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Antigen-Epitope Retrieval To Facilitate Proteomic Analysis of Formalin-Fixed Archival Brain Tissue

F. Rahimi,[†] C. E. Shepherd,[†] G. M. Halliday,[†] C. L. Geczy,[‡] and M. J. Raftery*[,§]

Prince of Wales Medical Research Institute, Inflammatory Diseases Research Unit, and Bioanalytical Mass Spectrometry Facility, University of New South Wales, Sydney, Australia

Formalin is a routine fixative facilitating tissue preservation and histopathology. Proteomic techniques require freshly frozen specimens, which are often difficult to procure, and methods facilitating proteomic analysis of archival formalin-fixed brain tissue are lacking. We employed antigen-epitope-retrieval principles to facilitate proteomic analysis of brain tissue that had been fixed and stored in formalin for 3−7 years. Twenty-micrometer-thick cryopreserved OCT-embedded sections from inferior temporal cortex of human (7 years in formalin) or mouse brain specimens (3 years in formalin) were hematoxylin-/eosin-stained. Approximately 16−64-mm^2 areas of the tissue sections were manually scraped off slides, or ∼2 mm^2 of human brain cortex was captured off membrane-coated slides using laser microdissection. Tissue was treated using various pH and temperature conditions prior to trypsin digestion and nano-LC−MS/MS. The largest number of proteins were retrieved by solubilization at pH 9 at 95 °C for 1 h; treatments at pH 4 or 6 at 25 or 65 °C were generally ineffective. Three-year formalin-fixed murine tissue did not yield more proteins compared to human tissue. Use of formalin-fixed tissue for proteomics is an invaluable tool for medical research. The combination of proteomics and microdissection enables selective enrichment and identification of novel, unique, or abundant proteins that may be important in pathogenesis.

Formalin fixation is the primary and universal method of tissue processing for light microscopy and archival preservation because it prevents tissue autolysis[1] and preserves histological and morphological architecture.[2] Some tissue repositories (e.g., brain banks) contain thousands of formalin-preserved or formalin-fixed paraffin-embedded specimens[3] representing an invaluable source for histopathological examination and retrospective clinical studies.[4] However, formalin fixation reduces immunohistochemical reactivity and has precluded efficient protein extraction for proteomic analyses possibly as a result of formalin-mediated covalent cross-linking.[5] Two formalin-induced cross-linking mechanisms are methylene bridges that can form in proteins[6] and nucleic acids[7] and the Mannich reaction in which tyrosine residues, often located at the epitopes or antigen-binding sites of proteins, are cross-linked to nearby arginine or lysine residues, thus compromising immunoreactivity.[8] The Mannich reaction and methylene bridges can be reverted by heating and high pH, forming the basis for antigen-epitope-retrieval (AER) methods.[6,8−10]

Because formalin fixation causes protein insolubility due to covalent cross-linking, studies involving proteomic analyses (e.g., one- or two-dimensional polyacrylamide gel electrophoresis, Western blotting, high-performance liquid chromatography, and amino acid sequencing) of formalin-fixed tissue are scant,[10−14] and although tissues fixed by alternative non-cross-linking fixatives (including ethanol, acetone, Carnoy's solution, Hepes−glutamic acid-mediated organic solvent protection effect fixation, and zinc-based fixatives) are more amenable to proteomic analyses,[15−19] these are not as routinely used. Moreover, many proteomic

* Corresponding author. Tel.: 61-2-9385-3758. Fax: 61-2-9662-4469. E-mail: m.raftery@unsw.edu.au.

[†] Prince of Wales Medical Research Institute.

[‡] Inflammatory Diseases Research Unit.

[§] Bioanalytical Mass Spectrometry Facility.

