Tissue proteomics using chemical immobilization and mass spectrometry

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Abstract

Proteomics analysis is important for characterizing tissues to gain biological and pathological insights, which could lead to the identification of disease-associated proteins for disease diagnostics or targeted therapy. However, tissues are commonly embedded in optimal cutting temperature medium (OCT) or are formalin-fixed and paraffin-embedded (FFPE) in order to maintain tissue morphology for histology evaluation. Although several tissue proteomic analyses have been performed on FFPE tissues using advanced mass spectrometry (MS) technologies, high-throughput proteomic analysis of OCT-embedded tissues has been difficult due to the interference of OCT in the MS analysis. In addition, molecules other than proteins present in tissues further complicate tissue proteomic analysis. Here, we report the development of a method using chemical immobilization of proteins for peptide extraction (CIPPE). In this method, proteins are chemically immobilized onto a solid support; interferences from tissues and OCT embedding are removed by extensive washing of proteins conjugated on the solid support. Peptides are then released from the solid phase by proteolysis, enabling MS analysis. This method was first validated by eliminating OCT interference from a standard protein, human serum albumin, where all of the unique peaks contributed by OCT contamination were eradicated. Finally, this method was applied for the proteomic analysis of frozen and OCT-embedded tissues using iTRAQ (isobaric tag for relative and absolute quantitation) labeling and two-dimensional liquid chromatography tandem mass spectrometry. The data showed reproducible extraction and quantitation of 10,284 proteins from 3996 protein groups and a minimal impact of OCT embedding on the analysis of the global proteome of the stored tissue samples.

Keywords: OCT, Proteomics, Protein chemical immobilization, Polymer contamination, Tissues

Tissue proteins play important roles in biological processes. The quantitative analysis of tissue proteins facilitates the understanding of molecular mechanisms that differentiate between normal and disease states. Disease-specific tissue-derived proteins can also be used as biomarkers for disease diagnosis or as novel drug targets for therapeutic drug development [1]. In the diseased state, tissues secrete or shed disease-specific proteins into the body fluids such as serum, which can be used as biomarkers. However, the secreted proteins from a diseased tissue are present in higher concentrations at the tissue site and become diluted in the serum, which contains proteins from other tissues [1,2]. If disease-specific proteins can be identified in tissues, tissue-targeted approaches can be used to detect tissue-derived proteins in serum [1]. An example of this type of approach was shown for the detection of prostate cancer proteins in serum using tandem time-of-flight (TOF/TOF) mass spectrometry (MS) [3].

Traditionally, tissue proteins are analyzed using immunoassays, which rely on the development of high-quality antibodies. Advances in MS and high-performance liquid chromatography (HPLC) systems have led to the increasingly widespread applicability of proteomics [4]. Increases in sensitivity, resolution, and speed

Abbreviations used: TOF/TOF, tandem time-of-flight; MS, mass spectrometry; HPLC, high-performance liquid chromatography; OCT, optimal cutting temperature medium; FFPE, formalin-fixed and paraffin-embedded; MALDI, matrix-assisted laser desorption/ionization; ESI, electrospray ionization; LC, liquid chromatography; MS/MS, tandem mass spectrometry; 2D DIGE, two-dimensional gel electrophoresis; SPEG, solid-phase extraction of glycopeptides; CIPPE, chemical immobilization of proteins for peptide extraction; HSA, human serum albumin; PBS, phosphate-buffered saline; DTT, dithiothreitol; BCA, bisinchoninic acid; RPLC, reversed-phase liquid chromatography; TEAB, triethyl ammonium bicarbonate; FDR, false discovery rate; PTM, post-translational modification.
of the mass spectrometers have enabled the rapid identification of large numbers of proteins with high confidence, thereby enabling the analysis of complex samples such as tissue. Analysis of the tissue proteome, located at the primary site of pathology, aids the understanding of the molecular mechanism of diseases and facilitates the identification of potential biomarkers and therapeutic targets.

