Communication

Apolipoprotein A-I Binds and Inhibits the Human Antibacterial/Cytotoxic Peptide LL-37*

(Received for publication, September 11, 1998)

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The antibacterial and cytotoxic activity of the human cathelicidin peptide LL-37 is inhibited by plasma. Because LL-37 does not undergo rapid degradation in human plasma, we postulated that this inhibition results from binding of LL-37 to unidentified proteins. An LL-37 binding plasma protein has now been isolated by affinity chromatography. SDS-polyacrylamide gel electrophoresis of proteins that bound to an LL-37 column revealed one band with a molecular mass of about 26 kDa, and amino acid sequence analysis identified the protein as apolipoprotein A-I (apoA-I). Biomolecular interaction analysis using surface plasmon resonance showed that LL-37 and isolated apoA-I bind with an apparent $K_d$ in the low micromolar range. 50 μM of apoA-I inhibits the antibacterial activity of 50 μM LL-37 by about 50% of the inhibition exhibited by plasma. In addition, anti-apoA-I IgG completely blocks the plasma inhibition of LL-37 antibacterial activity up to a peptide concentration of 25 μM and blocks most of the plasma inhibition at higher LL-37 concentrations. These results indicate that apoA-I is the main LL-37 binding protein in human plasma and may work as a scavenger of LL-37, thus suggesting a novel mechanism involved in the regulation of a cathelicidin peptide.

Naturally occurring antibacterial peptides are widespread and play an important role in host defense (1). These peptides are derived from gene-encoded precursors by proteolysis. Cathelicidins constitute one family of such peptides that has been identified in bovine, porcine, rabbit, mouse, ovine, and human tissues (2–4). Cathelicidins have a conserved N-terminal domain. The sequence of the proregion is similar to cathelicidin antimicrobial peptide gene is up-regulated during inflammatory skin disorders, whereas no expression was detected in normal skin (9). Furthermore, the gene is highly expressed in lung epithelia, and significant expression is also found in other epithelial tissues like the gastrointestinal tract (10), suggesting that LL-37 participates in the human first line of defense. LL-37 is highly cationic and transforms from an unordered to amphipathic α-helical structure in an anion-, pH-, and concentration-dependent manner; the antibacterial activity of LL-37 correlates with the α-helical content (11).

The minimal inhibitory concentration of LL-37 against Escherichia coli D21 is 5 μM, and LL-37 down to 15 μM is cytotoxic to eukaryotic cells (11). Therefore it seems likely that released LL-37 is harmful to host cells. However, in human plasma both the antibacterial and cytotoxic activity of LL-37 is inhibited, which is not caused by proteolysis (11). We have now isolated the main LL-37 binding protein from human plasma and identified it as apolipoprotein A-I (apoA-I). Investigations of the interactions between LL-37 and apoA-I suggest that apoA-I can function as a scavenger of LL-37 and thereby prevent host cell damage.

EXPERIMENTAL PROCEDURES

Materials—Streptavidin-agarose, human apoA-I, human albumin, and egg phosphatidylcholine (PC) were from Sigma. Sheep anti-human apoA-I IgG was from The Binding Site. Human plasma was from the Department of Transfusion Medicine at Karolinska Hospital. Phast gels were from Pharmacia, and Sep-Pak cartridges were from Waters. All chemicals for peptide synthesis were from Perkin-Elmer.

Peptide Synthesis—Biotinyl-Lys(Gly)LL-37 (biotinylated LL-37) was synthesized with tert-butylxycarbonyl chemistry in an ABI 430A peptide synthesizer (Perkin-Elmer), starting from 1 g of resin-bound LL-37 (11). First three Gly residues were added, and thereafter N'-biotinyl-Lys (Bachem) dissolved in dimethylformamide was added using double coupling. The protecting groups were removed, and the peptide was released from the resin by cleavage in anhydrous hydrogen fluoride/anisole/methanesulfide (10:1:1 v/v/v) for 80 min at 0 °C. After removal of scavenger and protecting groups with diethyl ether, the peptide was recovered in 30% acetic acid and lyophilized. Biotinylated LL-37 was isolated by reversed-phase HPLC using a C18 column and a linear gradient of acetonitrile in aqueous 0.1% trifluoroacetic acid. The mass of the purified peptide determined by matrix-assisted laser desorption/ionization mass spectrometry was 5023 Da (calculated 5019 Da). The antibacterial activity of biotinylated LL-37 is identical to that of LL-37, as determined by the inhibition zone assay (see below). The secondary structure of biotinylated LL-37 in 50 mM sodium phosphate buffer, pH 7.4 or pH 5.0, is indistinguishable from that of LL-37, as judged by CD spectroscopy.

