Chapter 33

Amyloid-Binding Proteins: Affinity-Based Separation, Proteomic Identification, and Optical Biosensor Validation

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Abstract

The amyloid-beta peptide is considered as a key player in the development and progression of Alzheimer's disease (AD). Although good evidence exists that amyloid-beta accumulates inside cells, intracellular brain amyloid-beta-binding proteins remain poorly characterized. Here we describe a protocol for affinity-based profiling of amyloid-beta-binding proteins of rat brain, their proteomic identification and validation by a surface plasmon resonance (SPR)-based analysis. It includes: (a) SPR-based selection of immobilization conditions for beta-amyloid coupling and choice of appropriate resin for preparation of an affinity sorbent; (b) immobilization of beta-amyloid on the selected resin; (c) preparation of biological samples (rat brain homogenate) for affinity-based separation; (d) isolation of rat brain proteins by affinity chromatography using amyloid-beta as the affinity ligand; (e) sample preparation for proteomic analysis; (f) proteomic analysis and protein identification; (g) SPR-based validation of the interaction of identified proteins with immobilized amyloid-beta.

Key words Amyloid-beta, Amyloid-beta-binding proteins, Affinity-based proteomic profiling, Rat brain

1 Introduction

The amyloid-beta peptide 1–42 formed during proteolytic processing of the amyloid precursor protein (APP) is considered as a key player in the development or progression of Alzheimer's disease (AD) and other pathologies associated with formation of protein aggregates in the central nervous system [1–6]. Although much attention is paid to formation of extracellular amyloid plaques and protein aggregates as well as to corresponding mechanisms of their toxicity, good evidence exists that intracellular amyloid-beta can accumulate intraneuronally and contribute to disease progression [4, 7–13]. This suggests importance of amyloid-beta interaction with particular intracellular targets. Indeed, interaction of amyloid-beta with intracellular catalase caused inactivation of this enzyme and accumulation of hydrogen peroxide inside cells [14].

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This implies that oxidative stress induced in the cells exposed to amyloid-beta may be (at least partially) associated with reduced degradation of intracellular hydrogen peroxide [14].

In the cell amyloid-beta was found in different intracellular compartments [4]. The latter suggests possibility of amyloid interaction with a broad range of proteins, which may be thus denominated as amyloid-beta-binding proteins. Although there are reports on the interaction of amyloid-beta peptide with various intracellular proteins [14, 15] and development of protocols for affinity isolation of amyloid-beta-binding proteins [16], the affinity-based proteomic profiling of brain proteins interacting with immobilized amyloid-beta, their identification has not been performed yet.

Here we describe a protocol for affinity-based profiling of amyloid-beta-binding proteins of rat brain, their proteomic identification and validation by a surface plasmon resonance (SPR)-based analysis. It includes several interrelated blocks (Fig. 1):

(a) SPR-based selection of immobilization conditions for amyloid-beta coupling and choice of appropriate resin for preparation of an affinity sorbent.

(b) Immobilization of amyloid-beta on the selected resin.

(c) Preparation of biological samples (rat brain homogenate) for affinity-based separation.