Tissue proteomics requires tissues to be stored by snap-freezing. However, flash-frozen tissues without embedding medium are difficult to section, thereby making histopathology or immunohistochemistry evaluation difficult. Instead, tissues are embedded in optimal cutting temperature medium (OCT) or are formalin-fixed and paraffin-embedded (FFPE) to retain their morphology [5]. Recently, proteomic analyses have been performed on FFPE-embedded tissues [6–8]. However, FFPE tissues undergo extensive protein/DNA/RNA cross-linking during formalin fixation, which creates inter- and intra-cross-linked proteins [5,9,10]. Some of the peptide modifications that occur in the proteomic analysis of FFPE tissues are methylol derivatives, Schiff bases, and methylene bridges [9]. The duration of FFPE processing and storage can also lead to different levels of protein degradation and protein modifications. In contrast, OCT-embedded tissues are instantly frozen and stored for histological studies; therefore, the protein content likely remains unchanged and is representative of the tissue proteome.

OCT is widely used for tissue embedding prior to storage. However, the proteomic analysis of OCT-embedded tissues is difficult, largely due to the presence of water-soluble synthetic polymers. OCT can compete with peptides for ionization during MS analysis [11]. OCT can also generate ion suppression in matrix-assisted laser desorption/ionization (MALDI) MS and ionization competition in electrospray ionization (ESI) MS [12]. In addition, OCT creates a deleterious effect on the peptide chromatographic separation required for tissue proteomics. OCT has a high affinity to the reversed-phase stationary medium commonly used in shotgun proteomics. OCT competes with peptides for binding to the C18 reversed-phase column. OCT also decreases the sensitivity of detection due to its propensity to coelute with peptides during chromatographic separation. Thus, it is necessary to remove OCT prior to the liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis of tissues.

Due to the malicious effect of OCT on the mass spectrometer, only a small number of proteomic studies have been performed on OCT-embedded tissues [13–17]. OCT-embedded tissues are studied using either two-dimensional gel electrophoresis (2D DIGE) technology or shotgun proteomics using LC–MS/MS. 2D DIGE can separate proteins from OCT; however, most of the LC–MS/MS studies of OCT-embedded tissue did not completely remove OCT, resulting in fewer protein identifications [15–17]. Recently, we demonstrated that OCT-embedded tissues could be used for glycoproteomic analysis using solid-phase extraction of glycopeptides (SPEG) [18]. The glycopeptides were chemically immobilized to the solid support using oxidized glycan tags, whereas the non-glycopeptides and OCT were removed from the immobilized peptides before the enzymatic release of N-glycopeptides. Whereas the SPEG method was used to effectively remove the OCT interference prior to glycoprotein analysis, proteins without glycans were not conjugated to the solid phase and were excluded from the proteomic analysis.

In this study, we describe a method using chemical immobilization of proteins for peptide extraction (CIPPE) from tissues for tissue proteomic analysis. In this method, proteins are chemically immobilized onto a solid support, which allows for sample cleaning to remove interferences from tissues before peptides are released from the solid support using proteolysis. We applied the method to the quantitative analysis of frozen and OCT-embedded tissues to determine the impact of OCT on the MS-based detection of the tissue proteome.

Materials and methods

Materials

Human serum albumin (HSA), 1× PBS (phosphate-buffered saline) buffer, dithiothreitol (DTT), sodium cyanoborohydride, and iodoacetamide were purchased from Sigma–Aldrich (St. Louis, MO, USA). A protein estimation BCA (bisinchoninic acid) kit, poly-acrylamide desalting spin columns, and AminoLink coupling resin were purchased from Thermo Scientific (Rockford, IL, USA). Sequencing-grade trypsin was purchased from Promega (Madison, WI, USA). ITRAQ 4-plex reagents were purchased from AB Sciex (Framingham, MA, USA). PNGase F was obtained from New England Biolabs (Ipswich, MA, USA).

Protein extraction

Mouse kidney tissue was collected from NIH01a mice and snap-frozen in Christopher Kemp’s laboratory at the Fred Hutchinson Cancer Research Center [19,20]. Mouse kidney tissue was cut in half. One half was embedded in OCT, followed by storage at −80 °C. The other half was stored as fresh-frozen tissue. The OCT-embedded or frozen mouse kidney tissue was lysed in 1 ml of RIPA buffer (100 mM sodium citrate, 50 mM sodium carbonate, and 1% NP-40) by vortexing for 2 to 3 min and sonating for 4 min in an ice bath to homogenize the tissues. After the tissues were homogenized, the buffer of the extracted proteins was exchanged with 1 ml of pH 10 binding solution (40 mM sodium citrate and 20 mM sodium carbonate) using polyacrylamide desalting spin columns. The protein concentration was determined by BCA assay. Protein (1 mg) from each tissue was used for AminoLink resin capture using the chemical immobilization of proteins to beads protocol as described previously [21,22]. Briefly, AminoLink resin (800 μl) was loaded onto snap-cap spin columns and centrifuged at 2000g for 1 min. Resin was washed with 800 μl of pH 10 buffer (100 mM sodium citrate and 50 mM sodium carbonate), followed by centrifugation. The wash step was repeated twice. The sample in pH 10 buffer (1 mg:200 μl, protein/beads) was added to the washed AminoLink resin.