Isolation and Analysis of LL-37 Binding Proteins from Human Plasma—Biotinylated LL-37 (50 nmol) in 1 ml of 0.1 mM sodium phosphate buffer, pH 7.4, was mixed with 1 ml of streptavidin-agarose suspension. The mixture was incubated at room temperature for 20 min and then
poured into a glass column (inner diameter, 0.6 cm). After equilibration with 0.1 M sodium phosphate buffer, pH 7.4, 2 ml of human plasma was added, and the column was incubated for 20 min. Thereafter the plasma was eluted, the column was washed with 10 ml of 0.1 M sodium phosphate, pH 7.4, and finally eluted with 2 ml of 0.1 M sodium phosphate buffer, pH 5.0. The low pH eluate was desalted on a reversed-phase Sep-Pak C18 column (125 Å pore size) equilibrated with 0.1% trifluoroacetic acid. After washing with 0.1% trifluoroacetic acid, the protein was eluted with 80% acetonitrile/0.1% trifluoroacetic acid. The Sep-Pak eluate was analyzed by SDS-PAGE and sequence analysis. SDS-PAGE was performed in 10–15% gradient gels with the Phast-system (Pharmacia). Amino acid sequence analysis was carried out in an ABI 494 Protein Sequencer (Perkin-Elmer), and phenylthiohydantoin derivatives were identified by HPLC.

**Studies of Plasma and ApoA-I Effects on LL-37 Antibacterial Activity**—For antibacterial assay, LL-37 was dissolved at 25–220 μM in different solutions, including (i) water, (ii) human plasma, (iii) 50 μM apoA-I in buffer A (50 mM sodium phosphate buffer, pH 7.4), (iv) 670 μM albumin in buffer A, (v) apoA-I (50 μM), and albumin (670 μM) in buffer A, (vi) PC (0.3–1.5 mM) in buffer A, (vii) apoA-I (35 μM) and PC (600 μM) in buffer A, (viii) human plasma (which contains 35–70 μM apoA-I concentration in human plasma) applied) 3 different solutions, including (i) water, (ii) human plasma, (iii) 50 μM apoA-I in buffer A, (iv) 670 μM albumin in buffer A, (v) apoA-I (50 μM), and albumin (670 μM) in buffer A, (vi) PC (0.3–1.5 mM) in buffer A, (vii) apoA-I (35 μM) and PC (600 μM) in buffer A, (viii) human plasma (which contains 35–70 μM apoA-I concentration in human plasma) applied). A continuous flow (30 °C) was maintained over the sensor surface. ApoA-I was diluted in buffer to concentrations from 2.5 to 15 μM. Surfactant P20 (Biacore AB), pH 7.3, was maintained over the sensor surface. LL-37 (and biotinylated LL-37) still exhibits 70% of the helical content observed at pH 7.4. Therefore pH 5.0 buffer was chosen for elution because at this pH protein side chain carboxylate anions (presumed to be involved in charge interactions with polycationic LL-37) start to get protonated, but xylose-containing glycosylated apoA-I (Fig. 2). The LL-37 binding protein is apolipoprotein A-I. Amino acid sequences of human apoA-I and that determined of the LL-37 binding protein were compared. The sequence is identical to that of human apoA-I residues 1–20. Residues that were not unambiguously determined are indicated by X. The Pro amount was 19 pmol in cycle 3 and 17 pmol in cycle 4, strongly indicating Pro also at position 4. At cycle 6 only Ser increases, as judged by visual inspection of the chromatogram, but integration of the peak area does not unambiguously support this assignment.

**ApoA-I Binds to Immobilized LL-37**—Human plasma proteins that bind to biotinylated LL-37 were purified by affinity chromatography. The bound proteins were eluted from the column by 0.1 M sodium phosphate buffer, pH 5.0. pH 5.0 was chosen for elution because at this pH plasma side chain carboxylate anions (presumed to be involved in charge interactions with polycationic LL-37) start to get protonated, but LL-37 (and biotinylated LL-37) still exhibits 70% of the helical content observed at pH 7.4. Therefore pH 5.0 buffer was expected to decrease charge interactions between LL-37 and bound protein while retaining a mainly helical conformation of the bound protein. LL-37 Inhibition by ApoA-I—To find out whether apoA-I isolated from human plasma inhibits LL-37 antibacterial activity, LL-37 at different concentrations was mixed with 50 μM apoA-I, and the activity against E. coli D21 was analyzed. At 220 μM of LL-37, apoA-I exhibited 30% of the inhibitory capacity of plasma, whereas at 50 μM of LL-37, 50% of the plasma inhibition was reached, as determined from the inhibition zone diameters, where a doubled LL-37 concentration yields an increase in zone diameter of 1 mm. The discrepancy in inhibitory capacity between isolated apoA-I and plasma could be caused by apoA-I being removed from its plasma environment or by the existence of additional LL-37 binding plasma proteins not detected by affinity chromatography. The following two control experiments indicate that the former alternative (isolated apoA-I being less effective than apoA-I in plasma) is the most likely explanation: (i) Addition of anti-apoA-I IgG to human plasma restores the LL-37 antibacterial activity to that observed in water (Fig. 3). This strongly indicates that no other plasma protein contributes significantly to plasma inhibition of LL-37. The incomplete recovery at high
Ka 

