wells of microtest III™ flexible assay plates were incubated overnight at 4°C with collagen types I to V at concentrations of 100 μg/ml in 0.1 M $NaHCO_3$, pH 8.2, washed and blocked with 0.2% BSA in 0.1 M $NaHCO_3$, pH 8.2, for 1 h at room temperature. Wells were then washed with PBS, containing 0.2% BSA (binding buffer), incubated with ^{125}I-labeled N-CAM 110 or ^{125}I-labeled MAG 90 (70 μl, diluted in binding buffer) for 5 h at room temperature and again washed with binding buffer. Bound radioactivity was determined by cutting out the bottoms of the plastic wells and placing them directly into vials for counting.

To determine the binding efficiencies of N-CAM and MAG during colla-

gen fibril formation 20 μl ^{125}I-labeled N-CAM 110 (50 nM) or ^{125}I-labeled MAG 90 (100 nM) were added to 1 ml collagen solution (150 μg/ml collagen type I in PBS) in Eppendorf tubes at different time points and incubated for 2 h at room temperature. In parallel, fibrillogenesis was monitored turbidimetrically (see above). Collagen fibrils were collected by centrifugation (5 min at 10,000 *g*), washed once with PBS, and recentrifuged. Fibril-bound radioactivity was determined by cutting off the tip of the Eppendorf tube and placing it directly into a counting vial.

Results

Purification of Soluble Forms of MAG and N-CAM

The molecular forms of MAG and N-CAM soluble in detergent-free solutions were isolated by immunoaffinity chromatography from supernatants of an adult mouse brain membrane fraction after incubation for 2 h at 37°C in cell culture medium. Both molecules were stable in detergent-free buffers. By SDS-PAGE, soluble MAG migrated as a broad smear with an apparent molecular mass of 90 kD (MAG 90, Fig. 1 *a*) and soluble N-CAM as a band of 110 kD (N-CAM 110, Fig. 1 *b*). In comparison, when these molecules were purified from detergent lysates of the mouse brain membrane fraction and analyzed by SDS-PAGE, MAG consisted of a broad smear at 100 kD (Fig. 1 *c*) and N-CAM consisted of the three bands at 180, 140, and 120 kD (Fig. 1 *d*). Determination of the NH_2-terminal amino acid sequence of MAG 90 and N-CAM 110 revealed the same NH_2-terminal amino acid sequence as the membrane-bound forms.

Collagen Fibrillogenesis

Since both MAG and N-CAM were found associated with collagen fibrils in the peripheral nervous system in situ (Martini and Schachner, 1986) and isolated MAG 90 and N-CAM 110 interacted in vitro with different collagen types (Fahrig et al., 1987; Probstmeier et al., 1989), we investigated if the binding of MAG 90 and N-CAM 110 to collagen type I could influence fibril structure and/or rate of fibril formation.

MAG 90 and N-CAM 110 were incubated with collagen type I for 2–3 h at 30°C, since this time period is sufficient for fibril formation without additives. The structure of collagen fibrils formed in the presence of MAG 90 or N-CAM 110 was investigated electron microscopically after staining of fibrils with sodium phosphotungstate. The diameter of the collagen fibrils formed was measured on micrographs, examples of which are shown in Fig. 2 (*D–F*). The mean control collagen diameters were in the range of 55 ± 22 nm (Fig. 2 *A*). These values are comparable to those found by other investigators (see, for example, Wood and Keech, 1960; Valli et al. 1986). In the presence of MAG 90 (Fig. 2 *E*) or N-CAM 110 (Fig. 2 *F*) at concentrations of 10^{-6} M the diameter of collagen fibrils was significantly increased over the control values (Fig. 2 *A*). The mean diameter of collagen fibrils increased to 144 ± 40 nm in the presence of MAG 90 (Fig. 2 *B*) and to 155 ± 60 nm in the presence of N-CAM 110 (Fig. 2 *C*). The maximal diameters observed were 130 ± 10 nm in the control, 230 ± 10 nm in the presence of MAG 90, and 290 ± 10 nm in the presence of N-CAM 110 (Fig. 2, *A–C*). Thus, the mean diameter of collagen fibrils was increased by a factor of approximately three and the maximal diameter by a factor of approximately two in the presence of the two molecules. No changes in the banding pattern of collagen fibrils was observed (Fig. 2, *G–I*).