The sample–resin mixture was incubated at room temperature overnight on a mixer. The mixture was centrifuged at 2000g to remove any unbound protein. The resin was rinsed three times with 450 μl of 1× PBS buffer. Then, 50 mM sodium cyanoborohydride in PBS (400 μl) was added to the resin. After a 4-h incubation, the supernatant was removed via centrifugation (2000g), and 400 μl of 1 M Tris–HCl (pH 7.6) in the presence of 50 mM sodium cyanoborohydride was added to block any unreacted aldehyde sites on the resin. The blocking process was terminated after 1 h. Then, the beads were washed twice with 1× PBS, twice with 1.5 M NaCl, and three times with deionized water.

Peptide extraction by proteolysis

Proteins bound to the beads were treated with 10 mM DTT in 50 mM ammonium bicarbonate for 30 min at 60 °C, followed by a wash with 50 mM ammonium bicarbonate. Afterward, the beads were treated for 1 h with 15 mM iodoacetamide in 50 mM ammonium bicarbonate in the dark. Finally, proteins were digested using trypsin (1:50, enzyme/substrate) in the presence of 50 mM ammonium bicarbonate. The proteins were digested at 37 °C overnight. The released peptides were collected from the supernatant of the
beads and the following water wash step of the beads. iTRAQ labeling was performed according to the manufacturer’s protocol [23].

In-solution digestion

HSA protein with and without OCT was incubated with 10 mM DTT at 60 °C for 1 h and alkylated for 30 min with 10 mM iodoacetamide in the dark at room temperature. The pH of the solution was adjusted to 7.5 with 50 mM NH₄HCO₃, and protein was enzymatically digested with trypsin (1:50, enzyme/substrate) with overnight incubation at 37 °C.

MS analysis of peptides by direct infusion to TSQ Quantum

A TSQ Quantum Ultra triple quadrupole mass spectrometer (Thermo Scientific) with an ESI source was used for the analysis of HSA peptides using direct infusion. The flow rate was set at 5 μl/min. Peptides were scanned from m/z 300 to 1000 using a spray voltage of 3000 V and a capillary temperature of 180 °C.

High-pH RPLC fractionation

iTRAQ-labeled peptides (200 μg) were subjected to high-pH RPLC (reversed-phase liquid chromatography) fractionation with a 1200 Infinity LC system (Agilent Technology, Santa Clara, CA, USA) and a 4.6 × 100-mm BEH130 C18 column (Waters, Milford, MA, USA). Samples were adjusted to a basic pH using 1% ammonium hydroxide and injected into 2 ml of solvent A (7 mM triethyl ammonium bicarbonate [TEAB] in water). Solvent B was 7 mM TEAB in 90% acetonitrile.

The separation gradient was set as follows: 0% B in 18 min, 0 to 31% B in 42 min, 31 to 50% B in 10 min, 75 to 100% B in 15 min, and 100% B in an additional 10 min. A total of 96 fractions were collected throughout the LC separation and were concatenated into 24 fractions according to the following scheme: fractions 1, 25, 49, and 73; fractions 2, 26, 50, and 74, and so forth. The samples were dried in a SpeedVac and stored at −80 °C until LC–MS/MS analysis.

LC–MS/MS analysis

A Dionex Ultimate 3000 RSLC nano system (Thermo Scientific) was used with a 75 μm × 25-cm Acclaim PepMap100 separating column (Thermo Scientific). Peptides were separated using a flow rate of 300 nl/min with mobile phases A (0.1% formic acid in water) and B (0.1% formic acid in 95% acetonitrile). The gradient profile was set as follows: 4 to 35% B in 70 min and 35 to 95% B in 5 min. MS analysis was performed using a Q Exactive mass spectrometer (Thermo Scientific). The spray voltage was set at 2.0 kV. Orbitrap spectra were collected at a resolution of 70 K followed by data-dependent HCD (higher energy collisional dissociation) MS/MS (at a resolution of 17,500 and collision energy of 33) of the 20 most abundant ions. A dynamic exclusion time of 25 s was used.

