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Interaction Between Alzheimer's Disease βA4 Precursor Protein (APP) and the Extracellular Matrix: Evidence for the Participation of Heparan Sulfate Proteoglycans

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Abstract The interaction between the Alzheimer amyloid precursor protein (APP) and an intact extracellular matrix (ECM), matrigel, obtained from Engelbreth-Holm-Swarm tumors was evaluated. Based on quantitative analyses of the binding data obtained from solid phase binding assays, two binding sites on the ECM were identified for [^{125}I]-APP (with apparent Kd_1 of 1.0×10^{-11} M and Kd_2 of 1.6×10^{-9} M respectively). Over 70% of [^{125}I]-APP was displaced by heparin and N-desulfated heparin but not by chondroitin sulfate. Pretreatment of matrigel with heparitinase decreased the binding of [^{125}I]-APP by 80%. β-amyloid peptides (residues 1-40, 1-28, and 1-16) containing a heparin binding domain also displaced 80% of bound [^{125}I]-APP, which was totally displaced by intact APP. The binding of [^{125}I]-APP to matrigel increased by 210% with a decrease in the pH. These observations suggest that [^{125}I]-APP interacts mainly with heparan sulfate proteoglycan present in the ECM. The binding of [^{125}I]-APP to individual ECM components was also analyzed. [^{125}I]-APP was found to bind laminin and collagen type IV but not fibronectin. However, when these ECM constituents were combined, the extent of APP-binding decreased significantly, to levels comparable to those obtained with intact matrigel, suggesting that multiple interactions may occur between ECM constituents and [^{125}I]-APP. The results are discussed in terms of APP function and amyloidogenesis. J. Cell. Biochem. 65:145–158.

Key words: Alzheimer's disease; heparan sulfate proteoglycans; β-amyloid; extracellular matrix

Alzheimer's disease (AD) is characterized by the deposition of amyloid in the extracellular compartments of the cerebral cortex. Extracellular amyloid contains a 40–43 amino acids protein (β-amyloid) [Glenner et al., 1984; Master et al., 1985], which is derived from the larger amyloid precursor protein (APP), and is found together with several components normally present in the extracellular matrix (ECM) [Klier et al., 1990; Brandan and Inestrosa, 1993]. APP has the characteristics of an integral membrane protein, with a single transmembrane domain of hydrophobic amino acid residues close to its COOH-terminus [Goldgaber et al., 1976; Kang et al., 1987; Tanzi et al., 1988]. The precise function of the APP is still unknown yet. Cleavage of APP by an APP secretase, within the amyloidogenic sequence, results in a disruption of the β-amyloid sequence and the release of the ectodomain of APP which includes residues 1 to 17 of the β-amyloid protein. This secreted APP form has a relative molecular mass (Mr) of 110–120 K [Palmert et al., 1989; Weidemann et al., 1989; Sisodia et al., 1990; Small et al., 1991].

Many studies have attempted to elucidate the functional significance of APP in various biological processes. Several such studies suggest that secreted APP can bind different components of the ECM, such as heparan sulfate proteoglycans [Schubert et al., 1989; Narindrasorasak et al., 1991, 1995; Snow et al., 1994, 1995], laminin [Narindrasorasak et al., 1992], fibronectin [Narindrasorasak et al., 1995], and collagen type IV [Breen, 1986; Narindrasorasak et al., 1995].

Several other forms of amyloid not related to AD also exist such as the immunoglobulin-related amyloid and the pancreatic islet-cell amyloid found in adult-onset diabetes mellitus. These forms of amyloid contain heparan sulfate

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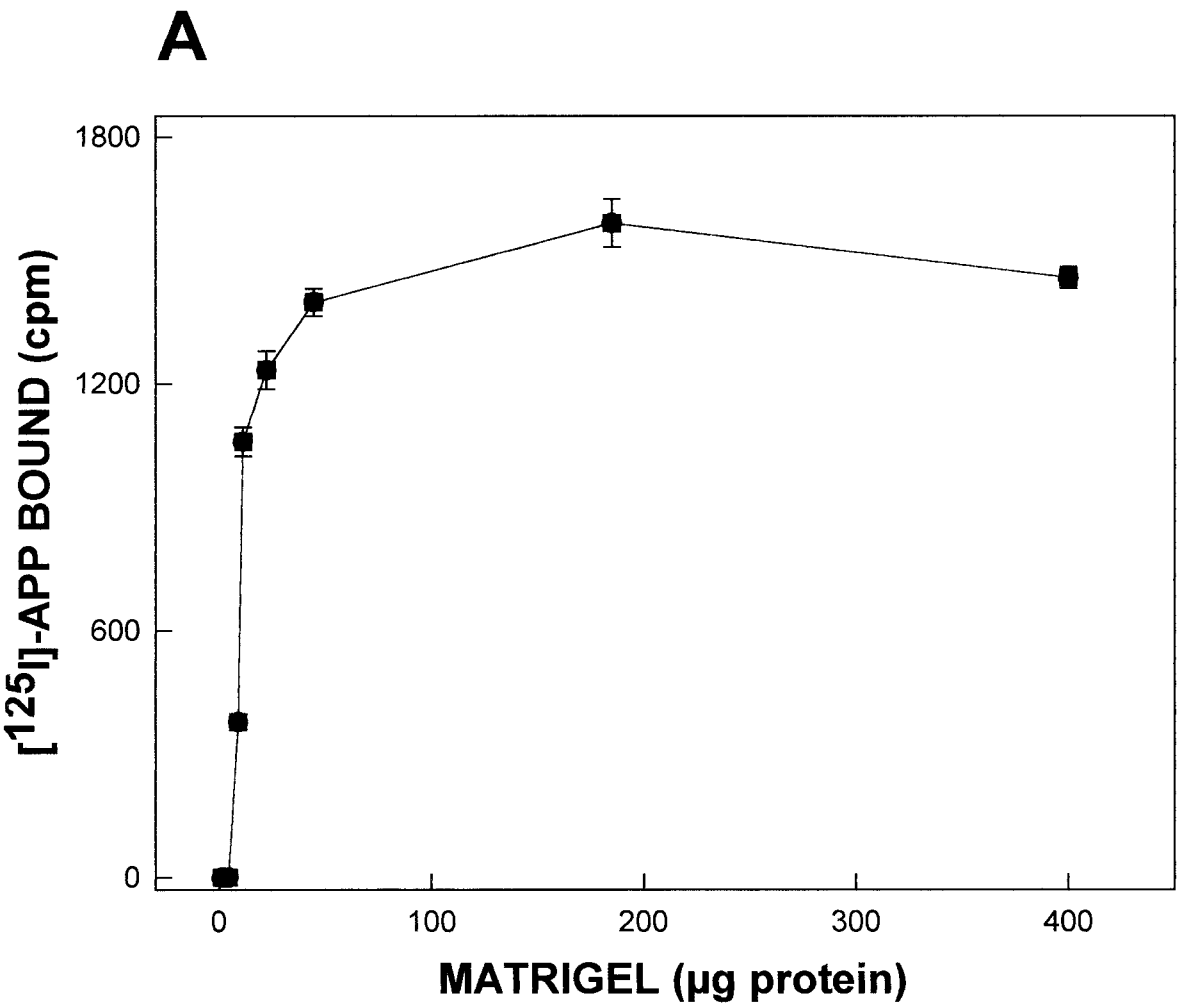

Fig. 1. Binding of [^{125}I]-APP to matrigel. **A:** Wells containing the indicated amount of matrigel were incubated with [^{125}I]-APP for 12 h at room temperature. The radioactivity bound was determined as explained under Materials and Methods. Values correspond to the mean of four experiments ± standard deviation. **B:** A constant amount of 50 µg of matrigel was applied to each well and the equivalent of 30 ng of [^{125}I]-APP were added. At the indicated times, the amount of bound [^{125}I]-APP was determined. Values correspond to duplicate samples.

proteoglycans [Young et al., 1989, 1992]. Moreover, every form of amyloid examined to date contains highly sulfated glycosaminoglycans (GAGs) [Snow et al., 1987; Brandan and Inestrosa, 1993].