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techniques, including liquid chromatography and mass spectrometry generally require fresh/fresh-frozen specimens, but their availability, collection, and preservation are often costly and problematic, thereby highlighting the need for effective, simple, and economical tools for proteomic analyses of untapped archival formalin-fixed specimens. Development of strategies allowing proteomic analysis of formalin-fixed tissue is advantageous, particularly when selective laser microdissection techniques, combined with powerful and sensitive mass spectrometry-based proteomics, could be applied to investigate cellular or microstructural proteomes in normal/diseased specimens and could lead to discoveries of pathological/prognostic markers of (e.g., brain) diseases and potential therapeutic targets. Here we describe optimization of a simple and cost-effective solubilization method to facilitate mass spectrometric analysis of human and mouse brain tissue archived in formalin and retrieved by macrodissection or laser microdissection.

EXPERIMENTAL SECTION

Materials. Hematoxylin, eosin, and poly(L-lysine) were obtained from Sigma (St. Louis, MO). Protease inhibitor mixture was from Roche Applied Science (Mannheim, Germany). Poly-(ethylene−naphthalene) (PEN)-coated slides were purchased from P.A.L.M Microlaser Technologies (Bernried, Germany) and optimal-cutting-temperature (OCT) compound from ProSciTech (Thuringowa, Qld, Australia).

Tissue Preparation. Postmortem human brain tissue, obtained in 1999 as part of an ethically approved donor program managed by the Prince of Wales Medical Research Institute Tissue Resource Center, was fixed in 15% formalin for 2 weeks and then sliced and transferred to 10% phosphate-buffered formalin (pH 7.2−7.4) for long-term archiving. Mouse brain specimen had been stored in 10% formalin for 3 years. Gradual oxidation of formalin to formic acid[2] and consequent pH alteration was circumvented by refreshing the formalin solution every 2 years. Blocks from human inferior temporal cortex or mouse brain were cryopreserved in 30% sucrose in Tris-HCl (100 mM, pH 7.5, ∼48−72 h) before embedding in OCT and cryomicrotomy at 20 μm. Sections were either mounted on ethanol-washed normal glass slides or PEN-coated slides pretreated with ultraviolet light and poly(L-lysine) according to manufacturer's instructions. Staining used a rapid protocol for laser-capture microscopy[20] with some modifications consisting of sequential incubations in 70% ethanol (1 min), hematoxylin (1−3 min), Scott's water (10% magnesium sulfate, 7% sodium bicarbonate, 10 s), and eosin (10 s) and gradual dehydration in 70 (30 s) and 100% ethanol (2 × 30 s) with brief MilliQ water rinses between most steps; slides were subsequently air-dried. Hematoxylin and eosin contained protease inhibitors. Stained human sections were scraped off slides (16, 64 mm^2) with a small sterile scalpel and treated at pH 4, 6, and 9 at 25, 65, or 95 °C for 1, 2, 3, 5, or 14 h in 25 mM Tris-HCl. Alternatively, random areas of human cortical tissue were captured off PEN-coated slides using a Robot-MicroBeam (P.A.L.M microlaser technologies) coupled onto an inverted Axiovert 135 microscope (Carl Zeiss, Göttingen, Germany) according to standard procedures using the PALMRobo software (laser power, 79; laser focus, 41). Areas of ∼3000−6000 μm^2 were rectangularly circumscribed (Figure 1) and ablated using the RoboLPC software command and collected by 1−2 laser pressure catapulting shots into a cap containing 50 μL of MilliQ water. Approximately 300−400 elements were collected to obtain 2−3 mm^2 of tissue. After lyophilization, tissue was treated at pH 9, for 1 h at 95 °C and tissue equivalent to ∼0.2 mm^2 was analyzed by mass spectrometry.