Database search

Data generated using Orbitrap were searched using SEQUEST with Proteome Discoverer 1.3 (Thermo Scientific) against the IPI mouse version 3.87 database containing 59,534 protein entries. Peptides were searched with fully tryptic specificity allowing only two missed cleavages. The search parameters used were 10 ppm precursor tolerance and 0.06 Da fragment ion tolerance, a static modification of 4-plex iTRAQ at the N terminus, and carboxymethylation of cysteine. The following variable modifications were used: oxidation of methionine, deamidation of asparagine, and 4-plex iTRAQ modification of lysine. Filters used for data analysis included peptide rank 1, 2 peptides per protein, and 2% FDR (false discovery rate) threshold.

Data analysis

Peaks were selected from ESI spectra acquired on a TSQ Quantum mass spectrometer with a threshold of 20% intensity of the base peak intensity. Peaks were obtained from HSA protein digested in the presence of OCT, without OCT, and with OCT followed by the removal of OCT. Afterward, the spectra were aligned and compared. The comparison was performed among HSA, HSA with OCT, and HSA with OCT followed by OCT removal using CIPPE.

The Pearson’s correlation coefficient of the peptide spectra obtained between replicate analyses of OCT-embedded tissues (iTRAQ 116 and 117) and frozen tissue (iTRAQ 114 and 115) using CIPPE was calculated to assess the reproducibility of the OCT removal method. Protein expression from the OCT-embedded tissue (116 and 117) and frozen tissue (114 and 115) was quantified and normalized using Proteome Discoverer 1.3. The log₂ ratios between replicates 116 and 117 were used as the “null” distribution, and the values for the 5% cutoff (2.5th and 97.5th percentiles) of the histogram were selected as the thresholds for increased and decreased expression. Similarly, the Pearson’s correlation coefficient of the peptide spectra between the OCT-embedded tissues/frozen tissues (116/114 and 117/115) was calculated to assess the impact of embedding the tissue in OCT.

Results and discussion

To analyze the global proteome of tissues, therefore, we employed the CIPPE method based on the capture of proteins by conjugation of amino groups of proteins to beads containing aldehyde groups (Fig. 1). After washing, the proteins conjugated to beads were reduced, carbamidomethylated, and proteolyzed to release the peptides for proteomic analysis (Fig. 1). Using this method, proteins were extracted from tissues and chemically immobilized onto the solid phase by reductive amination, and peptides were released from solid phase by proteolytic digestion. However, OCT polymers from OCT-embedding and other MS interfering molecules from tissues were not immobilized on the beads or not released from beads. Therefore, they were removed by washing the beads.

To develop a procedure to remove the OCT, HSA was used as a model protein. The tryptic peptides from HSA with OCT contamination were directly analyzed by a TSQ Quantum mass spectrometer using direct infusion ESI. Fig. 2A shows the ESI spectrum of OCT-contaminated HSA digested with trypsin demonstrating an MS pattern with a regular bell-shaped curve with mass values that are 44, 22, and 14.6 Da apart. These readily observed peaks correspond to different charge states of the polyethylene glycol present in OCT. The OCT polymer peaks mask those of the HSA peptides. In MS, OCT dominates the mass spectrum, indicating the preferential ionization of OCT compared with the HSA peptides in a set mixture of OCT to the HSA peptide. At 20% of the base peak intensity, only 11 peptide HSA peaks were detected in the OCT-contaminated HSA (10% OCT volume/HSA weight) (Fig. 2A). In contrast, OCT-contaminated HSA had 46 unique polymer peaks that suppressed the ionization of HSA peptides and overshadowed these peptides in the mass spectrum. To remove OCT interferences from the sample, first OCT-contaminated HSA was chemically immobilized onto beads using reductive amination, then the beads were then washed with various conditions, and finally the immobilized HSA was digested using trypsin. The released peptides were analyzed (Fig. 2B). We found that after washing the beads with PBS, 1.5 M NaCl, and
water, no OCT peaks were detected and the HSA tryptic peptide peaks were visible in the mass spectra. None of the 46 polymer peaks uniquely observed in the OCT sample was observed after CIPPE. In this method, proteins were bound to the solid phase and released by trypsin. The OCT polymers were removed from released HSA tryptic peptides, resulting in the complete removal of OCT from chemically immobilized proteins. The results showed that CIPPE removed OCT contaminants from the protein sample, thereby enabling the MS analysis of proteins with OCT contamination.