The ECM is a highly organized structure found in all multicellular organisms. Its macromolecullar constituents generally contain multiple binding domains for various proteins [Ruoslahti, 1988, Adams and Watt, 1993]. For instance, laminin contains structural domains for interaction with: heparan sulfate proteoglycan, entactin and more than one cellular receptor type [Tryggvason, 1993]. Because APP is known to interact with several isolated components of the ECM, the aim of the present study was to examine the interaction of soluble APP with an intact ECM, matrigel, obtained from Engelbreth-Holm-Swarm tumors. This ECM has been shown to be a successful model for the study of the interactions between cell components and the ECM [Lin and Bissell, 1993; Roskelly et al., 1995]. This approach is particularly interesting since it has been proposed that transmembrane APP could serve directly as a cell adhesion molecule [Breen et al., 1986; Mönning et al., 1992], while secreted APP may be involved in the regulation of cell-cell interactions by generating intracellular signals via unknown mechanisms. We report here that soluble APP interacts specifically with matrigel mainly through heparan sulfate proteoglycans. The extent of this interaction, which is prob-

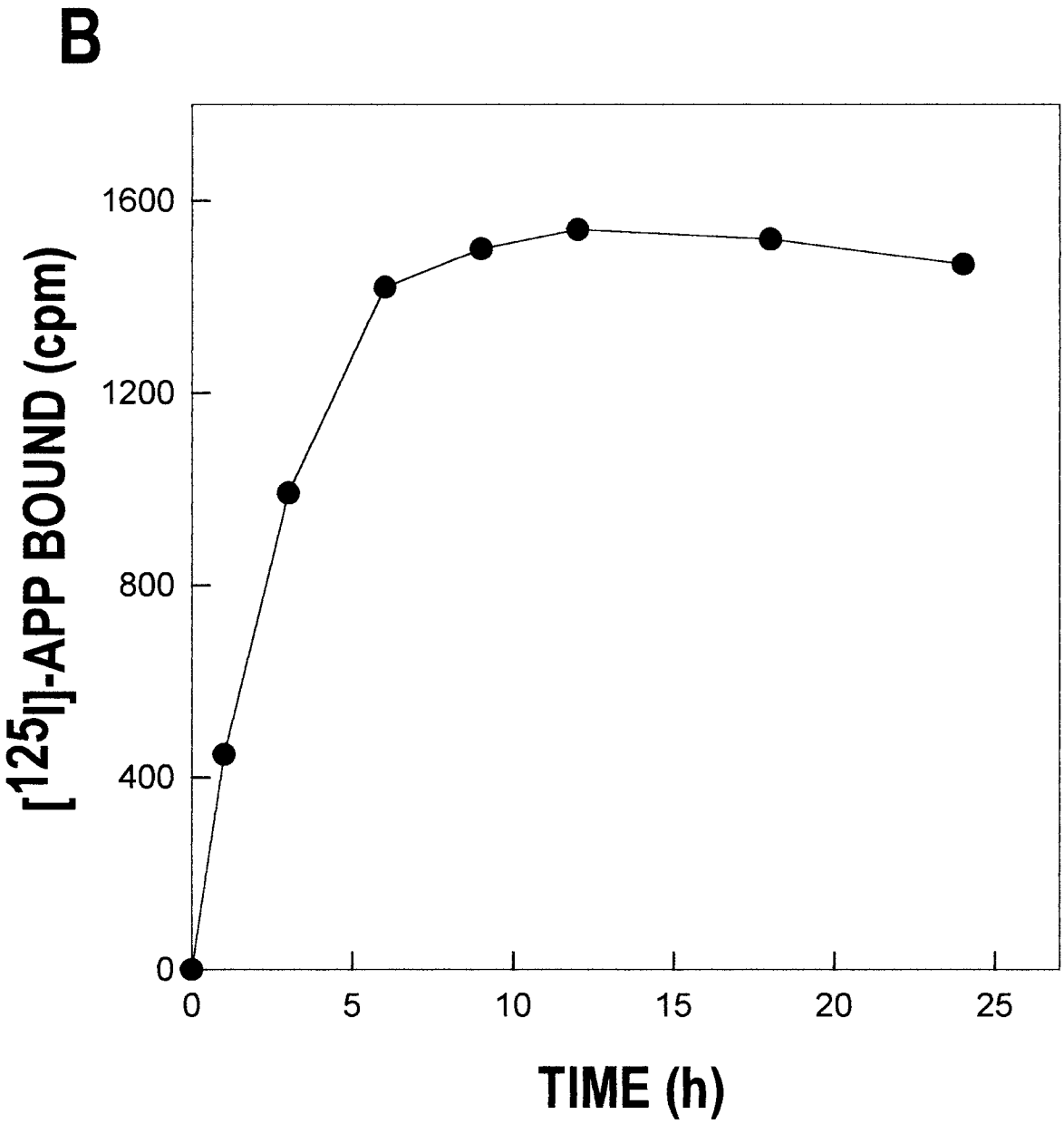

Figure 1. (Continued.)

ably mediated by the heparin binding domain present near the COOH-terminus of APP, is greatly diminished when an organized ECM is used instead of isolated matrix components.

MATERIALS AND METHODS

Materials

Matrigel was obtained from Collaborative Research, Bedford, MA. Heparin, N-desulfated heparin, chondroitin sulfate, β amyloid peptides 1–16, 1–28, 1–40, Sephadex G-25, and bovine serum albumin were obtained from Sigma Chemical Co., St. Louis, MO. Iodogen was obtained from Pierce, Rockford, IL. Heparitinase was obtained from Seikagaku Co., Japan. The APP_{695} form of the amyloid precursor protein was kindly donated from Athena Neuroscience, South San Francisco, CA. $Na^{125}I$ was obtained from the Chilean Nuclear Commission, Santiago, Chile.