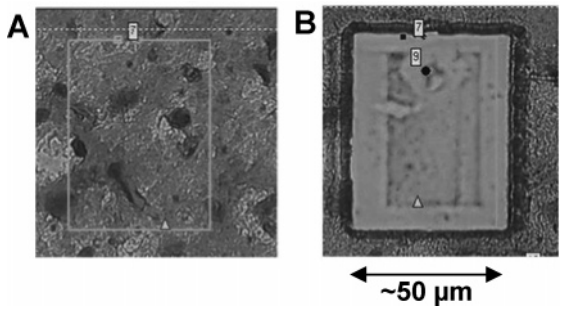

Figure 1. Screen shot of the P.A.L.M RoboLPC software showing rectangularly circumscribed random areas of the human cortex before (A) and after (B) laser capture (40×). Area captured in (A) approximates 3500 μm^2. Approximately 300−400 elements approximating an area of 2 mm^2 were captured and equivalent to 0.2 mm^2 was used for nanoLC MS analysis.

Mass Spectrometry. Samples (20−30 μL, 25 mM Tris-HCl) were incubated with trypsin (15 ng/μL; Promega) after pH adjustment (∼8.5) and digested overnight at 37 °C. Two microliters of digest was diluted in 8 μL of formic acid (1% v/v in MilliQ water), and 5 μL was concentrated and desalted using a micro C18 precolumn (500 μm × 2 mm, Michrom Bioresources, Auburn, CA) with H_2O/CH_3CN (98:2, 0.1% formic acid) at 20 μL/min. After a 4-min wash, the precolumn was automatically switched (Valco 10-port valve, Houston, TX) into line with a fritless nanocolumn.[21] Digested peptides were separated by nano-LC using a Cap-LC autosampler system (Waters, Milford MA) and eluted using a linear gradient of H_2O/CH_3CN (98:2, 0.1% formic acid) to H_2O/CH_3CN (50:50, 0.1% formic acid) at ∼200 nL/min over 30 min. The precolumn was connected via a fused-silica capillary (10 cm, 25 μm) to a low-volume tee (Upchurch Scientific) where 2600 V was applied and the column tip positioned ∼1 cm from the Z-spray inlet of a QTOF Ultima API hybrid tandem mass spectrometer (Micromass, Manchester, UK). Positive ions were generated by electrospray, and the QTOF was operated in data-dependent acquisition mode. A TOF MS survey scan was acquired (m/z 350−1700, 1 s), and the two largest multiply charged ions (counts, >20) were sequentially selected by Q1 for MS/MS analysis. Argon was used as the collision gas and an optimum collision energy chosen based on charge state and mass. Tandem mass spectra were accumulated for up to 6 s (m/z 50−2000).

Database Searching. Peak lists were generated by MassLynx (version 4 SP1, Micromass) using the Mass Measure program to automatically baseline-subtract, smooth, and centroid the data before submission to the database-searching program, Mascot (version 2.1, Matrix Science, London, UK), with proteins identified by correlation of mass spectra to entries in the NCBInr database (July 2006). Mascot MS/MS ion search criteria was as follows: taxonomy—*Homo sapiens* (for human data) and *Mammalia* (for

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mouse data), trypsin digestion allowing up to one miscleavage, variable modification—oxidation of methionine, peptide tolerance of 0.25 Da, and MS/MS tolerance of 0.2 Da. A probability-based Mowse score of >35 for human data and >41 for mouse data was assigned by the Mascot software and indicated identity or extensive homology (p <0.05). The ion cutoff was set to 30, excluding the low-scoring peptides form the result summary. At least one distinct peptide enabled differentiation between various protein isotypes that shared peptide sequences.

