Structural features of apolipoprotein B synthetic peptides that inhibit lipoprotein(a) assembly

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Abstract Lipoprotein(a) [Lp(a)] is assembled via an initial noncovalent interaction between apolipoprotein B100 (apoB) and apolipoprotein(a) [apo(a)] that facilitates the formation of a disulfide bond between the two proteins. We previously reported that a lysine-rich, α-helical peptide spanning human apoB amino acids 4372–4392 was an effective inhibitor of Lp(a) assembly in vitro. To identify the important structural features required for inhibitory action, new variants of the apoB4372-4392 peptide were investigated. Introduction of a central leucine to proline substitution abolished the α-helical structure of the peptide and disrupted apo(a) binding and inhibition of Lp(a) formation. Substitution of hydrophobic residues in the apoB4372-4392 peptide disrupted apo(a) binding and inhibition of Lp(a) assembly without disrupting the α-helical structure. Substitution of all four lysine residues in the peptide with arginine decreased the IC50 from 40 μM to 5 μM. Complexing of the arginine-substituted peptide to dimyristoylphosphatidylcholine improved its activity further, yielding an IC50 of 1 μM. We conclude that the α-helical structure of apoB4372-4392 in combination with hydrophobic residues at the lipid/water interface, is crucial for its interaction with apo(a). Furthermore, the interaction of apoB4372-4392 with apo(a) is not lysine specific, because substitutions with arginine result in a more effective inhibitor.—Sharp, R. J., M. A. Perugini, S. M. Marcovina, and S. P. A. McCormick. Structural features of apolipoprotein B synthetic peptides that inhibit lipoprotein(a) assembly. J. Lipid Res. 2004. 45: 2227–2234.

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Lipoprotein(a) [Lp(a)] is an atherogenic lipoprotein comprised of apolipoprotein(a) [apo(a)] attached to the apolipoprotein B100 (apoB) of a LDL (1). Large clinical trials have identified high plasma levels of Lp(a) as a risk factor for developing heart disease (2–4). The deposition of Lp(a) in vascular lesions has been well documented in humans (5, 6) and in animal models (7). Unfortunately, Lp(a) has proven resistant to traditional lipid-lowering therapies (8, 9), except high doses of niacin (10). Estrogen dramatically reduces Lp(a) levels (11), but a safe and effective agent for lowering Lp(a) levels has yet to be developed.

The majority of evidence suggests that Lp(a) assembly occurs in circulation (12) after secretion of apo(a) from the liver and metabolism of VLDL to LDL in the circulation. Lp(a) assembly is thought to be a two-step process (13, 14) with noncovalent interactions between apo(a) and apoB preceding the formation of the disulfide bond between apo(a) Cys4057 (13, 15) and apoB Cys4326 (16, 17). The two-step model of Lp(a) assembly was confirmed in a study of a human apoB100 mutant lacking Cys4326, which still showed binding to apo(a), despite being unable to form the disulfide bond (18).

Recent research has focused on characterizing the apoB residues involved in the noncovalent interaction with apo(a). Lysine residues on apoB have been implicated in the noncovalent interaction with apo(a), because lysine analogues disrupt Lp(a) assembly in vitro (19–21) and deletion of lysine-binding sites in apo(a) kringles IV domains interferes with Lp(a) formation (22–24). A number of apoB sequences that noncovalently bind apo(a) have been reported. Becker et al. (25) identified an apoB lysine residue in the N terminus, apoB680, that mediates the noncovalent binding of an apoB18 fragment to apo(a); in their study, a synthetic peptide spanning the Lys680 residue was shown to inhibit Lp(a) formation at similar concentrations to lysine analogues.

Sequences in the carboxyl terminus of apoB have also been implicated in the noncovalent interaction with apo(a).

Abbreviations: apo(a), apolipoprotein(a); apoB, apolipoprotein B100; CD, circular dichroism; DMPC, dimyristoylphosphatidylcholine; Lp(a), lipoprotein(a).