Methods

APP iodination. APP_{695} was iodinated using the chloramine T method [Carpenter and

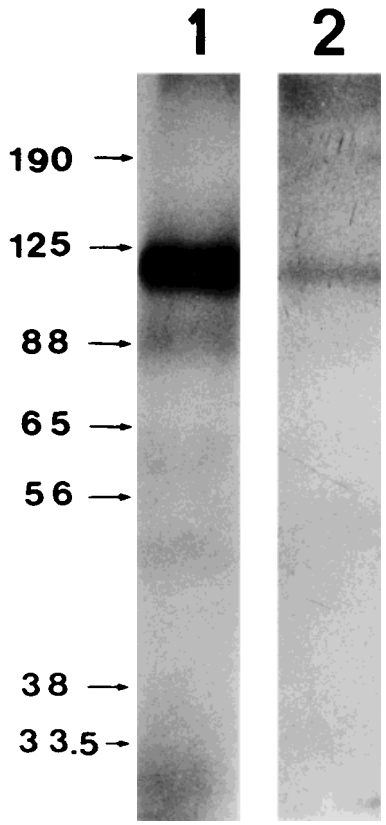

Fig. 2. The radioactive material recovered after binding of [^{125}I]-APP to matrigel is equivalent from the material applied. Five matrigel coated wells, containing 50 µg each, were incubated with [^{125}I]-APP (44,000 c.p.m.) for 12 h at room temperature. After rinsing, the wells were incubated with 5% sodium dodecyl sulfate containing 0.1 M NaOH for 15 min. The released material (**lane 1**) together with the applied [^{125}I]-APP (**lane 2**) were separated on a 8% SDS-PAGE, dried, and exposed by autoradiography. The values listed on the left correspond to molecular weight standards (kDa).

Cohen, 1976]. ^{125}I-APP was separated from free ^{125}I, using a Sephadex G-25 column. [^{125}I]-APP was analyzed by electrophoresis on 8% SDS-PAGE and detected by fluorography as described previously [Carlson and Wight, 1987].

Binding of [^{125}I]-APP to matrigel. Individual wells from Microtest III plates containing 96 wells were incubated with the indicated amounts of matrigel and left to dry for 24 h at 37°C. Each well was washed three times with phosphate buffered saline (PBS) and incubated for 1 h with 20 mg/ml of BSA solution to block unspecific binding sites. Then, each well was routinely incubated for the indicated times or during 12 h at room temperature with either 30 ng or the indicated concentration of [^{125}I]-APP (100 c.p.m./ng of [^{125}I]-APP), dissolved in PBS. At the end of each incubation period, unbound material was removed from the wells which were washed four times with PBS. The bound c.p.m. were determined using a gamma counter. In parallel experiments unspecific binding was determined by adding a 100-fold excess of APP to displace the [^{125}I]-APP bound to the matrigel coated wells. These values were subtracted from the total [^{125}I]-APP bound in order to obtain specific binding. The binding of APP to BSA coated wells was lower than 200 c.p.m.

Displacement of [^{125}I]-APP bound to matrigel by glycosaminoglycans and β-amyloid peptides. Glycosaminoglycans and β-amyloid peptides were used to displace the [^{125}I]-APP bound to matrigel wells. For this, [^{125}I]-APP was incubated with matrigel as above after which the wells were washed. Heparin, N-desulfated heparin and chondroitin sulfate dissolved in PBS at 1 mg/ml were then added for 12 h at room temperature. The remainder of [^{125}I]-APP bound to matrigel was determined as above. When β-amyloid peptides and unlabeled-APP were used, these agents were added to the wells at a concentration of 100 µg/ml and the [^{125}I]-APP bound to matrigel determined after 1, 3, 6, 9, and 12 h respectively.

Heparitinase treatment of matrigel. Wells covered with 50 µg of matrigel were incubated with 5 mU of heparitinase in the presence of 10 mM Tris-HCl pH 7.0, 1 mM $CaCl_2$ at 37°C for 3 h.

Effect of pH on the binding of [^{125}I]-APP to matrigel. Wells covered with 50 µg of matrigel were incubated with [^{125}I]-APP in the presence of 20 mM sodium acetate pH 4 and 5 with 150 mM NaCl, and 20 mM sodium phosphate pH 6, 7, and 8, containing 150 mM NaCl. The incubation time was 12 h, after which the wells were washed with the corresponding buffers, and radioactivity was determined as above.

Binding of [^{125}I]-APP to laminin, collagen type IV, and fibronectin. 10 µg of laminin, collagen type IV, and fibronectin dissolved in 0.2 M $NaHCO_3$ pH 9.6 were dried on the wells. The latter were then blocked with BSA and incubated with 50 ng of [^{125}I]-APP for 12 h at room temperature. Finally the wells were washed four times with PBS and bound radioactivity was determined as above.

RESULTS

In order to analyze the association of the APP with the ECM, a solid-phase binding assay was developed in which matrigel, a highly enriched

ECM fraction derived from the Engelbreth-Holm-Swarm tumor, was immobilized onto the wells of microtiter plates. [^{125}I]-APP was subsequently added, and bound [^{125}I]-APP was quantified by measuring the amount of radioactivity that remained associated to the microtiter dish. Figure 1A demonstrates the binding of [^{125}I]-APP to matrigel. The time dependence of this binding is shown in Figure 1B. Maximal binding occurred between 5–10 h incubation at room temperature after which the [^{125}I]-APP bound reached a plateau and did not change for up 24 h incubation. Figure 2, lane 1 shows the SDS-electrophoresis of the [^{125}I]-APP used in the binding assays, compared to the radioactive material released by 5% SDS after the binding assay (Fig. 2, lane 2). In both cases, the apparent molecular weight of [^{125}I]-APP is unchanged: 117,000. The finding that [^{125}I]-APP binds matrigel in a saturable manner is shown in Figure 3A. A Scatchard plot of this data (Fig. 3B) reveals that the association of [^{125}I]-APP with matrigel is probably mediated by multiple non-equivalent binding sites. From the curve two binding sites for APP with values of Kd_1 1.9×10^{-11} M and Kd_2 of 1.6×10^{-9} M can be discerned.

Since it has been shown that different forms of APP are able to interact with isolated components of the ECM, we decided to identify the component of the matrigel ECM to which [^{125}I]-APP was binding. The approach used consisted of incubating the matrigel with [^{125}I]-APP, removing the unbound [^{125}I]-APP and then incubating in the presence of various GAGs. As shown in Figure 4, heparin and N-desulfated heparin were able to displace over a 70% of bound [^{125}I]-APP from the matrigel wells. On the other hand, chondroitin sulfate did not displace bound [^{125}I]-APP. Having shown that the binding of [^{125}I]-APP to matrigel was inhibited by heparin, it was important to determine the kinetics of this competitive binding. Figure 5 shows that heparin was able to displace over 80% of the [^{125}I]-APP bound to matrigel, with 50% released after 4 h incubation. To further evaluate the participation of heparan sulfate proteoglycans in the binding of [^{125}I]-APP to matrigel the latter was pre-incubated with heparitinase prior to carrying out the binding assays. Table I indicates that this treatment abolished the binding of [^{125}I]-APP by 80% compared to untreated matrigel. These results suggest that APP probably interacts with heparan sulfate proteoglycans present in matrigel.

The [^{125}I]-APP used in these studies contained at least two potential heparin binding consensus sequences, one in the COOH-terminal of the molecule which includes residues 1–17 of the β-amyloid peptide, and the other close to its NH_2-terminal [Small et al., 1994]. We therefore decided to investigate whether the interaction observed between [^{125}I]-APP and the heparan sulfate proteoglycans present in the matrigel was mediated by the COOH-terminal heparin binding domain of the APP. For this, matrigel was incubated with [^{125}I]-APP for 12 h and, having removed unbound [^{125}I]-APP, was further incubated with three β-amyloid peptides of different sizes. Figure 6 shows that the [^{125}I]-APP bound was displaced by the three amyloid fragments tested (residues 1–40, 1–28, and 1–16). These peptides were able to displace about 75% of the [^{125}I]-APP bound to matrigel. The same figure also shows that an excess of unlabeled APP completely displaced the [^{125}I]-APP bound to matrigel. These results suggest that APP interacts with matrigel mainly through the heparin binding site present in the β-amyloid containing sequence of APP. However, further interactions are also likely occur to through other domains such as the one located at the NH_2-terminal of the APP protein.

