FEBS Letters 355 (1994) 151–154

FEBS 14789

Interaction between the zinc(II) and the heparin binding site of the Alzheimer's disease βA4 amyloid precursor protein (APP)

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Received 19 October 1994

Abstract The Alzheimer's disease βA4 amyloid precursor protein (APP) has been suggested to be involved in regulation of cell growth, neurite outgrowth and adhesiveness through binding to heparin sulfate proteoglycans. In order to unravel the molecular mechanisms underlying those functions in vitro we show that APP binds in a time dependent and saturable manner to the glycosaminoglycan side-chains of proteoglycans but not to chondroitinsulfate. We also demonstrate an interaction between the high affinity heparin binding site within the carbohydrate domain of APP and the zinc(II) binding site of APP. We show that the affinity for heparin is increased two- to four-fold in the presence of micromolar zinc(II). Thus micromolar concentrations of zinc(II) appear to be able to modulate the binding of APP to heparin side-chains of proteoglycans and as shown previously [Science 265 (1994) 1464–1467] to induce the aggregation of soluble amyloid βA4 protein.

Key words: Alzheimer's disease; Heparin binding; Zinc binding; βA4 amyloid

1. Introduction

The amyloid precursor protein (APP) from which the 39–43 amino acid polypeptide βA4 is derived represents the prototype protein of a superfamily of proteins that are generated from alternatively spliced transcripts [2]. APP is synthesized as an integral membrane glycoprotein which secretory forms are derived by proteolytic cleavage within the ectodomain [3,4]. APP exists in at least eight distinct isoforms. These are termed L-APP_{677}, APP_{695}, L-APP_{696}, APP_{714}, L-APP_{733}, APP_{751}, L-APP_{752} and APP_{770} [5–10]. The βA4 protein is the subunit of the extracellular amyloid deposits in Alzheimer's disease.

Regarding the putative function of APP, APP_{751} and APP_{770} isoforms which contain a region homologous to the Kunitz protease inhibitor (KPI) consensus sequence have a role in regulation of extracellular protease activity [11]. The secreted or membrane-associated forms of APP have also been shown to be involved in cell growth regulation, to regulate neurite length and to participate in neuronal cell and cell-matrix adhesion [12–14]. The ability of APP to stimulate cell adhesion and growth does not depend on the presence of the KPI domain and may derive from its high affinities for heparin, heparin sulfate proteoglycans [15], laminin and collagen type IV [16–20]. A growth-promoting activity on A-1 fibroblasts has been mapped to residues 328–332 of the APP_{695} isoform which lacks the KPI domain [21]. APP has been shown to bind zinc(II) and copper(II) at two distinct sites [17,22]. The zinc(II) binding site of APP has been shown to reside within residues 181–200 and to be encoded by exon 5. A copper(II) binding peptide of APP containing the consensus motif for type II copper binding proteins corresponds to residues 135–155 which are encoded within exon 4. Zinc(II) and copper(II) binding of APP are suggested to control APP conformation and stability [17,22].

We have analyzed the specificity of binding to the glycosaminoglycans heparin and chondroitinsulfate, and the effect of zinc(II) on heparin binding of APP. Using surface plasmon resonance (SPR) we were able to show that zinc(II) binding strengthens the binding to heparin thus demonstrating an interaction of residues involved in ligand binding which are located in different domains. This suggests that APP is an allosteric protein.

2. Experimental

2.1. Purification and radioiodination of APP

APP695 was isolated from rat brain as described previously [17]. Radio-iodination of purified rat brain APP was done with the Iodo-Beads iodination reagent (Pierce). Free iodine was removed by affinity chromatography on heparin Sepharose CL6B (Pharmacia). The specific activity of the radioiodinated APP was 5×10^5 to 1×10^6 cpm/pmol.

2.2. APP-heparin binding

To determine the dissociation constant of APP-heparin binding, 20 μl of heparin-Sepharose was incubated with increasing concentrations (10^{-9} to 10^{-12} M) of ^{125}I-APP (200 μl of 150 mM NaCl, 10 mM Tris-HCl, pH 7.5, 0.1% BSA). The amount of APP bound was quantified by liquid scintillation counting. Values are corrected for non-specific binding to Sepharose CL-6B.