CIPPE is a chemical immobilization method for protein immobilization via protein amino groups and peptide release by protease digestion. To compare the peptide spectral pattern from proteins directly digested by trypsin in solution and the peptide spectral pattern released from immobilized protein, HSA without OCT contamination was digested in solution. The tryptic peptides from HSA digested in solution were directly digested by trypsin in solution and the peptide spectral digestion. To compare the peptide spectral pattern from protein digestion using direct infusion ESI, and the peptides were digested in solution were directly analyzed by a TSQ Quantum tamination was digested in solution. The tryptic peptides from HSA (Fig. 2C). The difference may have resulted from the OCT contamination.

The results show that there are no significant losses of Lys-containing peptides from the on-bead digested samples using the CIPPE method. However, peptides prepared from on-bead digestion using the CIPPE method and in-solution digestion showed different patterns (Fig. 2). This could be due to the fact that protein amino groups are randomly conjugated to the solid support and conjugated peptides could be lost in abundance, but the Lys-containing peptides are still detectable and identified due to random conjugation on beads. In the CIPPE method, the proteolytic digestion of proteins will not release chemically immobilized amino terminal or Lys-containing peptides. However, proteins might not be immobilized onto the beads at the same amino group-containing peptides. Our data show that identification of most of the peptides on the immobilized protein is possible provided that there are various amino groups present in the protein. In a complex sample analyzed via a shotgun proteomic approach, commonly only two peptides are required for protein identification. In addition, protease digestion efficiency could differ between on-bead digestion and in-solution digestion based on the availability of different protease digestion sites, which could result in the altered quantitation of different peptides between on-bead digestion and in-solution digestion. However, this random conjugation of amino group-containing peptides and changes in protease digestion efficiency will not affect the relative quantitation among the protein samples because different protein samples are expected to have similar conjugation and protease digestion efficiency.

To determine the impact of embedding tissue with OCT prior to tissue proteomic analysis, we applied the developed method to OCT-embedded tissues and frozen tissues. A complex biological tissue, mouse kidney, was analyzed. Mouse kidney tissue was divided into two halves. One half was embedded in OCT, and the other half was directly frozen. An equal amount of protein from the four tissues (1 mg) was used for quantitative proteomic profiling using chemical immobilization and iTRAQ methodology (Fig. 3). Protein from each sample was first bound to beads. The quantity of unbound protein was determined by BCA assay and was estimated to be less than 5% of the protein input for protein immobilization. Beads containing sample were rigorously washed. Proteins were further reduced and alkylated on the beads. Finally, proteins were released from the beads using proteolysis, and all of the released peptides were iTRAQ labeled and mixed. Technical replicates of an OCT-embedded tissue (labeled with iTRAQ 116 and 117) and technical replicates of a frozen tissue (labeled with iTRAQ 114 and 115) were lysed. Here, 200 μg of sample was used for global proteomic analysis. In the global proteomic analysis, basic RPLC was used to generate 24 offline fractions, and each fraction was subjected to LC–MS/MS analysis using a Q Exactive mass spectrometer. From the proteomic analysis of the iTRAQ-labeled tryptic peptides, 10,284 proteins belonging to 3996 protein groups were identified on the basis of at least two unique peptides exceeding a threshold score of 2% FDR (see Supplemental Table 2 in supple-
The quantification results are depicted in Fig. 4. Each dot represents an identified peptide. The replicates of 114/115 and 116/117 showed little variance. The correlation between channels 116 and 117 was 0.990 for the replicate global proteomic analysis of OCT-embedded tissues. Similarly, the correlation between iTRAQ channels 114 and 115 was 0.996 for the replicate proteomic analysis of frozen tissues. From the analysis of replicate OCT-embedded tissues using CIPPE and MS/MS, we estimated that 95% of proteins had a ratio between 0.850 and 1.180, which indicated good analytical reproducibility of the frozen and OCT-embedded tissues using the CIPPE method based on the chemical immobilization of tissue proteins. Our results showed that accurate quantitation could be achieved on OCT-embedded tissues and frozen tissues using chemical immobilization, iTRAQ labeling, and MS/MS.