It has been previously demonstrated that the interaction between β-amyloid peptide and heparin is pH dependent [Brunden et al., 1993]. For this reason we decided to evaluate the association of [^{125}I]-APP with matrigel at pH values ranging from four to eight. An analysis of the binding data is shown in Figure 7. A higher degree of binding was observed at low pH values compared to high pHs. The pH-dependence of the APP-matrigel association was not due to a change in the solubility of [^{125}I]-APP, as sedimentation of [^{125}I]-APP at different pHs was not observed (data not shown). These results are consistent with the involvement of one or more histidine residues, present in the heparin binding domains of the APP, in the interaction of this molecule with the ECM [Brunden et al., 1993].

Finally, the ability of [^{125}I]-APP to interact with other components present in the ECM was evaluated. Figure 8 shows that [^{125}I]-APP was

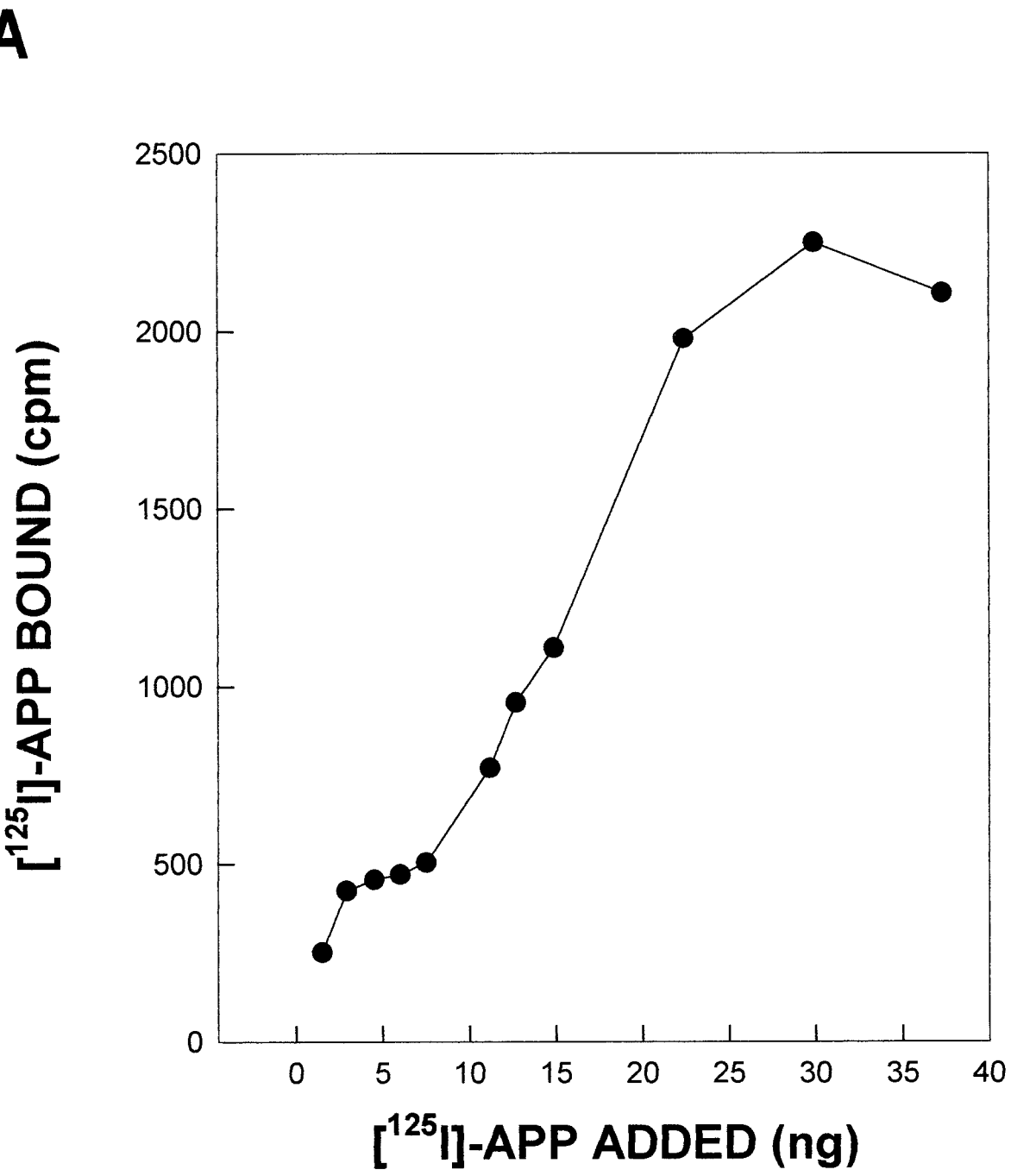

Fig. 3. The binding of [^{125}I]-APP to matrigel is time-dependent. **A:** The matrigel coated wells (50 µg) were incubated with [^{125}I]-APP at various concentrations in phosphate buffered saline, for 12 h at room temperature. Unspecific binding was from the curve values subtracted. **B:** Scatchard plot for the binding curve data. The two slopes of the curve correspond to Kd_1 of 1.0×10^{-11} M and Kd_2 of 1.6×10^{-9} M respectively.

able to interact with laminin and collagen type IV, whereas low levels of binding were observed with fibronectin. Further studies revealed that the binding of [^{125}I]-APP to laminin and collagen type IV was saturable, confirming previous data [Narindrasorasak et al., 1995]. Interestingly in comparison, binding of [^{125}I]-APP to a mixture of these ECM components was remarkably low. This diminished binding was also observed when laminin or collagen type IV were

