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Full-length apolipoprotein E protects against the neurotoxicity of an apoE-related peptide

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Abstract

Apolipoprotein E was found to protect against the neurotoxic effects of a dimeric peptide derived from the receptor-binding region of this protein (residues 141–149). Both apoE3 and apoE4 conferred protection but the major N-terminal fragment of each isoform did not. Nor was significant protection provided by bovine serum albumin or apoA-I. Full-length apoE3 and apoE4 also inhibited the uptake of a fluorescent-labeled derivative of the peptide, suggesting that the mechanism of inhibition might involve competition for cell surface receptors/proteoglycans that mediate endocytosis and/or signaling pathways. These results might bear on the question of the role of apoE in neuronal degeneration, such as occurs in Alzheimer's disease where apoE4 confers a significantly greater risk of pathology.

Keywords

Alzheimer's disease; neurotoxicity; neuroprotection; LRP; proteolysis

a. Introduction

Apolipoprotein E has been shown to exert an isoform-specific contribution to the risk of Alzheimer's disease and has been implicated in other conditions where the presence of apoE4 usually confers greater risk [15,44]. Although a number of hypotheses have arisen from studies of simplified tissue culture systems and various animal models, there is no consensus as to which, if any, of the proposed mechanisms are relevant to the etiology or progression of disease. In the case of Alzheimer's disease, many of the hypotheses assume that apoE plays an indirect role through its influence on the production, toxicity, deposition and/or clearance of amyloid, as recently reviewed by Bu [8]. An alternative hypothesis is that apoE plays a more direct role that might include isoform-specific influences on neuronal viability, regardless of its role in amyloid pathology [15,44,52,68].

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One proposed mechanism through which apoE may contribute to neuronal degeneration involves proteolysis of the full-length protein to generate fragments that have deleterious effects on neuronal function. Two groups have found that different fragments of apoE can exert isoform-specific neurotoxic effects under certain conditions. One line of investigation has implicated N-terminal fragments of apoE [16,45,46,59,60]; the other has found evidence for the role of fragments that include more of the C-terminal domain [7,31–33]. The incidence and identity of the fragments present in human brain tissue is still under investigation and a recent study confirmed that apoE fragments are present in human brain and are more prevalent in apoE3 cases, but are not specifically associated with Alzheimer's disease [19].

Some of the reported effects of apoE fragments and related peptides appear to involve interactions with apoE receptors. For example, peptides derived from the receptor-binding domain of apoE can have neurotoxic or neuroprotective effects, depending on the specific peptide sequence, dose, and experimental conditions [2,3,6,11,16,23,24,26–28,37,39–42,49,57,59,60,63]. One of these peptides, a short tandem repeat of residues 141–149 (Stan), has been shown to exhibit dose-dependent neurotoxicity [26,59,60]. In addition, full-length apoE3 and apoE4, as well as N-terminal fragments of apoE3 and apoE4, have been reported to be neurotoxic under selected conditions [26,27,59].

In previous studies, the neurotoxicity of full-length apoE was associated with the generation of apoE fragments [59]. If apoE neurotoxicity is due to proteolytic fragments of apoE rather than to the full-length protein (which has been reported to be neuroprotective in some systems [3,35,43,53]), one possible explanation for variable results is that the full-length protein can protect against neurotoxic fragments. We sought to test this hypothesis by examining the influence of full-length apoE on the toxicity of Stan. We find that both full-length apoE3 and apoE4, but not BSA or apoA-I, confer significant protection against Stan-related neurotoxicity, suggesting that the variability in the reported biological effects of apoE may be partly due to the extent to which apoE fragments are present or produced under specific experimental conditions.

2. Results

Dissociated embryonic chick sympathetic neurons were treated with Stan alone or in combination with various concentrations of either full-length apoE3 or apoE4 or the N-terminal fragment of both isoforms. The peptide showed dose-dependent effects on neuronal survival with low concentrations (.5–2 μ M) causing slightly increased survival and higher concentrations causing neurotoxicity with a half-maximal effect ranging from 4–8 μ M (figure 1), consistent with previous studies [59,60].

