Sample Preparation for 2-D Gel-Based Expression Proteomics of an Alzheimer's Disease Mouse Model

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Introduction

Alzheimer's disease (AD) is an age-related disorder affecting more than 30% of people over 80 years of age. The pathogenesis of this progressive dementia remains to be fully understood, but symptomatic disease is associated histopathologically with dense extracellular amyloid plaques, neurofibrillary tangles, and neuronal loss, primarily in the brain. The core of the amyloid plaque is β-amyloid peptide, a derivative of the amyloid precursor protein (APP). Animal models that have been generated to study the neuropathology of AD include a transgenic mouse that overexpresses human APP. In this mouse model, overexpression of the human APP transgene in neurons is sufficient to produce dense extracellular amyloid plaques, neurofibrillary tangles, and neuronal degeneration in the aged animals similar to those observed in the AD brain. However, little is known about the protein processing pathways involved in β-amyloid peptide aggregation. Therefore, a 2-D gel-based differential protein expression analysis may shed light on the underlying processes of AD and reveal potential biomarkers for its diagnosis.

Sample preparation is a key to successful 2-D separations, and we were interested in developing a reliable, convenient, and reproducible method for the 2-D analysis of proteins from brain tissue. We applied a combination of premixed sample preparation kits to maximize the resolution and reproducibility obtained from the 2-D protein profiles of the brains of 3-month-old APP-overexpressing and control mice:

- The ReadyPrep™ total protein extraction kit was used to maximize the number of solubilized proteins for 2-D analysis.
- The ReadyPrep reduction/alkylation kit was used to break and block the formation of inter- and intramolecular disulfide bonds.
- The ReadyPrep 2-D cleanup kit was used to remove substances that interfere with isoelectric focusing (IEF), including excess salts, lipid, and nucleic acids.

As typical AD symptoms do not appear in 3-month-old transgenic mice, this research may lead to the identification of potential biomarkers for the early disease mechanism of AD.

Methods

Each experiment (sample preparation through 2-D analysis) was performed three times. For each experiment, samples were analyzed in triplicate.

Sample: Left brains (~250 mg) of 3-month-old female nontransgenic control and human APP-overexpressing mice (Sun Health Research Institute, AZ)

Sample preparation:

- Homogenization and protein extraction in 2 ml lysis buffer from a modified ReadyPrep total protein extraction kit (7 M urea, 2 M thiourea, 2% CHAPS, 40 mM Tris, 3 mM TBP) and concentration measurement with the *RC DC*™ protein assay
- Treatment with the ReadyPrep reduction/alkylation kit
- Treatment with the ReadyPrep 2-D cleanup kit and resuspension of protein pellets in IPG rehydration buffer (7 M urea, 2 M thiourea, 2% CHAPS, 3 mM TBP, 0.2% Bio-Lyte® ampholyte, pH 3–10) and concentration measurement with the *RC DC* protein assay

2-D gel electrophoresis:

- First-dimension IEF: 500 µg total protein separated on 24 cm ReadyStrip™ IPG strips, pH 3–10 NL. IEF was performed with a PROTEAN® IEF cell using the program: 250 V (rapid ramp) for 20 min; 10,000 V (rapid ramp), 80,000 V–hr
- Second-dimension SDS-PAGE: PROTEAN® Plus precast gels and a PROTEAN Plus Dodeca™ cell run at 200 V for 15 hr
- Gel staining: SYPRO Ruby protein gel stain (Invitrogen)
- Imaging: Molecular Imager FX™ Pro multiimager system
- Image analysis: PDQuest™ v. 7.4 2-D analysis software

Results

To compare the protein profiles of the brains from control and APP-overexpressing (APP) mice, 500 µg protein from each sample was separated on an IPG strip (pH 3–10 NL), followed by SDS-PAGE on a large format gel.

- Figure 1 shows two representative 2-D gels of the control and APP mouse brain proteins, which exhibit similar, highly resolved patterns of spots. PDQuest analysis identified over 900 distinctive protein spots in these gels (Figure 2).
- Figure 3 highlights the same region of four different gels. Within this area, resolution of protein spots was very high, and the correlation coefficient between the control and APP groups was 0.95, signifying high gel-to-gel reproducibility and strong similarity between the protein profiles of these groups.
- Similar results were obtained over the course of three independent experiments and demonstrate that our sample preparation and 2-D gel methods yield highly consistent, reproducible results.

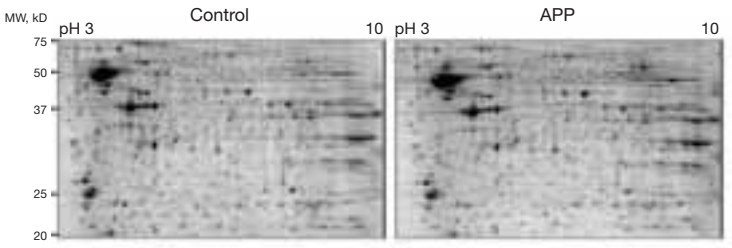

Fig. 1. Representative 2-D gel separations of protein samples obtained from control and APP-overexpressing mice.