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We recently reported that a synthetic peptide spanning apoB amino acids 4372–4392, bound apo(a) and inhibited Lp(a) assembly in vitro (26). Note that the apoB_{4372-4392} peptide was 250-fold more effective than the lysine analogue, e-aminocaprylic acid, at inhibiting Lp(a) assembly (26). This work suggested that the apoB_{4372-4392} sequence constitutes an apo(a) binding site that is important for Lp(a) inhibitory studies. 

**EXPERIMENTAL PROCEDURES**

**Peptides**

Five new synthetic apoB peptides based on the human apo B4372–4392 sequence were synthesized by Chiron Mimotopes using an Applied Biosystems model 430A synthesizer. Peptides were purified by HPLC and stock solutions solubilized in Milli-Q water for inhibitory studies. Table 1 gives the amino acid sequences and associated characteristics of the new series of peptides. The importance of the α-helical structure for activity was tested with the apoB_{4372-92ARG} peptide, which contains lysine to alanine substitutions at Lys4372 and Lys4392, respectively. The importance of a cluster of four conserved hydrophobic residues surrounding the Lys4392 residue was tested in the apoB_{4372–4392} sequence. To elucidate the key structural features responsible for the inhibitory properties of the apoB_{4372-4392} peptide, a series of apoB peptides based on the apoB 4372–4392 sequence were designed and tested for their ability to bind apo(a) and inhibit Lp(a) assembly.

**CD spectroscopy**

The CD spectra of the apoB peptides in the absence and presence of DMPC were recorded at 25°C on an Aviv 62DS CD spectrophotometer using a 1 nm pathlength quartz cuvette. Spectra were collected between the wavelengths of 195 nm and 250 nm, with a step size of 0.5 nm and a slit bandwidth of 1.5 nm. The temperature was maintained at 30°C, above the phase transition temperature of DMPC (~24°C), using a jacketed vial with a circulating water bath (30). The vesicles were centrifuged at 2,250 × g for 5 min to pellet titanium originating from the sonicator probe. Phospholipid concentrations were determined at 20°C using an enzymatic phospholipid assay kit (Roche, Australia).

**Preparation of phospholipid vesicles**

Small unilamellar vesicles of DMPC (Sigma-Aldrich, St. Louis, MO) were prepared using a method similar to that described by New (29). The DMPC was suspended in 10 mM Tris-HCl, pH 7.4, and sonicated using a 9.5 mm probe (Soniprep 150, MSE Scientific Instruments, Sussex, England) at 20 MHz for 10 × 60 s with 30 s breaks in between. The temperature was maintained at 30°C, above the phase transition temperature of DMPC (~24°C), using a jacketed vial with a circulating water bath (30). The vesicles were centrifuged at 2,250 × g for 5 min to pellet titanium originating from the sonicator probe. Phospholipid concentrations were determined at 20°C using an enzymatic phospholipid assay kit (Roche, Australia).

**TABLE 1. Characteristics of the ApoB_{4372-4392} peptide series**

| Peptide name       | Amino acid sequence | Purity (%) | Mₐ (dmol cm⁻²) | % α-Helix² | -DMPC | +DMPC |
|--------------------|---------------------|------------|-----------------|------------|------|-------|
| ApoB_{4372-4392}   | KYELEEEKIVSLKNLVALK | 86         | 2,507           |            | 68   | 88    |
| ApoB_{4372-92ARG}  | KYELEEKIVSLKNLVALK | 95         | 4,491           | 1          | 6    | 1     |
| ApoB_{4372-92HYDRO}| KYELEEEKIVSLKNLVALK| 89         | 2,540           | 63         | 49   | 66    |
| ApoB_{4372-92ARG}  | KYELEEEKIVSLKNLVALK| 95         | 2,540           | 87         | 66   | 87    |
| ApoB_{4372-92HYDRO}| KYELEEEKIVSLKNLVALK| 90         | 2,317           | 94         | 72   | 100   |

DMPC, dimyristoylphosphatidylcholine; Mₐ, relative molecular mass.