tained data were used for calculation of apparent association between LL-37 and apoA-I. An overlay plot of sensorgrams for were studied by surface plasmon resonance detection using thus possible that LL-37-phospholipid interactions can contrib-

ute partly inhibit the antibacterial activity of LL-37. It is 

clude that apoA-I is the main, or only, LL-37 binding protein in 

not affect LL-37 antibacterial activity, and addition of albumin 

also with phospholipids (see below). (ii) Albumin at 670 

m 

is 13 mm.

LL-37 concentrations (Fig. 3) may be caused by LL-37 compet-

ting with antibodies for apoA-I binding or by LL-37 interacting 

also with phospholipids (see below). (ii) Albumin at 670 

m 

not affect LL-37 antibacterial activity, and addition of albumin to 

apoA-I does not increase the inhibitory capacity. We con-

clude that apoA-I is the main, or only, LL-37 binding protein in 

human plasma. However, PCapoA-I complexes as well as PC 

alone partly inhibit the antibacterial activity of LL-37. It is 

thus possible that LL-37-phospholipid interactions can contrib-

t to the plasma inhibition of LL-37.

ApoA-I Binds LL-37 with a Low Micromolar Dissocia-

tion Constant—Real time interactions between LL-37 and apoA-I 

were studied by surface plasmon resonance detection using 

immobilized biotinylated LL-37. Confirming the results from 

affinity chromatography, biosensor studies showed a binding 

between LL-37 and apoA-I. An overlay plot of sensorgrams for 

different concentrations of apoA-I is shown in Fig. 4. The 

obtained data were used for calculation of apparent association 

(Ka ) and dissociation (KD) constants. A 1:1 interaction model 

was found to be the most relevant, as judged from the residuals 

between fitted curves and experimental data. Global fitting of 

data from three experiments, covering three to five apoA-I 

concentrations each, was applied for simultaneous calculation 

of apparent association and dissociation rate constants. KD for 

the LL-37-apoA-I interaction was calculated from the kinetic 

rate constants (k on = 0.7–1.6 × 10^8 M \(^{-1}\) s \(^{-1}\); k off = 1.0–2.3 × 

10^-7 s \(^{-1}\) and was in the range 0.6–2.4 

m 

was in the range 

0.4–1.5 × 10^8 M \(^{-1}\).

FIG. 3. Plasma inhibition of LL-37 is mediated by apoA-I. Inhibition zone assay against E. coli D21 with LL-37 at different concentra-

tions dissolved in human plasma + anti-apoA-I IgG, human plasma, 
or water. 3 

m 

each, was applied for simultaneous calculation 

data from three experiments, covering three to five apoA-I 

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10^-7 s \(^{-1}\) and was in the range 0.6–2.4 

m 

was in the range 

0.4–1.5 × 10^8 M \(^{-1}\).

FIG. 4. LL-37-apoA-I interactions detected by surface plasmon resonance. Overlay plots of sensorgrams illustrating the interaction of 

apoA-I with immobilized LL-37. The concentrations of apoA-I were (from bottom to top): 2.5, 5, and 10 

m 

The rapid rise at the start of the association phase and the corresponding drop at the end are due to refractive index changes caused by stock buffer when the samples are diluted in running buffer.

