BIOCHEMISTRY
including biophysical chemistry & molecular biology

Article

pubs.acs.org/biochemistry

The Positional Specificity of EXXK Motifs within an Amphipathic α-Helix Dictates Preferential Lysine Modification by Acrolein: Implications for the Design of High-Density Lipoprotein Mimetic Peptides

Ying Zheng,[†] Sea H. Kim,[‡] Arti B. Patel,[‡] Vasanthy Narayanaswami,[‡,§] Anthony T. Iavarone,[∥] Gregory L. Hura,[⊥] and John K. Bielicki*[,†]

[†]Life Sciences Division, Lawrence Berkeley National Laboratory, Donner Laboratory, University of California, Berkeley, California 94720, United States

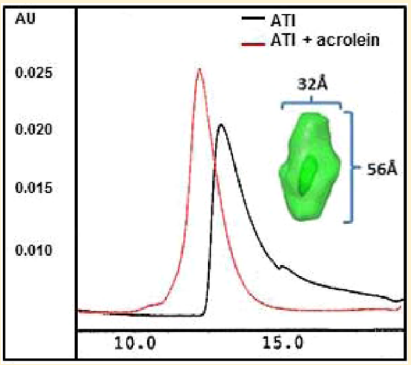
[‡]Department of Chemistry and Biochemistry, California State University Long Beach, Long Beach, California 90840, United States

[§]Children's Hospital of Oakland Research Institute, Oakland, California 94609, United States

[∥]Department of Chemistry, QB3 Mass Spectrometry Facility, University of California, Berkeley, California 94720, United States

[⊥]Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720, United States

ABSTRACT: Despite the ability of acrolein to damage proteins, factors governing its reactivity with the ε-amino group of lysine are poorly understood. We used a small 26-mer α-helical peptide (ATI-5261) to evaluate the influence of acidic glutamate (E) residues on site-specific lysine modification by acrolein and if this targeting played a major role in inhibiting the cholesterol efflux activity of the peptide. Exposure of ATI-5261 to acrolein resulted in *N*-(3-formyl-3,4-dehydropiperidino) (FDP)-lysine adducts at positions 5 and 25 and led to a concentration-dependent reduction in cholesterol efflux activity (55 ± 7 and 83 ± 3% decrease with 5:1 and 20:1 acrolein:peptide molar ratios, respectively). Amino acid substitution (K → R) experiments and mass spectrometry revealed neither K5 nor K25 was preferentially modified by acrolein, despite the location of K5 within a putative EXXK motif. Moreover, both lysine residues remained equally reactive when the lipidated peptide was exposed to acrolein. In contrast, placement of EXXK in the center of ATI-5261 resulted in site-specific modification of lysine. The latter was dependent on glutamate, thus establishing that acidic residues facilitate lysine modification and form the molecular basis of the EXXK motif. Preferential targeting of lysine, however, failed to augment the inhibitory effect of the aldehyde. Overall, the inhibitory effects of acrolein on cholesterol efflux activity were largely dependent on the number of lysine residue modifications and cross-linking of α-helical strands that restricted dissociation of the peptide to active forms.



Acrolein is a highly toxic substance widely produced from the combustion of organic matter, including fossil fuels, cooking oils, and tobacco products.[1,2] It represents a major constituent of both mainstream and sidestream tobacco smoke and poses significant health risks to smokers and nonsmokers.[3−8] The ubiquitous nature of acrolein is underscored by endogenous production from oxidation of polyunsaturated fatty acids and protein threonine via myeloperoxidase.[9−13] Consequently, acrolein modification of proteins has been implicated in the development of cardiovascular disease (i.e., atherosclerosis and diabetes) and neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease.[4,14−16]

The toxic effects of acrolein are mediated, in part, via modification of nucleophilic sites of proteins, such as amino acid side chains of lysine, cysteine, and histidine.[17] Recent studies suggest interactions between these sites and oxidants can be influenced by neighboring amino acids.[17−20] Such a mechanism may produce unique chemical signatures useful for early disease detection. Examples of this include structural modifications to high-density lipoproteins (HDL) that inhibit the antiatherogenic reverse cholesterol transport pathway.[21−26] Oxidative modifications of apolipoprotein A-I (apoA-I), the major protein of HDL, weaken its ability to stimulate cellular cholesterol efflux and activate lecithin:cholesterol acyltransferase (LCAT), two key events that protect against macrophage foam cell formation and atherosclerosis.[27−31]

ApoA-I possesses numerous lysine residues (21 total), but few are modified by acrolein in high abundance.[31] Thus, it has been suggested that unique features of amphipathic α-helices may enhance reaction of acrolein with specific lysine residues in the protein. These unique features take the form of salt-bridge configurations (i.e., EXXXKXXE and EXXK motifs) thought to

Received: May 14, 2012
Revised: July 13, 2012
Published: July 16, 2012

   dx.doi.org/10.1021/bi300626g | *Biochemistry* 2012, 51, 6400−6412

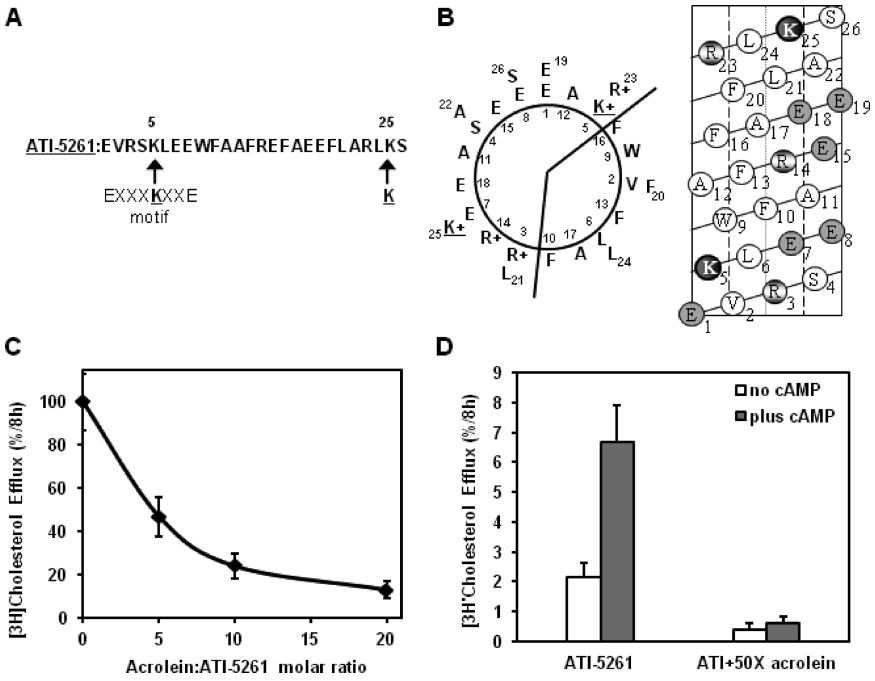


Figure 1. Acrolein inhibits the ability of ATI-5261 to stimulate cellular cholesterol efflux. (A) Primary amino acid sequence of ATI-5261 showing the location of lysine 5 (K5) within an acidic residue motif (i.e., EXXXKXXE motif) and lysine 25 (K25) at the C-terminus. X represents intervening residues. (B) Helical-wheel (left) and helical-net (right) diagrams depicting the location of lysine residues in relation to neighboring amino acids within the peptide secondary structure. Lysines 5 and 25 are underlined. (C) Acrolein inhibits the cholesterol efflux activity of ATI-5261 in a concentration-dependent manner. ATI-5261 at 1 mg/mL PBS was exposed (20 h) to increasing concentrations of acrolein, dialyzed to PBS, and then added to cultures of [^{3}H]cholesterol-labeled J774 cells treated with cAMP to upregulate ABCA1. (D) ABCA1-dependent cholesterol efflux activity of control and acrolein-treated peptides. ATI-5261 was exposed to a large molar excess (50×) of acrolein, dialyzed, and then added (10 μg/mL) to cultures of [^{3}H]cholesterol-labeled J774 cells treated with and without cAMP. Results in panels C and D are means ± SD (n = 3).

position acidic residues in the proximity of positively charged lysine, thereby enhancing its nucleophilicity. To date, there has been no definitive proof that these motifs facilitate site-specific modification of lysine in amphipathic α-helices. This issue has been difficult to address because of the relatively large number of EXXK motifs in apoA-I and their different positioning along various amphipathic α-helical repeats.

Recently, we developed a small HDL mimetic peptide (ATI-5261) that mimics α-helices of apoA-I and apoE.[32,33] This peptide is highly α-helical and forms a well-defined structure in solution. Moreover, the peptide stimulates cellular cholesterol efflux via ATP-binding cassette transporter A1 (ABCA1) like native apolipoproteins on a per molecule basis. For these reasons, ATI-5261 was utilized in this study to investigate relationships between EXXK motifs, lysine modification by acrolein, and impairment of peptide acceptor activity to stimulate ABCA1 cholesterol efflux. We found that acrolein modifies lysine in a site-specific manner when EXXK motifs are positioned in the center of the α-helix, i.e., down the length of the peptide away from the ends. However, preferential lysine modification contributed little to overall reductions in cholesterol efflux activity. The inhibitory effects of acrolein were largely dependent on the number of lysine residue modifications and chemical cross-linking that limited dissociation of the peptide to active biological forms.

EXPERIMENTAL PROCEDURES

Peptides. Peptides were synthesized (Biosynthesis Inc., Lewisville, TX) from all-L-amino acids, capped with N-terminal acetyl and C-terminal amide groups, isolated by high-performance liquid chromatography, and used at a purity of >95%. Stock solutions of 1−4 mg of peptide/mL of phosphate-buffered (pH 7.4) saline (PBS) were routinely prepared, filter-sterilized (Nalgene filtration capsules), and stored at 4 °C. Peptide concentrations were determined by the absorbance at 280 nm. ATI-5261−POPC complexes were prepared as described previously.[32] The homogeneity and size of ATI-5261−POPC complexes were assessed by nondenaturing gradient gel electrophoresis.[32] Small angle X-ray scattering was performed (beamline 12.3.1, http://sibyls.als.lbl.gov) to verify the size of complexes and provide information related to the relative conformation of peptide strands versus the lipid-free peptide in solution.[33]

Exposure of Peptides to Acrolein. Acrolein (90%, Aldrich) was diluted in ultrapure water (Invitrogen) immediately prior to use. Reaction mixtures consisted of 1 mg of peptide/mL of PBS and increasing amounts of acrolein. Samples were incubated for 1−20 h at 37 °C in a water bath shielded from light. Reactions were terminated by dialysis against PBS (unless otherwise indicated) using Spectra/Por Float-A-Lyzers (100−500 molecular weight cutoff). Peptide incubations (37 °C) in the absence of acrolein served as controls. In some experiments, peptide stock solutions kept at 4 °C were used as controls.