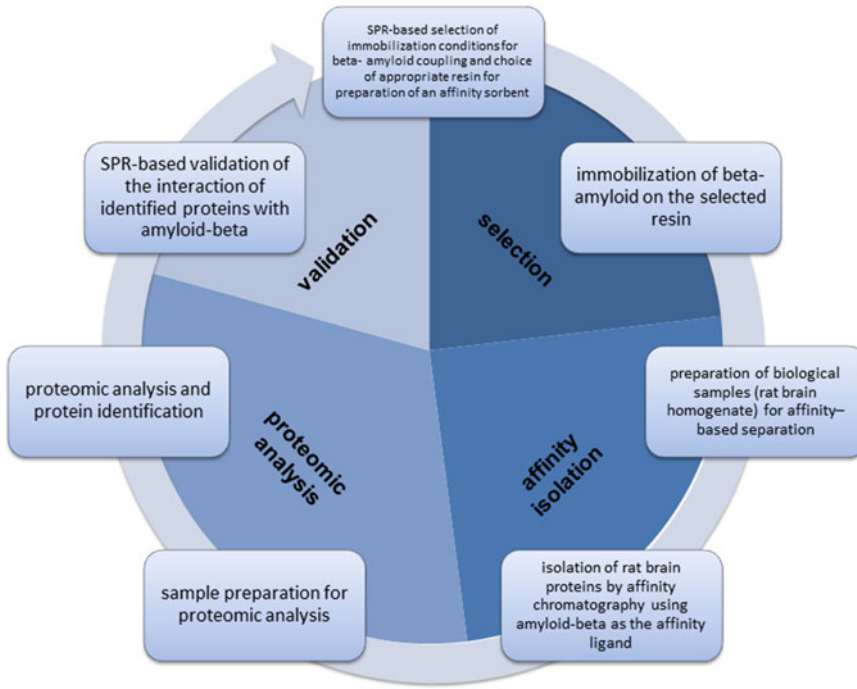

Fig. 1 Scheme illustrating the experimental design

(d) Isolation of rat brain proteins by affinity chromatography using amyloid-beta as the affinity ligand.

(e) Sample preparation for proteomic analysis.

(f) Proteomic analysis and protein identification.

(g) SPR-based validation of the interaction of identified proteins with amyloid-beta.

2 Materials

2.1 SPR-Based Biosensor

All SPR-based biosensor consumables are commercially available from GE Healthcare as ready to use dry substances, which should be dissolved using certain volumes of water.

1. Immobilization buffer A: Na-acetate buffer, pH 4.0. Prepare a 10× stock solution by adding 41 mL of 0.01 M glacial acetic acid to a 100 mL graduated cylinder containing 9 mL of 0.01 M sodium acetate.
2. Immobilization buffer B: 0.01 M potassium phosphate buffer, pH 4.75. Prepare a 10× stock solution. Transfer 88.7 mL of 0.1 M potassium phosphate monohydrate to a 100 mL graduated cylinder and add 11.3 mL of 0.1 M potassium phosphate dehydrate and filter through a 0.45 μm Whatman filter.
3. EDC: 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC).
4. NHS: *N*-hydroxysuccinimide (NHS).
5. Activating mixture: 0.2 M EDC, 0.05 M NHS.
6. HBS-EP buffer: 0.01 M HEPES, pH 7.4, 0.15 M NaCl, 0.03 M EDTA, 0.005 % surfactant P20 (Polyoxyethylene sorbitan).
7. Blocking solution: 1 M ethanolamine, pH 8.5.
8. Rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Sigma-Aldrich).
9. Running buffer: 0.05 M potassium phosphate buffer, pH 7.4. Transfer 80.2 mL of 0.1 M potassium phosphate monohydrate to a 100 mL graduated cylinder; add 19.8 mL of 0.1 M potassium phosphate dehydrate. Make up to 100 mL with water and filter through a 0.45 μm Whatman filter.
10. Washing buffer A: 1 M NaCl in running buffer.
11. Washing buffer B: 0.05 M glycine–HCl, pH 9.5. Prepare 5× stock solution. Weigh 47 g glycine, add 400 mL of deionized water, and adjust pH to 9.5 by 6 M HCl. After titration make up the volume to 500 mL with water.
12. Biacore 3000 with Control software (GE Healthcare).

2.2 Components for Affinity-Based Separation

1. Biological material (rat or mouse brains).
2. Human amyloid-beta protein fragment (1–42) of the highest purity available (Sigma-Aldrich).
3. Affi-Gel 10 (Bio-Rad).
4. Protease inhibitors: 0.5 % bacitracin water solution, 0.1 % aprotinin water solution, and 0.1 M phenylmethylsulfonyl fluoride (PMSF) solution in 96 % ethanol.
5. Immobilization buffer: 0.1 M Na-acetate buffer, pH 4.0.
6. Washing buffer: 1 M Tris-acetate buffer, pH 7.0. Weigh 12.11 of Tris-base and add 70 mL deionized water. Adjust pH to 7.0 by glacial acetic acid. Make up the volume to 100 mL with water.
7. Storage buffer: running buffer from 2.1 and add 0.02 % NaN_3.
8. Solubilization solution: 0.05 M potassium phosphate buffer, pH 7.4.
9. Triton X-100.
10. Elution buffer: 1 M glycine-HCl, pH 2.8, 0.15 M NaCl. Dissolve 7.56 g glycine and 8.76 g sodium chloride (NaCl) in 70 mL water and adjust pH to 2.8 by 6 M HCl. Make up the volume to 100 mL with water.