*Residues altered from the original apoB_{4372-4392} peptide are shown in lowercase boldface type.

The % α-helix was calculated using equation 1 and the [θ]_{222nm} obtained from the CD spectra; the average error was found to be ±2% for repeated scans of the same peptide.
Apo(a) binding studies

Each peptide was evaluated for its ability to bind apo(a) using a solid-phase enzyme-linked ligand sorbent assay (26). Briefly, 96-well polystyrene microtiter plates (Costar®, Corning Inc., Corning, NY) were coated with 5 µg per well of each apoB peptide. Apo(a) transgenic mouse plasma, and wild-type mouse plasma (negative control) were diluted 1 in 10 and 50 µl aliquots and incubated on the peptide-coated plates for 90 min at room temperature to allow binding of apo(a) to the peptides. Bound apo(a) was detected with the HRP-labeled MAb-a5 antibody (33), washed, and then developed with substrate solution [O-phenylenediamine dihydrochloride and hydrogen peroxide (Sigma-Aldrich)]. The level of background binding was determined from the wild-type plasma sample and this was subtracted from the apo(a) binding curves.

In vitro Lp(a) formation assays

The peptides were tested for their ability to act as inhibitors of Lp(a) assembly as described by Sharp et al. (26). Plasma (1 µl) from an apo(a) transgenic mouse was incubated with plasma (2 µl) from a human apoB transgenic mouse for 3 h at 37°C in the presence of increasing amounts (1–400 µM) of each apoB peptide. The amount of Lp(a) formed in each incubation was assessed after aliquots were size-fractionated on SDS/4% polyacrylamide gels under nonreducing conditions and the separated proteins quantified by an Lp(a)-specific ELISA, as detailed by Sharp et al. (26). Background absorbance was determined from incubations containing human apoB only and this was subtracted from all data points. The percent Lp(a) formed in each incubation was calculated relative to the incubation containing no inhibitor.

RESULTS

The WHEEL program (34) was used to produce a helical wheel diagram of the apoB\textsubscript{4372-4392} peptide (Fig. 1). Amino acids targeted for substitution in the new series of peptides are highlighted in Fig. 1. The secondary structures of the five human apoB peptides were determined by CD spectroscopy both in the absence and in the presence of DMPC and each was compared with the control apoB\textsubscript{4372-4392} peptide (Fig. 2). Using equation 1, the percent α-helical structure for each peptide was calculated from the ellipticity value at 222 nm and the data are reported in Table 1. The spectra for the control apoB\textsubscript{4372-4392} peptide (Fig. 2A) showed the characteristic double minima at 208 and 222 nm indicative of α-helical structure. The α-helicity of apoB\textsubscript{4372-4392} was further induced by the binding of the peptide to DMPC, as previously reported (26). As predicted, the proline-substituted peptide, apoB\textsubscript{4383P}, had lost all secondary structure and adopted largely random structure, as indicated by a single minimum at 205 nm (Fig. 2B). Furthermore, the addition of DMPC had little effect on the structure of this peptide (Fig. 2B). Substitution of the N-terminal lysine residue to alanine resulted in a 19% decrease in α helical content in the apoB\textsubscript{K4372A} peptide compared with apoB\textsubscript{4372-4392} (Fig. 2C). The addition of DMPC increased the helical content of the apoB\textsubscript{K4372A} peptide by 14%; however, the DMPC-complexed peptide was 25% less helical than apoB\textsubscript{4372-4392} in the presence of the same concentration of phospholipid. Substitution of the C-terminal lysine residue for alanine did not affect α-helical content; apoB\textsubscript{K4392A} showed a secondary structure similar to apoB\textsubscript{4372-4392} both in the absence and in the presence of DMPC (Fig. 2D). The hydrophobic replacement peptide, apoB\textsubscript{4372-4392HYDRO}, was slightly more helical than the original peptide, both in the absence (72% helical) and in the presence (94% helical) of DMPC (Fig. 2E).