Assessment of Cholesterol Efflux Activity. J774 macrophages were used to determine the ability of peptides to stimulate cholesterol efflux.[32−34] Cells were plated onto 24-well culture plates and labeled with [^{3}H]cholesterol (1 μCi/mL) in RPMI-1640 with 1% FBS for 48 h. A cAMP analogue (cpt-cAMP) was added (18 h) to some cells to upregulate ABCA1 expression. Cells were extensively rinsed and then exposed to lipid-free peptides or ATI-5261−POPC complexes in serum-free RPMI-1640 medium. The amount of [^{3}H]cholesterol

dx.doi.org/10.1021/bi300626g | Biochemistry 2012, 51, 6400−6412

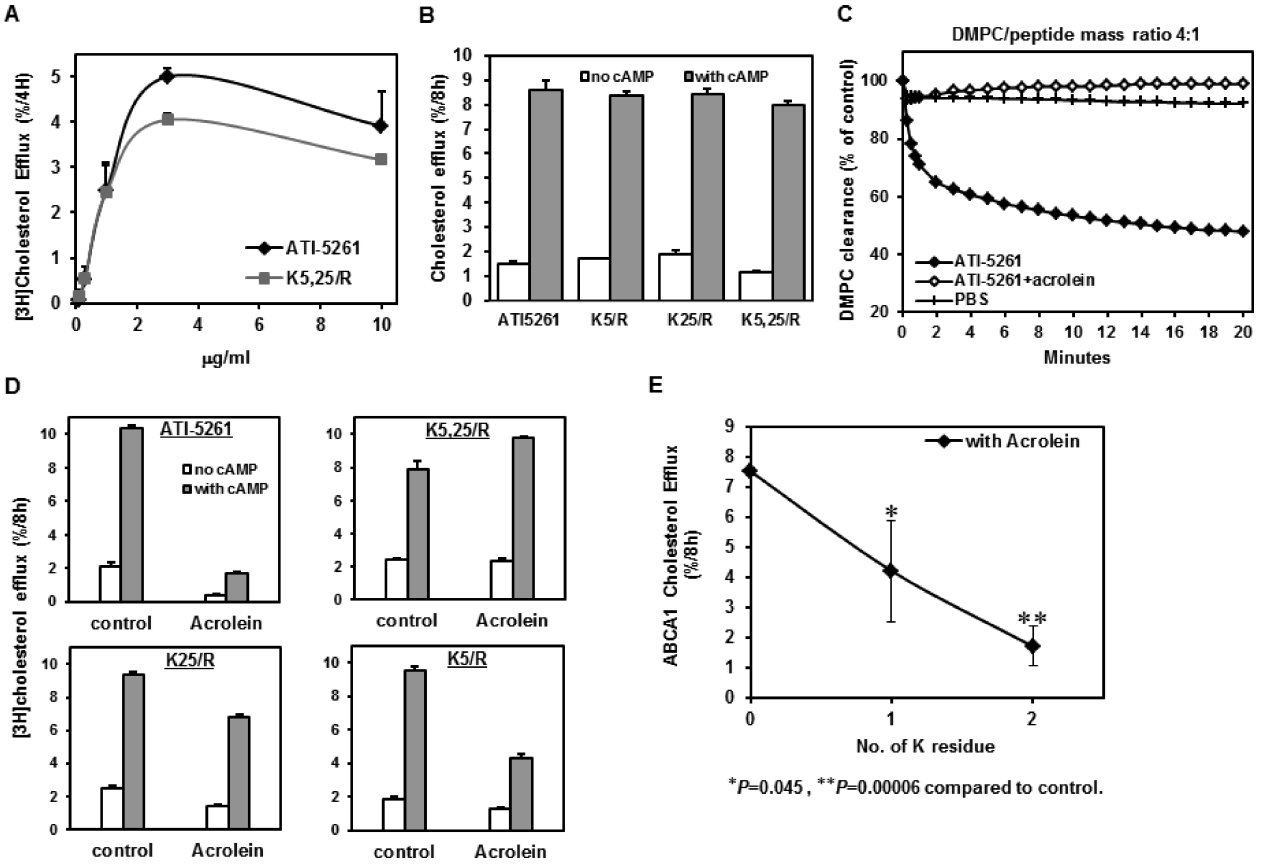


Figure 2. Evidence that acrolein inhibits the cholesterol efflux activity of ATI-5261 via modification of lysine residues. Single and double K → R substitutions within ATI-5261 were used to investigate whether lysine residues were necessary for the acrolein-induced loss of cholesterol efflux activity. (A) Control experiment demonstrating the K5,25→R double variant was a potent stimulator of efflux of cholesterol from J774 cells treated with cAMP. Cholesterol efflux (4 h) was assessed using increasing concentrations of lipid-free peptides. (B) ABCA1-dependent cholesterol efflux activity of peptides determined using [^{3}H]cholesterol-labeled J774 cells treated with and without cAMP. Single and double K → R variants of ATI-5261 were added to cells in lipid-free form at 10 μg/mL serum-free RPMI-1640 medium. Cholesterol efflux results (8 h) are representative of three experiments (means ± SD). (C) Lipid binding activity of ATI-5261 determined by the DMPC clearance assay. ATI-5261 was treated (20 h) with a 20-fold molar excess of acrolein, dialyzed, and then incubated (room temperature) with DMPC dispersions in PBS (pH 7.4) at the indicated mass ratio, and the decrease in turbidity was monitored at 405 nm. Results are representative of two experiments. (D) Acrolein inhibits the cholesterol efflux activity of ATI-5261 in a lysine-dependent manner. Peptides treated with a 20-fold molar excess of acrolein were added to [^{3}H]cholesterol-labeled J774 cells. Cholesterol efflux (8 h) was assessed using lipid-free peptides at a concentration of 3 μg/mL serum-free medium. Results are representative of three experiments (means ± SD). (E) Line graph summarizing the relationship between peptide lysine content and susceptibility to inactivation by acrolein (data from panel D). A Student's t test was used to evaluate statistical differences, using $p < 0.05$ as the criterion for significance.

appearing in the medium was expressed as a percentage of cell radioactivity at time zero. Background release of [^{3}H]-cholesterol to serum-free medium was subtracted from values obtained with peptides. The difference in [^{3}H]cholesterol efflux between cells treated with (gray bars in figures) and without (white bars) cpt-cAMP was defined as the ABCA1 component of cholesterol efflux.

Liquid Chromatography and Mass Spectrometry (LC–MS). Peptides were analyzed using an ultraperformance liquid chromatograph (UPLC, nanoAcquity, Waters, Milford, MA) connected in-line with a quadrupole time-of-flight mass spectrometer having a nanoelectrospray ionization (nanoESI) source (Q-Tof Premier, Waters). The UPLC was equipped with C_{18} trapping (20 mm × 180 μm, 5 μm, Waters Symmetry) and analytical (100 mm × 100 μm, 1.7 μm, Waters BEH130) columns and a 10 μL sample loop. Solvent A consisted of 99.9% water and 0.1% formic acid, and solvent B consisted of 99.9% acetonitrile and 0.1% (v/v) formic acid. Following sample injection, trapping was performed for 6 min with 100% A at a flow rate of 15 μL/min. A seal wash period of 5 min was used. The injection needle was washed with 500 μL each of solvents A and B after injection to avoid cross contamination between samples. The elution program consisted of a linear gradient from 15 to 95% B over 30 min, isocratic conditions at 95% B for 4 min, a linear gradient to 1% B over 0.33 min, and isocratic conditions at 1% B for 11.67 min, at a flow rate of 500 nL/min. The analytical column and sample compartment were maintained at 35 and 8 °C, respectively.

For mass spectrometry, the column exit was connected to a Universal NanoFlow Sprayer nanoESI emitter mounted in the nanoflow ion source of the Q-Tof Premier. The nanoESI source parameters were as follows: nanoESI capillary voltage, 2.4 kV; nebulizing gas (nitrogen) pressure, 0.15 mbar; sample cone voltage, 35 V; extraction cone and ion guide voltages, 4 V; and source block temperature, 80 °C. No cone gas was used. The collision cell contained argon gas at a pressure of 8×10^{-3} mbar. The Tof analyzer was operated in "V" mode. Under these conditions, a mass resolving power of 1.0×10^4