2.3 Proteomic Analysis

1. Lysis buffer: 0.1 M Tris–HCl, pH 8.5, 8 M urea, 0.015 M dithiothreitol. Add 0.12 g Tris-base, 4.8 g urea, 0.023 g dithiothreitol and water up to the total volume of 8 mL. Dissolve the urea completely. Adjust pH to 8.5 by 6 M HCl and make up the volume to 10 mL with water.
2. Alkylation solution: 0.05 M iodoacetamide. Dissolve 9.25 mg iodoacetamide in 1 mL water.
3. 0.05 M ammonium bicarbonate solution. Dissolve 39.5 mg of NH_4HCO_3 in 10 mL water.
4. Washing solution: 0.5 M NaCl. Dissolve 292 mg sodium chloride in 10 mL water.
5. Trypsin sequencing grade modified (specific activity 18,611 U/mg) (Promega).
6. Mobile phase A: 0.08 % formic acid and 0.015 % TFA in water.
7. Mobile phase B: 0.08 % formic acid and 0.015 % TFA in 80 % acetonitrile.
8. Mobile phase C: 0.08 % formic acid and 0.015 % TFA in 4 % acetonitrile.

2.4 Instrumentation

1. Optical biosensor Biacore 3000 and the Control software (GE Healthcare) for instrument operation.
2. Research grade sensor chips CM5 (GE HealthCare).

3. Homogenizer Ultra-Turrax T 10 (Cole-Parmer).
4. Compact liquid chromatography system AKTAprime plus (GE Healthcare).
5. Polystyrene chromatographic columns Tricorn 5/20 (GE Healthcare).
6. Spectrophotometer Cary 50 (Varien).
7. Microcentrifuge Eppendorf 5415 R (Eppendorf).
8. Peristaltic pump P-1 (GE Healthcare).
9. Centrifuge Heraeus megafuge 40 R (Thermo Scientific).
10. Centrifugal filters Amicon Ultra-15 (10 K) and Microcon-10 (10 K) (Merck Millipore Ltd).
11. Thermostat 5320 (Eppendorf).

2.5 LC-MS and Data Evaluation

1. High resolution mass spectrometer Q Exactive (Thermo Scientific) equipped with Easy-Spray® ion source.
2. Column for reverse-phase chromatography PepMap® RSLC C18, 150 mm × 50 μm, particle size 2 μm, pore size 100 A (Thermo Scientific).
3. Chromatography RSLC-nano system Ultimate 3000 (Thermo Scientific) composed of nano-flow and loading pumps, column oven with integrated 10-port switching valve and micro-autosampler.
4. Data acquisition and analysis software Xcalibur version 2.2 SP1.48.
5. Data converter software Raw2MSM version 1.10.
6. Data analysis and evaluation software MASCOT server 16 CPU.

3 Methods

3.1 SPR-Based Selection of Immobilization Conditions for Beta-Amyloid Coupling

SPR-based selection of immobilization conditions and appropriate resin are required for preparation of an affinity sorbent. Most frequently, affinity-based sorbents are prepared using cyanogen bromide (CNBr)-activated Sepharose [16]. In this case CNBr-activated Sepharose coupling of ligands of interest occurs under alkaline condition via their amino groups. However, in alkaline conditions the beta-amyloid peptide (1–42) readily aggregates [17]. Taking into consideration our SPR-based data on poor immobilization of beta-amyloid peptide (1–42) on the optical biosensor chip surface at pH 4.0 (Fig. 2), it appears that CNBr-activated Sepharose is inapplicable for covalent immobilization of the monomeric form of this peptide (*see* **Note 1**). Thus, we have chosen Affi-Gel 10 as an alternative resin due to possibility of covalent immobilization under a broad range of pH values.

