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Enrichment of Cysteine-Containing Peptides from Tryptic Digests Using a Quaternary Amine Tag

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A new strategy for specifically targeting cysteine-containing peptides in a tryptic digest is described. The method is based on quantitatively derivatizing cysteine residues with a quaternary amine tag (QAT). Tags were introduced into proteins following reduction of disulfide bonds through derivatization of cysteine residues with (3-acrylamidopropyl)trimethylammonium chloride. After trypsin digestion, derivatized cysteine-containing peptides were enriched by strong cation exchange chromatography. The method was validated using model peptides and a protein. The QAT strategy has several advantages over other methods for the selection of cysteine-containing peptides. One is that it increases the ionization efficiency of cysteine-containing peptides. The other is that chromatographic selection is achieved with simple, robust cation exchange chromatography columns. As a result, this new strategy provides a simple way to facilitate enrichment of cysteine-containing peptides, thereby reducing sample complexity in bottom-up proteomics.

Bottom-up, signature peptide, and shotgun proteomics are terms used to describe a sequential process in which the proteins in a proteome are first digested and then fractionated by several chromatographic steps and the parent proteins finally identified using mass spectra obtained from a few signature peptides. The problem with this strategy is that the peptides produced in the initial proteolysis step overwhelm the analytical capacity of current LC/MS systems, both in number and in dynamic range.[1] To address this issue, various affinity selection methods, targeting either post-translational modifications (PTM) or specific amino acid residues, have been applied. Immobilized lectin affinity chromatography has been used to target glycosylation[2−4] while Ga(III)-IMAC[5,6] or specific antibodies[7] have been used to select phosphorylated peptides. A number of amino acid residues, viz. cysteine,[8−10] histidine,[11,12] methionine,[13] and N-terminal serine/threonine,[14] have also been selectively targeted to reduce sample complexity. An advantage of targeting specific amino acid residues is that knowledge of their presence can constrain database searches,[15] often to 10−20% of the peptides derived from a proteome.

Of the various amino acid residues targeted, cysteine is the most popular due to the ease with which it is derivatized and low abundance in various proteomes. In silico studies of *E. coli*, yeast, and the human proteome show that over 80% of proteins in each of these proteomes contain a cysteine residue. However, this 80% is represented by less than 20% of the tryptic peptides.[16] This suggests that by selecting cysteine-containing peptides, a substantial decrease in sample complexity can be achieved at minimal loss in protein coverage of a proteome.

Reduction and alkylation of cysteine residues is a critical component of proteomics since peptides are identified primarily through their tryptic peptides and proteolysis is greatly enhanced by cleaving disulfide bonds. A number of reagents such as 4-vinylpyridine, acrylamide, iodoacetamide, and *N*-ethylmaleimide have been used to alkylate sulfydryl groups.[15,17] A popular approach to the selection of cysteine-containing peptides is to append a tag to an alkylating agent that after reacting with the peptides will dramatically enhance chromatographic selection. This is most often accomplished by alkylating sulfhydryl groups with a biotinylated reagent followed by subsequent selection and enrichment with avidin affinity chromatography.[8,18] Although avidin selects biotinylated species with enormous affinity, this approach has disadvantages. Avidin columns are of relatively low capacity and are costly. Other concerns are that biotinylated peptides can be irreversibly bound to avidin chromatography

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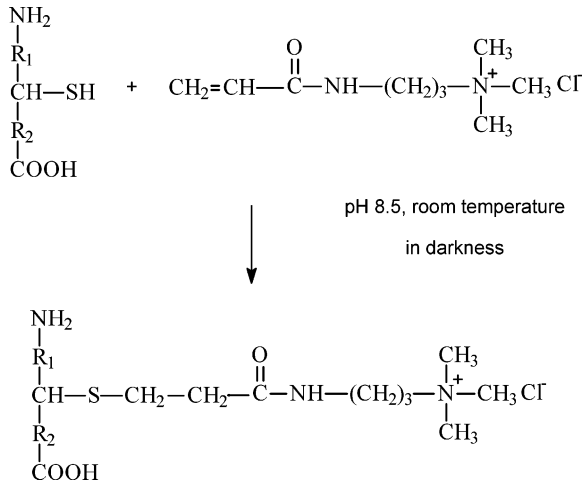

Figure 1. The reaction of (3-acrylamidopropyl)trimethylammonium chloride (APTA) with a cysteine residue in a peptide.

columns[19] and hydrophobic peptides containing no cysteine residues are also captured.[20]

The work present here tests the utility of strong cation exchange (SCX) chromatography columns in the selection of peptides with cysteine residues that have been derivatized with a quaternary amine tag. It demonstrates that this is possible because SCX chromatography columns do not retain nonderivatized tryptic peptides at pH values higher than 6.0.

EXPERIMENTAL SECTION

Materials and Reagents. Synthetic peptides HCKFWW, CDPGYIGSR, and CGYGPKKKRKVGG were purchased from BACHEM Bioscience Inc. (King of Prussia, PA). Apo-transferrin (human), lysozyme (egg), TPCK-treated trypsin (bovine), urea, dithiothreitol (DTT), iodoacetic acid (IAA), *N*-(2-hydrozyethyl) piperazine-*N*′-(2-ethanesulfonic acid) (HEPES), (2-[*N*-morpholino] ethanesulfonic acid) hydrate (MES), *N*-tosyl-L-lysylchloromethyl ketone (TLCK), sinapinic acid, calcium chloride, and (3-acrylamidopropyl)trimethylammonium chloride (APTA) (75 wt % solution in water) were purchased from Sigma-Aldrich (St. Louis, MO). Trifluoroacetic acid (Sequanal Grade) and dialysis bags were acquired from Pierce (Rockford, IL). HPLC-grade acetonitrile (ACN) and sodium chloride were obtained from Mallinckrodt Baker, Inc. (Paris, KY).

C_{18} column (4.6 × 250 mm) was purchased from Vydac (Hesperia, CA). The TSK Chelate-5PW column (7.5 × 7.5 mm) was purchased from TosoHaas (Montgomeryville, PA). The low-pressure micro-splitter valve was from Upchurch Scientific (Oak Harbor, WA). Deionized water was produced by a Milli-Q A10 System from Millipore (Bedford, MA).

Derivatization of Cysteine with a Quaternary Amine Tag. Sulfhydryl groups were derivatized with (3-acrylamidopropyl)-trimethylammonium chloride (APTA) (Figure 1). This reaction is analogous to the well-known reaction of sulfhydryl groups with acrylamide and acrylic acid.[15,17] Reaction with APTA introduces a quaternary amine into cysteine-containing peptides. These quaternary amine tagged peptides will subsequently be referred to as QAT peptides.