dx.doi.org/10.1021/bi300626g | Biochemistry 2012, 51, 6400–6412

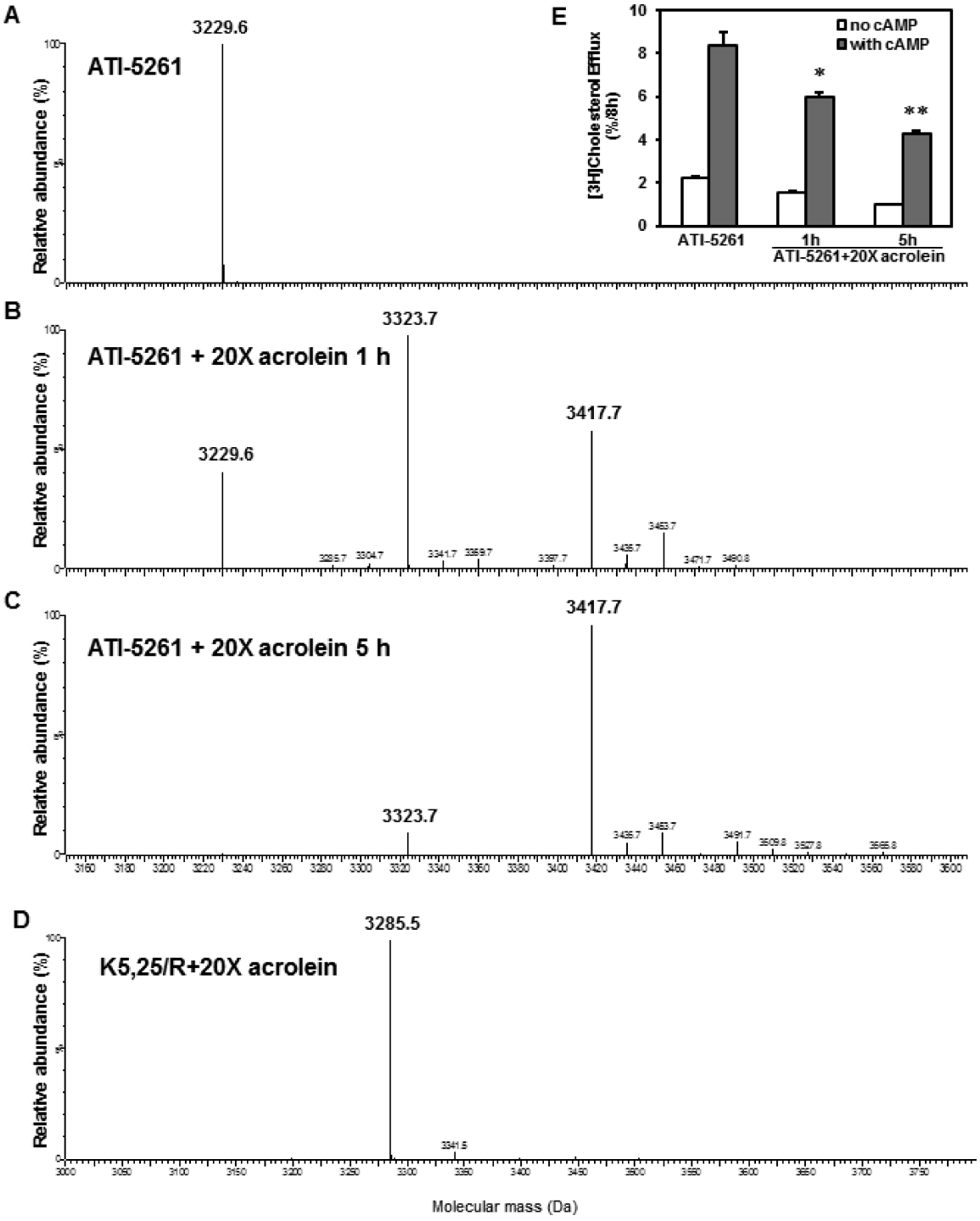


Figure 3. LC−MS analysis of lipid-free ATI-5261 exposed to acrolein. ATI-5261 was incubated (1 and 5 h) with and without a 20-fold molar excess of acrolein, dialyzed to PBS, and subjected to mass spectrometric (MS) analysis and activity assessments. (A) Control ATI-5261 peptide incubated for 5 h in PBS alone (no acrolein). Note the presence of a single mass species of 3229.6 Da corresponding to unmodified ATI-5261. (B) Peptide mass species obtained following a 1 h incubation of ATI-5261 with a 20-fold molar excess of acrolein. A major peak at 3323.7 Da corresponding to a 94 Da addition to ATI-5261 is shown, consistent with formation of a single FDP-lysine adduct. (C) Mass species obtained from a 5 h incubation of ATI-5261 with a 20-fold molar excess of acrolein. A major species at 3417.4 Da corresponding to a peptide with two FDP-lysine adducts (188 Da addition) was obtained. (D) Peptide K5,25→R that lacks lysine residues was exposed (5 h) to a 20-fold molar excess of acrolein at 37 °C, dialyzed, and then analyzed by LC−MS. Note the major peak at 3285.5 Da, corresponding to the unmodified peptide. (E) Cholesterol efflux activity of control and modified peptides (3 μg/mL) determined using [^{3}H]cholesterol-labeled J774 cells treated with (gray bars) and without (white bars) cAMP. Cholesterol efflux values (%/8 h) are means ± SD (n = 3).

(measured at m/z 771) was routinely achieved, which was sufficient to resolve the isotopic distributions of the singly and multiply charged peptide ions measured in this study. External mass calibration was performed immediately prior to analysis using a solution of sodium formate. Mass spectra were recorded in the positive ion mode over the range of m/z 400−2000, in

dx.doi.org/10.1021/bi300626g | *Biochemistry* 2012, 51, 6400−6412

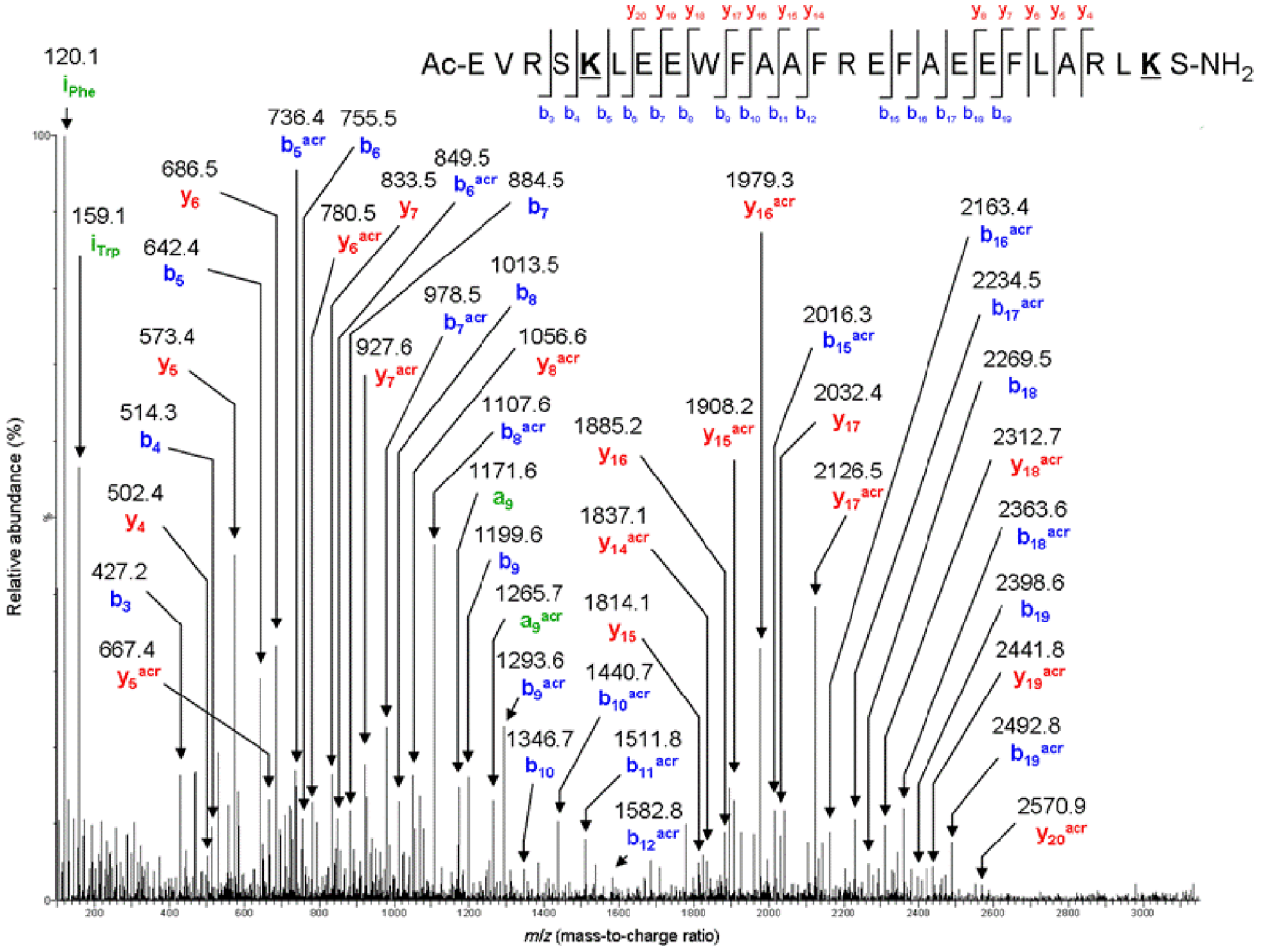


Figure 4. MS/MS reveals K5 and K25 within ATI-5261 were equally susceptible to modification by acrolein. Tandem mass spectrum and sequence map resulting from the ATI-5261 peptide modified by condensation of two acrolein molecules (i.e., 94 Da addition), measured for the 3323.7 Da mass species from a 1 h exposure to a 20-fold molar excess of acrolein. Lysine residues in the peptide sequence map, which are acrolein modification sites, are in bold font and underlined. Fragment ions containing the 94 Da addition, due to acrolein adduction, are denoted by acr. The results indicate the 3323.7 Da species consisted of a mixture of peptide molecules carrying the FDP-lysine adduct at either K5 or K25.

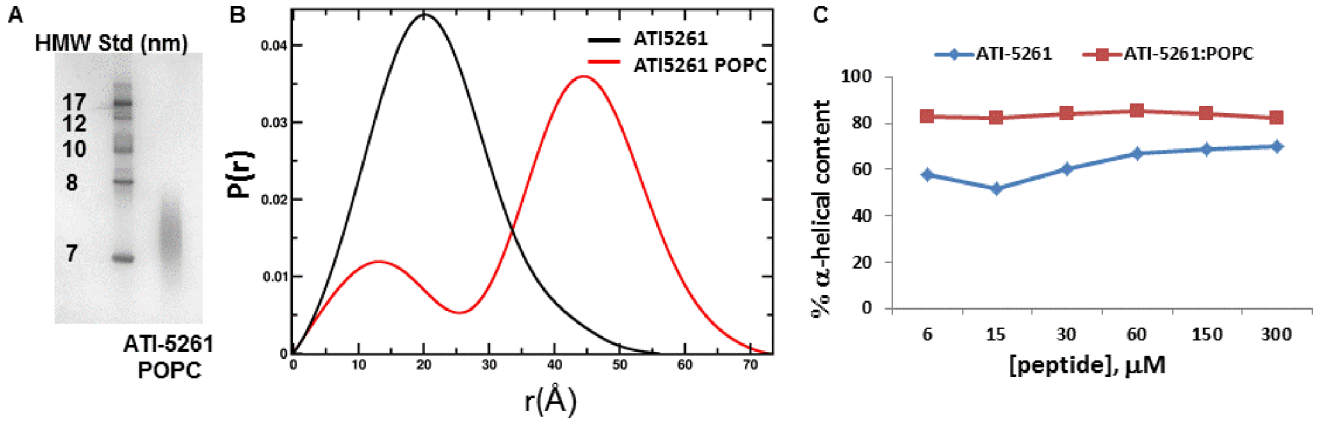


Figure 5. Biophysical properties of ATI-5261–POPC complexes. ATI-5261 was formulated with POPC by cholate dialysis. (A) Size and homogeneity of lipidated complexes determined by a nondenaturing gradient (4 to 20%) gel electrophoresis. The sample load was 3 μg of peptide per well. (B) Small angle X-ray scattering (SAXS)-derived electron pair histogram [$P(r)$] showing the difference in the spatial distribution of electron density for lipid-free ATI-5261 (black) and ATI-5261–POPC complexes (red). (C) Plot showing the relationship between the peptide secondary structure and the concentration of either lipid-free peptide (diamonds) or ATI-5261–POPC complexes (squares). Results are representative of two replicate experiments.

continuum data format, using a 0.95 s scan integration and a 0.05 s interscan delay.