2.3. Surface plasmon resonance (SPR)

Surface plasmon resonance analysis of heparin binding (BIAcore, Pharmacia; [23]) was done in HBS (10 mM HEPES, pH 7.4, 150 mM NaCl, 0.005% BIAcore Surfactant P20) at a flow rate of 5 μl/min according to the instructions of the manufacturer. For all experiments 200–300 ng of rat brain APP was used for coupling to the sensor chip (thiol coupling method) and gave 5,000–7,000 response units corresponding to 0.06 pmol APP/mm^2 of the sensor chip (coupling yield of 2–3%). Heparin bound to APP on the sensor chip surface was removed by injecting 20 μl of 1 M NaCl. Free heparin (Sigma, H-7005) and chondroitin sulfate A (Sigma, C-8529) dissolved in 1 × HBS were injected without and with 100 μM of the following metal ions: $ZnCl_2$, $CoCl_2$, $CaCl_2$, $MgCl_2$. Injections were done with one metal ion at a time. Injections were repeated two times independently and increase of

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SSDI 0014-5793(94)01176-1

response units taken from the dissociation phase averaged. Saturating effect of zinc(II) for heparin binding: the increase of heparin binding was measured after injecting 2.5 ng of free heparin together with increasing concentrations of zinc(II).

In SPR, the observed change in response units is either due to a conformational change of the immobilized protein or the binding of a ligand provided both are large enough to alter the refractive index at the sensor chip surface [17,23].

3. Results

3.1. Heparin binding of rat brain APP

A solid phase binding assay showed that binding of radioiodinated APP to heparin is time dependent (Fig. 1A) reaching half maximal binding after 8 min at 4°C. This interaction was concentration dependent over nanomolar to picomolar APP concentrations (Fig. 1B). Scatchard transformation of these data revealed the presence of a high affinity binding site of APP for heparin with an apparent dissociation constant of 300 pM. The measured kinetic parameters are dependent on the working method used and may only partially reflect the in vivo situation.

3.2. Zinc modulation of the heparin binding site in vitro

Since the stability of APP enriched by heparin affinity chromatography from plasma of patients with Alzheimer's disease was greatly reduced in the presence of 20 μM zinc(II) and since APP alone was found to bind $^{65}Zn^{2+}$ [22], we studied the influence of zinc(II) on APP heparin binding by surface plasmon resonance (SPR) using biosensor based technology. This technique allows to monitor at high sensitivity the binding of those ligands to the protein immobilized to the SPR sensor chip surface whose binding results in a change of the reflected monochromatic light. Briefly, proteins are covalently coupled via a dextrane matrix on a gold surface of a sensor chip. The sensor chip is mounted in a flow cartridge which allows continous flow of buffer and the sensor chip surface forms one wall of the flow cell. The refraction of light (760 nm) from the gold layer is recorded and compared with the reflection obtained after addition of ligands. The difference in response units is a measure of the deviation of light upon ligand binding. Such a difference is expected to be observed if the surface area of the ligand is large enough, or if in the case of a small ligand the protein itself undergoes a significant conformational change upon ligand binding. As shown in Fig. 2, heparin which belongs to the former group of large ligands fulfils the criteria to be analyzed for its binding to APP by SPR. A difference in refractive index is caused by mass changes on the sensor chip surface when the analyte heparin binds to immobilized APP. An increase of 70 response units (RU) and fast association was observed after injecting 75ng heparin (Fig. 2A). The interaction of APP and heparin could be shown to be specific because other negatively charged polymers like chondroitin sulfate A cannot replace heparin (Fig. 2B). Zinc(II) binding to APP does not alter the refractive index (Fig. 2C) and cannot be directly analyzed by SPR. If zinc(II) binding would alter APP conformation in such a way that heparin binding is influenced this should then be detected by SPR. Indeed, at low concentrations of zinc(II) (50 nM to 200 nM) the refractive index observed by heparin binding of rat brain APP increases (Fig. 3A). Since the zinc(II) binding site of APP was shown to have lower affinity to other divalent metal ions such as Ca^{2+}, Co^{2+} or Mg^{2+}, we compared the effect of these ions with that for zinc(II). This comparison