Optimization of Derivatization Reaction on Synthetic Peptides. Stock solutions (2.0 mg/mL) of the synthetic peptides HCKFWW, CDPGYIGSR, and CGYGPKKKRKVGG were prepared separately in 50 mM HEPES (pH 8.5). Each stock solution was further diluted with 50 mM HEPES buffer (pH 8.5) to a final concentration of 40 μg/mL in a 1-mL solution. A 1000-fold excess of alkylating reagent was added and the reaction was carried out in the dark for time periods of 5 min, 0.5 h, 1 h, 1.5 h, 2 h, 3 h, 4 h, and 8 h, respectively. Aliquots (100 μL) of each peptide solution at different reaction times were withdrawn and the reaction was stopped by freezing at −80 °C. Three peptide solutions were mixed immediately before injection for LC/MS analysis.

To optimize the derivatizing agent to peptide molar ratio, 100 μL of each peptide (40 μg/mL) was separately mixed with the derivatizing agent APTA in different molar excess. The peptide-to-APTA ratios examined were 1:1, 1:50, 1:100, 1:200, 1:500,1:1000, 1:2000, and 1:4000, respectively. Again, the reaction was carried out for 1 h in the dark and stopped by freezing at −80 °C.

Derivatization and Digestion of Transferrin. Apo-transferrin samples (10 mg) were dissolved in 1.0 mL of 50 mM HEPES buffer (pH 8.5) containing 20 mM $CaCl_2$ and 8 M urea (in the case of using thiourea, 2 M thiourea and 6 M urea were added). DTT was added to a final concentration of 20 mM to reduce disulfide bonds in the transferrin. The solution was then incubated for 2 h at 37 °C, after which it was cooled to room temperature. Derivatization of the cysteine residues was achieved by adding an excess of APTA to a final concentration of 2.0 M. The mixture was incubated in the dark for 2 h with subsequent dialysis overnight in 50 mM HEPES buffer (pH 8.0). The following day the derivatized protein was transferred to a fresh tube and trypsin was added (1/50 w:w ratio of enzyme to total protein). The digestion was allowed to proceed overnight at 37 °C and then stopped by adding TLCK in a slight molar excess over that of trypsin. The dialysis step is necessary to remove excess APTA and prevent it from displacing QAT peptides on the strong cation exchange chromatography column used in peptide selection.

Strong Cation Exchange Selection. A TSK-GEL SP-5PW column (7.5 × 7.5 mm) was used for selection of cysteine-containing peptides. All chromatographic steps were performed with a BioCAD 20 Micro-Analytical Workstation (Applied Biosystems, Framingham, MA). The flow rate was 1 mL/min with dual detection at 215 and 280 nm. To prepare the column for selection, the column was first cleaned with 7 bed volumes of a 50 mM MES buffer (pH 6.0, 1.0 M NaCl) and then equilibrated with 50 mM MES buffer at pH 6.0. Before being injected onto the strong cation exchange column, transferrin tryptic peptides were adjusted to pH 6.0. Cysteine-containing peptides were eluted from the SCX column with 50 mM MES buffer (pH 6.0, 0.5 M NaCl). The same procedure was applied to other tested pH buffers (sodium acetate buffer at pH 4.5 and HEPES buffer at pH 7.5).

LC/MS Analysis. Peptides from the transferrin tryptic digest were separated on a Vydac C18 column (4.6 × 250 mm) using an Integral Micro-Analytical Workstation (Applied Biosystems, Framingham, MA) at 1 mL/min. Solvent A was 0.01% TFA in deionized H_2O (dI H_2O) and solvent B was 95% CH_3CN/0.01% TFA in dI H_2O. A low-pressure postcolumn micro-splitter was used to direct 5−8% of the flow from the column to the QSTAR workstation (Applied Biosystems, Framingham, MA) equipped with an ESI

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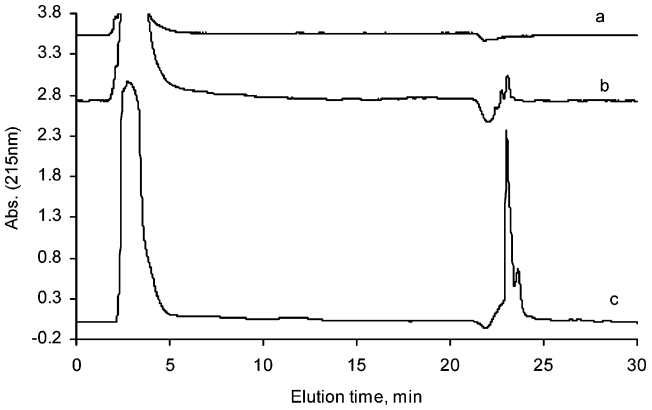

Figure 2. Elution profiles of transferrin tryptic peptides from strong cation exchange chromatography at different pH. Peptide loading was achieved at (a) 50 mM HEPES (pH 7.5), (b) 50 mM MES (pH 6.0), and (c) 50 mM sodium acetate (pH 4.5). Elution was performed after 15 min by switching to a corresponding pH buffer containing 0.5 M NaCl. Flow rate was maintained at 1.0 mL/min.

source. Flow from the HPLC was diverted to waste for 15−20 min after sample injection at 100% solvent A to remove salts, remaining derivatizing reagent APTA, and weakly adsorbed peptides. The QSTAR was then reconnected and peptides were separated in either a 15- or 60-min linear gradient (from 0% B to 50% B). MS spectra were obtained in the positive ion mode at a sampling rate of one spectrum per second.

MS/MS Analysis. Alternatively fractions from the LC were collected, dried, and stored for later analysis. At the time of analysis they were redissolved in CH_3OH/H_2O/acetic acid (50%/49%/1%) and injected into the QSTAR for MS/MS analysis. MS/MS analyses were done by flow injection at 3−5 μL/min. Lower collision energy was applied to QAT peptides than nonderivatized peptides. Identification of a peptide sequence was achieved by manual sequence matching using the SWISS−PROT database. The tandem MS of synthetic peptides and their corresponding QAT peptides were studied and compared.

MALDI-TOF MS Analysis. MALDI-TOF-MS mass spectra were obtained on a Voyager DE RP Biospectrometry Workstation (PerSeptive Biosystems, Framingham, MA)). All samples were collected in the positive linear mode using myoglobin as an external standard. A sinapinic acid (SA) matrix was prepared as a saturated, aqueous solution that contained 49.9% acetonitrile and 0.1% TFA. The sample was prepared by spotting 1 μL of protein solution first, followed by 1 μL of SA matrix immediately. The sample was allowed to air-dry before loading into the mass spectrometer. Spectra were obtained by averaging at least 200 laser shots.