Tandem Mass Spectrometry (MS/MS). Real-time deisotoping and charge state recognition were used to select

dx.doi.org/10.1021/bi300626g | *Biochemistry* 2012, 51, 6400–6412

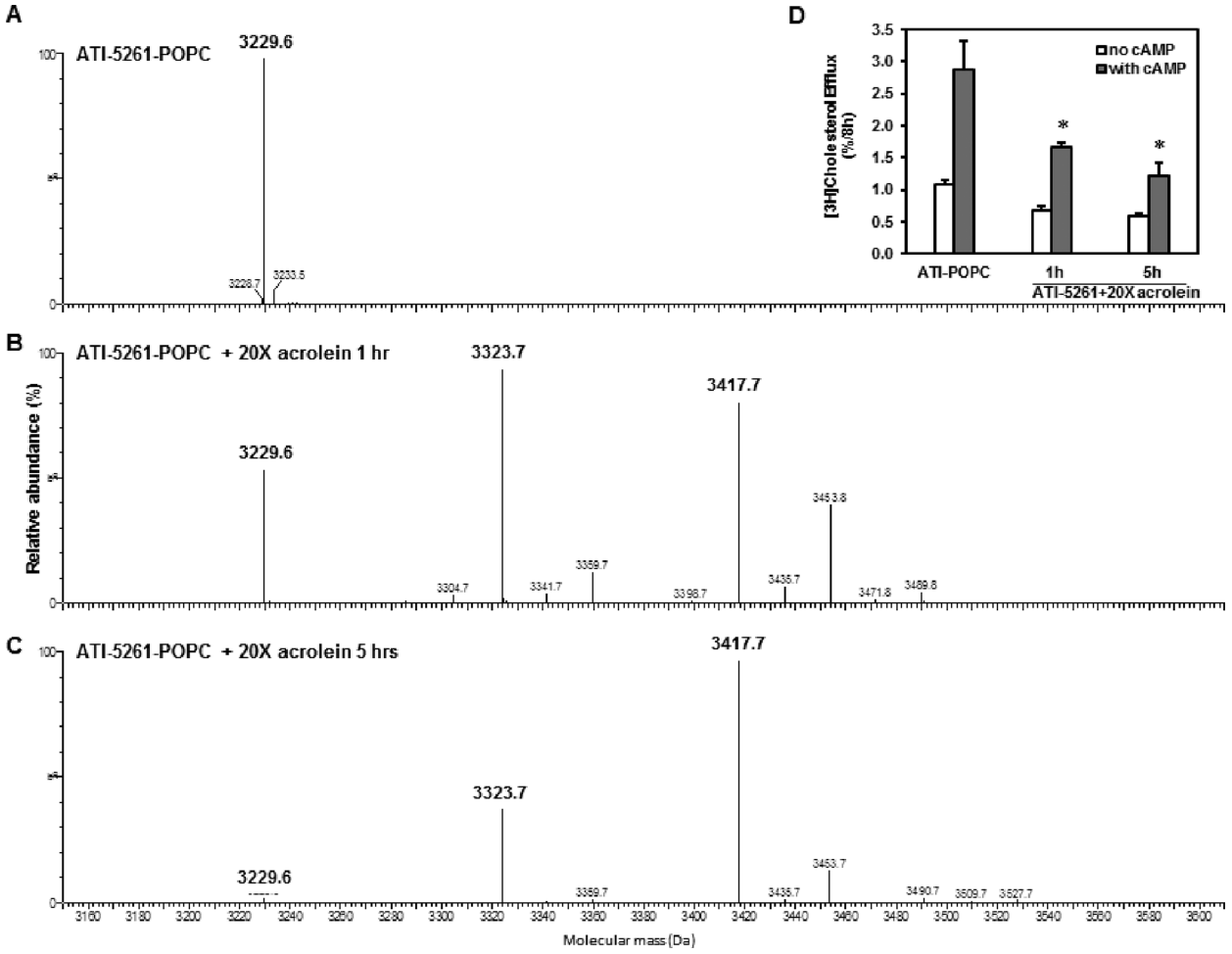


Figure 6. LC−MS analysis of ATI-5261−POPC complexes exposed to acrolein. ATI-5261−POPC complexes were incubated (37 °C for 1 and 5 h) with a 20-fold molar excess of acrolein, dialyzed to PBS, and subjected to mass spectrometric analysis and activity assessments. (A) Control complexes incubated for 5 h in PBS (no acrolein). Note the presence of a single mass species at 3229.6 Da corresponding to the molecular mass of unmodified ATI-5261. (B) Peptide mass species obtained following a 1 h incubation of the ATI-5261−POPC complexes with a 20-fold molar excess of acrolein. A major peak at 3323.7 Da corresponding to a 94 Da addition to ATI-5261 is shown, consistent with formation of a single FDP-lysine adduct. (C) Mass species obtained from a 5 h incubation of ATI-5261 with a 20-fold molar excess of acrolein. A major species at 3417.4 Da corresponding to the peptide with two FDP-lysine adducts (188 Da addition) was obtained. (D) Cholesterol efflux activity of control and modified peptides (3 μg/mL) determined using [^{3}H]cholesterol-labeled J774 cells treated with (gray bars) and without (white bars) cAMP. Cholesterol efflux values (%/8 h) are means ± SD (n = 3).

+5 charge state precursor ions of the ATI-5261 peptide for MS/MS. Collision energies for collisionally activated dissociation (CAD) were automatically selected on the basis of the mass and charge state of a given precursor ion. MS/MS spectra were acquired over the range of m/z 100−2500 using a 1.95 s scan integration and a 0.05 s interscan delay. Ions were fragmented to achieve a minimal total ion current (TIC) of 800000 cps in the cumulative MS/MS spectrum for a maximum of 60 s. The $[M + 5H]^{5+}$ ions of the ATI-5261 peptide in unmodified form and those modified by a 94 Da mass addition (i.e., by the condensation of two acrolein molecules), at m/z 646.7 and 665.5, respectively, were selected as precursor ions for MS/MS. Data processing was performed using MassLynx (version 4.1, Waters). Mass spectral deisotoping to transform multiply charged ions onto a singly charged x-axis was performed using the maximum entropy algorithm, MaxEnt 3, in MassLynx.

Other Methods. A turbid solution of dimyristoylphosphatidylcholine (DMPC) was used to assess the capacity of peptides to solubilize phospholipid. DMPC was used at a final PBS (pH 7.4) concentration of 0.16 mg/mL, as described previously.[35] Circular dichroism (CD) spectroscopy was conducted on a Jasco 810 spectropolarimeter at 25 °C averaging four scans (20 nm/min per scan) per peptide per run and using lipid-free peptide at 0.2 mg/mL, i.e., dialyzed to 10 mM sodium phosphate buffer (pH 7.4) following treatments.[32,33] To assess the cross-linking of the peptide following acrolein exposure, samples were mixed with 3× SDS loading buffer (Cell Signaling Technology, Inc.) and then boiled for 1 min. The supernatants were loaded onto a 10−20% Tris-Tricine sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) gel and electrophoresed, and cross-linked species were visualized with SimplyBlue SafeStain.

Statistics. Where indicated, data were expressed as means ± the standard deviation (SD) of at least three independent experiments and statistical analyses performed using a Student's t test, with $p < 0.05$ as a criterion for determining significance.

RESULTS

ATI-5261 possesses two lysine residues that could serve as sites for acrolein modification (Figure 1A,B). Lysine 5 (K5) is

dx.doi.org/10.1021/bi300626g | Biochemistry 2012, 51, 6400−6412

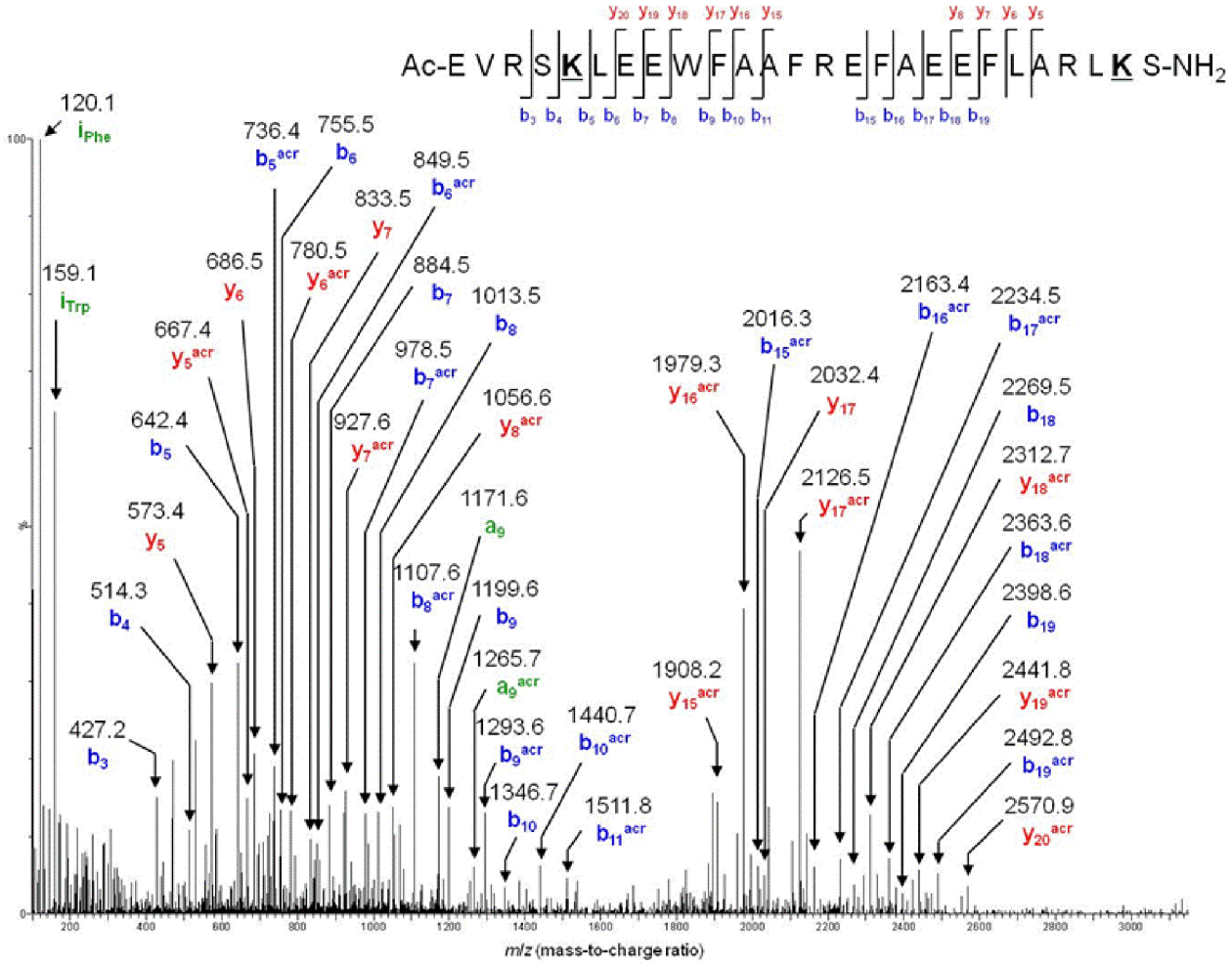


Figure 7. MS/MS reveals K5 and K25 within ATI-5261−POPC complexes were equally susceptible to modification by acrolein. Tandem mass spectrum and sequence map of the ATI-5261 peptide within POPC complexes modified by condensation of two acrolein molecules (94 Da addition), measured for the 3323.7 Da mass species from a 1 h exposure to acrolein. Lysine residues in the peptide sequence map, which are acrolein modification sites, are shown in bold and underlined. Fragment ions containing the 94 Da addition, due to acrolein adduction, are denoted by acr. The results indicate the 3323.7 Da species consisted of a mixture of peptide molecules carrying the FDP-lysine adduct at either K5 or K25.

sandwiched between two acidic residues each spaced one helical turn on either side of its position, denoted as EXXXKXXE, whereas K25 is situated two or more turns from nearest acidic residues. These differences suggested ATI-5261 may be useful for determining whether acrolein modifies lysine residues in a site-specific manner. Acrolein treatment of ATI-5261 produced a concentration-dependent reduction in the cholesterol efflux activity of the peptide (Figure 1C). Nearly complete reductions of ABCA1 cholesterol efflux activity were seen at >20:1 acrolein:peptide molar ratios, as determined using J774 cells (Figure 1C,D).