The presence of full-length apoE resulted in significant reduction in neurotoxicity of the apoE peptide. Representative images of cells exposed to the peptide in the presence and absence of full-length apoE are shown in Figure 2. The majority of cells were killed by peptide concentrations of 8 μ M and higher with an LD50 around 5 μ M. In the presence of either apoE3 or apoE4, there was a clear reduction in toxicity such that many cells were spared even at a peptide concentration of 12 μ M. Both full-length apoE3 and apoE4 showed protection when present at approximately a 1:1 (m:m) stoichiometry (apoE:Stan). Full-length apoE did not show any evidence of neurotoxicity by itself in these experiments.

Due to variability across experiments (usually due to variations in initial plating density), the results of 3–9 different experiments were combined and analyzed after normalizing the amount of toxicity relative the number of surviving (control) cells. These results are shown in Figure 3. Statistical analysis demonstrated an overall group effect such that both apoE3 and apoE4

resulted in significant reduction in peptide-induced neurotoxicity. Although there was a trend for greater protection with apoE4, this was not statistically significant.

The dose-dependent protective effect of apoE3 was tested using different concentrations of full-length apoE3 in the presence of a concentration of Stan that usually kills 60–80% of the neurons. Increasing concentrations of the full-length protein resulted in greater protection against the peptide, as shown in Figure 4. The extent of protection varied with different preparations of full-length apoE and from experiment to experiment.

The N-terminal fragments of apoE3 and apoE4 and bovine serum albumin did not show significant protection against the peptide when used at concentrations comparable to those at which full-length apoE showed significant protection. In some experiments, there was toxicity associated with the apoE4 N-terminal fragment as found in previous studies [60]. BSA did not show toxicity or protection at comparable concentrations, and only showed modest protection in some experiments at high concentrations (25 μ M). ApoA-I, which is another exchangeable amphipathic apolipoprotein with similarities to apoE, also showed no consistent protection against the toxicity of the peptide.

To assess the possibility that the presence of the peptide altered the uptake and/or intracellular metabolism of full-length apoE, Western blots were prepared of cultures that had been exposed to full-length apoE3 in the presence or absence of different concentrations of the peptide. As shown in Figure 5, the amount of full-length apoE recovered from the intracellular pool was not affected by the presence of the peptide. In addition, although there was a faint band corresponding to a lower molecular weight fragment of apoE in the extracellular pool, there was no corresponding band in the intracellular pool, possibly because the overall levels were less.

Evidence that the peptide accumulates in neurons is provided by the use of the fluorescein-labeled derivative, which accumulated in the neurons in a time-dependent manner (Figure 6). Rapid uptake of the labeled peptide was observed in the majority of the cells so that by 1 hour virtually all of the neurons had become fluorescent. However, there was a range of fluorescence intensity such that initially only a few cells were intensely fluorescent. The number of intensely fluorescent cells increased with the duration of exposure or concentration of the peptide. The intensely fluorescent cells also appeared to be round and less phase-bright than the cells with less intense fluorescence, exhibiting morphologies consistent with degenerative changes.

Next, we determined whether the full-length protein might interfere with the cellular uptake of the peptide. This was carried out by monitoring the labeling of the neurons with the fluorescein-labeled peptide in the presence and absence of apoE, BSA, or suramin, the latter being an established inhibitor of ligand binding to the LDLr [55,61]. The rate and extent of labeling was dramatically reduced by the presence of full-length apoE and suramin but was minimally affected by BSA (Figure 7). The uptake of fluorescein-Stan was also minimally affected by apoAI (results not shown).

Previously we showed that heparin blocked peptide toxicity (both synthetic peptide and the 22 kDa NT fragment) in cultured primary neurons, which suggested involvement of HSPG in the neurotoxicity [60]. To evaluate this possibility, we examined the direct interaction of Stan with heparin *in vitro* (Figure 8) on a heparin-Sepharose column in comparison with the N-terminal fragment of apoE3. Following loading, the bound peptide (or protein) was eluted by ionic competition with an increasing concentration of NaCl applied as a linear gradient. Stan displayed tighter binding than the N-terminal fragment of apoE3 in that ~ 600 mM NaCl was required to elute heparin-bound Stan compared with 400 mM required to elute apoE3 N-terminal fragment.

3. Discussion

There is substantial evidence that apoE4 contributes to increased risk of neurological dysfunction and neurodegenerative disease [1,15,68,69]. However, the mechanism through which apoE4 exerts these effects is unknown. The normal role of apoE in neurological function, regardless of isoform, remains enigmatic. Genetic studies in mice have provided evidence that the absence of apoE can be detrimental or beneficial. For example, Laskowitz et al. [38] showed neuroprotective effects of apoE in an animal model of ischemic stroke. Similar results have been found in subsequent studies [30,36,56].