monas aeruginosa and E. coli (10, 11). In addition, LL-37 lyses 

human erythrocytes at the cytotoxic concentrations.2 The 

LL-37 precursor is present in plasma (14), and the mature 

peptide is found in bronchoalveolar lavage fluid.3 This and the 

fact that other cathelicidins are released from neutrophil gran-

ulocytes (15) suggest that LL-37 acts extracellularly. Further-

more, LL-37 is up-regulated during different inflammatory 

skin disorders, giving a high concentration at inflammatory 
sites (9). If this applies to inflammatory disorders in general, 

the existence of mechanisms that protect against LL-37 cyto-

toxicity appear relevant. LL-37 and apoA-I could interact both 
at inflammatory sites and in plasma, because the blood vessels 

are permeable to both proteins and because LL-37 could be 

released from the precursor in plasma. The apparent KD for the 

LL-37-apoA-I binding implies that physiological apoA-I plasma 

concentrations (50 

m 

will scavenge >90% of LL-37 at concen-

trations (25 

m 

where it is cytotoxic to eukaryotic cells. Thus, 

our data suggest that apoA-I can function as a scavenger of 

LL-37 and thereby prevent host cell damage secondary to 

LL-37 release at inflammatory sites. This is the first report 
describing inhibitory mechanisms involved in the regulation of 
cathelicidin antibacterial peptides. However, defensins bind to 

activated α2-macroglobulin in plasma, which was suggested as a 

scavenging mechanism in inflamed tissues (16).

ApoA-I apparently has several antiinflammatory functions 

in addition to its possible role in scavenging LL-37, it is in-

volved in the protection against endotoxins (17), binds and 
inhibits polymerization of complement factor C9 (18), and 
inhibits IgG-induced neutrophil activation as measured by 
degranulation and superoxide production (19). The latter function 
is mediated by free apoA-I but not by high density lipoprotein 
particles, which may in turn serve as a regulatory mechanism 
because acute phase proteins may displace apoA-I from high 
density lipoprotein particles during inflammation (19).

ApoA-I contains amphipathic α-helical regions and comple-

ment factor C9 and LL-37 likewise contain amphipathic heli-

ces. It is tempting to speculate that the amphipathic helices of 

apoA-I, which are central for its ability to interact with phos-

pholipids, also serve to bind amphipathic peptides. Several 
cathelicidin peptides are amphipathic and may also be subject 
to scavenging by apoA-I or other amphipathic plasma proteins. 

Notably, the antibacterial activity of the porcine cathelicidin

DISCUSSION

A protein responsible for plasma inhibition of LL-37 antibac-
terial/lytic activity was isolated by affinity chromatography 

and identified as apolipoprotein A-I. ApoA-I is concluded to be 

the main, or only, plasma protein that binds and inhibits LL-

37: (i) isolated apoA-I inhibits the antibacterial activity of 

LL-37 to 50% compared with the inhibition exerted by whole 

plasma and (ii) addition of anti-apoA-I IgG to plasma com-

pletely blocks inhibition of the antibacterial activity up to 25 

m 

LL-37. In addition, real time binding analysis confirmed the 

results from affinity chromatography and showed that the LL-

37-apoA-I binding has a KD of about 1–2 

m 

LL-37 is cytotoxic to human leukocytes at concentrations 

(15–25 

m 

that are three to five times its minimal inhibitory 

concentration value against bacteria, including Pseudo-



1 Y. Wang, B. Agerberth, and J. Johansson, unpublished observation.

2 B. Agerberth, J. Grunewald, E. Castaños-Velez, B. Olsson, H. Jörnvell, H. Wigzell, A. Eklund, and G. H. Gudmundsson, manuscript in preparation.
PR-39, which is neither lytic nor amphipathic (20, 21), is not affected by plasma (11).

In conclusion we show that apolipoprotein A-I binds LL-37, suggesting that this could be a means of attenuating cytotoxic effects at inflammatory sites.

Acknowledgments—Drs. Mats Andersson (Department of Medical Biochemistry and Biophysics, Karolinska Institutet), Gudmundur H. Gudmundsson (Microbiology and Tumor Biology Center, Karolinska Institutet), and Joakim Lundahl (Department of Clinical Immunology, Karolinska Institutet) are gratefully acknowledged for constructive discussions.

REFERENCES

1. Boman, H. G. (1995) Annu. Rev. Immunol. 13, 61–92
2. Zanetti, M., Gennaro, R., and Romeo, D. (1995) FEBS Lett. 374, 1–5
3. Gallo, R. L., Kim, K. J., Bernfield, M., Kozak, C. A., Zanetti, M., Merluzzi, L., and Gennaro, R. (1997) J. Biol. Chem. 272, 13088–13093
4. Mahoney, M. M., Lee, A. Y., Brezinski-Caliguri, D. J., and Huttner, K. M. (1995) FEBS Lett. 377, 519–522
5. Boman, H. G., Agerberth, B., and Boman, A. (1993) Infect. Immun. 61, 2978–2984
6. Cabiaux, V., Agerberth, B., Johansson, J., Homblié, F., Goormaghtigh, E., and Ruyschaert, J.-M. (1994) Eur. J. Biochem. 224, 1019–1027
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J. Biol. Chem. 1998, 273:33115-33118. doi: 10.1074/jbc.273.50.33115

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