Variants of ATI-5261 with amino acid substitutions (K → R) were used to identify the role of lysine residues in the acrolein-induced loss of activity. Single lysine to arginine (K5 → R or K25 → R) or double K5 and K25 → R (K5,25→R) substitutions in ATI-5261 had little impact on the cholesterol efflux activity in the absence of acrolein (Figure 2A,B). Moreover, the use of arginine to replace lysine did not alter the peptide secondary structure [α-helicities of 63 ± 7 and 64 ± 3% for ATI-5261 and K5,25→R, respectively (n = 6)] or lipid binding activity [$t_{1/2}$ values from the DMPC clearance assay of 1.53 ± 0.38 and 1.46 ± 0.48 Δabs/min for ATI-5261 and K5,25→R, respectively (n = 3)], indicating the peptides were suitable for further study. Acrolein inhibited the lipid binding activity of ATI-5261 (Figure 2C), consistent with the loss of cholesterol efflux activity (Figure 1). In contrast, the K5,25→R peptide retained cholesterol efflux (Figure 2D) and lipid binding activities (not shown) in the presence of a large molar excess of acrolein. Cholesterol efflux to peptides with single K → R substitutions was only partially reduced by excess acrolein (Figure 2D). Thus, the inhibitory effect of acrolein appeared to be dependent on the presence of both K5 and K25 within ATI-5261 (Figure 2E).

LC−MS was performed to verify that both lysine residues in ATI-5261 were susceptible to modification by acrolein. A brief 1 h exposure of ATI-5261 to a 20-fold molar excess of acrolein produced a major shift in peptide mass from 3229.6 Da (unmodified) to 3323.7 Da, corresponding to the addition of a single FDP-lysine adduct per peptide (Figure 3, panel A vs panel B). A minor component at 1 h having a mass of 3417.7 Da was also detected, consistent with two FDP-lysine adducts per peptide. By 5 h of exposure to acrolein, the 3417.7 Da species was the major product (Figure 3C). No modified forms were detected using the K5,25→R peptide (Figure 3D), consistent with the activity data indicating the substitution of arginine for lysine conferred resistance to

dx.doi.org/10.1021/bi300626g | Biochemistry 2012, 51, 6400−6412

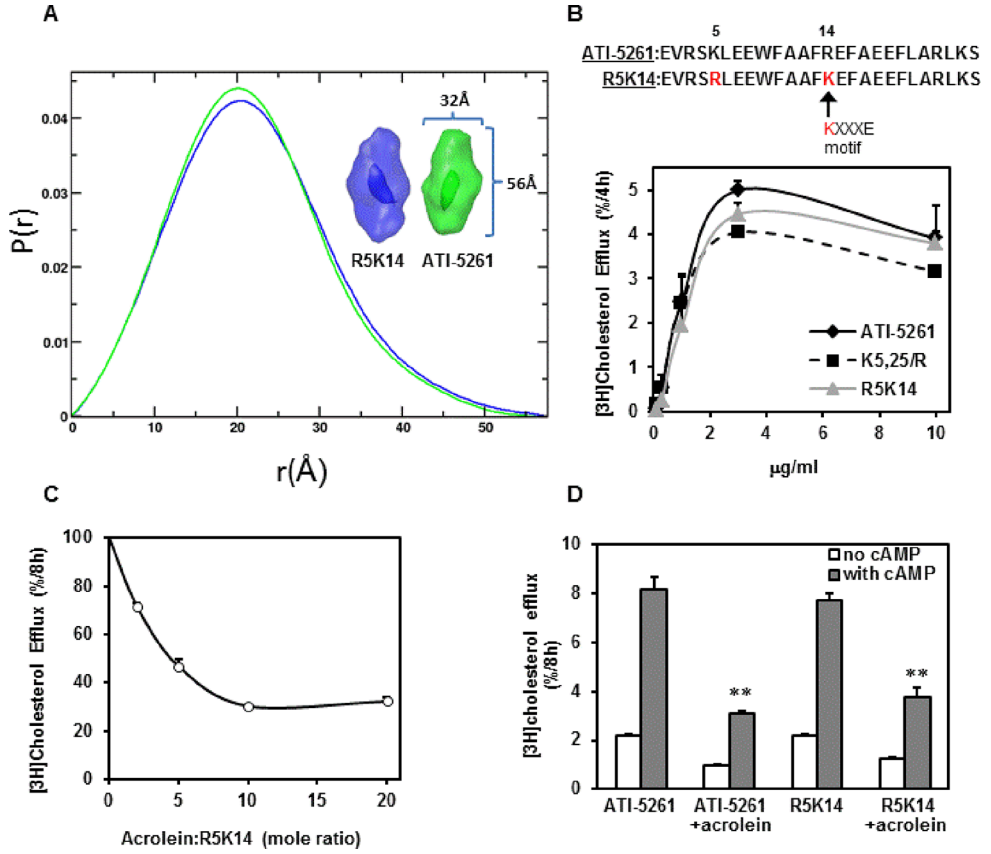


Figure 8. Characteristics of the R5K14 exchange variant of ATI-5261. A variant of ATI-5261 was created whereby the positions of K5 and R14 were switched. (A) Solution structure of the resulting R5K14 variant determined by SAXS compared to parent ATI-5261. Shown are electron density plots for lipid-free peptides, with reconstruction of their molecular envelopes (inset). (B) Sequences of peptides showing the location of the EXXK motif in the center of peptide R5K14. The plot shows the dependence of cholesterol efflux (%/4 h) on the concentration of lipid-free peptides, using [^{3}H]cholesterol-labeled J774 cells treated with cAMP. (C) Acrolein inhibits the cholesterol efflux activity of R5K14 in a concentration-dependent manner. R5K14 was exposed (20 h) to increasing concentrations of acrolein, dialyzed to PBS, and then added (3 μg/mL) to cultures of [^{3}H]cholesterol-labeled J774 cells treated with cAMP. Cholesterol efflux values are representative of two experiments. (D) Acrolein treatment (20-fold molar excess for 20 h) impairs the ability of peptides to stimulate ABCA1-dependent cholesterol efflux, determined using [^{3}H]cholesterol-labeled J774 cells treated with (gray bars) and without (white bars) cAMP. Lipid-free peptides were evaluated at a concentration of 3 μg/mL. Cholesterol efflux values (%/8 h) are means ± SD (n = 3).

acrolein. Formation of a modified ATI-5261 peptide correlated with a decrease in ABCA1 cholesterol efflux activity (Figure 3E). Tandem MS (MS/MS) was performed to identify the modification site(s) on the peptide. Analysis of the single-adduct species (3323.7 Da) following a 1 h treatment of ATI-5261 with acrolein revealed a mixture of peptides in solution carrying the FDP-lysine adduct at either position K5 or K25 (Figure 4). Moreover, the collective peak heights of b and y fragments harboring acrolein were nearly identical, indicating both K5 and K25 were equally susceptible to modification upon initial exposure to acrolein.

Lipidation of ATI-5261 with POPC produced a homogeneous population of small ~7 nm particles as determined by nondenaturing gradient gel electrophoresis (Figure 5A). These particles displayed an electron density map consistent with the arrangement of peptide strands on the outer edge of discoidal-like lipid particles,[36] i.e., distinctly different from that of the lipid-free peptide, as revealed by SAXS (Figure 5B). Moreover, lipidation of ATI-5261 increased the α-helical content of the peptide by ~20% (Figure 5C). Despite the increase in the level of secondary structure and the difference in the configuration of peptide strands, lysine modification by acrolein occurred within ATI-5261–POPC complexes in a manner analogous to that seen using the lipid-free peptide (Figure 3 vs Figure 6). By 1 and 5 h exposure of ATI-5261–POPC complexes to acrolein, modified forms with one (3323.7 Da) and two (3417.7 Da) FDP-lysine adducts were detected by LC–MS. In addition, MS/MS performed on the 3323.7 Da mass species revealed a mixture of peptide molecules in solution carrying FDP-lysine adducts at either position K5 or K25 (Figure 7), indicating both lysine residues were similarly reactive toward acrolein when ATI-5261 was bound to the lipid.

To test whether the location of lysine within ATI-5261 influenced the reactivity with acrolein, the positions of positively charged K5 and R14 were switched to create an R5K14 variant (Figure 8). This moved the motif to the center (KXXXE, i.e., K14–E18) of the peptide away from the N-terminus (Figure 8B). Both R5K14 and ATI-5261 self-associated similarly in the absence of lipid, as revealed by SAXS (Figure 8A); therefore, the arrangement of peptide strands in solution was apparently identical for the two peptides. The secondary structure of R5K14 was also similar to that of ATI-5261 (α-helicity of 66% for R5K14 vs 68% for ATI-5261), as was its ability to promote cholesterol efflux in the absence of acrolein (Figure 8B). Exposure of R5K14 to acrolein produced a decrease in cholesterol efflux activity not unlike that seen using