On the other hand, there is some evidence that the presence of apoE can have negative effects. For example, the absence of apoE conferred greater neuroprotection following cycad exposure [66] and longer survival in an SOD model of ALS [51] where increased expression of apoE is associated with neuronal degeneration [25]. Studies in which different isoforms of human apoE are expressed generally support the conclusion that apoE4 has more negative, or less positive, influences on phenotype than apoE3. In the case of transgenic models of Alzheimer's disease, the broad conclusion is that the presence of apoE4 leads to greater amyloid deposition [4,5,29,34], which is considered by many investigators to be the most salient feature of the disease.

Full-length apoE has also been reported to exhibit isoform-specific effects on neurite outgrowth and neuronal viability [27,35,48,54,62]. DeMattos et al. [17] showed that a minimally-lipidated form of apoE3 enhances neurite growth from Neuro-2a cells whereas a similar lipidated form of apoE4 showed no effect. Most evidence indicates that the intact protein is protective against various toxic insults such as amyloid [43]. However, apoE4, in particular, has been reported to be neurotoxic under some conditions [47,48,54,59]. One possibility is that the neurotoxic effects of apoE4 are mediated by proteolytic fragments that include the receptor-binding domain; the same region on which the neurotoxic peptides are based [46,59].

According to this hypothesis, proteolysis of the full-length protein results in the production of fragments with variable degrees of C-terminal truncation. In one study, the major N-terminal fragment of apoE generated by thrombin digestion was found to exhibit isoform-specific neurotoxicity with the apoE4 fragment exhibiting greater toxicity than the apoE3 fragment [45]. Another group has reported that a C-terminal truncated fragment of apoE4 is more toxic than the corresponding apoE3 fragment [31–33]. However, the evidence for the production and accumulation of such fragments in the human brain is inconsistent [12,33,46,67]. A recent report indicates that the incidence of such fragments is not affected by the disease but seems to be higher with apoE3 genotype [19]. Carrette et al. [9] found evidence for apoE fragments in 2 year-old transgenic mouse cortex in which mutant APP is overexpressed by itself or in combination with mutant presenilin 1.

Our previous work has implicated the generation of neurotoxic fragments in those cases where the full-length protein exerts neurotoxicity [59]. In our own studies, as well as the literature, the neurotoxicity of the full-length protein is not consistently detected. The reasons for this variability might include differences in the protein preparation as well as culture conditions. If, the full-length protein is not in itself toxic, but can give rise to neurotoxic fragments, the variability might be partly explained by antagonistic effects of the full-length protein and its fragments, a possibility investigated in this study.

The peptide used for these experiments, a tandem repeat of residues 141-149 of apoE, was first reported by Dyer et al. [18] to have cytostatic effects on mitogen-activated T cells. Subsequent work by Clay et al. [13] demonstrated a cytotoxic effect of the same peptide on IL2-dependent T cells and on embryonic chick sympathetic neurons [16]. The present results confirm previous reports of dose-dependent neurotoxicity. Although low concentrations of the peptide result in slightly enhanced neuronal survival compared with untreated cells, higher concentrations result

in dose-dependent cell death. The relatively steep dose-response curve over approximately a 4-fold range of concentrations indicates that the toxicity represents a rapid and irreversible cellular event, perhaps relating to calcium influx [59,65]. This peptide was also reported to stimulate endocytosis [64] in cultured rat cortical neurons and the present results are consistent with a relatively rapid internalization of the fluorescent peptide over a time course similar to that reported for increased endocytosis.

Although the mechanism of toxicity is unknown, some evidence implicates members of the LDL receptor family and/or cell surface proteoglycans. Initial work indicated that the neurotoxic effect of the peptide is mediated by LRP, based on inhibition of the effect by RAP, an antagonist of LDL receptor family ligands, and an anti-LRP antibody [60]. Subsequent work, however, reported that RAP did not inhibit toxicity of a related peptide [26,49] but did inhibit toxicity associated with the full-length protein. On the other hand, this peptide has been reported to bind LRP with high affinity [14] and the isoform-specific effects of apoE on neurite growth have been reported to be mediated by LRP [50].