To determine the impact of OCT embedding on tissue proteomics, we further analyzed the quantitative data from the OCT-embedded tissues and compared them with the data from the frozen tissues. The scatter plot of intensities of the iTRAQ channels 116 versus 114 and 117 versus 115 (OCT-embedded tissue vs. frozen tissue) showed similar patterns as the technical replicates of the OCT-embedded tissues (Fig. 4). The quantitative distribution is roughly symmetrical, with only a minimal spread from the $R^2 = 1$ line in the scatter plot, indicating high quantitative similarity between the frozen and OCT-embedded tissues. A vast majority of the proteins from OCT-embedded tissue versus frozen tissue showed a 1:1 ratio, with 91.0% (117/115) and 82.6% (116/114) of the proteins exhibiting a ratio within the 0.850 to 1.180 cutoff determined from the replicate analysis of OCT-embedded tissues. The correlation of 0.923 between 114 and 116 and of 0.956 between 115 and 117 is similar to the replicate analysis of the OCT-embedded tissues. The data showed that there were no significant differences between the OCT-embedded and frozen tissues.

Our results demonstrate that quantitative proteomic analysis of OCT-embedded tissue is feasible using CIPPE. Using this method, we successfully removed the interference from OCT and identified thousands of tissue proteins from OCT-embedded tissues. CIPPE has the potential to be used for PTM (post-translational modification) analysis such as glycosylation, phosphorylation, ubiquitination, and acetylation.

In addition to the removal of OCT from OCT-embedded tissues, this method could be used to extract peptides from tissue proteins...
for tissue proteomics. Compared with the proteins from body fluids, the proteins from tissues are more difficult to extract in order to obtain a complete proteome due to the three-dimensional structures of tissues and solubility of certain tissue proteins. During the proteomic analysis of tissues, detergents such as sodium dodecyl sulfate, NP-40, and Triton X-100 are often used to solubilize the membrane proteins from tissues for protein extraction (NP-40 was included in the RIPA buffer to extract kidney tissue proteins in this study). However, detergents also impede the MS detection of peptides, similar to the spectra from the OCT-contaminated HSA (Fig. 2A). In addition, these detergents, similar to OCT, bind to reversed-phase columns that are commonly used online with mass spectrometers, further impairing the capability of tissue proteomics using LC–MS–MS/MS. The CIPPE method described here is also capable of removing detergents from the tissue samples that were introduced during the protein extraction for proteomic analysis.

One of the limitations of this method is the incomplete release of all tryptic peptides from the beads. After the proteins are chemically immobilized onto the beads and washed, peptides are then released from the beads using trypsin digestion. We have shown that for protein identification and quantification, it is not necessary to recover all tryptic peptides. However, in the cases where all tryptic peptides are required for proteomic analysis, a cleavable linker to the solid phase could be used to capture and release all peptides. Another limitation of the current method is using the same amino group of the Lys residue for protein capture and the relative quantitation. The captured Lys residues are not cleaved by trypsin and so are blocked for labeling reagents such as iTRAQ. This will cause reduced quantity of Lys-containing peptides. However, the relative quantitation of the Lys-containing peptides from different samples still remains quantitative given the fact that Lys residues from each sample have similar coupling efficiency. For absolute quantitation of specific Lys-containing peptides, other amino acid residues such as cysteine can be used for coupling of the proteins.

Fig. 4. Quantitative analysis of proteins isolated from tissues using CIPPE. The scatter plot represents iTRAQ channels 115 and 114 (A), 116 and 117 (B), 114 and 116 (C), and 115 and 117 (D). The 116 and 117 channels were used for the quantitative analysis of two OCT-embedded tissues using CIPPE. The intensities observed for peptides in the 114:115, 116:117, 114:116, and 115:117 iTRAQ channels are plotted on the x axis and y axis, respectively, for each peptide spectrum match. The scatter plot represents the quantitative linearity between reporter ion groups. The sample and the reporter ion intensity scatter plot are clustered around a 45° line, indicating the symmetric distribution of fold change across the scatter plot.
Conclusion

This study shows that tissue proteins can be analyzed using shotgun proteomics incorporating CIPPE and MS/MS. The CIPPE methodology described here was used to conduct global proteomic analyses of OCT-embedded tissues and frozen tissues. This method is highly efficient in the removal of contaminants and other molecules and in the extraction of peptides for shotgun proteomics using MS. Our data indicate that OCT does not appear to affect the tissue proteome. Therefore, CIPPE can be used for the proteomic and PTM analysis of OCT-embedded tissues, leading to the possibility of the tissue proteomics of OCT-embedded tissues.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ab.2014.09.017.

References