dx.doi.org/10.1021/bi300626g | Biochemistry 2012, 51, 6400–6412

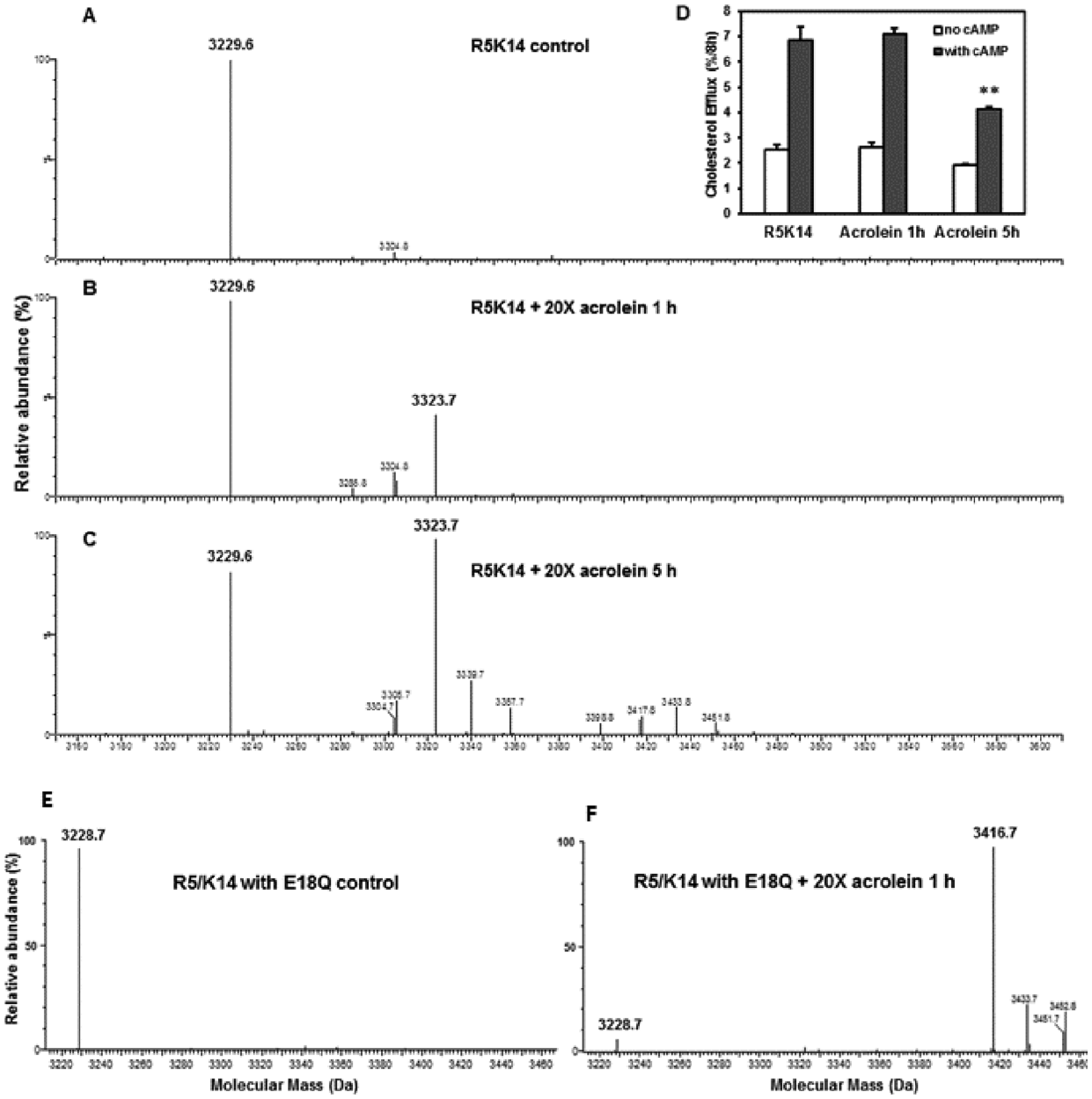


Figure 9. LC−MS analysis of peptide R5K14 following exposure to acrolein. R5K14 was exposed at 37 °C to a 20-fold molar excess of acrolein for 1 and 5 h, dialyzed to PBS, and subjected to mass spectrometric analysis and activity assessments. (A) Control peptide incubated for 5 h in PBS (no acrolein), dialyzed, and subjected to LC−MS. Note the presence of a single mass species at 3229 Da corresponding to the molecular mass of the unmodified peptide. (B) Mass species obtained following a 1 h incubation of R5K14 with a 20-fold molar excess of acrolein. An additional peak at 3323.7 Da corresponding to a 94 Da addition to R5K14 is shown, consistent with the formation of a single FDP-lysine adduct. (C) Mass species obtained from a 5 h incubation of R5K14 with a 20-fold molar excess of acrolein, showing the 3323 Da species with a single FDP adduct is the major form of the peptide. (D) Cholesterol efflux activity of control and modified peptides (3 μg/mL) determined using [^{3}H]cholesterol-labeled J774 cells treated with (gray bars) and without (white bars) cAMP. Cholesterol efflux values (%/8 h) are means ± SD (n = 3). (E) LC−MS analysis of peptide R5K14 harboring an E to Q substitution at position 18. The mass of the unmodified peptide (3228.7 Da) is shown for the control (no acrolein) incubation (1 h at 37 °C). (F) Rapid appearance of two adduct species (i.e., 3416.7 Da) following a brief 1 h exposure of the EQ peptide (R5K14 with E18Q) to acrolein. The data indicate the loss of selective modification of a single site (K14) upon removal of an acidic residue.

ATI-5261 (Figure 8C vs Figure 1C). Similarly, acrolein inhibited the ability of R5K14 to stimulate ABCA1-dependent cholesterol efflux (Figure 8D).

In contrast to results with ATI-5261, reaction of R5K14 with excess acrolein yielded a peptide (3327.7 Da) with a single FDP-lysine adduct after 1 and 5 h exposures (Figure 9A−C). MS/MS analysis of the 3323.7 Da species (1 h treatment with acrolein) resulted in peptide fragment ions with FDP-lysine adducts at position K14 only, indicating K14, located in the center of ATI-5261, was selectively modified (Figure 10). Similar results were obtained with the 5 h peptide sample (data not shown). Removal of the acidic residue from the motif via substitution with glutamine (E18Q substitution in R5K14) resulted in the rapid appearance of two FDP-lysine adducts (3416.7 Da species, i.e., modification of both K14 and K25) following a brief 1 h exposure to acrolein (Figure 9, panel E vs panel F), indicating the acidic residue within the EXXK motif was essential for selective modification of K14 within the R5K14 peptide.

dx.doi.org/10.1021/bi300626g | *Biochemistry* 2012, 51, 6400−6412

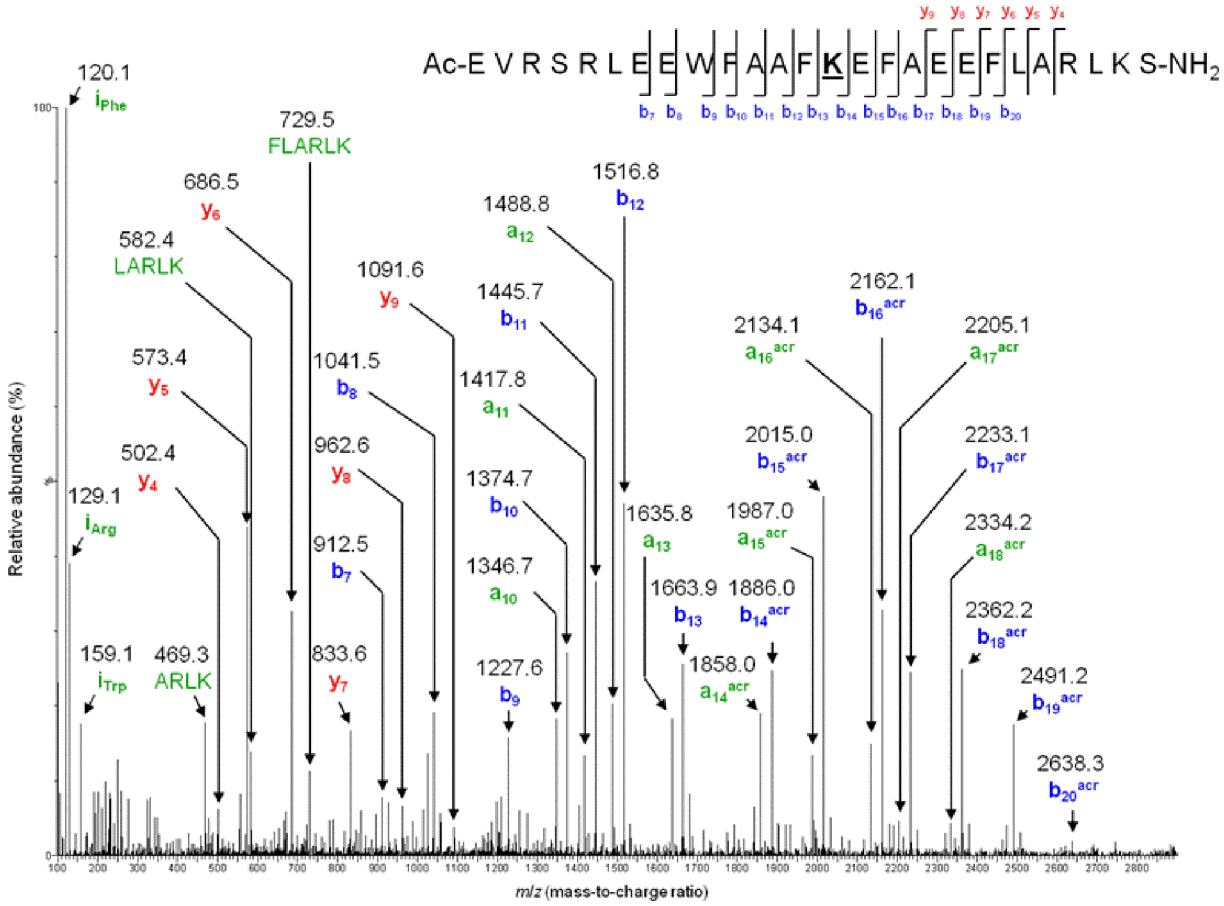


Figure 10. Selective modification of K14 following exposure of peptide R5K14 to acrolein. Tandem mass spectrum and sequence map of R5K14 modified by condensation of two acrolein molecules (i.e., 94 Da addition), measured for the 3323.7 Da mass species following a 1 h exposure to a 20-fold molar excess of acrolein. Lysine residues in the peptide sequence map, which are acrolein modification sites, are shown in bold and underlined. Fragment ions containing the 94 Da addition, due to acrolein adduction, are denoted by acr.

To further investigate mechanisms by which acrolein inhibited the cholesterol efflux activity of ATI-5261, biophysical studies were performed. Treatment of ATI-5261 with a 20-fold molar excess of acrolein produced a modest decrease in α-helical content; thus, loss of secondary structure did not appear to be a major determinant for inhibition of efflux activity (Figure 11A). Moreover, removal of a positive charge using glutamine (i.e., K25 → Q substitution) had little impact, indicating covalent addition of acrolein to the peptide was required to inhibit efflux activity. Formation of acrolein adducts was accompanied by interchain cross-linking of α-helical peptide strands visualized by SDS−PAGE (Figure 11C). Cross-linking was observed using various forms of ATI-5261 (i.e., R5K14 and ATI-5261−POPC complexes) except the double K → R peptide, which was resistant to acrolein. Previously, we found that ATI-5261 self-associates to form a tetrameric assembly in solution,[33] displaying a pattern of cross-linking with dimethylsuberimidate not unlike that seen here with acrolein (Figure 11C). As shown by FPLC, tetramers of ATI-5261 dissociate to low-molecular mass forms upon dilution (Figure 11D). This is consistent with our previous work indicating ATI-5261 monomers of high α-helical content are the active form of the peptide.[33] However, the acrolein-treated peptide eluted as a single symmetrical peak with a retention volume identical to that of the tetrameric assembly (RT = 12.28 mL), indicating that interchain cross-linking of ATI-5261 locked the peptide in a self-associated state and prevented its dissociation to active forms (Figure 11D).