If binding to an LDLr-type lipoprotein receptor is involved, it is consistent with evidence that heparin, heparan sulfate and polysaccharides derived from heparan sulfate are also protective [60], because apoE also normally interacts with cell surface HSPG. This interaction is mediated primarily via residues K143 and K146, which are present in Stan. It is likely that the presence of a tandem repeat of K143 and K146 leads to an increased (and therefore higher affinity) ionic interaction with the negatively charged moieties in heparin. In an analogous situation, Fisher and colleagues employed a tandem repeat of the apoE LDLr binding sites on a single polypeptide chain to evaluate the binding with the LDLr [20]. They showed that more than one receptor binding site on a single lipoprotein particle was required for high-affinity binding to the LDLr. The enhancement in binding affinity was attributed to the presence of proximal multivalent ligands. Thus, the toxicity associated with Stan may be related to its high affinity interaction with HSPG. The protective effects of other agents, such as MK-801, cobalt, and potassium chloride seem to implicate pathways that depend on fluxes in ions such as calcium. The signaling effects of apoE and related peptides are complex. For example, Caruso and colleagues [10] reported that apoE4 is more effective than apoE2 or apoE3 in inhibiting Wnt signaling in undifferentiated PC12 cells.

Other peptide sequences derived from the receptor-binding domain of apoE appear to mimic the full-length protein. For example, apoE₁₃₃₋₁₄₉ has been shown to exhibit anti-inflammatory activity [37] and to inhibit $\alpha 7$ nicotinic acetylcholine receptors [23,24]. The C-terminal half of this sequence (141-148) was about as effective in the latter assay as the whole peptide. Other related peptides have been reported to be effective in models of neurological injury such as subarachnoid hemorrhage [22]. This same peptide (apoE₁₃₃₋₁₄₉) has been reported to block NMDA receptors through an interaction with LRP [57]. It is not clear why monomeric presentation of a sequence should result in such opposite effects as that caused by dimeric forms. In general, tandem dimers have been proposed to be more active due to the stability of their alpha-helical structure. Whether these different peptides are acting at different sites or competing for common sites remains to be determined.

The present findings suggest that full-length apoE3 or apoE4 can also protect against this neurotoxic peptide. There are two broad mechanisms that might account for this effect. One is that full-length apoE and the peptide bind non-specifically, thereby reducing the effective concentration of the peptide. However, bovine serum albumin provided only modest protection when used at much higher concentrations and the N-terminal fragment of apoE did not confer protection at concentrations at which the full-length protein was effective. In fact, the apoE4 fragment caused additional toxicity in some experiments. ApoAI, which is an amphipathic apolipoprotein with similarities to apoE, also failed to provide protection.

The other possibility, already alluded to, is that apoE and the peptide exert antagonistic effects through competitive interactions at cell surface receptors such as LRP or HSPG or through activation of different intracellular pathways. The possible interactions of apoE with cell surface receptors are complex. The fact that the peptide is based on the receptor binding domain, as well as its affinity for LRP and heparin, is consistent with a competitive mechanism. However, the extent to which the competition is at the level of receptor binding or to downstream effects secondary to receptor activation or internalization is unknown.

Since the assay used in these experiments involved the use of exogenous application of reagents, the mechanism of toxicity must necessarily involve some interaction with the neuronal cell surface. The fact that expression of apoE fragments in neurons gives rise to a degenerative phenotype [31] suggests that such fragments can exert a neurotoxic effect through intracellular mechanisms. Additional studies will be needed to determine whether the effects of exogenously-applied peptides shares intersects with the mechanisms of endogenous fragments.

In light of the isoform-specific contribution of apoE to the risk of neurodegenerative disease, it is of interest to know whether there are isoform differences in protection against the neurotoxicity of the peptide. Although there was a trend for full-length apoE4 to be more protective than apoE3, the variability across experiments masked any significant effect that might be present. Therefore, any isoform difference, if present, would be modest, at best, at least under the conditions used here. The fact that the N-terminal fragment of apoE3 and apoE4 conferred no protection, and sometimes enhanced toxicity, is also consistent with previous findings that such fragments are likely to be toxic themselves [45]. Differences in the level of expression of apoE isoforms might also be relevant although variations in the production and/or retention of fragments may make it difficult to draw final conclusions.