The ability of each new human apoB peptide to bind apo(a) was assessed using a solid-phase binding assay (Fig. 3). The original apoB\textsubscript{4372-4392} peptide had previously been shown to bind apo(a) in a saturable manner (26). Both the apoB\textsubscript{K4372A} and apoB\textsubscript{K4392A} peptides showed minimal binding to apo(a) (Fig. 3). The apoB\textsubscript{4383P} and apoB\textsubscript{4372-4392HYDRO} peptides did not demonstrate any apo(a) binding beyond background levels. The apoB\textsubscript{4372-4392ARG} peptide was the only peptide shown to effectively bind apo(a) from transgenic mouse plasma. ApoB\textsubscript{4372-4392ARG} bound apo(a) to a saturable level in a manner similar to that seen for the original apoB\textsubscript{4372-4392} peptide (26).
The capacity of each apoB peptide in Table 1 to inhibit Lp(a) assembly in vitro was investigated. Increasing amounts of each peptide were incubated with fixed amounts of apo(a) and human apoB in an Lp(a) formation assay. The amount of Lp(a) formed was visualized by Western blot analysis (Fig. 4A–F). The inhibitory effect of the apoB4372-4392 control peptide is shown in Fig. 4A. Compared with the control (Fig. 4A), the apoB4383P peptide (Fig. 4B) had little effect on Lp(a) assembly in vitro, suggesting that the loss in α-helical structure had significantly reduced its ability to inhibit Lp(a) assembly. The apoB K4372A peptide (Fig. 4C) showed only a slight disruption to Lp(a) assembly, and apoB K4392A (Fig. 4D) showed very little effect. The apoB 4372-92HYDRO peptide also showed no effect on Lp(a) assembly, despite having retained its α-helical structure (Fig. 4E). The apoB 4372-92ARG peptide also showed no effect on Lp(a) assembly, despite having retained its α-helical structure (Fig. 4F). The apoB 4372-92ARG peptide proved to be a highly effective inhibitor of Lp(a) assembly. In the incubations containing apoB 4372-92ARG, Lp(a) levels decreased with increasing amounts of the peptide, and a corresponding increase in free apo(a) was seen (Fig. 4F). Indeed, the apoB 4372-92ARG peptide was more effective than the original apoB 4372-4392 peptide as an inhibitor, showing complete inhibition of Lp(a) assembly at a concentration of 25 μM (compared with 100 μM for apoB 4372-4392).

In addition to Western blot analyses, the amount of Lp(a) formed in each incubation was quantified by an Lp(a) ELISA and the results were used to determine IC50 values for each of the peptides (Fig. 5). The original apoB 4372-4392 peptide showed an IC50 value of 40 μM, as previously reported (26). The apoB 4383P peptide had little inhibitory capacity, with an IC50 value > 400 μM. The hydrophobic replacement peptide, apoB 4372-92HYDRO, also showed very little inhibitory capacity, with an IC50 value > 400 μM, whereas both apoB K4372A and apoB K4392A showed similar IC50 values of approximately 100 μM. By contrast, the arginine-substituted peptide, apoB 4372-92ARG, was the most potent inhibitor of Lp(a) formation, with an IC50 of 5 μM, 84-fold more effective than the apoB 4372-4392 peptide. The inhibi-

Fig. 2. Circular dichroism (CD) spectra of the apoB 4372-92 peptide variants. A: apoB 4372-4392. B: apoB 4383P. C: apoB K4372A. D: apoB K4392A. E: apoB 4372-92HYDRO. F: apoB 4372-92ARG. The CD spectra of the variant apoB peptides (150 μg/ml, dissolved in 10 mM Tris, pH 7.4) in the presence (solid symbols) and absence (open symbols) of dimyristoylphosphatidylcholine (DMPC) vesicles are shown compared with the original apoB 4372-4392 peptide. Spectra were recorded on an Aviv62DS spectrophotometer at 25°C using a step size of 0.5 nm and signal averaging time of 1 s. The mean residue ellipticity (θ) is plotted as a function of wavelength (nm) for each peptide.