RESULTS AND DISCUSSION

Principle of the Quaternary Amine Tag (QAT) Strategy. It is a common practice in shotgun proteomics to use SCX chromatography columns to separate peptides from a tryptic digest of a proteome. At acidic pH, all tryptic peptides carry a net positive charge and are adsorbed to a SCX column. While working with this mode of separation, the observation was made that many tryptic peptides from transferrin do not adsorb onto a SCX chromatography column being eluted with a weakly acidic or neutral mobile phase (Figure 2). LC/MS analysis of a transferrin

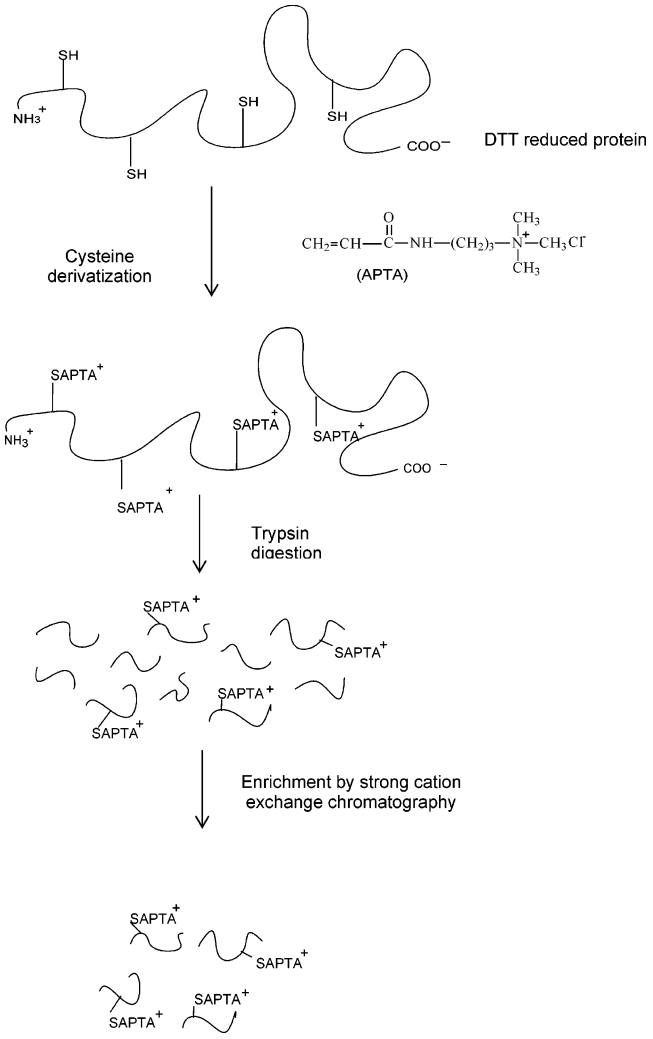

Figure 3. QAT strategy for derivatization of cysteine residues and enrichment of cysteine-containing peptides by strong cation exchange chromatography.

digest showed that although 26 peptides were retained at pH 4.5, only eight were adsorbed at pH 6.0. Of these eight, **K**SCHTGLG**R**, APNHAVVT**R**, AVAVV**KK**, AVGNL**RK**, SCHTGLG**R**, APNHAVVT**RK**, V**K**AVGNL**R**, and DSAHGFL**K**VPP**R**, five contain histidine and six had an additional basic amino acid resulting from a miscleavage by trypsin. Clearly, multiple basic amino acids in a peptide increase retention on an SCX column. In contrast, no peptides from this transferrin digest were retained at pH 7.5. Most tryptic peptides appear not to carry sufficient positive charge at higher pH to be retained by the SCX column. This led to the hypothesis that attaching additional positive charge to peptides through derivatization could cause them to be selected during SCX chromatography.

Actually, introduction of charged groups into proteins is a common practice. As part of alkylating proteins after reduction to prevent reformation of disulfide bonds, cysteine residues of proteins are frequently derivatized with iodoacetic acid or 4-vinylpyridine. But neither of these derivatizing agents introduce the requisite positive charge to enhance peptide capture by an SCX column at neutral pH. Although derivatization of proteins with 4-vinylpyridine introduces a positive charge, the pK_a of 4-vinylpyridine is sufficiently low (5.62) that it is not charged at neutral