To the extent that the peptide used here mimics the neurotoxicity of proteolytic fragments of apoE, these results indicate that the presence of full-length apoE may modify the activity of any neurotoxic fragments that might otherwise be present. If so, this could account for the variability that has been reported in some studies regarding the toxicity of apoE. In cases where the full-length protein exceeds the concentration of fragments (or when no fragments are present), little if any toxicity would be expected. If the proportion of fragments is greater, more toxicity might be expected. Furthermore, it is possible that apoE fragments exert dose-dependent effects on survival, similar to the biphasic effects on survival obtained with Stan (Figure 1). The proportion and concentration of fragments relative to the full-length protein may, therefore, be relevant to the role of apoE in neurodegeneration. In fact, the neuroprotective effects observed at low concentrations of the peptide point to the possibility of biphasic effects of apoE fragments, emphasizing the need to determine whether or not such fragments are consistently associated with pathology. Until these possibilities are explored in more detail, it remains unclear to what extent therapeutic strategies that rely on increased expression of apolipoprotein E can be expected to succeed.

4. Experimental Procedure

Cell culture assay

White Leghorn chick embryos (embryonic day 9 or day 10) were dissected in Ham's F12 medium and lumbar sympathetic chain ganglia were removed. Tissue was dissociated by treatment with 0.25% trypsin for 30 minutes and subsequently blocked with 100% FBS for 5 minutes, followed by 3 washes with F12. Neurons were dissociated by gentle trituration with flamed pipets and plated in neurobasal media (NB) on a 96-well poly-dl-ornithine (PORN)-coated plate overnight. The following day, the NB was removed and the appropriate treatments (e.g., Stan or Stan + apoE) in a total volume of 50 μ l were added to each well. These treatments

were left on the cells for 18–20 hours. Treatments were then removed and replaced with F12 and vital dye for imaging (see below). The treatment solutions (extracellular samples) were maintained frozen for subsequent analysis by Western blotting.

Imaging

Dishes with neurons were loaded with vital dye (5-carboxyfluorescein diacetate acetoxyethyl ester) for 30 minutes to 1 hour in F12 media at 37° C. Using a Nikon diaphot microscope, the cells were imaged with the QCapture program and a QImaging Micropublisher 5.0 RTV camera. In addition, cultures were grown directly on cover slips. This was carried out by first coating glass cover slips with PORN and placing them in a 24-well culture dish. Neurons were prepared as described above and plated onto the cover slips in NB for 1–3 days, at which time they were treated for different times with labeled peptide in the presence and absence of candidate inhibitors of peptide toxicity. The cells were then fixed with paraformaldehyde, rinsed, and the cover slips mounted on microscope slides with Fluoromount G (Electron microscopy Sciences, Hatfield, PA). The cells were subsequently imaged with a Zeiss confocal microscope.

Neurotoxicity assay

A fluorescent image was taken of each well using the 4x objective of a Nikon Diaphot microscope. The image was captured through the system described above. Automated counting of cells within the captured image was accomplished with the NIH Image J program using a macro developed by Dr. David B. Pettigrew. Each treatment was carried out on a minimum of 3 wells and the average number of vital dye-stained cells present in each well was calculated for each treatment. Intragroup comparisons were carried out using Kruskal-Wallis (Statview). Sigmoidal models were fit to log transformed group means (Prism). Fit models were compared using F-test. Due to high variability across experiments arising from variations in initial cell plating density, statistical comparisons were based on normalized results in which the number of surviving cells following treatments was expressed as the percentage of surviving cells without treatment.

Immunoblots

Extracellular medium was collected following the 18–20 hour cell treatment. In order to detect intracellular proteins, the remaining cells were initially lysed by freeze-thawing, followed by treatment with 6X Laemmli buffer. Samples were boiled in Laemmli buffer and resolved using SDS-PAGE and transferred onto a PVDF membrane. Membranes were blocked in 10% milk in TBS-T for 1 h on a shaker at 25 °C, and then incubated with goat anti-apoE pAB (Calbiochem), followed by secondary, anti-goat IgG (HRP) (Calbiochem). Blots were developed using ECL-plus.

Heparin binding analysis

The relative ability of Stan to interact with heparan sulfate proteoglycan (HSPG) was assessed in comparison with that of the N-terminal fragment of apoE3 as described earlier [58]. The binding was assessed using a Hi-Trap heparin-Sepharose column (Amersham/GE Healthcare) attached to a ÄKTA FPLC system. A solution containing 100 µg Stan or the N-terminal fragment of apoE3, that has been previously incubated with dithiothreitol (DTT) to reduce any inter molecular disulfide bond, was prepared in 20 mM sodium phosphate, pH 7.4. The solution was injected onto a 1 ml column at a flow rate of 0.5 ml/min. The bound samples were eluted using an increasing salt gradient from 0 to 2 M NaCl in 20 mM sodium phosphate, pH 7.4.