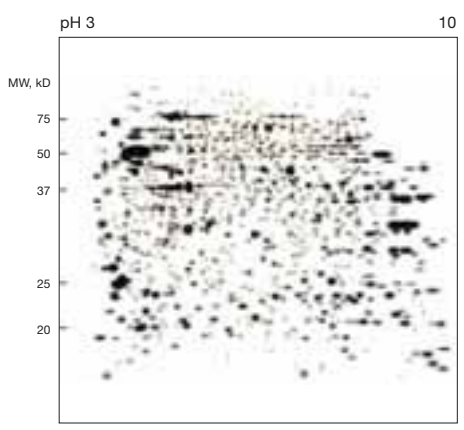

Fig. 2. Gaussian image of a representative 2-D gel from a control sample showing over 900 distinct spots.

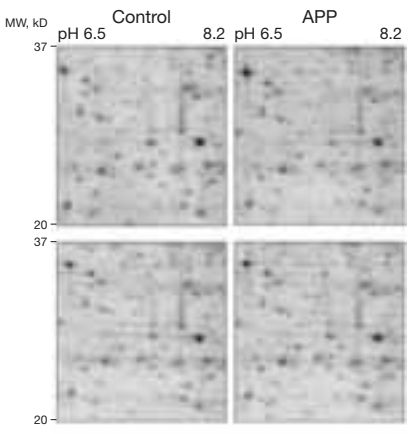

Fig. 3. Paired 2-D gel separations of protein samples obtained from control and APP-overexpressing transgenic mice. The same areas of the gels are shown and the correlation coefficient between the control and APP gel groups was 0.95.

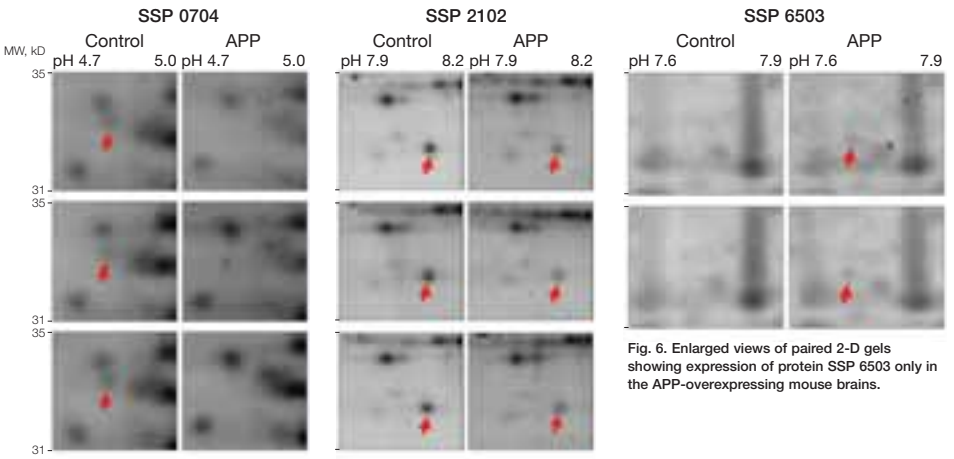

Fig. 4. Enlarged views of triplicate 2-D gels showing expression of protein SSP 0704 only in the control mouse brains.

Fig. 5. Enlarged views of triplicate 2-D gels showing higher level of expression of protein SSP 2102 in the control than in the APP-overexpressing mouse brains.

Fig. 6. Enlarged views of paired 2-D gels showing expression of protein SSP 6503 only in the APP-overexpressing mouse brains.

- Three protein spots exhibited distinct expression patterns between the control and the APP gels (Table 1). In all three experiments, protein SSP 0704 appeared in the control but not in the APP mouse brains (Figure 4) and the level of SSP 2102 decreased in the APP mouse brain (Figure 5). In one of the three experiments, protein SSP 6503 appeared in the APP but not in the control mouse brain (Figure 6). All three proteins, especially SSP 6503, were among the faintest spots observed. Indeed, the extremely low abundance of protein SSP 6503 may account for its detection in only one of three experiments.

Conclusions

- The workflow described here for 2-D gel-based expression proteomics employs consistent sample preparation tools and reproducible, highly resolved 2-D gels for biomarker discovery.
- Three proteins were differentially expressed between the control and APP overexpressing mouse brains. Since typical AD symptoms do not appear in the 3-month-old APP-overexpressing mouse, these proteins may be potential biomarkers for the early mechanisms of Alzheimer's disease. Validation of these findings should be pursued using other cell biology and molecular biology approaches, such as real-time PCR, cell staining, etc.
- The three differentially expressed proteins are present in very low abundance, which makes their identification by mass spectrometry difficult. Thus, sample fractionation will be needed to enrich these proteins for identification as well as for analytical purposes. Our ongoing studies apply chemical solubilization, ion exchange chromatography, and preparative IEF with the MicroRotofor™ cell to fractionate the samples to enrich the candidates for protein identification. These fractionation tools will also facilitate comparison of the expression profiles of other low-abundance proteins in the control and APP-overexpressing mouse brains.

Table 1. Summary of the differential protein expression in control and APP-overexpressing mouse brains.

	Mouse Model	
Protein	Control	APP
SSP 0704	+	–
SSP 2102	+	↓
SSP 6503	–	+

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