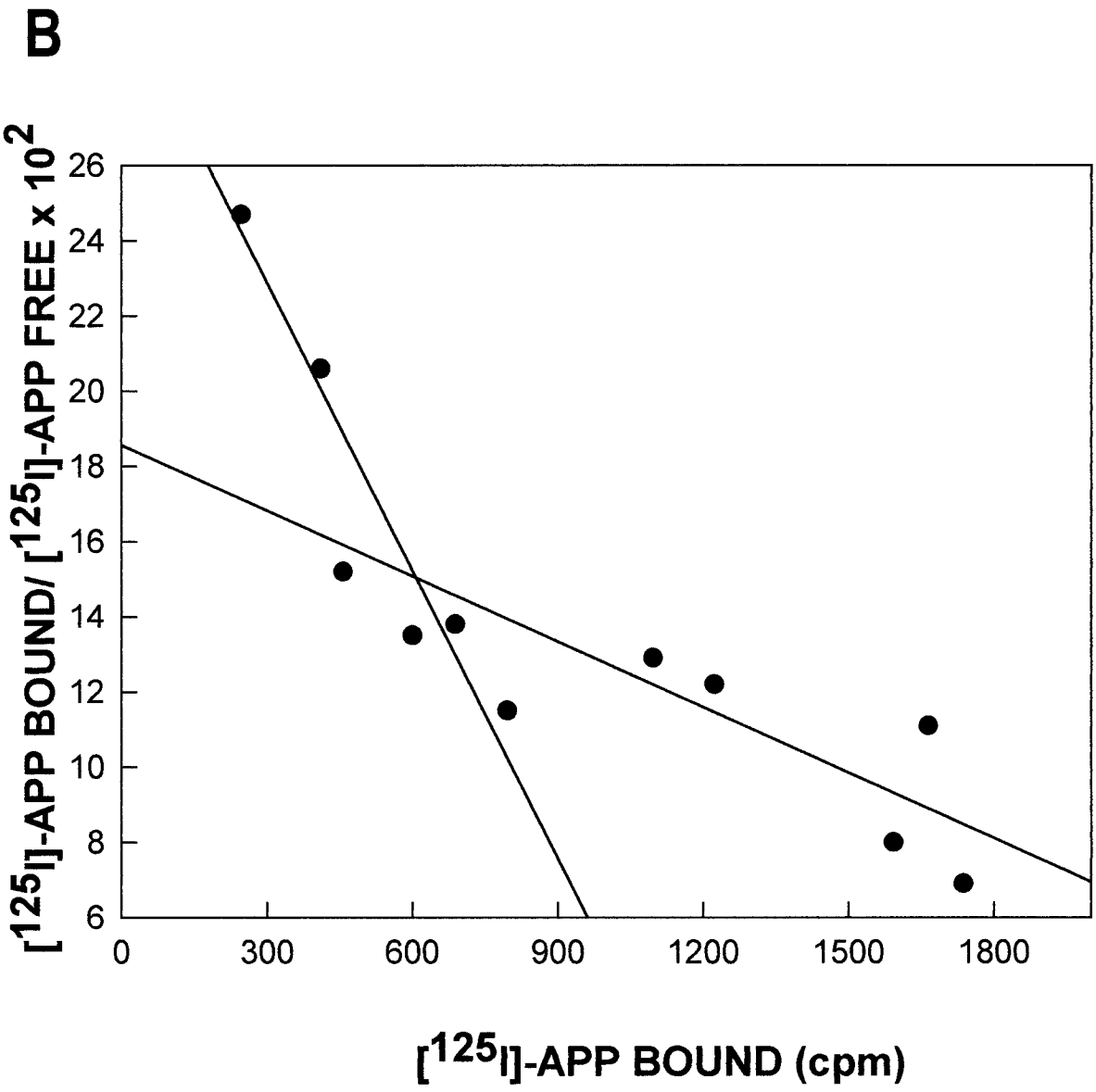

Figure 3. (Continued.)

combined with fibronectin. In Figure 8, the binding of [^{125}I]-APP to matrigel is also shown for the purpose of comparison. These results suggest that the interaction between APP and the ECM is complex and rapid conclusions cannot be drawn from experiments using isolated compounds.

DISCUSSION

In the present work, the direct interaction between APP and matrigel, an enriched fraction of ECM obtained from the Engelbreth-Holm-Swarm tumors [Terranova et al., 1986; Boudreau et al., 1995], was evaluated. The form of APP_{695} used clearly had a very high affinity for the matrigel material. Analysis of the binding data obtained suggested the presence of two sites for APP, with Kds values of Kd_1 1.9×10^{-11} M and Kd_2 of $1{,}6 \times 10^{-9}$ M respectively. These results indicated that APP was likely to interact strongly with two ECM proteins. The magnitude of these affinities suggested that very specific interactions probably exist between APP and the ECM. The potential significance of these results compared to other studies [Narindrasorasak et al., 1991, 1992; Snow et al., 1995],

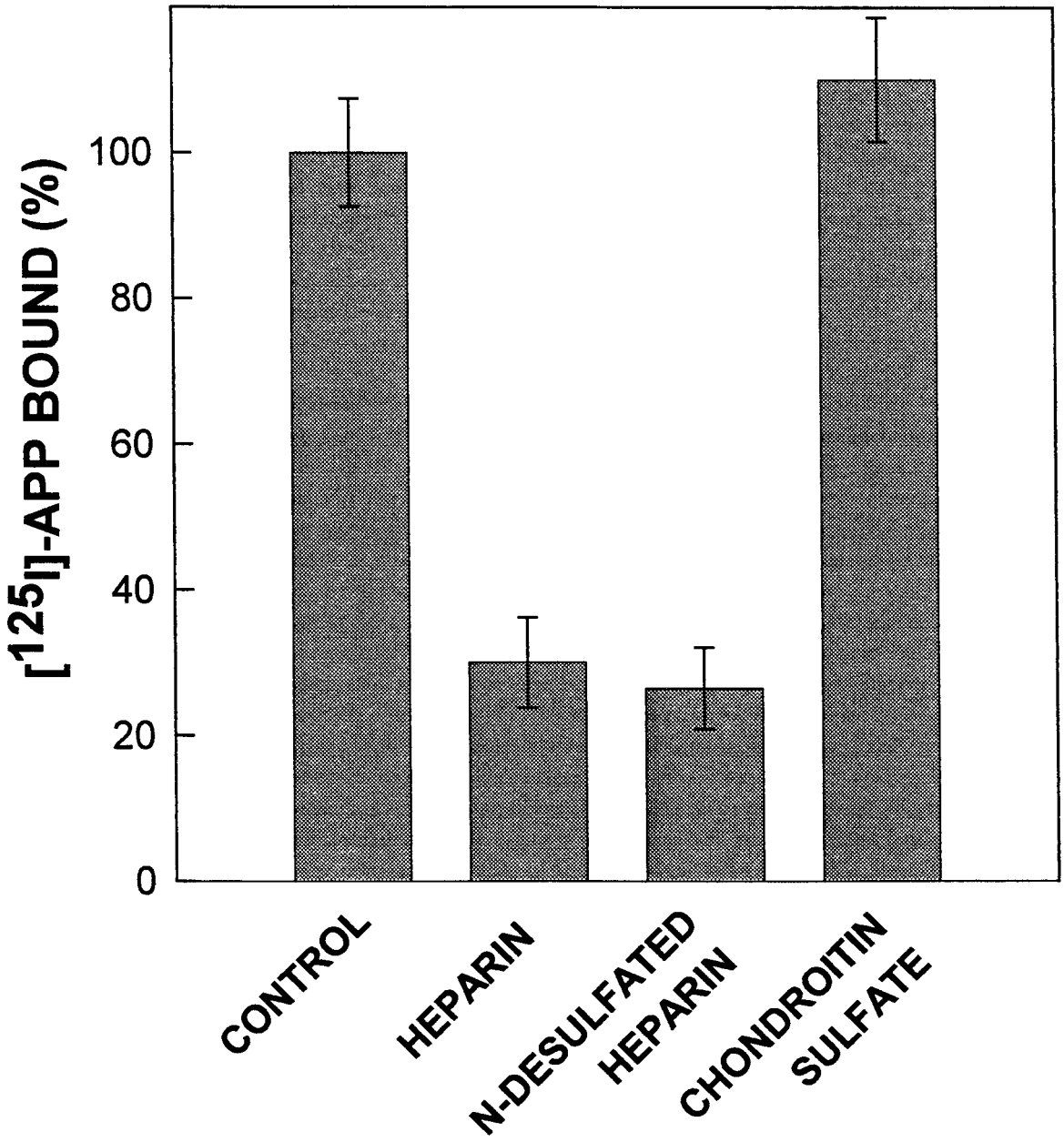

Fig. 4. Displacement of [^{125}I]-APP bound to matrigel by glycosaminoglycans. Matrigel coated wells (50 µg) were incubated with [^{125}I]-APP for 12 h at room temperature. After rinsing, the wells were incubated with the indicated glycosaminoglycans at 1.0 mg/ml for another 12 h and the amount of [^{125}I]-APP bound determined. Values correspond to the mean of three different determinations ± standard deviation.

resides in the fact that these experiments were carried out using a complete ECM immobilized on a solid phase rather than isolated compounds.