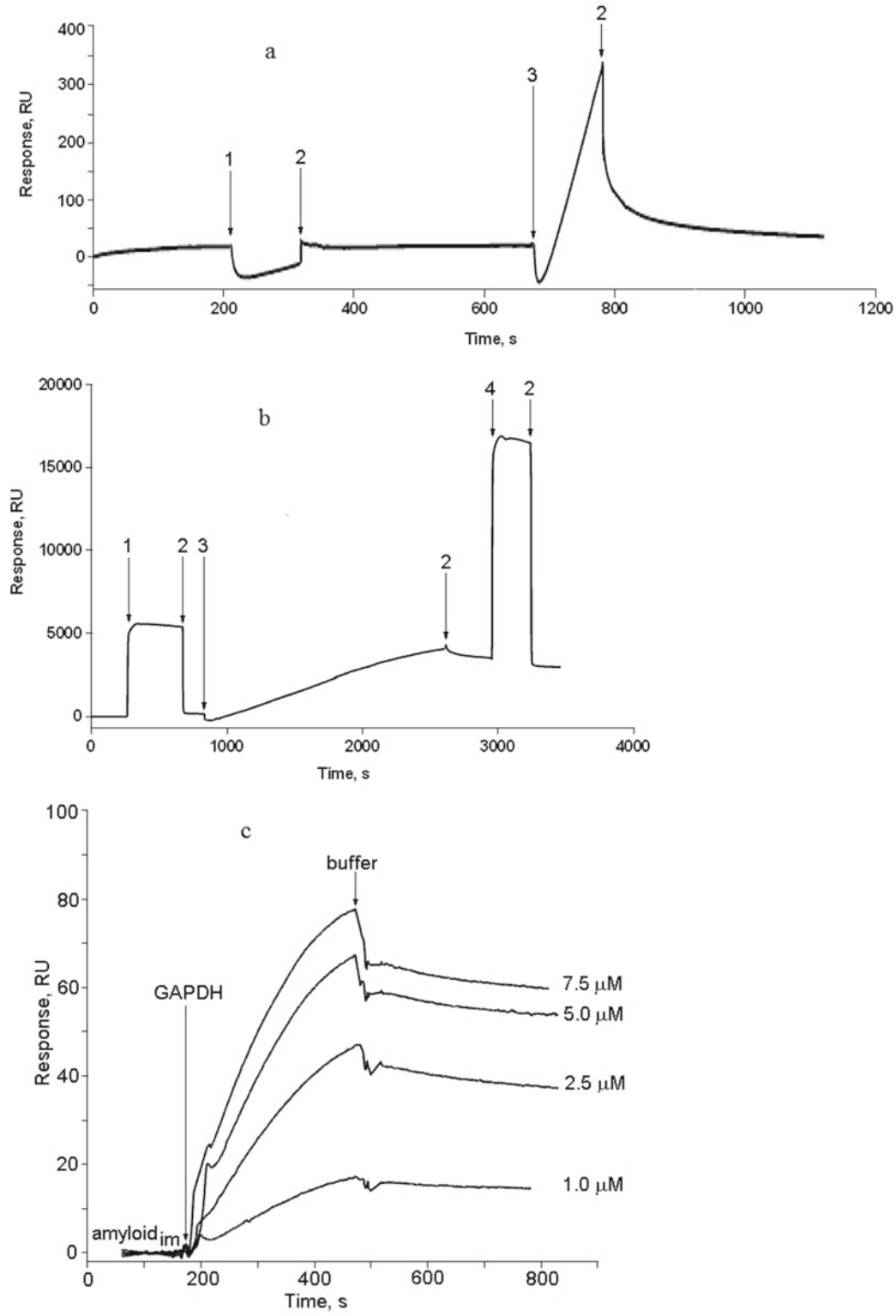

Fig. 2 SPR-based selection of immobilization conditions for amyloid-beta coupling and validation of amyloid-beta interaction with glyceraldehyde-3-phosphate dehydrogenase. (**a**) Optimization of pH for buffer used for amyloid-beta immobilization: *1*—amyloid-beta peptide (100 μg/mL) in immobilization buffer B, *2*—HBS-EP

3.1.1 Optimization of pH for Immobilization

1. Prepare human amyloid-beta 1–42 peptide 100 μg/mL solution in immobilization buffers A and B.
2. Inject the peptide solution, using a contact time of 2 min.
3. Set report points just before the start and end of the injection to determine the level of electrostatically bound peptide.

3.2 Immobilization of Amyloid-Beta Peptide

1. Activate sensor chips CM5 surface (GE HealthCare) by the activating mixture.
2. Inject amyloid-beta (100 μg/mL in immobilization buffer A or B) at a flow rate of 5 μL/min for 30 min.
3. Wash 5 min with HBS-EP buffer.
4. Passivate active groups by injection of blocking solution for 1 min.

