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SIMPLIFIED ELECTROPHORETIC SEPARATION OF AMYLOID-β PEPTIDES

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Several variants of amyloid-β peptides, differing in their carboxy terminus, have been identified as the major component of cerebral deposits of amyloid found in the brains of patients with Alzheimer's disease. Recently, we have refined a simple and inexpensive method for analysis and separation of amyloid-β peptides based on modification of discontinuous SDS-PAGE electrophoresis with utilization of Tricine as a trailing ion. Clear resolution was achieved by addition of high concentration of urea to the separation and stacking gel. The obtained data confirmed that described gel electrophoretic system is a superior procedure for the analysis of amyloid-β peptides providing enhanced resolution even for peptides which differ only in few amino acids in the length of polypeptide chain.

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Introduction

The progressive neurodegeneration in Alzheimer's disease (AD) is associated with the accumulation of extracellular deposits of amyloid found in brain tissue. The principal proteinaceous component of these senile plaques is the amyloid-β peptide (Aβ peptide), a proteolytic cleavage product of the membrane-bound, amyloid precursor protein (APP). This 4-kDa peptide contains 39-42(43) amino acid residues (Fig. 1) [1]. The Aβ peptides comprise a heterogeneous set of N- and C-terminally truncated peptides [2] and the three best known C-terminated Aβ peptides are $A\beta_{1-38}$, $A\beta_{1-40}$, and $A\beta_{1-42}$. $A\beta_{1-40}$ is the major soluble peptide secreted, while $A\beta_{1-42}$ accounts for approximately 10% [3]. Since both these peptides are directly involved in pathologic events of AD, synthetic $A\beta_{1-42}$ forms amyloid fibrils *in vitro* much more readily than $A\beta_{1-40}$ [4], and there is good evidence that $A\beta_{1-42}$ is deposited early and selectively in senile plaques of AD [5].

1 5 10 15 20 25 30 35 40
Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala

Fig. 1 Amino acid sequence of amyloid-β peptides in three-letter code (source: ExPASy web server)

Because of pathogenic role of Aβ amyloid deposits, the capabilities of identifying, characterizing and even quantifying of Aβ soluble forms and its variants may gain high importance. Rapid method for detection of the presence of each isoform would be an advantage in research and can even help in screening diagnostics due to determination of Aβ peptides from biological fluids.

Various gel electrophoretic techniques remain the simplest and most frequently used powerful tool for analytical separation and identification of peptides. Polyacrylamide gel systems capable of resolving Aβ peptide were introduced [6-13] and used for analyzing Aβ peptides from natural sources as CSF [14-16], cell culture media [2,15,17,18], brain homogenates [15,16] or even plasma [19]. Previously presented electrophoretic separations of Aβ peptides that differ in length by few amino acids most often used the effective urea version of the bicine/bistris/Tris/sulphate SDS-PAGE system of Wiltfang *et al.* [7].

In this paper we describe a rapid and elementary electrophoretic method for separation of individual β-amyloid peptides. The system presented here is based on the Tris-Tricine SDS-PAGE method of Schägger and von Jagow [20] with high concentration of urea too. This system was chosen since its great convenience for small protein/peptide fragments separation was proved.

Experimental

Tris, Tricine and other common chemicals were supplied by Sigma Aldrich (St. Louis, MO, USA). The unstained molecular weight marker (Precision Plus Unstained Standard), Premixed Electrophoresis Running Buffer and Tris-Tricine sample buffer were purchased from BioRAD (Hercules, CA, USA). Synthetic amyloid-β peptides $A\beta_{1-38}$, $A\beta_{1-40}$, and $A\beta_{-42}$ were obtained from Sigma Aldrich (St. Louis, MO, USA). The purity of synthetic peptides was at least 90 %, as specified by the producer. All the other chemicals were reagent grade and were used without further modification.

All electrophoresis procedures were performed using the Mini Protean3 Electrophoresis gel system (BioRAD, USA).