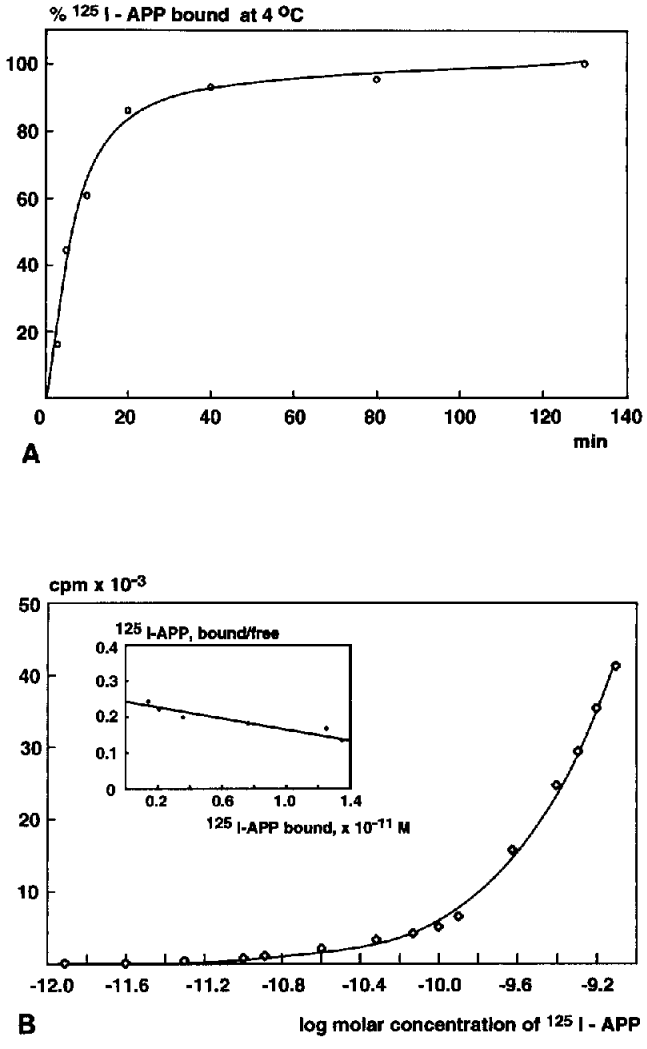

Fig. 1. Time course of ^{125}I-APP binding to heparin Sepharose (A). Maximal binding corresponds to 100%. Equilibrium measurement of ^{125}I-APP to heparin (B). Data represent mean values of two independent experiments. Scatchard analysis of APP binding to heparin reveals a dissociation constant of 0.3 nM for APP_{695} (inset).

showed that the increase of heparin binding to APP is strongest for zinc(II), followed by Co^{2+}, Ca^{2+} and Mg^{2+} (Fig. 3B). The latter metal ions are half as effective as zinc(II) which is in good agreement with their binding constants for the zinc(II) binding site of APP [22]. The saturation of the modulating effect of zinc(II) on heparin binding of APP is reached at 70 μM zinc(II) (Fig. 3A). This concentration of zinc(II) is within the physiological levels of zinc(II) in brain. We have shown previously [22] that direct binding of zinc(II) to heparin does occur below these concentrations and therefore cannot account for the effect.

The zinc(II) concentrations in the brain interstitium fluctuate from an average level of 0.15 μM to 300 μM during synaptic transmission [24–26]. Hence, the modulation of APP binding to heparin occurs over a range of zinc(II) concentrations that would be way below those encountered physiologically. The APP binding site for zinc(II) has been previously identified. It includes residues 181–200 of APP_{695} encoded within exon 5 [22]. It is located N-terminal to the heparin site that is encoded by exons 9 and 10 (G. Multhaup, unpublished).