DISCUSSION

In this study, we utilized α-helical peptide ATI-5261 to investigate mechanisms by which acrolein modifies lysine residues and produces an inhibition of acceptor activity to stimulate cellular cholesterol efflux. We found that brief exposures of ATI-5261 to acrolein produced FDP-lysine adducts at positions K5 and K25, i.e., the only two lysine residues in the peptide. This is consistent with a previous report demonstrating formation of FDP-lysine adducts with peptides exposed to acrolein.[31] ATI-5261 lysine modification by acrolein was time- and concentration-dependent and was accompanied by an inhibition of cholesterol efflux activity.

The two lysine residues of ATI-5261 are located at opposite ends of the peptide. Despite the difference in amino acids surrounding K5 and K25 in peptide secondary structure, both lysine residues were equally susceptible to modification by acrolein. Evidence of this was obtained using peptide variants with single K → R substitutions (i.e., partially resistant to acrolein) as well as LC−MS. The latter revealed ATI-5261 was modified with one or two FDP-lysine adducts following a brief (1 h) exposure to acrolein, regardless of whether the peptide was bound to the lipid. Moreover, MS/MS analysis of the single adduct species (3323.7 Da) revealed mixtures of peptide molecules in solution carrying the FDP-lysine adduct at either K5 or K25. These observations indicate that lysine residues located toward the ends of ATI-5261 were highly susceptible to

dx.doi.org/10.1021/bi300626g | Biochemistry 2012, 51, 6400−6412

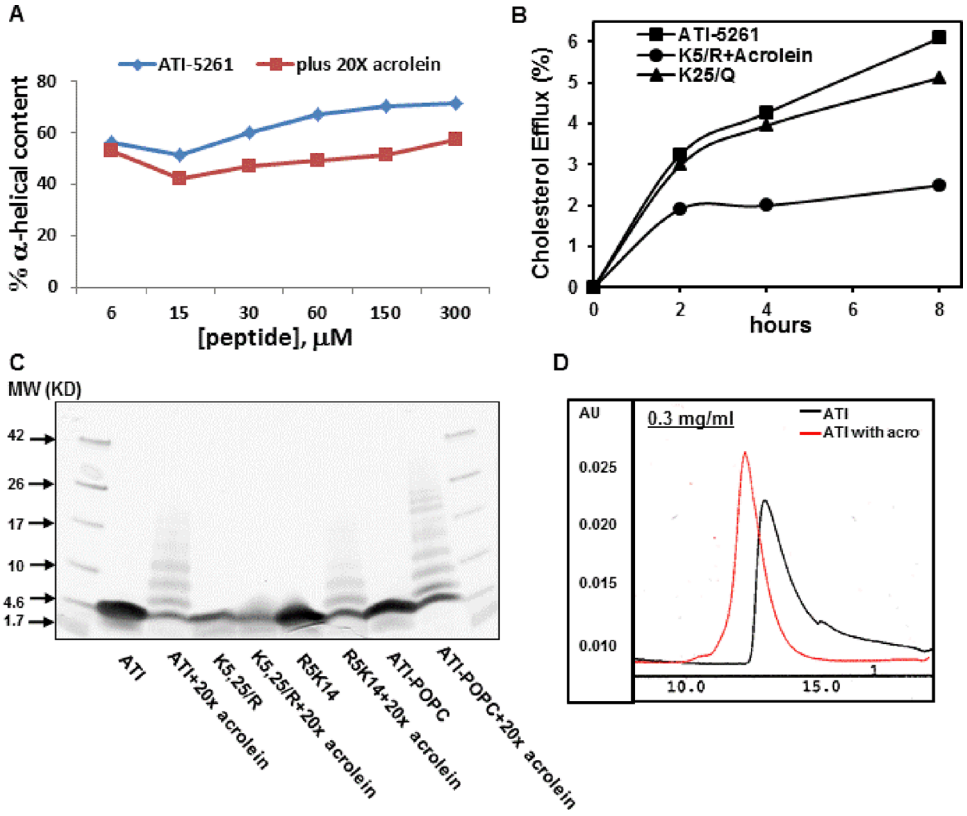


Figure 11. Impact of acrolein on α-helicity, cross-linking, and dynamics of ATI-5261. Lipid-free ATI-5261 and the indicated peptide variants in PBS (pH 7.4) were exposed to a 20-fold molar excess of acrolein for 20 h. (A) α-Helicity of the peptide at various concentrations determined by circular dichroism spectroscopy. Results are representative of two identical experiments. (B) Removing positive charge had little impact on cholesterol efflux activity, as judged using the K25Q substitution peptide. Shown for comparison is the cholesterol efflux activity (●) of a peptide with the FDP-lysine adduct at position K25 (K5R+Acrolein) as well as lipid-free ATI-5261 (■, no acrolein). Cholesterol efflux activity was assessed using saturating peptide concentrations (3 μg/mL). (C) Cross-linking of ATI-5261, ATI-5261−POPC complexes, and the R5K14 peptide variant following a 20 h exposure to a 20-fold molar excess of acrolein. A stained 4 to 20% SDS−PA gel is shown; protein loads were 3 μg/well. No detectable cross-linking was obtained using the K5,25R oxidation-resistant form of ATI-5261. (D) FPLC of lipid-free ATI-5261 treated with and without acrolein (20-fold molar excess). A Superdex75 column was used. The peptide concentration (0.3 mg/mL) was selected to demonstrate dissociation of the tetramer to low-molecular mass (LMW) species, as shown for control ATI-5261 [i.e., peak retention volume (RV) of 13.62 mL]. In contrast, the acrolein-treated sample exhibited an RV of 12.28 mL, similar to that of the control ATI-5261 tetramer at a high concentration of 1 mg/mL (RV = 12.32 mL). The K5,25R oxidation-resistant peptide exposed to acrolein retained the ability to dissociate to LMW forms [RVs of 13.69 mL vs 12.61 mL for a control K5,25R not exposed to acrolein (not shown in the figure)]. Results are representative of two independent experiments with different batches of peptide and multiple FPLC runs over a dilution series from 1 to 0.3 mg/mL.

acrolein modification regardless of the presence of putative EXXK motifs. This nonspecific reactivity produced a dramatic decrease in cholesterol efflux activity that directly correlated with the number of lysine residue modifications (Figure 2E).

To test whether the position of the EXXK motifs within ATI-5261 influenced reactivity toward acrolein, we created a unique R5K14 variant. The R5K14 peptide exhibited self-association, secondary structure, and cholesterol efflux activity similar to those of ATI-5261. Surprisingly, EXXK located in the center of R5K14, i.e., away from the ends, was preferentially modified by acrolein. The molecular basis for this specificity is currently not known. Lysine 14 is positioned at the lipid−water interface of the amphipathic α-helix, similar to the lysine residues (K5 and K25) in ATI-5261 when viewed via helical-wheel projections (Figure 1). Thus, preferential modification of lysine within the R5K14 peptide cannot be attributed to differences in location toward the lipid surface and/or exposure to the aqueous environment, i.e., compared to lysine residues in ATI-5261. However, it is generally believed the ends of small α-helical peptides are more dynamic and less helical than the central region. The high α-helical content in the center of ATI-5261 could optimize the proximity of the neighboring acidic residue to K14, thereby increasing its nucleophilicity and creating a site specifically targeted by acrolein. In support of this, removal of the acidic residue (E18Q) from the central motif in R5K14 abolished site-specific targeting of lysine, thus validating the role of the motif structure in promoting lysine modification (Figure 9E,F). Interestingly, modification of the central lysine (K14) within the R5K14 peptide was associated with only modest reductions in cholesterol efflux activity (Figure 9D). Thus, the inhibitory effects of acrolein appeared to correlate most strongly with the overall number of lysine modifications even in the presence of EXXK motifs.

The α-helical content of ATI-5261 was minimally impacted by acrolein, and removal of positive charge (K25 → Q) alone had little impact on cholesterol efflux activity. The latter observation suggests covalent modifications induced by acrolein were required to inhibit efflux activity, which was accompanied by intermolecular cross-linking of peptide strands. These experiments were conducted using concentrations (0.5 or 1 mg of peptide/mL) of lipid-free ATI-5261 that favor self-association. Under these conditions, ATI-5261 adopts a tetramer form in

dx.doi.org/10.1021/bi300626g | *Biochemistry* 2012, 51, 6400−6412

solution, as revealed by SAXS[33] and verified here (Figure 8). Tetramers of ATI-5261 dissociate to low-molecular mass species (mostly monomers) upon dilution over the active biological range for mediating cellular cholesterol efflux. Treatment with acrolein, however, locked ATI-5261 in a self-associated state, preventing dissociation to low-molecular mass species (Figure 11D). These self-associated forms seen (SDS−PAGE) with excess acrolein treatment (i.e., 20 h) also contained small amounts of an aggregated peptide larger than tetramers, which may also have prevented dissociation to active forms. Collectively, these observations indicate the availability of peptide monomers is required for mediating ABCA1 cholesterol efflux efficiently, as we have previously suggested.[33] Such a mechanism is consistent with exposure of ATI-5261 hydrophobic surfaces being necessary for mediating lipid interactions and cell cholesterol efflux.

It is interesting to note that approximately two molecules of acrolein per lysine was sufficient to produce ∼50% inhibition of ATI-5261 cholesterol efflux activity (5:1 acrolein:peptide molar ratio; two lysine residues per molecule). This is very similar to the behavior of apoA-I (50:1 acrolein:apoA-I molar ratio; 21 lysine residues per molecule), which possesses numerous lysine residues within different types of EXXK motifs.[31] Moreover, the lipid-free and -bound forms of ATI-5261 (this study) appear to be equally sensitive to oxidative damage, which also appears to be the case for apoA-I.[31] Thus, both ATI-5261 and apoA-I appear to be highly sensitive to acrolein, which is thought to be produced in vivo from lipid peroxidation and/or MPO-mediated events associated with macrophage foam cells and atherosclerosis development. As a result, oxidation-resistant forms of HDL mimetic peptides and/or apoA-I may prove to be highly effective in reducing the plaque lipid burden and atherosclerosis under conditions of oxidative stress and inflammation.

AUTHOR INFORMATION

Corresponding Author

*Lawrence Berkeley National Laboratory, Donner Laboratory MS1-267, One Cyclotron Road, Berkeley, CA 94720. Phone: (510) 495-2208. Fax: (510) 486-6488. E-mail: jkbielicki@lbl.gov.

Funding

The work was supported by Tobacco-Related Disease Research Program (TRDRP) of the state of California Grants 17RT-0082 (J.K.B.) and 17RT-0165 (V.N.). The contribution of G.L.H. was supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, under Contract DE-AC02-05CH11231.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The work was conducted, in part, at Lawrence Berkeley National Laboratory through the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, under Contract DE-AC02-05CH11231.

ABBREVIATIONS

ABCA1, ATP-binding cassette transporter A1; SAXS, small-angle X-ray scattering; MS, mass spectrometry; LC, liquid chromatography; FDP, *N*-(3-formyl-3,4-dehydropiperidino); POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; CD, circular dichroism; FPLC, fast protein liquid chromatography; HDL, high-density lipoproteins; apoA-I, apolipoprotein A-I; LCAT, lecithin:cholesterol acyltransferase.