RESULTS AND DISCUSSION

Optimization of Solubilization Protocol. According to many reports, DNA and RNA remain stable in, and extractable from, paraffin-embedded, formalin-fixed tissue and can be amplified by polymerase chain reaction.[22−25] However, few studies report proteomic analyses of archived formalin-fixed tissue. Amyloid A in formalin-fixed, paraffin-embedded, amyloid-containing tissue, for example, was detected by the Shtrasburg method, possibly because this protein is not modified by formalin and retains its typical electrophoretic, chromatographic, and immunogenic characteristics even after 30 years of fixation and paraffin-embedding.[11,26] Similarly, some nuclear, cytosolic, and membrane-bound proteins were extracted from fixed paraffin-embedded tissue by conventional detergent-containing (2% SDS) RIPA buffer after heating at 100 °C for 20 min followed by 60 °C for 2 h[14] and detected by SDS−PAGE and immunoblotting. Amino acid sequencing identified peptides derived from an immunoglobulin light-chain polypeptide in 2-year-old formalin-fixed cerebrovascular amyloid deposits in SDS-/urea-containing electrophoresis buffer after treatment at 90 °C for 15 min.[13] In all these studies, methods based on AER principles were employed. Heat-based AER of formalin-fixed tissue is routine for immunohistochemistry;[27] heat treatment disrupts cross-links, appropriate pH facilitates proper epitope refolding,[10] and different pH conditions may facilitate detection of different antigenic epitopes.[28] The simplicity of the AER technique is ideal for routine, cost-effective, and reproducible immunohistochemistry.[29] Therefore, to facilitate proteomic analysis of nonparaffin-embedded archival formalin-fixed tissue, a simple approach similar to AER strategy was used. We first assessed the effects of various pH and heat treatments using formalin-fixed human brain tissue in a simple buffer (Tris-HCl, 25 mM). Laser microdissection, which requires preservation of tissue morphology, was considered as a preceding step. Therefore, hematoxylin and eosin staining was performed but paraffin-embedding excluded as whole formalin-fixed archival tissue (stored in formalin for ∼7 years) was available. This made xylene treatment unnecessary, but OCT-embedding and cryopreservation was required to facilitate cryomicrotomy. Manually scraped tissue (∼16 mm^2, 20 μm thick) was incubated at pH 4, 6, or 9 for 1 h at 25, 65, or 95 °C, then trypsin-digested, and analyzed by mass spectrometry. Figure 2 shows that no major peptide was retrieved in mixtures incubated at pH 4 or 6 at 25 °C, whereas five proteins (glial fibrillary acidic protein (GFAP) (4 peptide/Mowse score 155), ATP synthase α subunit (2/106), ferritin light subunit (1/83), β-actin (2/70), and guanine nucleotide-binding protein (1/50)) were identified using pH 9 and 25 °C. Treatment at pH 4 and 6 at 65 °C allowed identification of four proteins. However, 20 were identified if the pH was raised to 9 at 65 °C (Supporting Information Table 1A). The best results were obtained when the tissue was incubated at 95 °C, allowing identification of 24 proteins with high Mowse scores at pH 4 (Supporting Information Table 1B), 27 at pH 6 (Supporting Information Table 1C), and 33 at pH 9 (Supporting Information Table 1D).

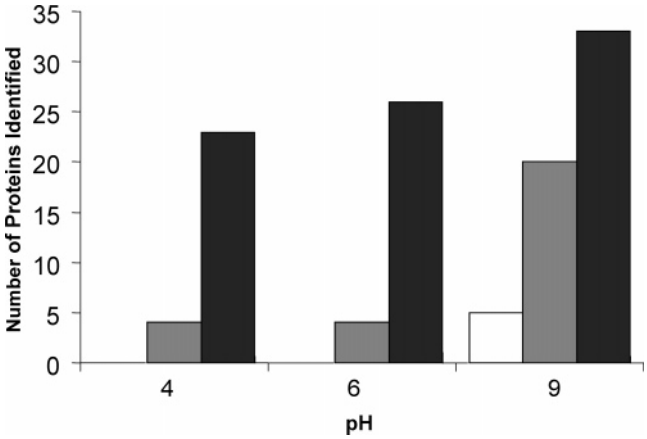

Figure 2. Analysis of formalin-fixed human brain tissue showing effects of pH (abscissa) and temperature vs number of proteins (y-axis) identified. Equivalent amounts (∼16 mm^2) of formalin-fixed archival brain tissue were manually scraped off untreated slides and incubated in 25 mM Tris-HCl buffers at pH 4, 6, and 9 at 25 (□), 65 (gray box solid), and 95 °C (■) for 1 h; pH was adjusted to ∼8.5, proteins digested with trypsin, peptides analyzed by nanoLC tandem MS, and proteins identified by Mascot searches.