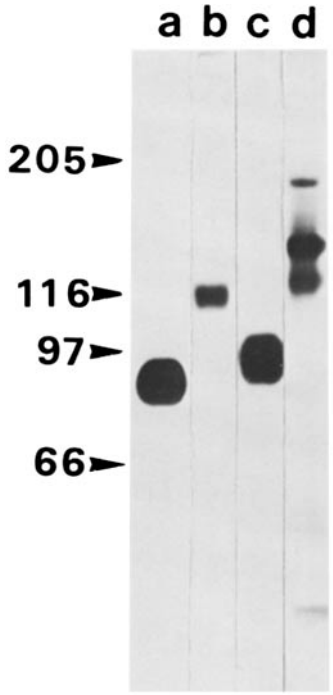

Figure 1. SDS-PAGE analysis of the molecular forms of MAG (*a* and *c*) and N-CAM (*b* and *d*) immunoaffinity purified from the supernatant of a membrane fraction from adult mouse brains incubated in cell culture medium for 2 h at 37°C (*a* and *b*) or from detergent lysates of membrane fraction (*c* and *d*). Molecular masses (in kD) are indicated at the left margin.

Fibril formation was also measured turbidimetrically in parallel with the electron microscopic examination of the formation of collagen type I fibrils after negative staining. For these measurements, only MAG 90 was investigated, since the high amounts of protein needed for such experiments were not available for N-CAM 110. Turbidity measurements of collagen fibril formation reveal a lag and a growth phase (Gelman et al., 1979). The lag phase represents a period of linear growth of microfibrils, which can not be detected turbidimetrically, while the growth phase corresponds to the stage of collagen fibril formation by lateral association of microfibrils (Williams et al., 1978; Kadler et al., 1988). As a first estimate if the rate of fibril formation was changed, the time points at which half-maximal absorbance values are reached were compared (see, for example, Kleinman et al., 1981). These values do not in fact discriminate between changes in the lag and the growth phase.

MAG 90 retarded the rate of fibril formation in a concentration-dependent manner. At a concentration of 10^{-6} M, the rate of fibril formation was delayed by 5–6 min with respect to the control value when time points were compared at which half-maximal absorbance values were reached (Fig. 3, *A* and *B*). At a concentration of 10^{-7} M the delay was 1–2 min (Fig. 3, *A* and *B*). Additionally, absolute levels of absorbance were increased in the presence of MAG 90 (Fig. 3, *A* and *C*), indicating changes in extent or structure of collagen fibrils formed.

Heparin also delayed collagen fibril formation and was a more potent inhibitor of fibrillogenesis on a molar basis than MAG 90. At a heparin concentration of 10^{-8} M, the rate of fibril formation was delayed by 1–2 min (Fig. 3, *C* and *D*) and at a concentration of 10^{-7} M by approximately 18 min (not shown). In contrast, chondroitin sulfate at the same concentration increased the rate of fibril formation by ~2–3 min (not shown). BSA at a concentration of 10^{-6} M (Fig. 3, *C* and *D*) showed no effect on fibril formation. The slopes of the turbidimetric curves (defined as increase per min in ab-