3.2.1 Binding Measurements

Protein–peptide interactions are monitored by injecting various concentrations of a model protein, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (*see* **Note 2**), at a flow rate of 5 μL/min for 5 min; GAPDH was dissolved in running buffer at 144 μg/mL–1.44 mg/mL. Regenerate the sensor surface after each measurement cycle by sequential injections of washing A and washing buffer B with for 0.5 min at a flow rate of 50 μL/min (Fig. 2).

3.3 Immobilization of Amyloid Beta Protein Fragment 1–42 on Affi-Gel 10

1. Dissolve 1 mg amyloid beta protein fragment 1–42 in 250 μL distilled water.
2. Add 250 μL immobilization buffer (*see* Subheading 2.2) and mix gently.
3. Wash Affi-Gel 10 resin (0.5 mL) on a glass filter with 10 volumes of distilled water and 2 volumes of the immobilization buffer A.
4. Transfer the resin to a 1.6 mL Eppendorf tube and eliminate the buffer by aspiration after centrifuging of resin slurry at 1,485 × *g* for 3 min.
5. Add amyloid beta protein fragment to the resin at a ratio of 1:1 (v/v) and incubate at 4 °C overnight at a gentle stirring.
6. Remove the excess of the protein fragment by centrifuging 4–5 times in washing buffer, at 1,485 × *g* for 3 min.

Fig. 2 (continued) buffer, pH 7.4, *3*—amyloid-beta peptide (100 μg/mL) in immobilization buffer A. At pH 4.0 (immobilization buffer A) amyloid-beta demonstrates much better interaction with the surface of the optical biosensor chip. (**b**) Immobilization of amyloid-beta peptide (100 μg/mL) in 10 mM sodium acetate, pH 4.0 onto the surface of the optical biosensor chip. *Arrows* indicate injections: *1*—EDC/NHS; 2—HBS-EP buffer; *3*—100 μg/mL amyloid-beta in immobilization buffer A; *4*—blocking solution. (**c**) Interaction of GAPDH (glyceraldehyde-3-phosphate dehydrogenase) with immobilized amyloid-beta peptide. Results are corrected versus control channel (without amyloid-beta peptide)

7. Add the same buffer to the resin up to the suspension 1:1 (v/v) and incubate at 4 °C for 4 h at a gentle stirring.
8. Wash 4–5 times with the same buffer and resuspend the resin in storage buffer.
9. Store Affi-Gel 10 with the immobilized amyloid beta protein fragment at 4 °C. The immobilized protein fragment can be kept under such conditions for more than 4 weeks.
10. Perform all the above-mentioned incubations (except the addition of amyloid beta protein fragment) with the control Affi-Gel 10.

3.4 Preparation of Rat Brain Homogenate for Affinity-Based Separation

1. Decapitate rats under light ether anesthesia.
2. Immediately dissect the brains and homogenize at a low speed in solubilization buffer (1:3) (w/v), using an Ultra-Turrax T 10 homogenizer.
3. Measure protein concentration using Bradford protein assay and spectrophotometer Cary 50 (Varian).
4. Add Triton X-100 (final concentration 3 %) to brain homogenates and incubate at 4 °C for 1 h.
5. Dilute the brain homogenate lysates threefold with solubilization buffer and remove Triton-insoluble components by centrifugation at 23,755 × *g* for 20 min at 4 °C.