To determine the effect of lengthy tissue treatment, in a separate experiment, human brain tissue scrapings (∼64 mm^2) were incubated in Tris-HCl buffer (pH 9 and 95 °C) for 2, 3, 5, or 14 h. Twenty proteins were identified after 2-h incubation (Figure 3, Supporting Information Table 2A) and 16 proteins after 3 h (Figure 3, Supporting Information Table 2B). However, the numbers of protein identifications decreased thereafter and only four (GFAP, 10/626; mutant β-actin (β'-actin), 1/58; α-tubulin, 1/44; and neurofilament L, 1/42) were identified after 5 h. Longer incubation of up to 14 h either at 95 (Figure 3) or 25 °C (not shown) did not yield any confident protein identification. Generally, the more abundant proteins were identified after 3- or 5-h treatment at pH 9/95 °C, and the Mowse scores decreased with incubation time; suggesting some degradation of the peptides occurred with longer treatment. Overall, these data indicate that the optimal condition was 1 h incubation at pH 9 and 95 °C; high pH alone was insufficient in solubilizing proteins without heating, and heating (95 °C) at pH 6 or 9 was apparently sufficient (Figure 2).

To compare species differences and possible differences in the duration of formalin fixation, murine formalin-fixed archival brain

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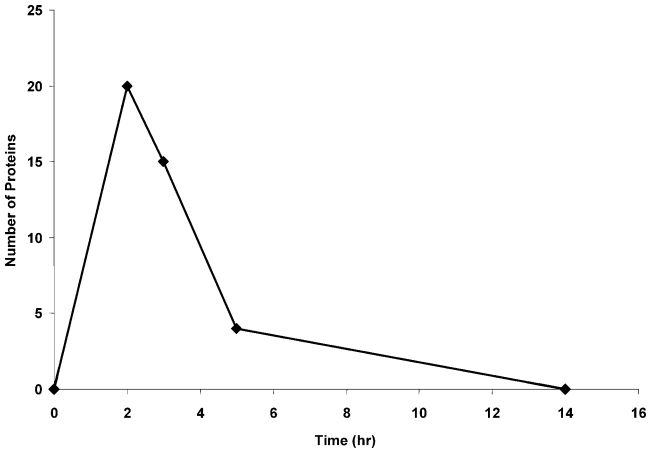

Figure 3. Analysis of formalin-fixed brain tissue showing the effect of incubation time (abscissa) vs number of proteins identified (*y*-axis). Approximately equivalent amounts of formalin-fixed human brain tissue were incubated at pH 9 and samples analyzed following 2, 3, 5, and 14 h heating at 95 °C. pH was adjusted to ~8.5, proteins digested with trypsin, peptides analyzed by nanoLC tandem MS, and proteins identified by Mascot searches.

tissue, which was stored in formalin for 3 years, was used. Approximately 16-mm^2 mouse tissue scrapings were incubated at 95 °C for 30 and 60 min at pH 9 and analyzed by nano-LC and mass spectrometry after trypsin digestion. Twenty-two proteins (Figure 4), with generally high Mowse scores indicating better matches and fragmentation spectra,[30] were identified after 60 min (Supporting Information Table 3). A similar number of proteins, but with generally lower Mowse scores, were identified after 30-min incubation (not shown). Three-year fixed murine tissue (Supporting Information Table 3) did not yield more protein identifications than those identified in 7-year formalin-fixed human tissue treated similarly (Supporting Information Table 1D), indicating species differences had little effect and suggesting that formalin-induced cross-linking was well advanced after 3-year fixation in murine tissue. These results conform with previous mechanistic propositions suggesting that heat treatment disrupts formalin-generated intra- or intermolecular cross-links, thereby solubilizing proteins and unmasking antibody-binding sites.[10] Similarly, this procedure may generate structural changes that allow the enzymatic digesting action of trypsin and consequent peptide release.