Reagents

ApoE peptide, apoE₍₁₄₁₋₁₄₉₎² (Stan), was prepared as described previously [13]. The fluorescent derivative of Stan, fluorescein- labeled apoE₍₁₄₁₋₁₄₉₎² was obtained from Dr. Curtis Dobson. Full-length apoE was purchased from CalBiochem (San Diego, CA). The N-terminal fragment of apoE3 and apoE4 encompassing residues 1-183 was prepared as described previously [21].

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Figure 1. Stan causes dose-dependent effects on neuronal survival

This dose-response curve shows the effect of increasing concentrations of Stan on embryonic chick sympathetic neuron survival expressed as a percentage of the number of neurons surviving in the absence of peptide. Low concentrations of Stan (.5–2 μM) usually result in increased survival with concentrations above that resulting in dose-dependent neurotoxicity. The results show the average of 5–6 experiments for each concentration with the corresponding standard deviation.

Figure 2. Full-length apoE protects against Stan neurotoxicity

Surviving embryonic chick sympathetic neurons are revealed in these representative fluorescence micrographs after being labeled with a vital dye one day following treatment with the indicated conditions for each panel. The majority of neurons did not survive in the presence of either 8 or 12 μ M of Stan, as shown in the center panels. The presence of full-length apoE3 or apoE4 resulted in less Stan toxicity.

Figure 3. Quantitative analysis of neuroprotection by apoE3 and apoE4 against Stan toxicity
 Analysis of dose response curves of Stan with and without apoE3 or apoE4 reveal an overall treatment group effect (Kruskal-Wallis, $p = 0.0004$). Post hoc analysis was done using pairwise Mann-Whitney. Stan in the presence of apoE3 or apoE4 is significantly different from Stan alone (Stan vs Stan + E3, $p = 0.0004$, Stan vs Stan + E4, $p = 0.0009$, Stan + E3 vs Stan + E4, $p = 0.24$). Analysis did not reveal an apoE isoform difference. Data collected from 3–9 experiments.

Figure 4. Increasing concentrations of full-length apoE3 show increased protection against Stan toxicity

This graph shows the dose-dependent protection of different concentrations of full-length apoE3 against 8 μM Stan toxicity expressed as the percentage of survival obtained in control wells in each experiment. There was no significant effect of apoE3 by itself on neuronal survival at any concentration (top line). The amount of Stan toxicity in the absence of apoE3 is indicated by the dotted line. Data were collected and analyzed from 3 experiments.

Figure 5. Western blot of apoE shows no effect of Stan on uptake or metabolism

Extracellular and intracellular materials were prepared following treatment of neurons with full-length apoE3 in the presence and absence of different concentrations of Stan. Although there is some indication of a low molecular weight fragment of apoE in the extracellular samples, this is not evident for the intracellular samples.

Figure 6. Fluorescein-labeled Stan accumulates in neurons

Fluorescence (upper panels) and DIC microscopy (lower panels) show time-dependent changes in the extent and incidence of fluorescent peptide accumulation and morphological changes in neurons exposed for the times indicated.

Figure 7. Full-length apoE reduces intracellular accumulation of fluorescein-Stan

Cultured neurons were treated for 2 hours with Stan (8 μ M) in the presence and absence of the reagents indicated in the panels. Fluorescence micrographs and their corresponding DIC images are shown side-by-side. Both suramin (20 μ M) and full-length apoE3 (10 μ M) reduced the extent of labeling whereas BSA (25 μ M) had no obvious effect.

Figure 8. Heparin binding interaction of Stan or apoE3 N-terminal fragment
Stan (broken line) or apoE3 N-terminal fragment (solid line) were solubilized in 20 mM sodium phosphate pH 7.4 and applied onto a heparin-Sepharose column in an ÄKTA FPLC system. The column flow rate was 0.5 ml/min. A linear salt gradient consisting of 0–2 M NaCl was applied to elute the bound proteins. Elution of the peptide was monitored at 220 nm and that of the protein at 280 nm.