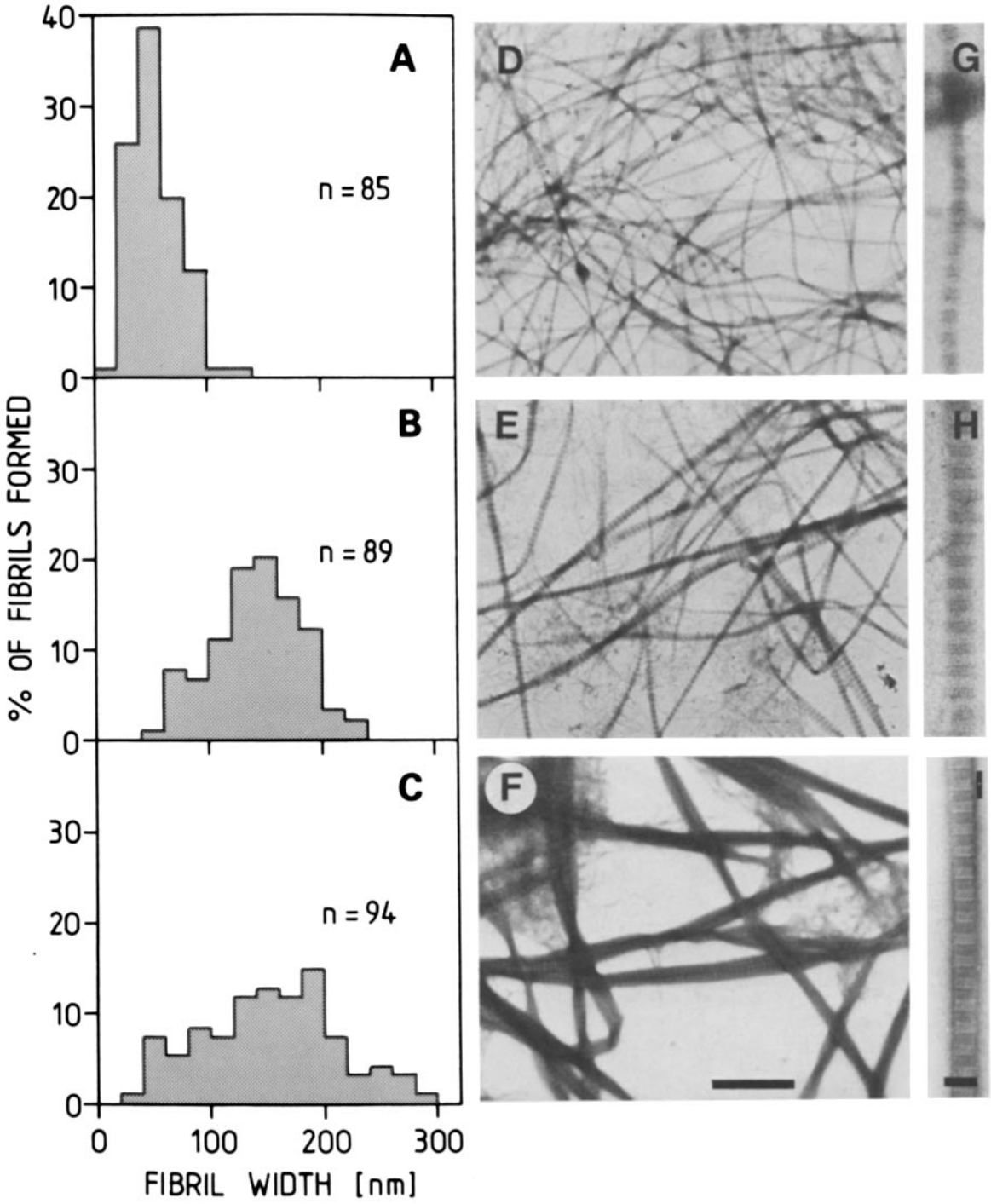

Figure 2. Influence of MAG 90 and N-CAM 110 on the electron microscopic appearance of collagen type I fibrils. Collagen fibrils were allowed to form with no additives (*A*, *D*, and *G*) and in the presence of 10^{-6} M MAG 90 (*B*, *E*, and *H*) or 10^{-6} M N-CAM 110 (*C*, *F*, and *I*). (*A–C*) Histograms of the relative frequency distribution of the diameters of collagen type I fibrils. For each fibril, the maximal diameter was chosen for determination of the diameter. (*D–F*) Representative electron micrographs of sodium phosphotungstate–stained collagen type I fibrils. (*G–I*) Higher magnification of collagen fibrils to resolve the banding pattern. Bars: (*D–F*) 1 μm; (*G–I*) 50 nm.

sorbance between 40% and 60% of the maximal absorbance) did not show large variations between the various additives and were in the range of 6.5×10^{-3}–$10.0 \times 10^{-3}\Delta E$/min. The lag periods (indicated by the intersection of the tangent to the maximal growth part of the curve with the x-axis) were 1.5 ± 0.1 min in the control and 1.6 ± 0.2 min in the presence of 10^{-6} M BSA. In the presence of 10^{-7} M MAG 90, the lag period was increased to 2.0 ± 0.1 min and in the presence of 10^{-6} M MAG 90 to 4.4 ± 0.3 min. These values were obtained from two to three experiments and are indicated as means ± SD. When MAG at a concentration of 10^{-6} M and BSA, heparin, or chondroitin sulfate at concentrations of 10^{-6} and 10^{-5} M were incubated alone for 2 h at 30°C, no change in the optic density at 315 nm was observed.