4. Discussion

Heparin binding to APP occurs very rapidly irrespective of the immobilized ligand, either heparin coupled to Sepharose or APP immobilized to the SPR sensor chip. Maximal binding was reached after approximately 30 min at 4°C and after one minute at 25°C.

The high salt concentration at which APP elutes from heparin-Sepharose is greater than physiological (620–1,000 mM NaCl; G. Multhaup, unpublished) and is therefore already an indication of heparin affinity and useful for comparative purposes. This approximates to the highest values found in the literature i.e. for antithrombin, a heparin-binding serine proteinase inhibitor that elutes at 750 mM NaCl [27]. More rigorous measurements revealed the apparent dissociation constant derived from Scatchard transformation as 3×10^{-10} M for

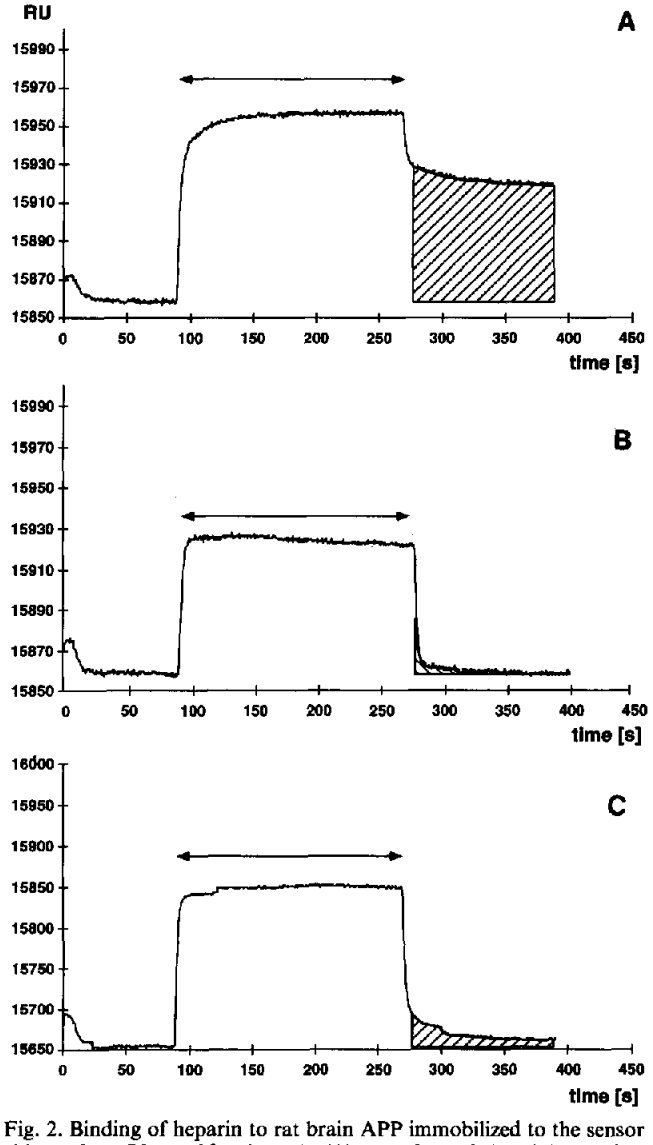

Fig. 2. Binding of heparin to rat brain APP immobilized to the sensor chip surface. 75 ng of free heparin (A) and 75 ng of chondoitin sulfate A (B) were injected for the time indicated by the arrow. The dissociation of heparin from APP (x-axis) and the amount of heparin bound (y-axis) are given by the hatching (A). Injection of chondroitin sulfate A and HBS containing 100 μM zinc(II) did not result in a significant increase of response units above the baseline (B and C).