Proteins Identified in Specimens Retrieved by Laser Microdissection. Our main aim was to develop sensitive and specific methods applicable to laser-microdissected human brain specimens. Initial experiments indicated that few proteins were identified by simply digesting with trypsin (not shown). To determine the applicability of AER methods, the sensitivity of mass spectrometery analysis with these types of samples and to assess whether this method could be used to analyze microdissected specimens, cortical tissue was randomly microdissected, heated at 95 °C at pH 9 for 1 h, trypsin digested, and the equivalent of 0.2-mm^2 tissue analyzed by nano-LC and mass spectrometry. Similar to previous results, 31 proteins were identified and the most abundant brain proteins had high scores (Table 1). The abundant GFAP had the best sequence coverage of 30% (Table 1) and a Mowse score of 806. Thirty-six percent of total proteins identified by laser microdissection were structural proteins, 39% enzymes, 6% nuclear, and other proteins comprised 19% (Figure 5). A similar technical report from Agilent Technologies by Sauber et al. (Application Note entitled "Identification of Proteins in Post-mortem Human Brain Tissue by Laser Microdissection/Pressure Catapult and Nano-LC/MS/MS", available at www.agilent.com/chem, document 5989-0895EN) used fresh snap-frozen human cerebellar tissue. In that study, 1-, 10-, and 20-mm^2 random areas containing white matter and cortex were collected using a P.A.L.M laser microdissection system and subjected to two lysis protocols before trypsin digestion. In a comparative workup using the Spectrum Mill MS proteomics workbench, the authors detected 8 proteins using urea (6 M) and 11 using a mixed organic solvent system (50% 1,1,1-trifluoroacetic acid and 50% 30 mM ammonium carbonate in water, 10 mM DTT, pH ~8) in ~10-mm^2 (10−15 μm thick) microdissected frozen cerebellar tissue. According to their estimates, 10^5 cells were present in 10-mm^2 tissue, each contributing 4 ng of protein, leading to 400 μg of total protein. In a separate experiment, they detected 26 proteins of which GFAP (25 peptides) was the most abundant, with a sequence coverage of 50%. Using laser microdissection, we detected 31 proteins in archival formalin-fixed human brain tissue using ~50-fold less total protein (~8 ng estimated as above) and a simple heat treatment procedure in 25 mM Tris buffer at pH 9. Therefore, our findings, despite using substantially less protein, compare well with those using fresh frozen brain tissue.

Tissue type and duration of formalin fixation, rather than paraffin-embedded storage, may be important factors affecting proteomic analyses. Recently, proteomic analysis of formalin-fixed, paraffin-embedded prostate cancer tissue was performed. A novel tissue microdissection technique optimized for extraction of cells from formalin-fixed, paraffin-embedded tissue and a proprietary protocol from Expression Pathology (Gaithersburg, MD) optimized for recovery and solubilization of proteins and peptides from formalin-fixed tissue for downstream nanoRPLC-MS/MS analyses were employed.[31] Microdissected tissue areas used in that study ranged from 5.3 to 35 mm^2 equating to 30 000−200 000 cells (considering an average cell diameter of 15 μm);[31] however, duration of formalin fixation of prostate tissue in that study was not reported. Similarly, Palmer-Toy et al. identified 123 proteins in four 8-μm-thick sections of a canalplasty specimen from a chronic-stenosing external-otitis case, which had been fixed in 10% formalin for 72 h and embedded in paraffin; however, section area was not reported in that study.[32] Crockett et al. recently successfully identified 324 unique proteins using trypsin digestion and proteomic LC−MS/MS analysis of a lymphoma-derived cell line fixed in formalin overnight and stored paraffin-embedded for 3 years as blocks containing ~10^7 cells[33] without prior heat treatment. However, proteomic analyses of formalin-fixed artificial cell blocks[33] may not be completely representative of fixed tissue blocks or tissues stored in formalin because the tissues are more