3.5 Isolation of Rat Brain Proteins by Affinity Chromatography Using Amyloid-Beta as the Affinity Ligand

1. Add the resultant supernatant (about 5 mg of protein/mL) to the suspension of the affinity sorbent (Affi-Gel 10 with the immobilized amyloid beta protein) (1:1, v/v).
2. Add protease inhibitors (bacitracin, aprotinin, and phenylmethylsulfonyl fluoride (PMSF)) up to the concentrations of 0.005 %, 0.01 %, and 0.001 M, respectively, and incubate the suspension overnight at 4 °C at a gentle stirring [18].
3. Perform the same incubations with the control Affi-Gel 10 without immobilized protein ligand to evaluate nonspecific binding of proteins (*see* **Note 3**).
4. After the incubation wash the sorbent by centrifuging with solubilization buffer up to the absence of the protein in the washing (evaluated by baseline at OD_{280} on spectrophotometer Cary 50 (Varian)).
5. Pack the sorbent using the polystyrene chromatographic columns Tricorn 5/20 (1 × 0.75 mL) (GE Healthcare) and eluate proteins by eluting buffer at a flow rate of 0.5 mL/min using compact liquid chromatography system AKTAprime plus (GE Healthcare) and peristaltic pump P-1 (GE Healthcare).
6. Concentrate the eluate up to 0.200 mL using Amicon Ultra-15 centrifugal filter devices (Heraeus megafuge 40 R, bucket-rotor, 2,121 × *g*, 25 min, 4 °C).

3.6 Sample Preparation for Proteomic Analysis

Extract, modify, and digest protein samples on filters using the recently published FASP protocol [19].

1. Precipitate the proteins using chloroform–methanol method [20]. Briefly, add methanol to the protein sample (1:2.5) vortex vigorously for 30 s and add chloroform (equal to the initial volume of the protein sample), vortex again and centrifuge the resultant mixture for 2 min at 23,755 × *g* at 20 °C for 2 min. Discard the upper layer and add a threefold excess (versus initial sample) of methanol, vortex and centrifuge at 20 °C for 3 min. Aspirate supernatant and dry the resultant sediment under air for 20–30 min.
2. Dissolve the sediment (of precipitated proteins) in 200 μL of lysis buffer for proteins denaturation and reducing of oxidized disulfide bonds.
3. Pipette the samples onto the centrifugal filters Microcon-10 (Merck Millipore Ltd) and centrifuge at 1,485 × *g* at 20 °C for 40 min.
4. Add 100 μL of alkylation solution in washing buffer and incubate at 20 °C for 30 min in darkness.
5. Centrifuge under the same conditions for 40 min.
6. Wash twice with 200 μL of washing solution (each time centrifuge under the same conditions for 40 min).
7. Add ammonium bicarbonate solution and sequencing grade trypsin (*see* **Notes 4** and **5**) at a ratio of 1:100 = total mass of enzyme:total mass of protein.
8. Incubate overnight at 37 °C using Thermostat 5320 (Eppendorf).
9. Centrifuge under the same conditions for 40 min.
10. Wash with 100 μL of washing solution.

3.7 Liquid Chromatography: Mass Spectrometry

Perform all mass spectrometry experiments using a high-resolution mass spectrometer Q Exactive (Thermo Scientific) equipped with Easy-Spray® ion source and RSLC-nano Ultimate 3000 chromatography system (Thermo Scientific) composed of nano-flow and loading pumps, column oven with integrated 10-port switching valve and micro-autosampler, or any high resolution mass spectrometer available.

1. Select the positive ionization mode.
2. Use data-dependent acquisition mode at 70 K resolution in MS and 35 K resolution in MS/MS.
3. Set the AGC targets at 1e6 ion for 20 ms and 5e4 ions for 75 ms at MS and MS/MS levels, respectively, with the underfill ratio of 10 %.

4. Set the instrument for top ten isolated MS peaks (window ±1 m/z) to be used for the further tandem MS spectra acquisition for full duty cycle.
5. Apply high energy collision dissociation (HCD) for peptides fragmentation with ±25 % stepped 30 eV normalized collision energy. Use 100 m/z as the first fixed mass for tandem spectra acquisition.
6. Use active dynamic exclusion mode for 40 s and chromatography peak filtering (width of 15 s).
7. Trigger exclusion charge states of ions at $z+1$ and $z>5+$.
8. Use nano-LC Ultimate 3000+ system with mobile phase A and mobile phase B for reverse-phase chromatographic separation of digested peptides was performed on PepMap® RSLC C18, 150 mm × 50 μm, particle size 2 μm, pore size 100 A (Thermo Scientific).
9. Load 1 μL of a sample digest for 4 min at a flow rate 5 μL/min in the isocratic mode using mobile phase C onto Acclaim PepMap C18 column (75 μm × 150 mm, 2 μm particle size, 100 A pore size).
10. Separate peptides by means of linear elution gradient of mobile phases A and B from 4 to 65 % of acetonitrile at a flow rate of 0.3 μL/min following by rapid increase (within 3 min) of the gradient to 98 % of mobile phase B and keep this mode remaining for 10 min. After the whole procedure perform column post-analysis reconstitution under initial conditions for 10 min.