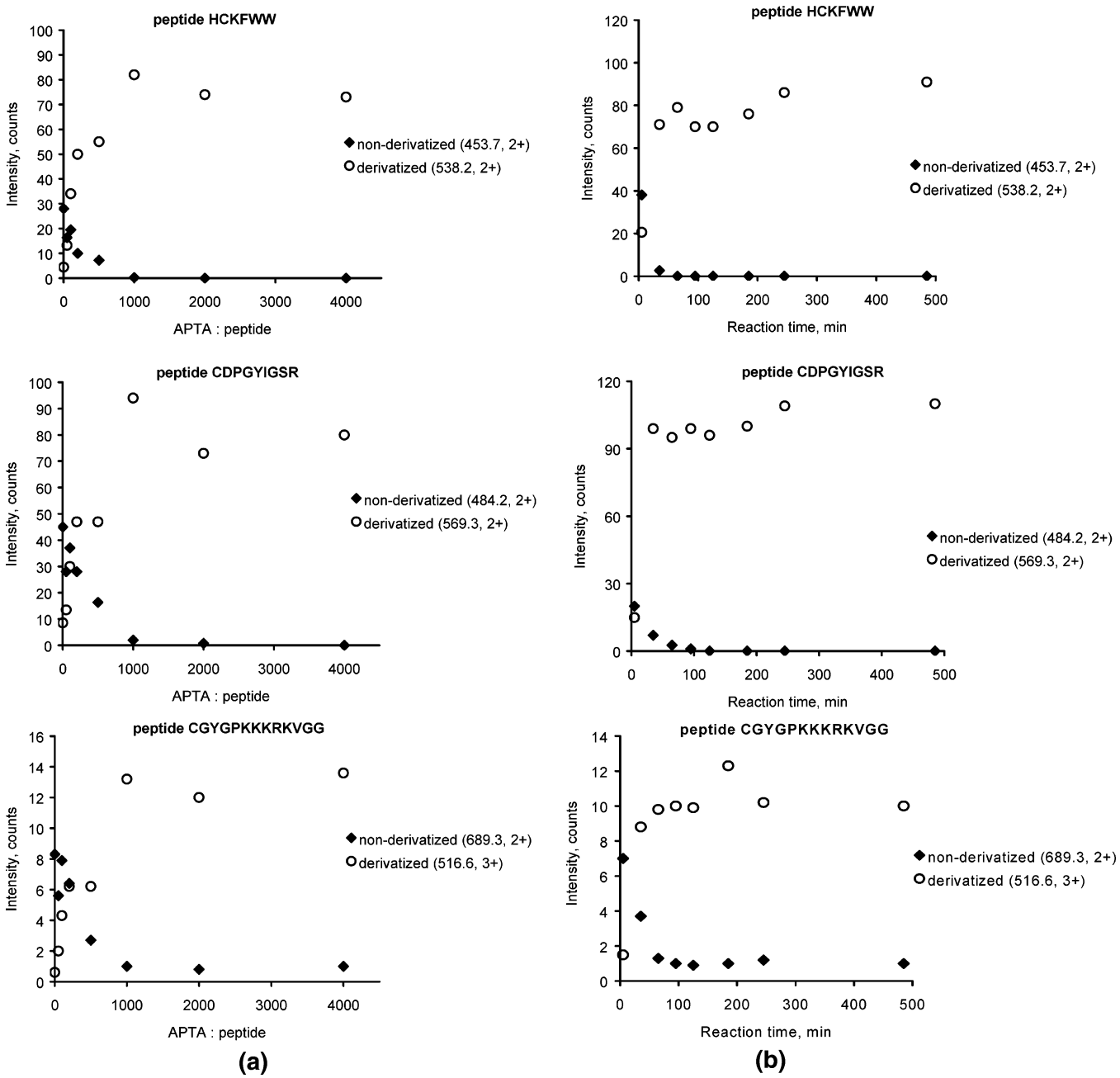

Figure 4. MS intensities of peptides after APTA derivatization at (a) various derivatizing agent-to-peptide ratios for a fixed an hour reaction time and (b) different reaction time periods at fixed 1000:1 APTA-to-peptide ratio.

pH.[21] Introduction of a quaternary amine would be much better.

Addition of a sulfhydryl group to a C=C bond is enhanced when the double bond is part of an α,β unsaturated system, as in the case of 4-vinylpyridine. The reagents (4-vinylbenzyl)trimethylammonium chloride and (3-acrylamidopropyl)trimethylammonium chloride (APTA) also meet this criterion and would be expected to react with sulfhydryl groups. Compared to commonly halogenated alkylating reagents for cysteine residue derivatization, acrylamide-based reagents have the advantage of not reacting with thiourea. It has been reported that addition of 1−2 M thiourea in protein solution can enhance solubilization of proteins, especially membrane and hydrophobic proteins. However, the interaction between thiourea with halogenated alkylating reagents gives partial cysteine residue alkylation.[22−24] Studies with the above two acrylamide-based reagents showed that only APTA reacted quantitatively with cysteine residues to introduce a quaternary amine tag (QAT) into proteins and peptides. Strong cation exchange properties of these QAT peptides were investigated below.

Figures 1 and 3 show the reaction schematic for tagging cysteine-containing proteins and the selective enrichment strategy of tryptic peptides derived from these proteins. The QAT tagging reagent APTA consists of two parts, (i) an acrylamide moiety that

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targets the sulfhydryl group of cysteine and (ii) a quaternary amine moiety for enhancing the interaction of tagged peptides with a SCX column. Quaternization also has the prospect of increasing ionization efficiency in the positive mode of mass spectrometry with both electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI). It is also important to note that derivatization with APTA increases the water solubility of proteins and peptides. No precipitation occurred during dialysis as is often the case when urea and other solubilizing agents are removed from samples.

Optimization of the Alkylation Reaction. Three synthetic peptides, HCKFWW, CDPGYIGSR, and CGYGPKKKRKVGG, were used to optimize reaction conditions for derivatizing cysteine residues with APTA (Figure 1). It is well-known that pH plays a key role in the sulfhydryl derivatization reaction.[25] At higher pH (>9.5), the primary amine on a lysine residue or at the N-terminus of a peptide will add to an acrylate.[25] But amines are sufficiently protonated several pH units below their pK_a that participation in this reaction is precluded. For this reason, the reaction was carried out at pH 8.5, which is also applied by other researchers without observing undesired side reaction products.[26,27] The degree to which participation of amine groups might be a problem with excess derivatization reagent APTA was examined using peptides with up to four lysine residues. Using LC/MS to analyze the reaction, although the ion signals of the dimers between intra- and inter-cysteine-containing peptides were observed, no undesired side reaction products at either lysine residues or at the N-terminus were detected, even with peptide CGYGP**KKK**R**K**VGG. Under a reducing environment such as DTT, the formation of dimers can be eliminated. It was concluded that quantitative derivatization of these peptides with APTA occurs in 1 h based on both total ion current chromatograms and reconstructed ion chromatograms (XIC) of the peptide and expected reaction products. Monoisotopic peak intensities of nonderivatized and derivatized forms of the three synthetic cysteine-containing peptides noted above were compared. Moreover, a fast gradient (15 min from 0 to 50% elution solution B) was used to minimize any solvent impact on ionization efficiency.

The optimum molar ratio of APTA to peptide was determined using molar ratios from 1:1 to 4000:1 in a 1-h reaction. Derivatization was found to be complete at a 1000:1 reagent−peptide ratio based on ESI-MS analysis of product formation (Figure 4a). No decomposition of QAT peptides was observed at higher ratios. The results were consistent for all three peptides investigated. Therefore, the 1000:1 molar ratio of APTA to peptide was applied in further studies.

Reaction time course was monitored from 5 min to 8 h, as shown in Figure 4b. Data from the three peptides examined revealed that the reaction proceeds to completion within an hour at the 1000:1 APTA-to-peptide ratio. Underivatized peptides were not detected in any of the studies. Neither were there any changes in peptide structure beyond the tagging of cysteine. However, cysteine-containing peptides were observed to form disulfide-linked intermolecular dimers in the absence of any reducing agent.