Our results indicated that almost 80% of APP bound to the matrigel could be displaced by either heparin or N-desulfated heparin, but not by chondroitin sulfate. A similar inhibition of the binding of APP to ECM was observed after heparitinase treatment of the ECM material, strongly suggesting that the interaction between APP and matrigel is mediated through heparan sulfate proteoglycans. The binding of APP to matrigel was found to have a high affinity constant (1.9×10^{-11} M) which was in very good agreement with values previously obtained for the interaction between APP and

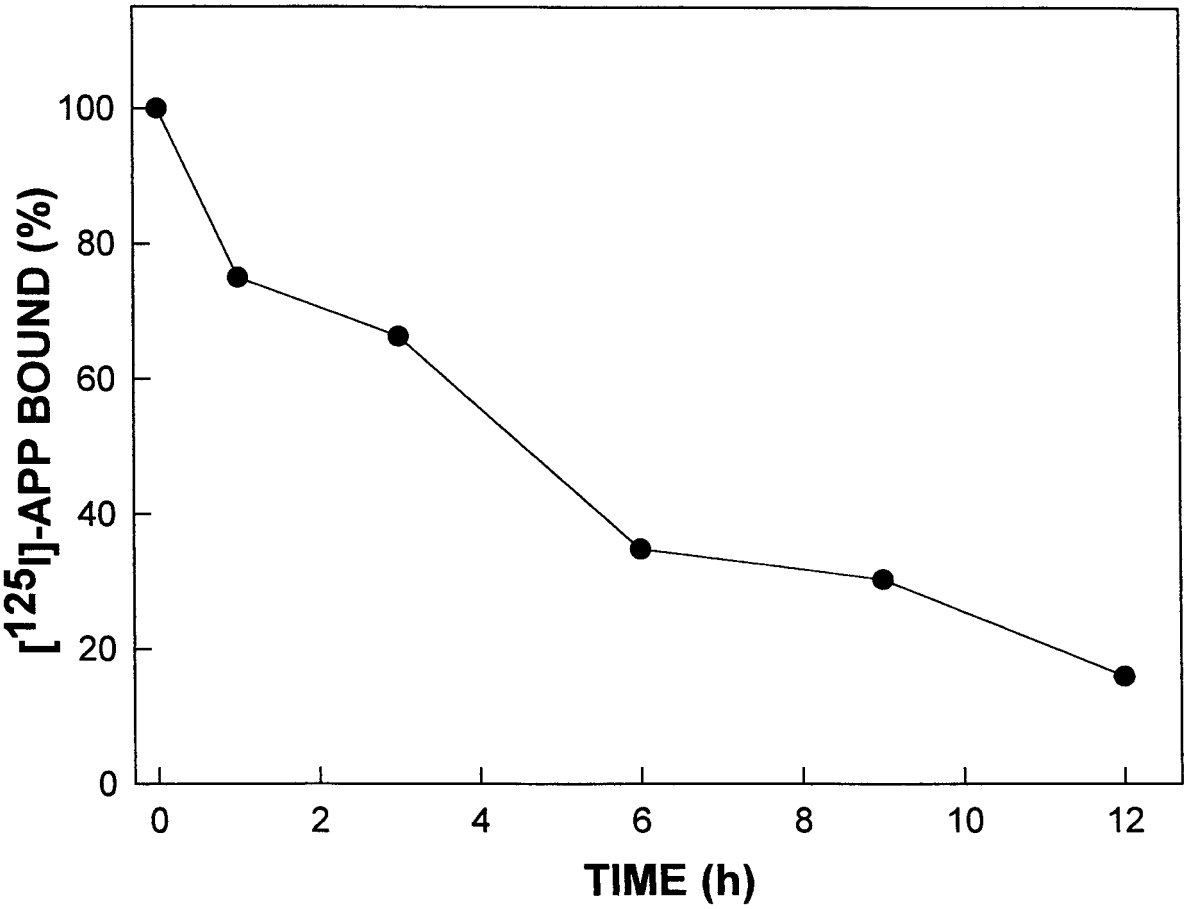

Fig. 5. Kinetic of the displacement of [^{125}I]-APP bound to matrigel by heparin. Matrigel coated wells (50 µg) were incubated for 12 h with [^{125}I]-APP at room temperature. After rinsing the wells, 1.0 mg/ml heparin was added and [^{125}I]-APP bound was determined at the indicated times. Values correspond to duplicate samples.

TABLE I. Inhibition of the Binding of APP to Matrigel by Heparitinase Pre-Treatment of the ECM*

	[^{125}I]-APP bound	
	c.p.m.	%
Control	1,732 ± 131	100
Heparitinase treated	329 ± 42	19

*The numbers correspond to an average ± S.D. of two different experiments done in triplicate.

isolated heparan sulfate proteoglycans [Narindrasorasak et al., 1991] as well as perlecan [Snow et al., 1995]. A number of different proteoglycans have now been found in association with the lesions characteristics of AD: neuritic plaques, congophilic angiopathy, and neurofibrillary tangles. Specifically perlecan, a heparan sulfate proteoglycan [Snow et al., 1988, 1990; Pelmutter et al., 1990] has been demonstrated to interact with APP with a high affinity in such lesions [Narindrasorasak et al., 1991; Snow et al., 1995]. The values of Kd reported here are in very good agreement with those found in the previous work.

Another interesting result was the fact that the matrigel bound APP could be significantly displaced by three derivatives of the β-amyloid peptide (1–40, 1–28, 1–16). These forms contained the consensus sequence for a putative heparin/heparan sulfate binding domain [Cardin and Weintraub, 1989; Brunden et al., 1993]. These results indicated that APP was likely to interact with the heparan sulfate proteoglycans of the ECM through the heparin binding domain present in the secreted form of APP. We found that the binding of APP to matrigel increased at least two-fold if the incubation was carried out at low pH instead of physiological pH. Similar results have been described for the