To determine if the interaction of MAG 90 and N-CAM

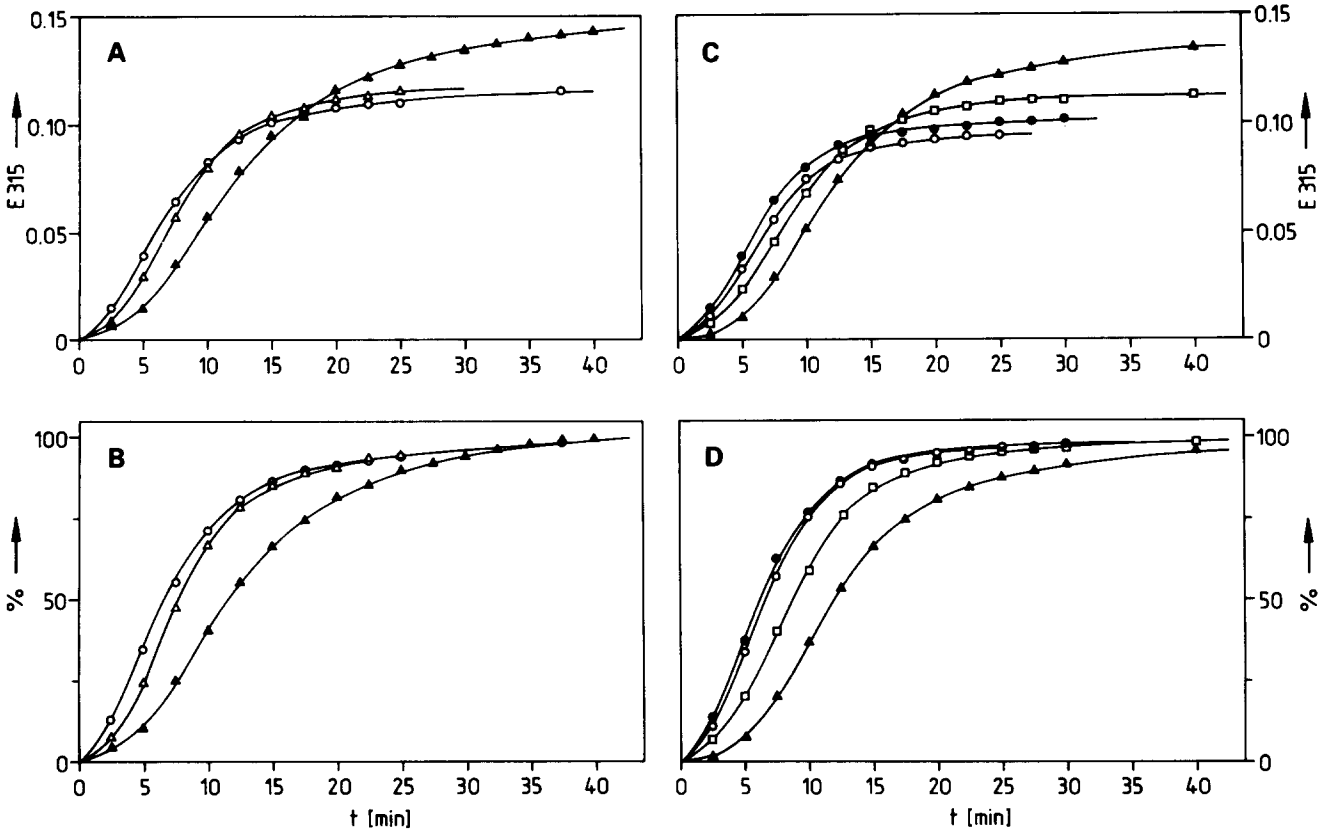

Figure 3. Effect of MAG 90, heparin, and BSA on the fibril formation of collagen type I as measured turbidimetrically by spectroscopic absorbance at 315 nm. Two independent experiments are shown in *A*, *B* and *C*, *D*. (*A* and *C*) Time course of change in absorbance (E 315). (*B* and *D*) Time course of the relative absorbance (% of maximal absorbance) E/E_{max} where E_{max} is the highest value of E 315 shown in *A* and *C*. Reaction mixtures contained: (▲) 10^{-6} M MAG 90, (△) 10^{-7} M MAG 90, (□) 10^{-8} M heparin, (●) 10^{-6} M BSA, (○) no additives.