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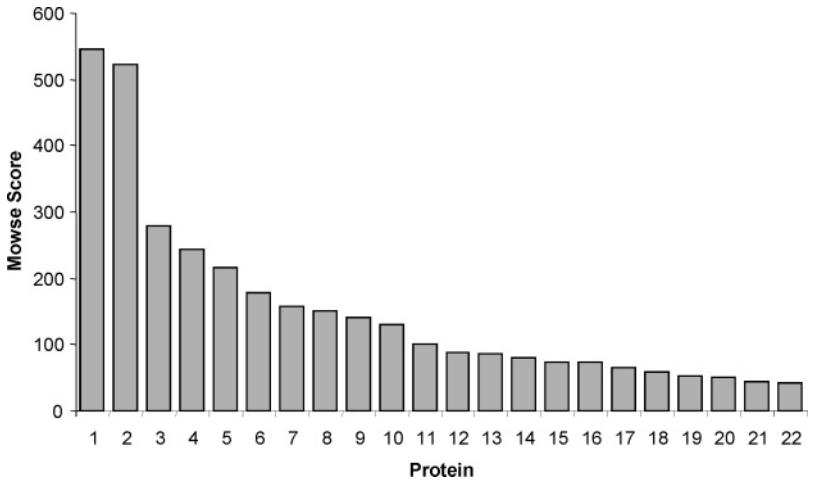

Figure 4. Analysis of formalin-fixed mouse brain tissue showing number of proteins (abscissa) and the corresponding Mowse scores (*y*-axis) identified in ~16-mm^2 tissue scrapings incubated at pH 9 at 95 °C for 60 min.

Table 1. Proteins Identified after NanoLC Tandem MS and Database Searches of Laser-Microdissected Archival Formalin-Fixed Human Brain Tissue after Treatment at pH 9, 95 °C for 60 min[a]

protein name	accession no.	theor MW/pI	distinct peptide matches	sequence coverage (%)	Mowse score
glial fibrillary acidic protein	gi|4503979	49850/5.42	16	30	806
tubulin, β 2*	gi|20809886	49776/4.76	9	15	303
tubulin, β polypeptide*	gi|18088719	49640/4.75	9	15	294
ATP synthase, H$^+$ transporting, mitochondrial F1 complex, α subunit precursor	gi|4757810	59714/9.16	4	9	236
class II β-tubulin isotype*	gi|27227551	49915.98/4.82	6	16	248
β-actin	gi|4501885	41710/5.29	4	12	173
2′,3′-cyclic-nucleotide 3′-phosphodiesterase (CNPI)	gi|219401	45070/8.73	3	6	162
ATP synthase, H$^+$ transporting, mitochondrial F1 complex, β polypeptide	gi|16741373	56525/5.26	3	8	147
neurofilament, light polypeptide 68 kDa	gi|105990539	61479/4.64	3	4	142
tubulin α 6*	gi|14389309	49863/4.96	4	5	137
creatine kinase B	gi|180555	42460/5.34	4	8	126
histone H4/o	gi|49659923	11360/11.36	2	17	108
neurofilament 3	gi|67678152	102415/4.90	2	1	95
α-tubulin *	gi|32015	49761/4.95	2	5	94
chain G, twinning in crystals of human skeletal muscle D-glyceraldehyde-3-phosphate dehydrogenase	gi|230868	35853/6.60	2	4	87
ferritin light subunit	gi|182516	16384/5.65	1	10	76
guanine nucleotide-binding protein	gi|386750	40046/5.35	1	3	63
eukaryotic translation elongation factor 1 α 2	gi|12653327	50438/9.11	1	2	56
α II spectrin	gi|1805280	284891/5.22	1	0.1	52
GLAST1	gi|825663	59534/8.52	1	2	51
peroxiredoxin 6	gi|77744395	25019/6.00	1	4	50
calcium/calmodulin-dependent protein kinase II α-B subunit	gi|4836795	55227/7.32	1	2	50
dihydropyrimidinase-like 3	gi|4503379	61924/6.04	1	1	50
neuropolypeptide h3 (human, brain, peptide, 186 aa)	gi|913159	20913/7.42	1	7	48
cathepsin D (lysosomal aspartyl protease)	gi|54697170	44524/6.10	1	1	44
γ-enolase	gi|930101	47125/4.94	1	2	44
syntaxin binding protein 1 isoform a	gi|4507297	68692/6.32	1	1	42
aspartate aminotransferase 2, precursor	gi|12653507	47459/9.14	1	2	42
histone H2A.2	gi|31979	13899/10.21	1	7	38
peroxiredoxin 3, isoform a precursor	gi|14250063	27705/7.11	1	5	37
α-globin	gi|183801	3284/8.53	1	32	36