Fig. 3. Binding of the apoB 4372-92 peptides to apo(a). The binding capacity of each apoB peptide was measured using a solid-phase enzyme-linked ligand sorbent assay assay. Peptides were immobilized on microtiter plates and incubated with increasing amounts of apo(a) contained in diluted apo(a) transgenic mouse plasma. Bound apo(a) was detected with the MAb-a5 antibody. Data points represent the average of the absorbance values obtained from quadruplicate incubations. Error bars indicate the standard deviation.
tory capacity of a murine peptide spanning apoB 4372–4392, apoB4372-92MURINE, which shows conservation of two of the four lysines in the human sequence and an arginine at position 4392, was also tested. ApoB4372-92MURINE was found to inhibit Lp(a) similarly to the human peptide, with an IC50 value of 45 μM.

As apoB4372-92ARG proved to be the only peptide in the new series effective at inhibiting Lp(a) assembly, further studies were performed to investigate the inhibitory capacity of this peptide in the presence of phospholipid. The addition of DMPC to the apoB4372-4392 peptide had previously been shown to improve the effectiveness of the peptide as an inhibitor of Lp(a) assembly (26). Similarly, we show that apoB4372-92ARG in the presence of DMPC im-

Fig. 4. Inhibition of lipoprotein(a) [Lp(a)] formation by apoB4372-92 peptides. A: apoB4372-4392. B: apoB4383P. C: apoB4372A. D: apoB4372HYDRO. E: apoB4372-92HYDRO. F: apoB4372-92ARG. Increasing amounts of each apoB peptide were added to incubations containing human apo(a) transgenic mouse plasma and human apoB transgenic mouse plasma. The original apoB4372-4392 peptide was included as a control. The amount of Lp(a) formed in each incubation was assessed by separation of the incubation mix on SDS/4% polyacrylamide gels under nonreducing conditions and Western blot analysis with the MAb-a5 antibody.

Fig. 5. Inhibitory capacity of the apoB4372-92 peptides. Increasing amounts of each apoB peptide were added to incubations containing human apo(a) transgenic mouse plasma and human apoB transgenic mouse plasma. The apoB4372-4392 peptide was included as a control. The amount of Lp(a) formed in each incubation was quantified using an Lp(a)-specific sandwich ELISA. Each incubation was measured in quadruplicate and the average value was expressed as a percentage of Lp(a) formed relative to the incubation containing no inhibitor. Error bars indicate the standard deviation. The IC50 values for each of the peptides are shown.
proved its inhibitory capacity by 5-fold, shifting the IC$_{50}$ value from 5 µM to 1 µM (Fig. 6). This is supported by Western blot analyses (Fig. 6, inset) showing that the DMPC-bound apoB$_{4372-92ARG}$ peptide caused complete inhibition of Lp(a) assembly at a concentration of 5 µM. Complexing of the less effective apoB$_{K4372A}$ and apoB$_{K4392A}$ peptides with DMPC showed a similar decrease in IC$_{50}$ to that seen with the apoB$_{4372-4392}$ and apoB$_{4372-92ARG}$ peptides (data not shown), suggesting that complexing to DMPC has a general stabilizing effect on peptide structure that results in a more effective inhibitor.