110 with collagen type I may be altered during fibril formation, binding of ^{125}I-labeled MAG 90 or ^{125}I-labeled N-CAM 110 to collagen was determined at different time points during fibril formation (Fig. 4). Fibrillogenesis was monitored in parallel by turbidity measurements (Fig. 4). Maximal binding values ranged between 13 and 15% of the total input counts for MAG 90 and 10–12% for N-CAM 110. While binding of N-CAM 110 was not significantly altered during fibrillogenesis, binding of MAG 90 decreased by ~50% with progression of collagen fibril formation. This decrease can be interpreted as an increasing inaccessibility of particular binding sites on collagen molecules with progression of fibril formation for MAG 90. To investigate this possibility we analyzed the binding of MAG 90 and N-CAM 110 to different collagen types by radioligand binding assays and Scatchard blot analysis.

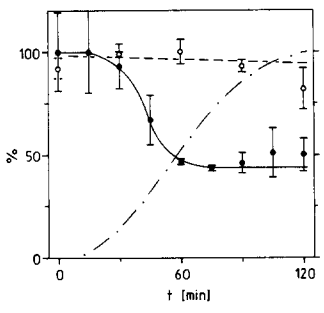

Figure 4. Binding of ^{125}I-labeled MAG 90 (●) or ^{125}I-labeled N-CAM 110 (○) to collagen type I at various stages of fibril formation. Fibril formation of collagen type I was measured in parallel by the change of the absorbance at 315 nm (·····). MAG 90 or N-CAM 110 were added at the indicated time points after initiation of fibrillogenesis (t = 0). Bound MAG 90 or N-CAM 110 were separated 2 h later from unbound molecules by collecting the fibrils by centrifugation. Mean values of double measurements ± SD from one experiment out of three are given. %, bound radioactive material (maximal binding at t = 0 is given as 100%).

Determination of Binding Sites for MAG 90 and N-CAM 110 by Solid Phase Radioligand Binding Assay

Direct binding of ^{125}I-labeled MAG 90 and N-CAM 110 to collagen type I was measured under physiological buffer conditions using a solid phase radioligand binding assay. In this kind of study we included the fibril forming collagen types II, III and V together with the nonfibril forming collagen type IV, which also represent potential binding partners for MAG and N-CAM in vivo (see, for example, Martini and Schachner, 1986, 1988). Binding curves and Scatchard plots (Scatchard, 1949) of the interaction between MAG 90 or N-CAM 110 and collagen type V are shown in Fig. 5. Scatchard plot analysis revealed linear plots for N-CAM 110 indicating a single class of binding sites and curve-shaped plots for MAG 90 suggesting at least two different classes of binding sites or negative cooperative effects during binding (Dahlquist, 1978). Similar binding curves and Scatchard plots were obtained for the binding of MAG 90 and N-CAM 110 to collagen types I–III (not shown). The K_D values for