3.8 Database Searching and Identification

For peptide identification, MS/MS data collected from LC-MS experiments are searched against Uniprot custom filtered database using MASCOT (version 2.2) search algorithm. Spectra are searched against Rat subset of SwissProt database.

1. Use the following search parameters: trypsin as the cutting enzyme, set mass tolerance for the monoisotopic peptide window at ±20 ppm (parts per million) and ±0.05 Da for the MS/MS tolerance window, one missed cleavage is allowed.
2. Perform parallel search of the data against decoy database, use the results to estimate the q-value using the Percolator algorithm and filter at 1 % FDR (false discovery rate).
3. Specify carbamidomethylation of cysteine as fixed modification and set methionine oxidation and cyclization of N-terminal glutamine as variable modifications.
4. Set the following criteria of positive identification: minimum score of 50, at least three positive identifications from three different runs.

3.9 SPR-Based Validation of the Interaction of Identified Proteins with Amyloid- Beta

Further validation of the interaction between immobilized amyloid-beta and proteins identified by mass spectrometry can be obtained using various methods. We recommend the SPR-based biosensor analysis with purified proteins of interest (*see* Figs. 1 and 2). Advantages of the SPR-based validation consist in the possibility of repeated use of the optical biosensor cuvette with immobilized amyloid-beta.

4 Notes

1. Recent results of isoelectric focusing studies indicate that low molecular forms of monomeric amyloid-beta (1–42 and 1–40) are characterized by a p*I* value of ca. 5, while at p*I* of 6–6.5 aggregates of high molecular weight are detected [17]. This suggests that it is better to use more acidic solutions of amyloid-beta to prevent its aggregation.
2. Several studies have recognized glyceraldehyde-3-phosphate dehydrogenase as the amyloid-binding protein (e.g., [18, 21]), and therefore it can be used as a probe for interaction with immobilized amyloid-beta.
3. Various intracellular highly abundant proteins (e.g., cytokeratins) may nonspecifically bind to a resin even without an immobilized affinity sorbent (*see* for example, ref. 22) and therefore a control batch of the ligand-free sorbent has to be subjected to all the same treatments but without amyloid-beta. The list of identified amyloid-binding proteins will have to be corrected for possible presence of proteins nonspecifically bound to the control sorbent.
4. Use sequencing grade trypsin only for mass spectrometry experiments due to its high specificity and low contamination of alpha-chymotrypsin. Also it is strongly recommended to use modified trypsin (with acetylated lysins) to prolong activity of enzyme and slow down its autolysis.
5. In the case of the use of non-sequencing grade trypsin its catalytic activity may be rather low; if so, it is recommended to add up to 0.003 M of calcium chloride to increase ionic strength of reaction mixture rather than sodium salts to minimize further cationization during LC-MS analysis. However, it should be noted that native trypsin may undergo autolysis, generating pseudotrypsin, which exhibits a broadened substrate specificity including chymotrypsin-like activity (*see* comments [23]). In addition, trypsin may be contaminated with chymotrypsin, and this fact has to be taken into consideration especially when high enzyme/substrate ratios are used.