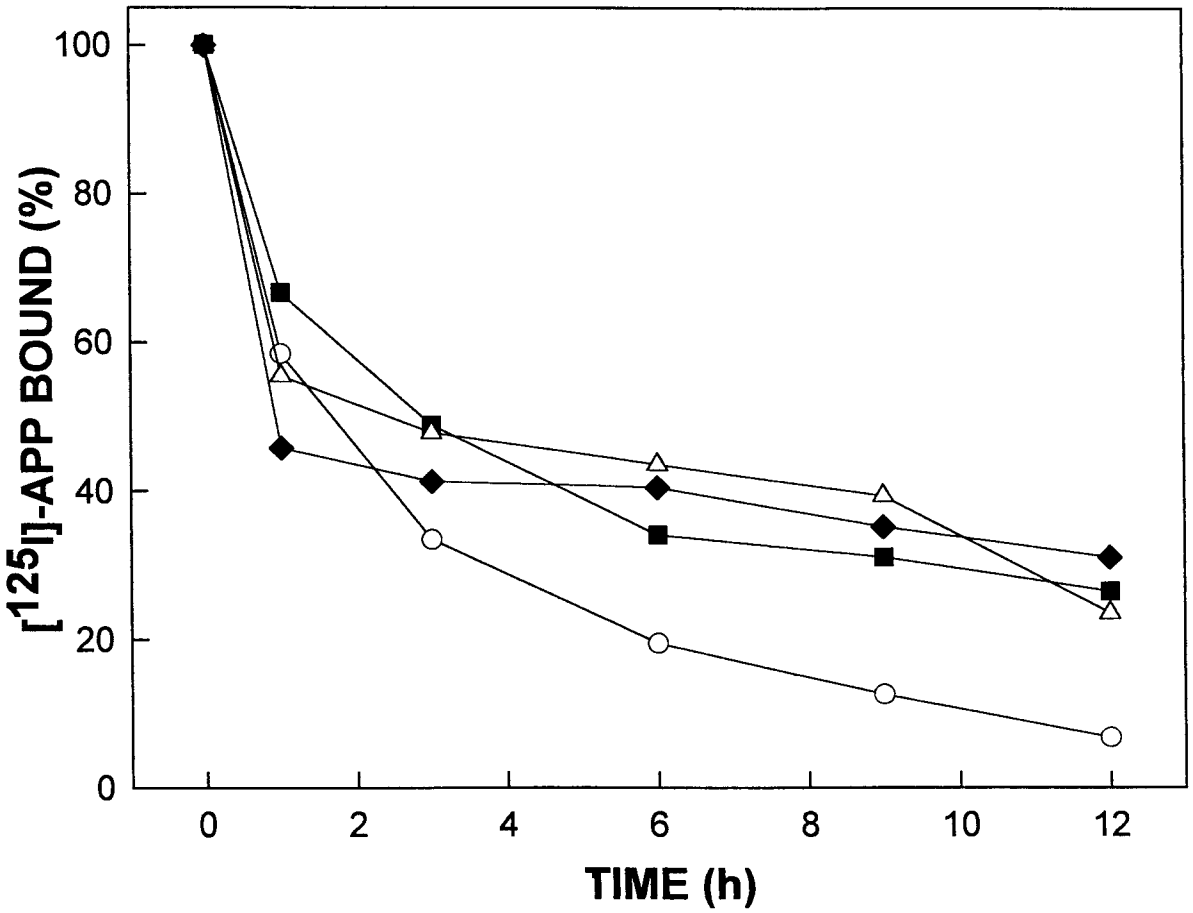

Fig. 6. Kinetics of the displacement of [^{125}I]-APP bound to matrigel by β-amyloid peptides and APP. Matrigel coated wells (50 µg) were incubated for 12 h with [^{125}I]-APP at room temperature. At the end of the incubation period unbound [^{125}I]-APP was removed and the wells incubated, for the indicated times, with 100 µg/ml of following β-amyloid peptides: residues 1–40, open triangles; 1–28, closed squares; 1–16, closed rhomboid; intact APP, open circles. The [^{125}I]-APP bound was determined as explained under Materials and Methods. Each value corresponds to an average of two samples.

interaction between β-amyloid and heparin and heparan sulfate [Brunden et al., 1993]. Together these observations suggest that the basic residues of heparin binding domain present in APP ($His_{13}His_{14}Gln_{15}Lys_{16}$) may be involved in the interaction between APP and perlecan and other heparan sulfate proteoglycan present in matrigel [Kleinman et al., 1982; Iozzo et al., 1994].

Almost 80% of the APP bound to matrigel was displaced by the three β-amyloid peptides or by heparin. Pre-treatment of matrigel with heparitinase decreased the binding between APP and matrigel by 80%. These values probably reflect the interaction between APP and the heparan sulfate proteoglycans present in matrigel. However, APP might also interact with additional components of the ECM. It has been shown that APP is able to interact with laminin [Narindrasorasak et al., 1992], collagen type IV, and fibronectin [Narindrasorasak et al., 1995] in isolated form. We found that APP was indeed able to bind laminin and collagen type IV, though almost no binding was observed with fibronectin. Interestingly, the binding of APP to individual components such as laminin and collagen type IV was three time higher on a weight basis, than the binding observed for APP to matrigel. Furthermore, the mixture of laminin with collagen type IV or fibronectin, or the combination of all three ECM constituents decreased APP binding to levels lower than for matrigel. Addition of laminin or collagen type IV to matrigel did not affect the binding of APP. These results indicate that the interaction between APP and a particular isolated compound does not necessarily reflect the extent of interaction that occurs be-