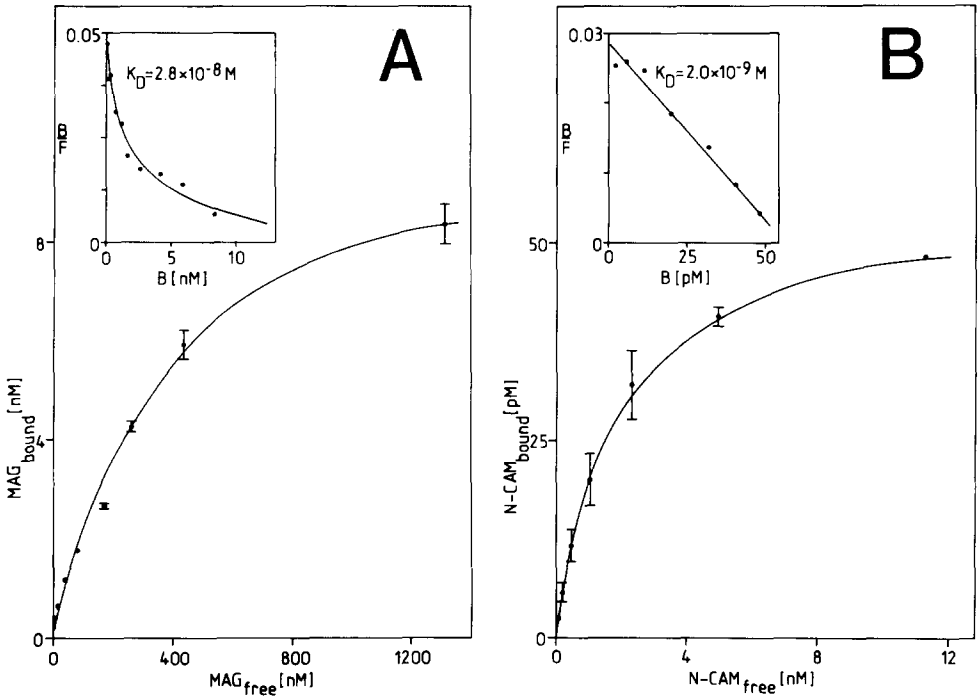

Figure 5. Solid phase radioligand binding assay. Binding of increasing amounts of ^{125}I-labeled MAG 90 (*A*) or N-CAM 110 (*B*) to collagen type V under isotonic buffer conditions. Scatchard plot analyses are given in the insets. Mean values of double measurements ± SD from one typical experiment are shown.

N-CAM 110 binding to the different collagens ranged from 2×10^{-8} M (collagen type II) to 2×10^{-9} M (collagen type V) (Table I). For MAG 90, K_D values obtained from the initial slopes of the curve-shaped Scatchard plots were in the range of 1×10^{-8} M–9×10^{-8} M and for the second slopes in the range of 2×10^{-7} M–8×10^{-7} M (Table I). Binding of MAG 90 and N-CAM 110 to collagen type IV was only 1.5 times above the background binding to BSA and analysis of binding data according to Scatchard was not applicable. In control experiments, the interaction of ^{125}I-labeled BSA with different collagen types was examined by the radioligand binding assay (data not shown). BSA interacted only weakly with collagen types I, II, III and V (signal-background ratio 2:1; the binding to BSA was set as a background binding) and not with collagen type IV (signal-background ratio 1.2:1). As an example, we analyzed the binding of ^{125}I-BSA to collagen type V via Scatchard plot analysis. The binding was not saturable and the attempt to analyze these bindings according to Scatchard resulted in a parallel to the x-axis, i.e., no K_D measurable.

Table I. K_D Values of the Binding of MAG 90 and N-CAM 110 to Different Collagen Types

	K_D values		
	MAG		N-CAM
Collagen type	First slope	Second slope	
I	6×10^{-8}	8×10^{-7}	1×10^{-8}
II	1×10^{-8}	2×10^{-7}	2×10^{-8}
III	9×10^{-8}	6×10^{-7}	2×10^{-8}
V	3×10^{-8}	5×10^{-7}	2×10^{-9}

Dissociation constants (K_D values) of binding of ^{125}I-labeled MAG 90 (MAG) and ^{125}I-labeled NCAM 110 (N-CAM) to collagen types I, II, III, and V were determined by Scatchard plot analysis on the basis of a solid phase radioligand binding assay in isotonic buffer. For determination of the two K_D values in the biphasic Scatchard plots for MAG 90, plots were fitted according to Rosenthal (1967) and Weder et al. (1974). Values are indicated in molar.

Discussion

In the present study we have investigated the interaction of soluble fragments of the two neural adhesion molecules MAG and N-CAM with collagen type I in the context of collagen fibril formation, an event likely to play an important role in the structuring of the ECM in mammalian peripheral nerves.