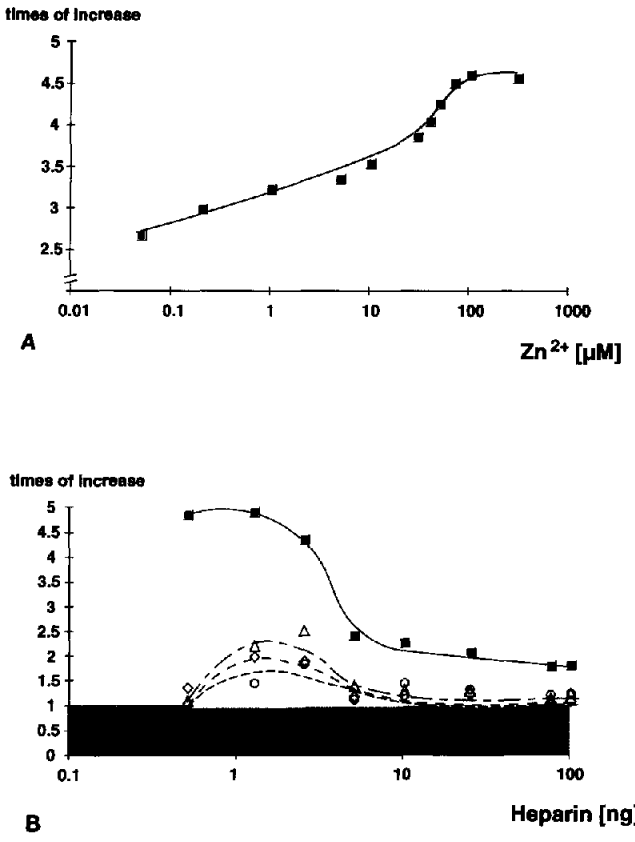

Fig. 3. Modulation of APP heparin binding by Zn^{2+} at fixed heparin concentration (2.5 ng) (A) and at 100 μM Zn^{2+} (filled squares), Ca^{2+} (diamonds) Co^{2+} (triangles) and Mg^{2+} (circles) at increasing heparin concentrations (B) analysed by surface plasmon resonance (SPR). Results are expressed as increase of relative response units in heparin binding upon addition of metal ions.

APP-binding to heparin-Sepharose. This is in good agreement with K_d values obtained by analysis of the binding of heparin to membranes isolated from LX-1 cells [28] but is approximately 60 times lower than that for binding of NCAM to heparin and even lower than that for homophilic binding of NCAM to NCAM [29].

APP binding to heparin in vitro suggests that APP growth stimulation may be mediated through this heparin-binding site in vivo. In addition, a low affinity APP heparin binding site located within residues 96–110 was shown to be involved in the regulation of neurite outgrowth [30]. This site binds three orders of magnitude less tightly to heparin than the high affinity site described here. But both sites appear to be associated with growth activity.

Zinc(II), known to occur in vesicles at concentrations of up to 300 μM in zinc(II)-sensitive mossy fibres of the hippocampus and to be released during neuronal activity [31] is shown here to strengthen the binding of APP to heparin. Zinc(II) is therefore in principle capable to modulate the interaction of APP with the heparan sulfate moiety of proteoglycans (HSPG). In brain HSPG's are exposed on the cell surface as receptors and are major constituents of the extracellular matrix [32].

A functional zinc(II) binding site on APP is of great importance because an abnormality of zinc metabolism in AD and Downs syndrome has been reported [33,34]. A disturbed homeostasis of extracellular zinc(II) in Alzheimer's disease [22] may

therefore interfere with the normal binding of APP to heparin-like molecules such as HSPG's [19] which are known to alter protein conformation, and the clearance and processing of bound proteins [35]. Zinc(II) binding may also influence APP processing since the stability of APP enriched by heparin affinity chromatography from plasma of patients with AD was greatly reduced at low concentrations of zinc [22]. Finally, at micromolar concentrations of free zinc(II) soluble βA4 molecules are precipitated [1]. This recent finding that zinc(II) binding to βA4 results in accelerated aggregation of synthetic βA4 peptides and in insoluble precipitates with the tinctorial properties of amyloid plaque deposits in Alzheimer's disease suggests that zinc(II) not only regulates the normal APP function but may also play a crucial role in amyloid depositions in Alzheimer's disease.

Acknowledgments: The authors thank Konrad Beyreuther for comments on the manuscript and Desiree Müller and Santosh Pinto for technical assistance.

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