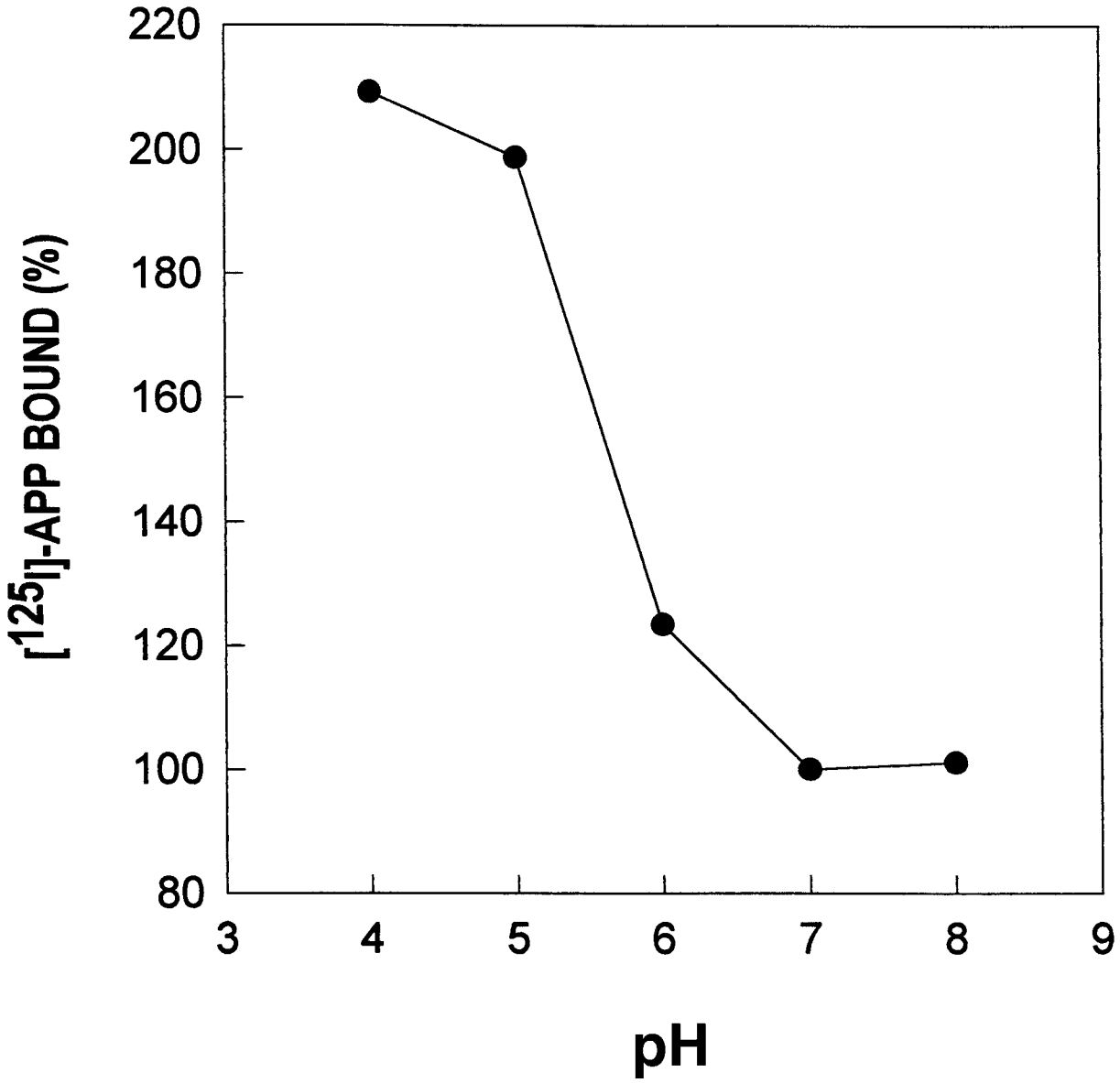

Fig. 7. Effect of pH on the binding of [^{125}I]-APP to matrigel. 30 ng of [^{125}I]-APP were incubated with immobilized matrigel (50 µg) in saline solutions at a variety of pH values as explained under Materials and Methods. After 12 h incubations, the [^{125}I]-APP bound was determined as a percentage of the value obtained at pH 7.0 (100%). Values correspond to duplicate samples.

tween APP and an intact ECM. Due to the existence of multiple binding domains on APP for other ECM compounds, one can expect the interaction between this protein and an organized ECM such matrigel to be different. Therefore the significance of the interaction between APP and isolated ECM constituents has to be considered with caution.

It is conceivable that the interaction between APP and the ECM might structurally influence the amyloidogenic generation of the β amyloid peptide from APP, a process which instigates amyloid deposition. Furthermore, as a result of the interaction between APP and ECM constituents alone, the heparan sulfate proteoglycans seems to accumulate in diffuse or early neuritic plaques, may preclude the effective formation of a structured basement membrane. Together these ideas give rise to the possibility that the nucleation process in amyloidogenesis might disturb the process of basement membrane formation, a phenomenon which is consistently

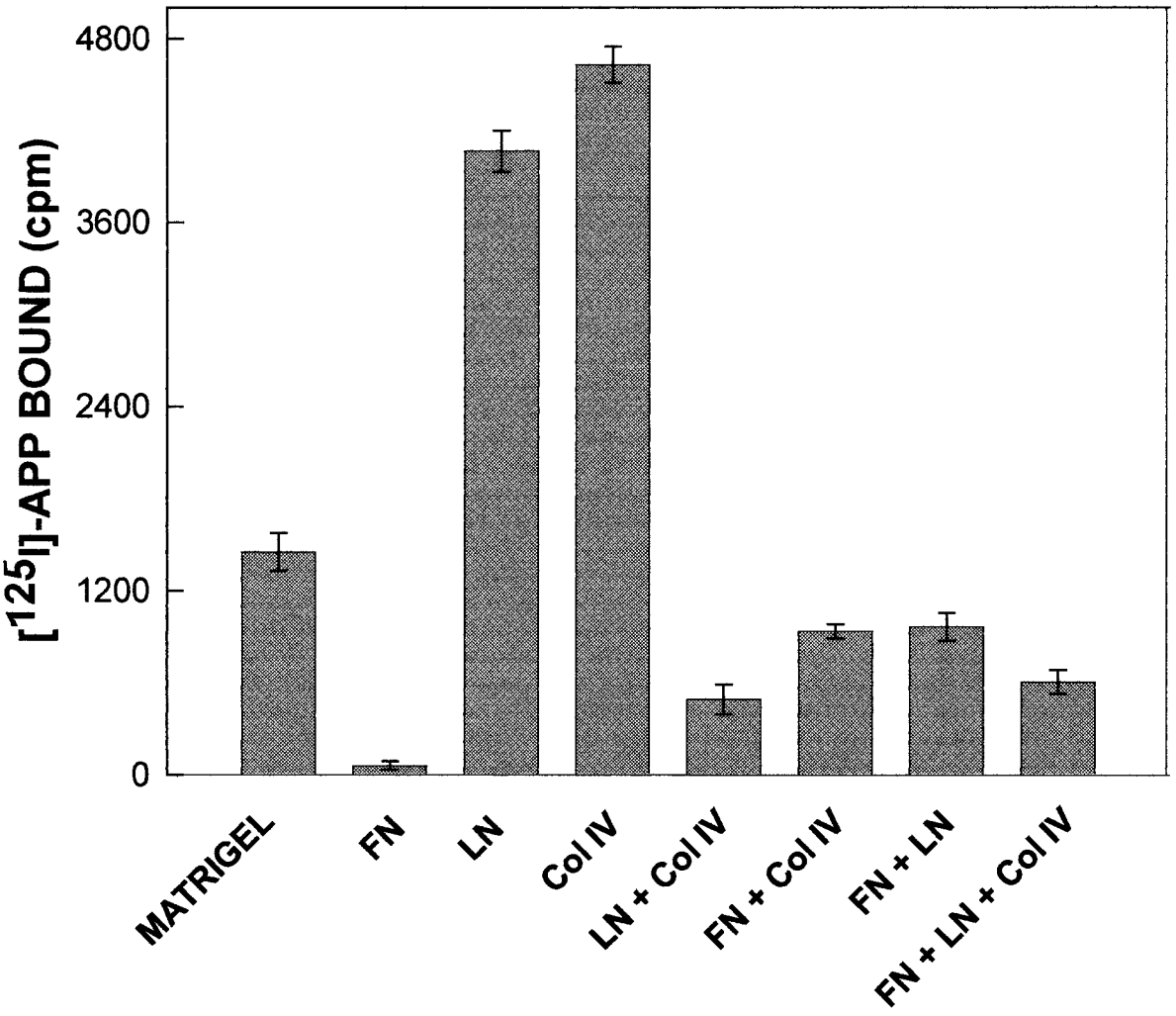

Fig. 8. Binding of [^{125}I]-APP to different immobilized substrates. Microtiter wells were covered with the indicated substrate or mixture, and then incubated with [^{125}I]-APP for 12 h at room temperature after which bound [^{125}I]-APP was determined. Values correspond to the mean of four different experiments ± standard deviation. FN, fibronectin; LN, laminin; Col IV, collagen type IV.

found in amyloid deposits where abnormalities in ECM structure have occurred [Schultz and Pintha, 1985; Schultz et al., 1985; Mooradian, 1988; Yagamuchi et al., 1992; Horiguchi et al., 1992; Brandan and Inestrosa, 1993]. An important inference of the observations of the present study, is the fact that APP, anchored to the plasma membrane, might be acting as a receptor for ECM constituents. Because both the heparin binding domains present in APP_{695} are facing the extracellular space, it is possible that this form of APP might be interacting with extracellular components thus serving an important role in cell adhesion and cell-cell interactions [Breen et al., 1991].

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