Indications as to the physiological role for the binding of MAG and N-CAM to collagen fibrils were derived from the observation that MAG and N-CAM influenced fibrillogenesis of collagen type I. The presence of MAG and N-CAM during fibrillogenesis leads to increased fibril diameter, while the two molecules do not alter the banding pattern of collagen fibrils. The fact that MAG increases the lag phase, but does not change the growth phase of fibril formation could mean that MAG influences the formation of microfibrils rather than their association. However, the binding specificities of MAG and N-CAM to fibril-forming collagen types seem to be quite different. First, Scatchard plot analysis of N-CAM binding to collagen types I, II, III, and V indicate a single class of binding sites, while Scatchard plots of MAG-binding infer at least two different classes of binding sites or negative cooperative effects during binding. Second, the efficiency of binding of MAG, but not of N-CAM, to collagen type I is reduced during fibrillogenesis. It is plausible to assume that one binding site for MAG becomes sterically blocked during fibril formation, while the other remains freely accessible in the growing fibril. Another explanation for the reduction in the binding efficiency of MAG during fibrillogenesis was proposed by some studies on the binding of glycosaminoglycans to fibril-forming collagen types, in which glycosaminoglycans have been found to interact only

transiently with the collagen monomers and are probably "squeezed out" during fibril formation (Scott, 1988 and references therein). Likewise, it is conceivable that MAG and N-CAM bind to collagen monomers, but these complexes can not initiate fibril formation and/or can not be integrated into growing fibrils. In fibrils, which have already been formed, however, binding of MAG and N-CAM is restricted to the fibril surface. In this model, we assume that MAG molecules, bound to the two different binding sites, have a different fate during fibril formation. While MAG molecules bound to the one binding site are "squeezed out" from the fibril, MAG molecules bound to the other can be stably integrated into collagen fibrils. Again, binding of the former type is prevented when fibrils have formed. Since binding of MAG and N-CAM was measured only after completion of fibrillogenesis we could detect only molecules that have not been "squeezed out". For N-CAM, the amount of bound molecules should be constant for all time points investigated. In contrast, one binding site for MAG becomes less accessible and the amount of bound molecules decreases during fibril formation.

The specificities of MAG and N-CAM interactions with different collagen types differ from those of other ECM glycoproteins. For example, binding of thrombospondin to collagen type V is Ca^{2+}-dependent (Galvin et al., 1987), whereas binding of MAG or N-CAM to collagen type V is Ca^{2+}-independent (Probstmeier, R., and T. Fahrig, unpublished observations). Laminin has been reported to bind to collagen type IV (Charonis et al., 1985), while MAG and N-CAM do not (Fahrig et al. 1987; Probstmeier et al., 1989).

MAG and N-CAM are not the only molecules that affect collagen fibrillogenesis. Fibronectin (Kleinman et al., 1981), the 59-kD connective tissue matrix protein (Hedbom and Heinegard, 1989), heparin and chondroitin sulfate (Wood, 1960; Öbrink, 1973) and different proteoglycans (see for example Vogel et al., 1984; Kuijer et al., 1988) have been reported to influence fibril formation, while other ECM molecules, such as laminin or chondronectin, have no effect on fibrillogenesis (Kleinman et al., 1981). Our results demonstrate for the first time that neural adhesion molecules can influence the structure of the ECM. This tenet is important from the point of view that structure and composition of the ECM can influence cell behavior. For instance, synthesis of DNA or collagen by fibroblasts, in the presence or absence of growth factors, depends on the composition of the ECM (Nakagawa et al., 1989). Shape and migration velocity of neural crest cells are influenced by the structure of the ECM they are in contact with (Tucker and Erickson, 1984). Heparin has been shown to destabilize collagen gels, such that their contraction by fibroblasts is drastically reduced (Guidry and Grinnell, 1987). The fact that the structure of collagen fibrils in different tissues changes during embryogenesis (Fleischmajer et al., 1983; 1987) suggests a functional significance. Since the interaction of MAG and N-CAM is not restricted to collagen type I, but includes other fibril forming collagens, it is possible that fibril formation of these collagen types could also be influenced by some adhesion molecules. Our studies have now given support to the notion that soluble forms of neural adhesion molecules can change the configuration of collagen fibrils, thus, calling for a more detailed investigation of the functional role of this structural change in cell–substrate interactions.

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