DISCUSSION

Although elevated levels of Lp(a) have been identified as an independent risk factor for developing vascular disease, there is currently no safe and effective Lp(a)-lowering therapy available. An understanding of the molecular interactions between apo(a) and apoB that initiate Lp(a) formation may lead to the design of novel Lp(a)-lowering agents. Previous research has established that apo(a) lysine-binding domains most likely interact with lysine residues on apoB to facilitate Lp(a) formation. A recent study indicates that the lysine-binding domains in apo(a) kringle IV$_5$ and IV$_6$ are the two most likely candidates to be involved in Lp(a) assembly (35). The apo(a) lysine-binding domains consist of an anionic center followed by a hydrophobic trough involving four hydrophobic residues. Lysine analogues interact with the pocket via the amino group of their side chain, making an ion-pair interaction with the anionic center; the aliphatic backbone interacts with the hydrophobic trough (36, 37).

We are interested in defining the apoB residues involved in the noncovalent interaction with apo(a). The current study aimed to identify the key structural features of a synthetic apoB peptide spanning amino acids 4372–4392, which was recently reported to bind apo(a) and effectively inhibit Lp(a) assembly in vitro (26). The apoB$_{4372–4392}$ sequence is predicted to lie within a class A amphipathic helix with lipid-binding capacity (38). Indeed, CD spectroscopy studies confirmed that the apoB$_{4372-4392}$ peptide is α-helical in nature and binds phospholipid in a saturable manner (26). The importance of the α-helical structure for the inhibitory action of apoB$_{4372-4392}$ was tested in this study by replacing the central leucine at position 4383 with proline to create the apoB$_{L4383P}$ peptide. CD spectroscopy of apoB$_{L4383P}$ showed a complete loss of α-helix structure.
with the L4383P substitution, which converted the peptide into a random coil structure (Fig. 2B). As a result, apo(a) binding studies showed that the peptide had lost the capacity to bind apo(a) (Fig. 3). Not surprisingly, the apoB<sub>4372-4392</sub> peptide also lost the ability to inhibit Lp(a) assembly showing an IC<sub>50</sub> > 400 μM. These data support the notion that the helical nature of the peptide is vital for its inhibitory capacity.

We also tested the importance of individual arginine residues in the apoB<sub>4372–4392</sub> sequence on Lp(a) assembly inhibition. We speculated that a lysine residue in close proximity to hydrophobic residues at the lipid/water interface could be important (Fig. 1). The Lys<sub>4372</sub> and Lys<sub>4392</sub> residues are highly conserved in species capable of binding apo(a) (27) and both lie close to hydrophobic residues at the lipid/aqueous interface. Relative to the control, CD spectroscopy studies showed a substantial reduction in the propensity for apoB<sub>K4372A</sub> to adopt an α-helical structure both in the absence and in the presence of DMPC (Fig. 2). In addition, only minimal binding of the apoB<sub>K4372A</sub> peptide to apo(a) was detected (Fig. 3); this was accompanied by a significant loss in the inhibition of Lp(a) assembly (Fig. 4). The apoB<sub>K4372A</sub> peptide had an IC<sub>50</sub> of 100 μM (Fig. 5), which is 2.5-fold higher than the original apoB<sub>4372–4392</sub> peptide. As apoB<sub>K4372A</sub> had lost some α-helical structure (Fig. 2C), it is possible that this may account for its loss of activity. Substitution of the Lys<sub>4392</sub> residue had no effect on secondary structure, but resulted in a similar loss of binding and inhibitory capacity to the apoB<sub>K4372A</sub> peptide, with an IC<sub>50</sub> of 100 μM. As the secondary structure of apoB<sub>4392A</sub> was unaffected, it is most likely the loss of positive charge at this position accounted for its reduced activity. We therefore propose that Lys<sub>4392</sub> is a likely candidate for binding to the anionic lysine binding pocket of apo(a). Because the apoB<sub>4392A</sub> peptide still shows inhibitory activity, however, other residues besides Lys<sub>4392</sub> must be important in binding apo(a).