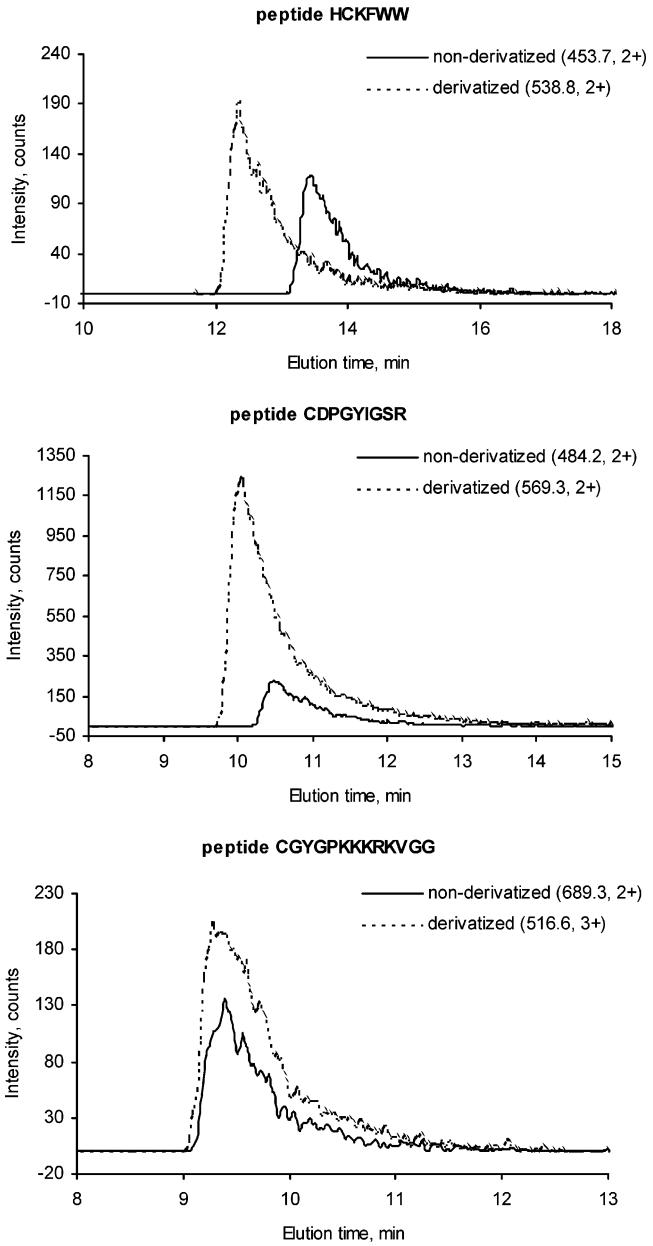

Figure 5. Comparison of ionization efficiency of nonderivatized (−) and derivatized (···) peptides from their reconstructed ion chromatograms (XIC).

Because of the permanent positive charge introduced into cysteine-containing peptides after quaternization, an increase in ionization efficiency was observed in the positive ion mode of both ESI-MS (Figure 5) and MALDI-MS (data not shown). But the increase in ionization efficiency was sequence-dependent. For example, ionization efficiency of CDPGYIGSR increased roughly 6-fold after quaternization whereas the increase for the peptides HCKFWW and CGYGPKKKRKVGG was only 2-fold. On the basis of these results, it is probable that cysteine quaternization will enhance the ionization efficiency of most cysteine-containing peptides, particularly those that are smaller and lack an arginine or histidine residue to convey positive charge.

Although the cysteine-containing model peptides were quantitatively derivatized, this reaction would be used in proteomic analyses at the protein level. Evaluation of the degree to which cysteine residues in a protein will be derivatized by a 1000:1 molar

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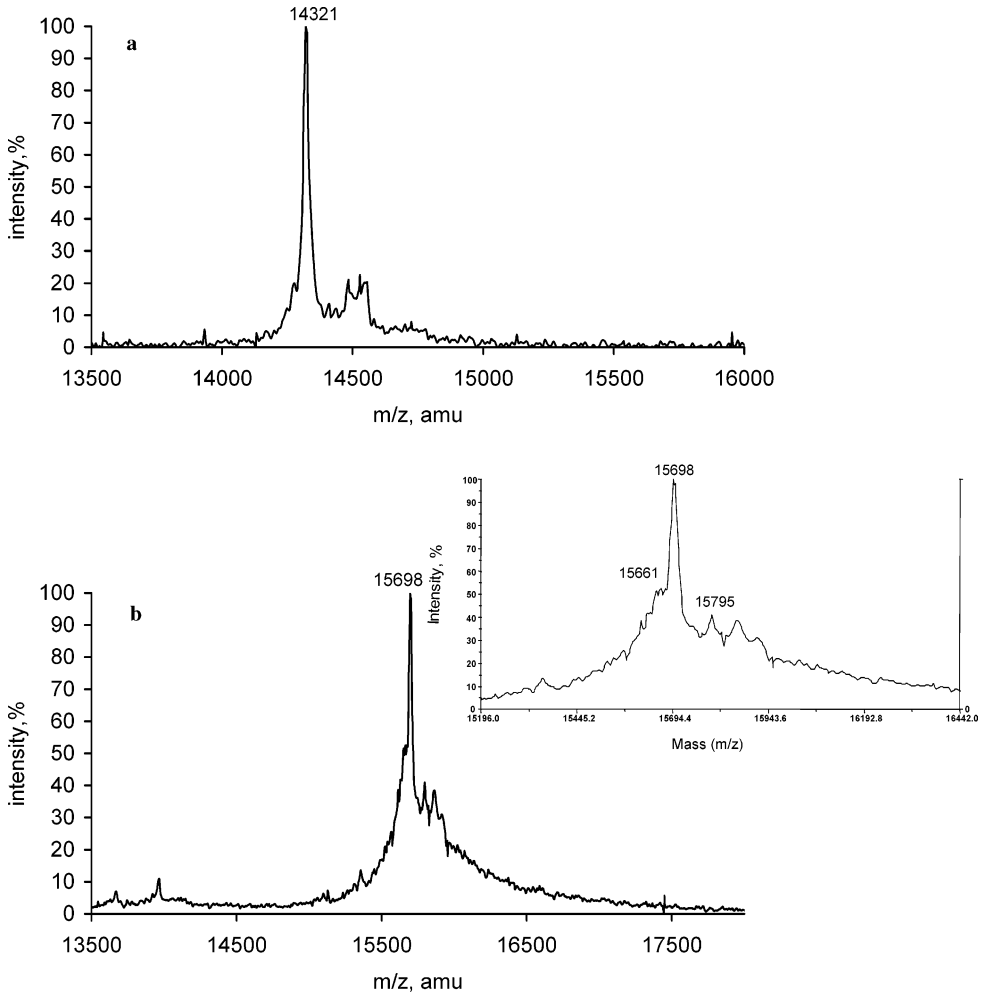


Figure 6. MALDI-MS spectra of (a) lysozyme and (b) APTA-derivatized lysozyme. The inset is the expanded spectrum of APTA-tagged lysozyme depicting the absence of any partially derivatized protein.