[a] Scores of >35 indicate identity or extensive homology (p <0.05). Asterisk (*) indicates that among other common peptides, at least one distinct peptide differentiated between various protein isotypes.

architecturally complex than cell blocks and prolonged in-formalin storage is problematic. Altogether, the above studies highlight that short formalin-fixation duration (<72 h) and lengthy paraffin-embedded storage of tissue that was fixed for shorter durations apparently does not complicate tissue proteomic analyses because stable formalin-mediated cross-links form time-dependently upon prolonged in-formalin storage.[29]

Formalin-mediated tissue fixation involves an "addition reaction" between an amine and an aldehyde and, finally, a "secondary condensation" reaction resulting in intra- and intermolecular methylene bridge cross-links. The latter process is slow, taking up to weeks to generate stable methylene cross-linking.[29] Similarly, experiments using bovine pancreatic ribonuclease A indicate that oligomer formation (over 9 days) and thermostability enhance-

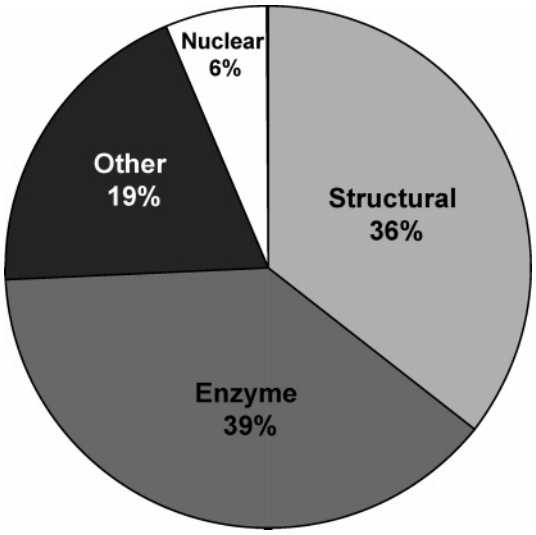

Figure 5. Pie chart representation of proteins identified in tissue captured by laser microdissection (equivalent to ~0.2 mm^2). Approximately 36% were structural proteins, 39% enzymes, 6% nuclear, and other proteins comprised 19%.

ment (over 200 h), which are afforded by protein cross-linking, are gradually progressive but saturate with time,[6] indicating that the duration of formalin fixation or prolonged storage of tissue in formalin may be a compounding factor hindering efficient proteomic analyses of fixed tissue/cells. The postmortem human brain tissue used in this study was obtained in 1999 and stored in 10% phosphate-buffered formalin for 7 years. To our knowledge, no study has previously attempted proteomic analysis of formalin-fixed, 7-year-old archival brain tissue.

In conclusion, our simple cost-effective solubilization strategies based on AER principles may have wider applications for mass spectrometry-based analyses of formalin-fixed brain or other preserved tissues. Laser microdissection may selectively capture cells or tissue microstructures, facilitating enrichment of proteins and detection of less abundant but cell type- or structure-specific proteins in conjunction with proteomic analysis. Development of such analytical methods allows analysis of vast global archives of formalin-fixed non-paraffin-embedded pathological or normal specimens.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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