The importance of a cluster of hydrophobic residues adjacent to the Lys<sub>4392</sub> residue was tested by creating a peptide, apoB<sub>4372–92HYDRO</sub>, in which four hydrophobic residues located on one face of the helix were replaced with alanines (Fig. 1; Table 1). Note that the apoB<sub>4372–92HYDRO</sub> peptide showed a complete loss of apo(a) binding and inhibitory capacity, with an IC<sub>50</sub> > 400 μM. Because the secondary structure of this peptide has remained α-helical (Fig. 2E), the loss in activity is likely due to the loss of hydrophobic residues, indicating that both the primary and secondary structure of the apoB<sub>4372–4392</sub> peptide are important for its inhibitory activity. We conclude that the substituted hydrophobic residues form part of the apo(a) binding motif in the apoB<sub>4372–4392</sub> peptide. One or more of these hydrophobic residues presumably help to anchor the peptide into the binding pocket along with the Lys<sub>4392</sub> residue.

Finally, to test whether the interaction of the apoB<sub>4372–4392</sub> peptide with apo(a) was specific to lysine residues, we replaced all four lysine residues in the apoB<sub>4372–4392</sub> peptide with arginines to create apoB<sub>4372–92ARG</sub>. CD spectroscopy studies of apoB<sub>4372–92ARG</sub> showed the peptide to be α-helical, with a significant increase in helical content in the presence of phospholipid (Fig. 2F). Apo(a) binding studies showed that apoB<sub>4372–92ARG</sub> specifically bound to apo(a) (Fig. 3). The ELISA analysis determined an IC<sub>50</sub> value for apoB<sub>4372–92ARG</sub> of 5 μM (Fig. 5) which is 8-fold more effective than the original apoB<sub>4372–4392</sub> peptide at inhibiting Lp(a) assembly. Furthermore, addition of the apoB<sub>4372–92ARG</sub> peptide to DMPC increased the inhibitory capacity 5-fold (IC<sub>50</sub> = 1 μM), resulting in a highly effective inhibitor. The results with the apoB<sub>4372–92ARG</sub> peptide suggest that the apo(a) lysine binding domains may have a stronger affinity for arginines than lysines. This is consistent with data showing that the addition of arginine can inhibit recombinant apo(a) derivatives from binding native LDL particles in vitro (39). We therefore propose that the guanidinium side chain of arginine may fit more tightly into the anionic binding pocket of apo(a). Note that the murine sequence contains an arginine at position 4392. A peptide spanning mouse apoB<sub>4372–4392</sub> was tested and found to inhibit Lp(a) formation, with an IC<sub>50</sub> similar to the human apoB<sub>4372–4392</sub> peptide (Fig. 7). Future studies investigating the importance of individual lysine to arginine substitutions in the context of the human apoB<sub>4372–4392</sub> sequence may help pinpoint the key lysine residue important for the interaction with the apo(a) lysine-binding pocket.

In summary, this study has determined several structural features that are responsible for the inhibitory action of the apoB<sub>4372–4392</sub> peptide on Lp(a) assembly. First, we show that the α-helical nature of this sequence is critical for inhibition, as demonstrated with the L4383P peptide, which shows a loss of helical structure coinciding with a loss in activity. This is also supported by data showing that the addition of phospholipid, which stabilizes the helical structure of the apoB<sub>4372–4392</sub> peptide, enhances the inhibition of Lp(a) assembly (26). Second, we show using the apoB<sub>K4392A</sub> and apoB<sub>4372–92HYDRO</sub> peptides that a positive charge at position 4392, along with hydrophobic residues at the interface of the amphipathic helix, is important for the interaction with apo(a). Finally, we show that substitutions of lysines for arginines in the sequence increases the inhibitory capacity of the peptide, suggesting a tighter fit of arginine in the anionic apo(a) binding cavity. The apoB<sub>4372–4392</sub> peptide has proved to be an excellent model for studying the nature of the noncovalent interactions between apoB and apo(a) in Lp(a) assembly and thus provides scope for the design of novel inhibitors of Lp(a) assembly.

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