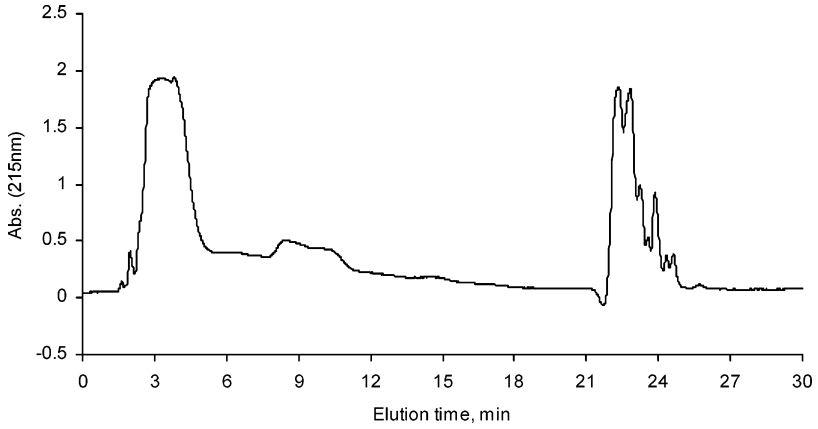
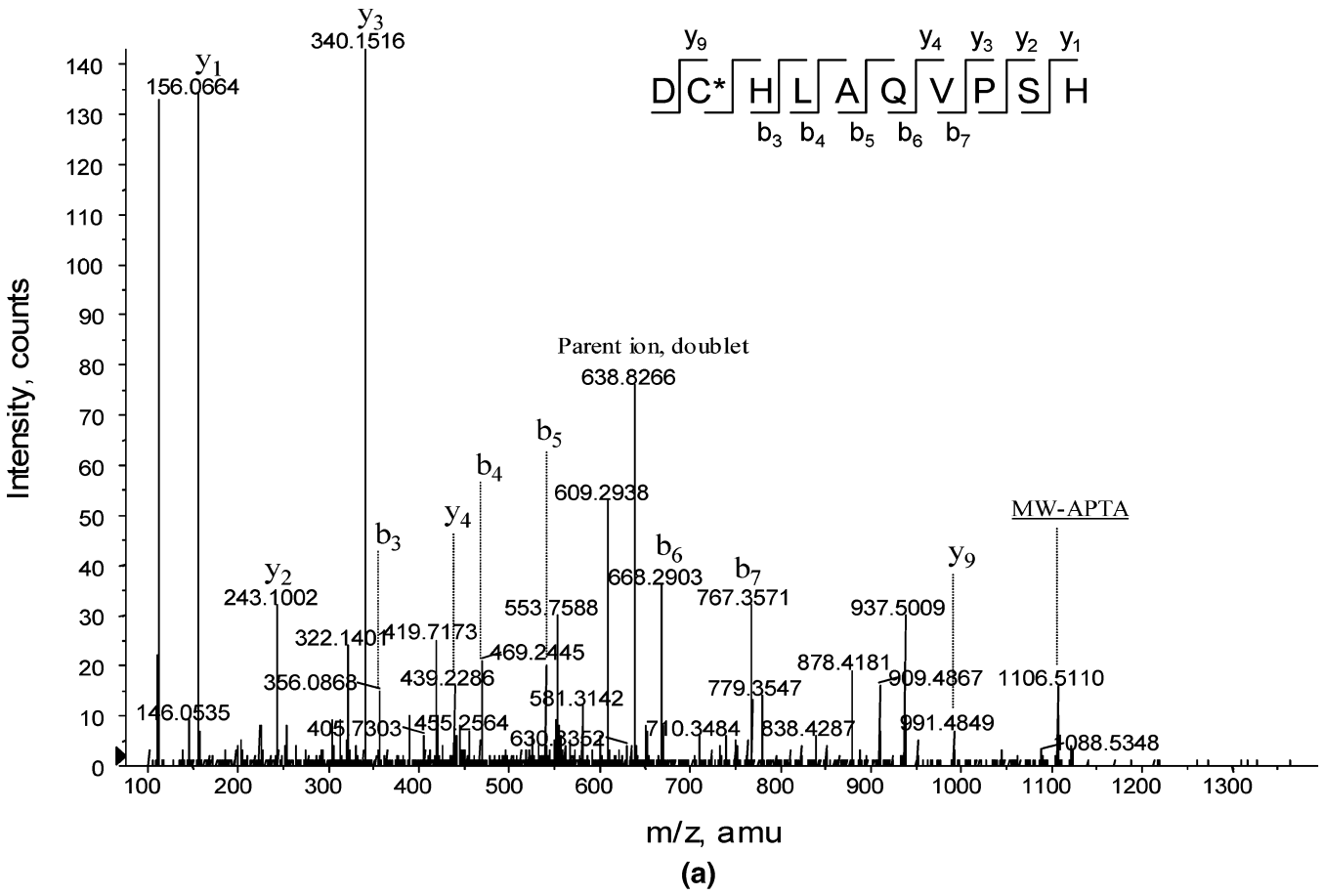


Figure 7. Strong cation exchange selection profile of APTA-derivatized transferrin tryptic peptides. Peptide loading was achieved at 50 mM MES (pH 6.0) buffer. The retained peptides were eluted after 15 min by switching to 0.5 M NaCl, 50 mM MES (pH 6.0) buffer. Flow rate was maintained at 1.0 mL/min.

ratio of APTA to protein was achieved using lysozyme as a model. After reduction, lysozyme was treated with APTA for an hour and then dialyzed. MALDI-MS spectra of both untreated and derivatized lysozyme were compared (Figure 6). It is seen in the figure that lysozyme and APTA-derivatized lysozyme vary by 1377 amu. Dividing the mass difference between the derivatized and underivatized protein by the mass of APTA (171.12 amu) gives a value of 8 for the number of cysteine residues derivatized in the protein. Clearly, all the eight cysteine residues in lysozyme were derivatized by APTA. Moreover, no additional peaks were seen in the MALDI-MS spectrum from partially derivatized lysozyme.