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References

1. Schmechel DE, Saunders AM, Strittmatter WJ, Crain BJ, Hulette CM, Joo SH, Pericak-Vance MA, Goldgaber D, Roses AD (1993) Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. Proc Natl Acad Sci U S A 90:9649–9653
2. Saido TC, Iwatsubo T, Mann DM, Shimada H, Ihara Y, Kawashima S (1995) Dominant and differential deposition of distinct beta-amyloid peptide species, A beta N3(pE), in senile plaques. Neuron 14:457–466
3. Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 297:353–356
4. LaFerla FM, Green KN, Oddo S (2007) Intracellular amyloid-β in Alzheimer's disease. Nat Rev Neurosci 8:499–509
5. Masters CL, Selkoe DJ (2012) Biochemistry of amyloid β-protein and amyloid deposits in Alzheimer disease. Cold Spring Harb Perspect Med 2(6):a006262
6. Upadhaya A, Kosterin I, Kumar S, von Arnim CA, Yamaguchi H, Fändrich M, Walter J, Thal DR (2014) Biochemical stages of amyloid-β peptide aggregation and accumulation in the human brain and their association with symptomatic and pathologically preclinical Alzheimer's disease. Brain 137(pt 3):887–903
7. Li QX, Maynard C, Cappai R, McLean CA, Cherny RA, Lynch T, Culvenor JG, Trevaskis J, Tanner JE, Bailey KA, Czech C, Bush AI, Beyreuther K, Masters CL (1999) Intracellular accumulation of detergent-soluble amyloidogenic A beta fragment of Alzheimer's disease precursor protein in the hippocampus of aged transgenic mice. J Neurochem 72:2479–2487
8. Wirths O, Multhaup G, Czech C, Blanchard V, Moussaoui S, Tremp G, Pradier L, Beyreuther K, Bayer TA (2001) Intraneuronal A beta accumulation precedes plaque formation in beta-amyloid precursor protein and presenilin-1 double-transgenic mice. Neurosci Lett 306: 116–120
9. Butterfield DA, Sultana R, Poon HF (2006) Redox proteomics: a new approach to investigate oxidative stress in Alzheimer's disease. In: Dalle-Donne I, Scaloni A, Butterfield DA (eds) Redox proteomics: from protein modifications to cellular dysfunction and diseases. Wiley, Hoboken, pp 563–603
10. Knobloch M, Konietzko U, Krebs DC, Nitsch RM (2007) Intracellular Aβ and cognitive deficits precede β-amyloid deposition in transgenic arcAβ mice. Neurobiol Aging 28:1297–1306
11. Reddy PH, Beal MF (2008) Amyloid beta, mitochondrial dysfunction and synaptic damage: implications for cognitive decline in aging and Alzheimer's disease. Trends Mol Med 14: 45–53
12. Spuch C, Ortolano S, Navarro C (2012) New insights in the amyloid-beta interaction with mitochondria. J Aging Res 2012:324968
13. Wu Z, Zhu Y, Cao X, Sun S, Zhao B (2014) Mitochondrial toxic effects of Aβ through mitofusins in the early pathogenesis of Alzheimer's disease. Mol Neurobiol 50(3):986–96
14. Habib L, Lee MTC, Yang J (2010) Inhibitors of catalase-amyloid interactions protect cells from β-amyloid-induced oxidative stress and toxicity. J Biol Chem 285:38933–38943
15. Hernandez-Zimbron LF, Luna-Munoz J, Mena R, Vazquez-Ramirez R, Kubli-Garfias C, Cribbs DH, Manoutcharian K, Gevorkian G (2012) Amyloid-β peptide binds to cytochrome C oxidase subunit 1. PLoS One 7(8):e42344
16. Calero M, Rostagno A, Ghiso J (2012) Search for amyloid-binding proteins by affinity chromatography. Methods Mol Biol 849:213–223
17. Wiberg H, Ek P, Ekholm PF, Lannfelt L, Emmer Å, Roeraade J (2010) Separation and characterization of aggregated species of amyloid-beta peptides. Anal Bioanal Chem 397:2357–2366
18. Schulze H, Schuler A, Stuber D, Dobeli H, Langen H, Huber G (1993) Rat brain glyceraldehyde-3-phosphate dehydrogenase interacts with the recombinant cytoplasmic domain of Alzheimer's beta-amyloid precursor protein. J Neurochem 60:1915–1922
19. Wisniewski J, Zougman A, Nagaraj N, Mann M (2009) Universal sample preparation method for proteome analysis. Nat Methods 6:359–362
20. Walker JM (ed) (2002) The protein protocol handbook. Humana Press Inc., Totowa, NY

21. Verdier Y, Földi I, Sergeant N, Fülöp L, Penke Z, Janáky T, Szücs M, Penke B (2008) Characterization of the interaction between Abeta 1-42 and glyceraldehyde phosphodehydrogenase. J Pept Sci 14:755–762
22. Buneeva O, Gnedenko O, Zgoda V, Kopylov A, Glover V, Ivanov A, Medvedev A, Archakov A (2010) Isatin binding proteins of rat and mouse brain: proteomic identification and optical biosensor validation. Proteomics 10: 23–37
23. Medvedev AE (2013) In macropore tryptic digestion at acidic pH and its implication for proteomics. Proteomics 13:3101–3102