REFERENCES

(1) Uchida, K., Kanematsu, M., Sakai, K., Matsuda, T., Hattori, N., Mizuno, Y., Suzuki, D., Miyata, T., Noguchi, N., Niki, E., and Osawa, T. (1998) Protein-bound acrolein: Potential markers of oxidative stress. *Proc. Natl. Acad. Sci. U.S.A. 95*, 4882−4887.

(2) Stevens, J. F., and Maier, C. S. (2008) Acrolein: Sources, metabolism, and biomolecular interactions relevant to human health and disease. *Mol. Nutr. Food Res. 52*, 7−25.

(3) Spiess, P. C., Deng, B., Hondal, R. J., Matthews, D. E., and van der Vliet, A. (2011) Proteomic profiling of acrolein adducts in human lung epithelial cells. *J. Proteomics 74*, 2380−2394.

(4) Aldini, G., Orioli, M., and Carini, M. (2011) Protein modification by acrolein: Relevance to pathological conditions and inhibition by aldehyde sequestering agents. *Mol. Nutr. Food Res. 55*, 1301−1319.

(5) Nazaroff, W. W., and Singer, B. C. (2004) Inhalation of hazardous air pollutants from environmental tobacco smoke in US residences. *J. Exposure Anal. Environ. Epidemiol. 14*, 571−577.

(6) Uchiyama, S., Inaba, Y., and Kunugita, N. (2010) Determination of acrolein and other carbonyls in cigarette smoke using coupled silica cartridges impregnated with hydroquinone and 2,4-dinitrophenylhydrazine. *J. Chromatogr., A 1217*, 4383−4388.

(7) Flouris, A. D., Vardavas, C. L., Metsios, G. S., Tsatsakis, A. M., and Koutedakis, Y. (2010) Biological evidence for the acute health effects of secondhand smoke exposure. *Am. J. Physiol. 298*, L3−L12.

(8) McCall, M. R., van den Berg, J. J., Kuypers, F. A., Tribble, D. L., Krauss, R. M., Knoff, L. J., and Forte, T. M. (1994) Modification of LCAT activity and HDL structure: New links between cigarette smoke and coronary heart disease risk. *Arterioscler. Thromb. 14*, 248−253.

(9) McCall, M. R., Tang, J. Y., Bielicki, J. K., and Forte, T. M. (1995) Inhibition of lecithin-cholesterol acyltransferase and modification of HDL apolipoproteins by aldehydes. *Arterioscler., Thromb., Vasc. Biol. 15*, 1599−1606.

(10) Bergt, C., Pennathur, S., Fu, X., Byun, J., O'Brien, K., McDonald, T. O., Singh, P., Anantharamaiah, G. M., Chait, A., Brunzell, J., Geary, R. L., Oram, J. M., and Heinecke, J. W. (2004) The myeloperoxidase product hypochlorous acid oxidizes HDL in the human artery wall and impairs ABCA1-dependent cholesterol transport. *Proc. Natl. Acad. Sci. U.S.A. 101*, 13032−13037.

(11) Undurti, A., Huang, Y., Lupica, J. A., Smith, J. D., Didonato, J. A., and Hazen, S. L. (2009) Modification of high density lipoprotein by myeloperoxidase generates a pro-inflammatory particle. *J. Biol. Chem. 284*, 30825−20835.

(12) Nicholls, S. J., and Hazen, S. L. (2009) Myeloperoxidase modified lipoproteins and atherogenesis. *J. Lipid Res. 50*, S346−S351.

(13) Shao, B., and Heinecke, J. W. (2011) Impact of HDL oxidation by the myeloperoxidase system on sterol efflux by the ABCA1 pathway. *J. Proteomics 74*, 2289−2299.

(14) Tamamizu-Kato, S., Wong, J. Y., Jairam, V., Uchida, K., Raussens, V., Kato, H., Ruysschaert, J.-M., and Narayanaswami, V. (2007) Modification by acrolein, a component of tobacco smoke and age-related oxidative stress, mediates functional impairment of human apolipoproteinE. *Biochemistry 46*, 8392−8400.

(15) Lovell, M. A., Xie, C., and Markesbery, W. R. (2000) Acrolein, a product of lipid peroxidation, inhibits glucose and glutamate uptake in primary neuronal cultures. *Free Radical Biol. Med. 29*, 714−720.

(16) Lovell, M. A., Xie, C., and Markesbery, W. R. (2001) Acrolein is increased in Alzheimer's disease brain and is toxic to primary hippocampal cultures. *Neurobiol. Aging 22*, 187−194.

(17) LoPachin, R. M., Gavin, T., Petersen, D. R., and Barber, D. S. (2009) Molecular mechanisms of 4-hydroxy-2-nonenal and acrolein toxicity: Nucleophilic targets and adduct formation. *Chem. Res. Toxicol. 22*, 1499−1508.

(18) Bergt, C., Fu, C., Huq, N. P., Kao, J., and Heinecke, J. W. (2004) Lysine residues direct the chlorination of tyrosine in YXXK motifs of apolipoprotein A-I when hypochlorous acid oxidizes high density lipoprotein. *J. Biol. Chem. 279*, 7856−7866.

(19) Zhang, H., Zeilonka, J., Sikora, A., Joseph, J., Xu, Y., and Kalyanaraman, B. (2009) The effect of neighboring methionine residue on tyrosine nitration and oxidation in peptides treated with MPO, H_2O_2 and NO_2 or peroxynitrite and bicarbonate: Role of intramolecular electron transfer mechanisms? *Arch. Biochem. Biophys. 484*, 134−145.
(20) Fu, X., Wang, Y., Kao, J., Irwin, A., d'Avignon, A., Mecham, R. P., Parks, W. C., and Heinecke, J. W. (2006) Specific sequence motifs direct the oxygenation and chlorination of tryptophan by myeloperoxidase. *Biochemistry 45*, 3961−3971.
(21) Morel, D. W. (1994) Reduced cholesterol efflux to mildly oxidized high density lipoprotein. *Biochem. Biophys. Res. Commun. 200*, 408−416.
(22) Bielicki, J. K., Forte, T. M., and McCall, M. R. (1996) Minimally oxidized LDL is a potent inhibitor of lecithin:cholesterol acyltrasnferase activity. *J. Lipid Res. 37*, 1012−1021.
(23) Bielicki, J. K., McCall, M. R., van den Berg, J. J., Kuypers, F. A., and Forte, T. M. (1995) Copper and gas-phase cigarette smoke inhibit plasma lecithin:cholesterol acyltransferase activity by different mechanisms. *J. Lipid Res. 36*, 322−331.
(24) Shao, B., Cavigiolio, G., Brot, N., Oda, M. N., and Heinecke, J. W. (2008) Methionine oxidation impairs reverse cholesterol transport by apolipoprotein A-I. *Proc. Natl. Acad. Sci. U.S.A. 105*, 12224−12229.
(25) Zheng, L., Nukuna, B., Brennan, M., Sun, M., Goornastic, G., Settle, M., Schmitt, D., Fu, X., Thomson, L., Fox, P. L., Ischiropoulos, H., Smith, J. D., Kinter, M., and Hazen, S. L. (2004) Apolipoprotein A-I is a selective target for myeloperoxidase-catalyzed oxidation and functional impairment in subjects with cardiovascular disease. *J. Clin. Invest. 114*, 529−541.
(26) Nicholls, S. J., Zheng, L., and Hazen, S. L. (2005) Formation of dysfunctional high-density lipoprotein by myeloperoxidase. *Trends Cardiovasc. Med. 15*, 212−219.
(27) Wu, Z., Wagner, M. A., Zheng, L., Parks, J. S., Shy, J. M., Smith, J. D., Gogonea, V., and Hazen, S. L. (2007) The refined structure of nascent HDL reveals a key functional domain for particle maturation and dysfunction. *Nat. Struct. Mol. Biol. 14*, 861−868.
(28) Shao, B., Tang, C., Heinecke, J. W., and Oram, J. F. (2010) Oxidation of apolipoprotein A-I by myeloperoxidase impairs the initial interaction with ABCA1 required for signaling and cholesterol export. *J. Lipid Res. 51*, 1849−1858.
(29) Peng, D., Brubaker, G., Wu, Z., Zheng, L., Willard, B., Kinter, M., Hazen, S. L., and Smith, J. D. (2008) Apoliporotein A-I tryptophan substitution lead to resistance to myeloperoxidase mediated loss of function. *Arterioscler., Thromb., Vasc. Biol. 28*, 2063−2070.
(30) Brubaker, G., Peng, D., Somerlot, B., Abdollahian, D. J., and Smith, J. D. (2006) Apolipoprotein A-I lysine modification: Effects of helical content, lipid binding and cholesterol acceptor activity. *Biochim. Biophys. Acta 1761*, 64−72.
(31) Shao, B., Fu, X., McDonald, T. O., Green, P. S., Uchida, K., O'Brien, K. D., Oram, J. F., and Heinecke, J. W. (2005) Acrolein impairs ATP binding cassette transporter A1-dependent cholesterol export from cells through site-specific modification of apolipoprotein A-I. *J. Biol. Chem. 280*, 36386−36396.
(32) Bielicki, J. K., Zhang, H., Cortez, Y., Zheng, Y., Narayanaswami, V., Patel, A., Johansson, J., and Azhar, S. (2010) A new HDL mimetic peptide that stimulates cellular cholesterol efflux with high efficiency greatly reduces atherosclerosis in mice. *J. Lipid Res. 51*, 1496−1503.
(33) Zheng, Y., Patel, A. B., Narayanaswami, V., Hura, G. L., Hang, B., and Bielicki, J. K. (2011) HDL mimetic peptide ATI-5261 forms an oligomeric assembly in solution that dissociates to monomers upon dilution. *Biochemistry 50*, 4068−4076.
(34) Vedhachalam, C., Narayanaswami, V., Neto, N., Forte, T. M., Phillips, M. C., Lund-Katz, S., and Bielicki, J. K. (2007) The C-terminal lipid-binding domain of apolipoprotein E is a highly efficient mediator of ABCA1-dependent cholesterol efflux that promotes the assembly of high density lipoproteins. *Biochemistry 46*, 2583−2593.
(35) Natarajan, P., Forte, T. M., Chu, B., Phillips, M. C., Oram, J. F., and Bielicki, J. K. (2004) Identification of an apolipoprotein A-I structural element that mediates cellular cholesterol efflux and stabilizes ATP binding cassette transporter A1. *J. Biol. Chem. 279*, 24044−24052.
(36) Peters-Libeu, C. A., Newhouse, Y., Hall, S. C., Witkowska, H. E., and Wesigraber, K. H. (2007) Apolipoprotein E:dipalmitoylphosphatidylcholine particles are ellipsoidal in solution. *J. Lipid Res. 48*, 1035−1044.