Enrichment and Identification of Cysteine-Containing Peptides from Transferrin. At pH 7.5, no tryptic peptides from apo-transferrin were retained by the SCX column, as illustrated in Figure 2a. However, at pH 6.0, peptides which have a net positive charge were retained as aforementioned. Therefore,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322.1401
419.7173
469.2445
878.4181
356.0868
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MW-APTA
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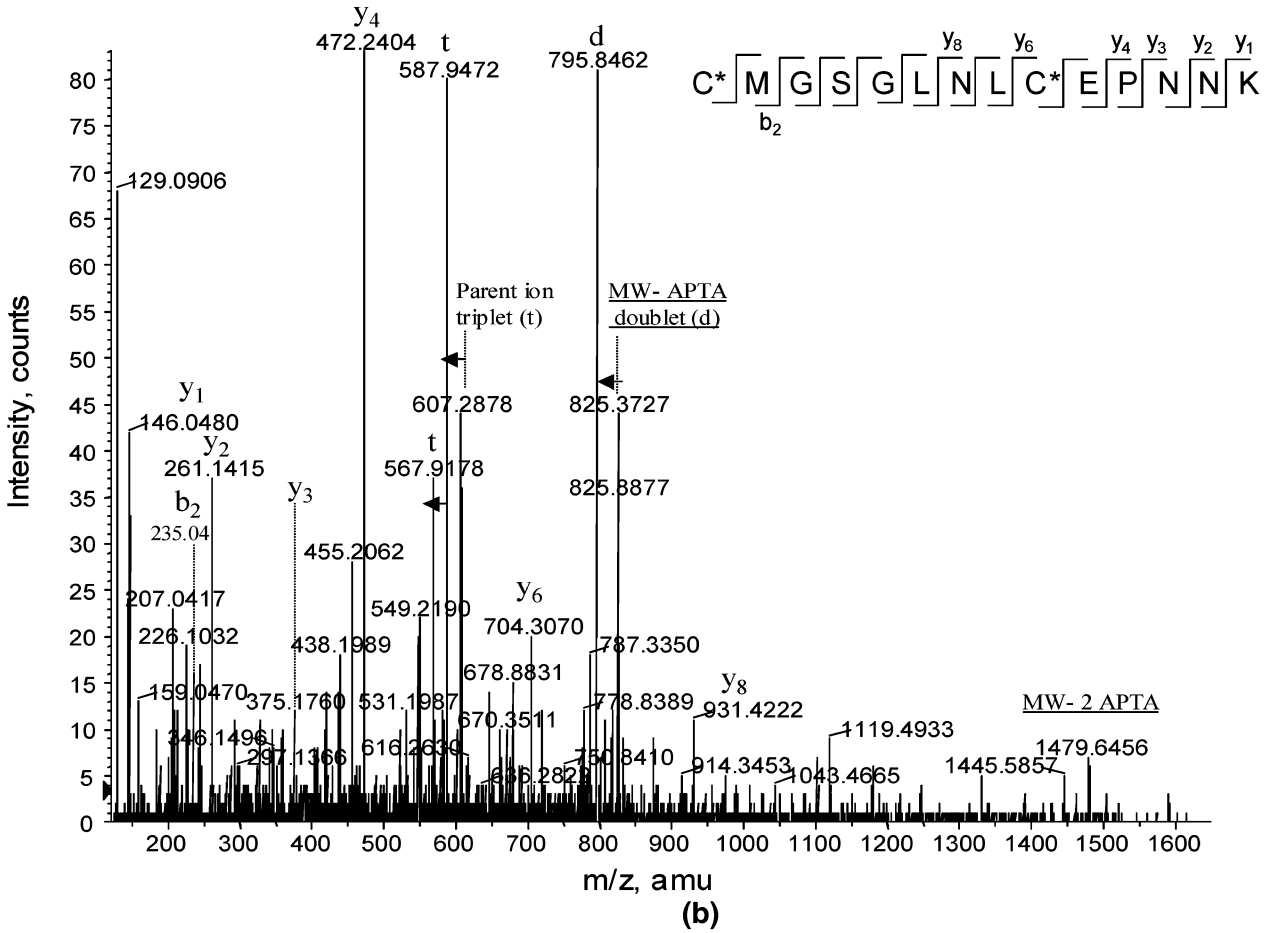
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m/z, amu

(b)

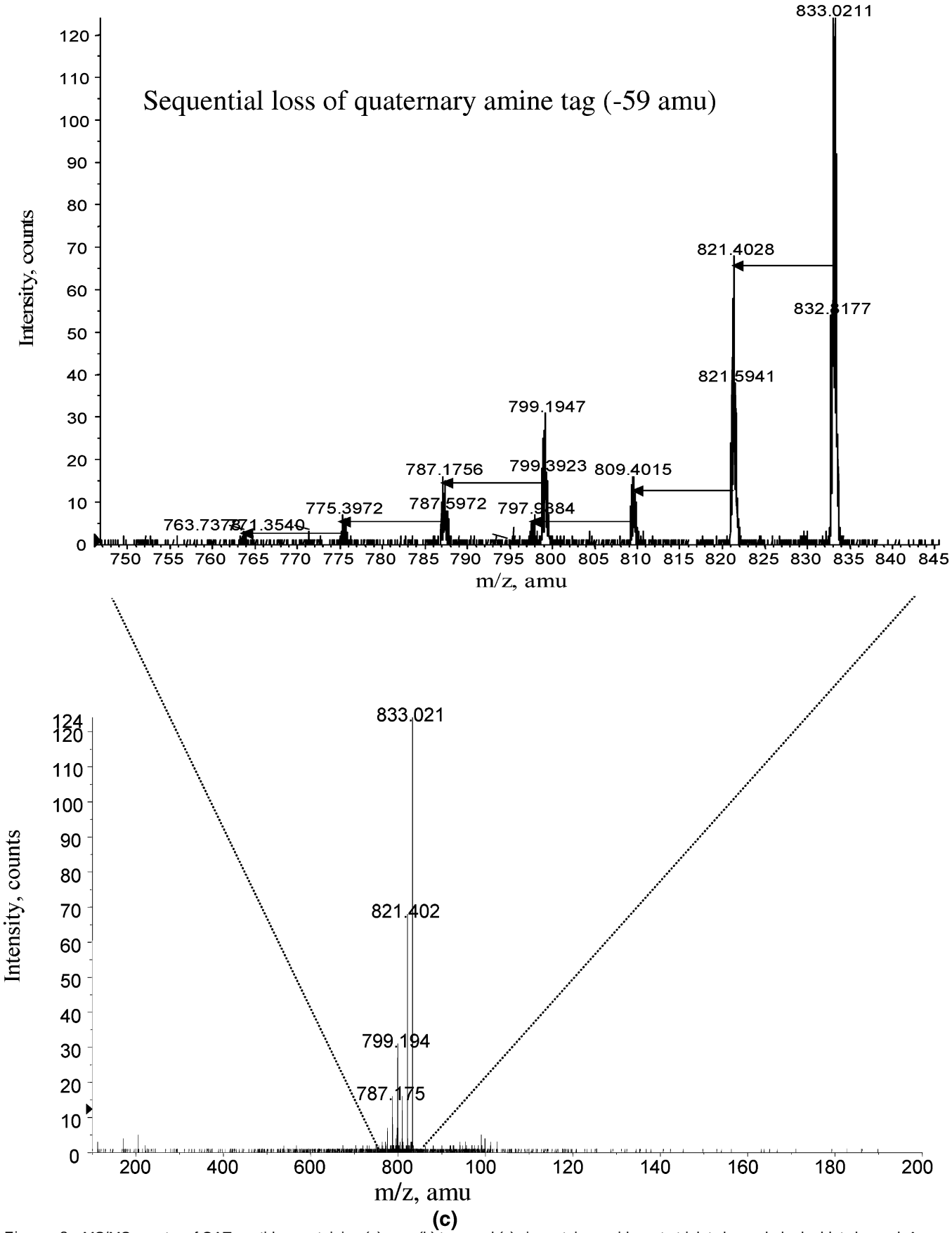

Figure 8. MS/MS spectra of QAT peptides containing (a) one, (b) two, and (c) six cysteine residues. t: triplet charged; d: doublet charged. Arrow indicates the loss of quaternary amine tag (−59 amu). Inset is the amplified graphic that depicts the loss of each quaternary amine tag (11.8 amu for a $z = 5+$ peptide).

specificity of cysteine peptide selection by an SCX column at pH 6.0 was evaluated using QAT peptides from apo-transferrin. Comparing the SCX profiles of the retained fraction with (Figure 7) and without (Figure 2b) APTA derivatization showed that more peptides were retained after derivatization.

Sequencing by tandem mass spectrometry and data from the SWISS−PROT database were used to identify the peptides selected by the SCX column. Figure 8 shows a few representative examples of derivatized cysteine-containing peptides with one, two, and six cysteine residues. It was found in MS/MS spectra that peptides containing a single cysteine residue loose the entire APTA derivatizing agent (−171.12 amu) from an $[M + 2H]^{2+}$ precursor ion. The peptide D**C***HLAQVPSH (Figure 8a) is an example. Peptides containing two cysteine residues were identified by a set of characteristic ions showing (1) the loss of two APTA (−342.24 amu) groups, (2) loss of one entire derivatizing agent APTA (−171.12 amu) and a quaternary amine moiety (−59 amu), and (3) the loss of two quaternary amine moieties sequentially from the $[M + 2H]^{2+}$ or $[M + 3H]^{3+}$ precursor ions (Figure 8b). With six cysteine residues (Figure 8c), as in the peptide SGS**C***AP**C***ADGTDFPQL**C***QL**C***PG**C***G**C***STLNQY, the most prominent ions in the spectrum came from the loss of multiple quaternary amine groups. The tandem mass spectrum obtained from the $[M + 5H]^{5+}$ precursor ion of this peptide showed a characteristic loss of 6 quaternary amine moieties (−59 amu). Identification of peptides with this many cysteine residues has to be based on computation of the molecular weight of the peptide and cysteine residue content. Fortunately, few peptides have three or more cysteine residues according to in silico analyses of databases and identification with only a peptide molecular weight and cysteine content is relatively easy. Loss of the quaternary amine tag, or the entire label during mass spectrometry, is a signature for the presence of cysteine. This observation is similar to that of phosphorylated peptides in which a loss of −80 amu ($H_2PO_3^-$) and −98 amu (H_3PO_4) in the MS/MS spectrum is a characteristic signature for the presence of phosphorylation.

It should be noted that, in the interpretation of APTA-derivatized peptide spectra, 170.12 amu is lost from peptides instead of the predicted 171.12 amu for APTA. This is thought to be due to the fact that because these peptides already carry a positive charge from the quaternary amine group, they have no need to acquire a proton during ionization. But when the entire APTA group (−171.12 amu) is lost, the peptide needs to pick up a proton to be ionized. This could explain the loss 170.12 amu instead of the expected 171.12 amu.

Table 1 lists the transferrin tryptic peptides retained on the SCX column after APTA derivatization. Twenty-eight of the 33 peptides identified contained cysteine. Of the 5 nonspecifically bound peptides, 4 contained a histidine residue. This is because histidine residues still have some positive charge at pH 6.0. Although these peptides can be eliminated by passing samples through a copper-loaded immobilized metal affinity chromatography (IMAC) column before selection with the SCX column,[23] sample loss may also incur. Since the number of nonspecific bound peptides is small, no further sample cleanup steps were applied.

Because this selection strategy is charge-dependent, it is anticipated that cysteine-containing peptides with multiple aspartic acid or glutamic acid residues will have a net negative charge and will not be selected by an SCX column as well. It should also be noted that a few hydrophilic cysteine-containing peptides may not be retained by a reversed phase chromatography (RPC) column, even though the SCX column selects them.

Table 1. Peptides Retained on the SCX Column from a Tryptic Digest of (3-Acrylamidopropyl)trimethylammonium Chloride (APTA)-Derivatized Transferrin[a]

derivatized peptide m/z	nonderivatized peptide m/z	mass difference	peptide sequence*
1099.61	929.48	170.13	DKEAC*VHK
954.52	784.39	170.13	ANC*HLAR
1000.52	830.39	170.13	SC*HTGLGR
810.42	640.29	170.13	C*QSFR
896.40	726.32	170.08	EAC*TFR
784.40	614.28	170.12	KINHC*
895.48	725.36	170.12	SGAFKC*L
751.37	581.25	170.12	VTDC*SG
640.29	470.16	170.13	YANC*
886.48	716.34	170.14	WC*ALSH
1308.67	1138.51	170.16	WC*ALSHHER
708.42	538.28	170.14	C*LFR
1209.60	1039.52	170.08	SLLEAC*TFR
1699.88	1529.75	170.13	KPVEEYANC*HLAR
1276.64	1106.51	170.13	DC*HLAQVPSH
1802.96	1632.79	170.17	DC*HLAQVPSHTVVAR
1122.62	952.45	170.17	C*ALSHHER
1353.72	1013.43	340.29	C*STSSLLEAC*
1454.72	1114.45	340.27	C*STSSLLEAC*T
1601.80	1261.54	340.26	C*STSSLLEAC*TF
1757.92	1417.65	340.27	C*STSSLLEAC*TFR
1886.04	1545.78	340.26	KC*STSSLLEAC*TFR
1819.90	1479.65	340.25	C*MGSGLNLC*EPNNK
1948.99	1608.73	340.26	C*MGSGLNLC*EPNNKE
1933.00	1592.74	340.26	LC*MGSGLNLC*EPNNK
1755.85	1245.46	510.39	C*PGC*GC*STLNQY
4160.00	3139.24	1020.76	SGSC*APC*ADGTDFPQLC*QLC*PGC*GC*STLNQY
4527.20	3506.42	1020.78	SGSC*APC*ADGTDFPQLC*QLC*PGC*GC*STLNQYFGY

[a] Asterisk indicates cysteine residue is derivatized with APTA.

CONCLUSION

It is concluded that APTA is an effective reagent for derivatizing cysteine residues that is complimentary to other derivatizing reagents commonly used in proteomics. It is further concluded that use of the APTA quaternary amine tag in combination with strong cation exchange chromatography (1) allows a relatively high degree of selectivity to be achieved in the selection of cysteine-containing peptides, (2) substantially simplifies tryptic digests in a single step, and (3) integrates easily with reversed phase chromatography (RPC). Similar to MudPIT, the fact that the SCX and RPC modes of chromatography are orthogonal in selectivity provide maximum separation. And the presence of QAT on peptides provide the possibility to increase ionization efficiency in mass spectrometry and QAT peptides can be easily identified in MS/MS spectra by their characteristic loss of either the entire APTA group (−171.12 amu) or the quaternary amine tag (−59 amu).

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