The polyacrylamide separation gel consisted of 16.5 % T/3 % C resolving gel over layered by a 4 % T/3 % C stacking gel. The concentration of urea added to the gels was 8 M (5 M) respectively. The precise compositions of gels used for Aβ peptides separation are given in Table I.

Table I Compositions of tris-tricine-urea SDS-PAGE stacking and separation gels

Reagents	Composition of	
	Stacking (comb) gel	Separating (resolving) gel
Acrylamide/bis 49.5 % T/3 % C	0.25 ml	1.666 ml
Gel buffer*	0.775 ml	1.666 ml
Urea	0.9 g	2.4 g
Deionized water	2.1 ml	1.666 ml
APS (10 %)	0.025 ml	0.025 ml
TEMED	0.0025 ml	0.0025 ml
Total volume (approx.)	3 ml	5 ml

* gel buffer: 3 M Tris-HCl buffer, pH 8.45, 3 % SDS

Lyophilized Aβ peptides were dissolved in PBS (pH 7.4) to the concentration of 1 mg ml^{-1} (stock solution) and for intended analysis were further diluted 25 times; 15 ml of diluted samples were mixed 1:1 (v/v) with the commercial Tris-Tricine sample buffer, in order to achieve a final protein quantity of 0.4 mg per well. The samples were incubated at 95 °C for 3 minutes and then loaded on gels; 5 ml of the molecular weight standards was used for each analysis. The gels were run with an initial voltage of 30 V and this voltage was maintained until the sample has completely entered the stacking gel (around 30 min) and this

was then increased to 90 V. Total run time was of approximately 2.5 hrs (as the tracking dye front approached the bottom of the gel). During separation, the electrophoretic system was kept at 4 °C and the running buffer was chilled to minimize urea breakdown in gels. The system conditions were constant for each run. Each electrophoresis experiment was repeated at least three times in independent experiments.

Gel fixation and staining of gels was accomplished using the ammoniacal silver staining procedure described by Oakley [21].

Results and Discussion

The conventional 16.5 % T/3 % C Tris-Tricine SDS-PAGE electrophoresis [20] (Fig. 2A) was originally intended for separation of small proteins and peptides and it was not found forcible in resolution of the synthetic Aβ fragments. These peptides were not well separated from each other and appeared as a single bold band when the peptides were mixed. In 1991 a very beneficial approach for peptides separation based on addition of high concentration of urea to the separation gel and using a multiphasic buffer system employing bicine and sulphate was presented (bicine/Tris/urea gels) [7]. This method without any further modification was for the first time used for Aβ separation by Klafki *et al.* in 1996 [12]. This group also compared the forementioned method with Tris-Tricine SDS-PAGE with 8 M urea added into the gel. Tris-Tricine-urea SDS-PAGE was found practical as well but Klafki also mentioned curving deformation of bands which can significantly complicate the identification of Ab fragments of similar length. The authors pointed out essential presence of urea and SDS in separation gel for clear separation. For everyday separation of truncated Aβ peptides there was a strong need to develop a simple method able to distinguish among several variants of Aβ fragments. We were able to extend the commonly used Tris-Tricine-SDS-PAGE procedure [20] through the addition of 5 M and 8 M urea into the stacking and separation gel, respectively. With the urea version of Tris-Tricine-SDS-PAGE we proved that the separation of Aβ peptides is possible and allows the superior resolution.

In high molarity urea environment (with or without SDS) the Aβ peptide becomes 100 % random coil and remains monomeric. Inclusion of 8M urea enables increases in the resolution for the separation of low molecular weight proteins [22,23]. With 8 M urea added into the Tris-Tricine-SDS separation gel a clear resolution of Aβ peptides was achieved (Fig. 2B). All the peptides are resolved as slightly diffused but clearly separated bands without curving. The precipitation of samples entering the gel was not visible either. But under these conditions different electrophoretic mobilities were observed, the peptides do not act up to the rule of mass-mobility relationship that is applied in common SDS-

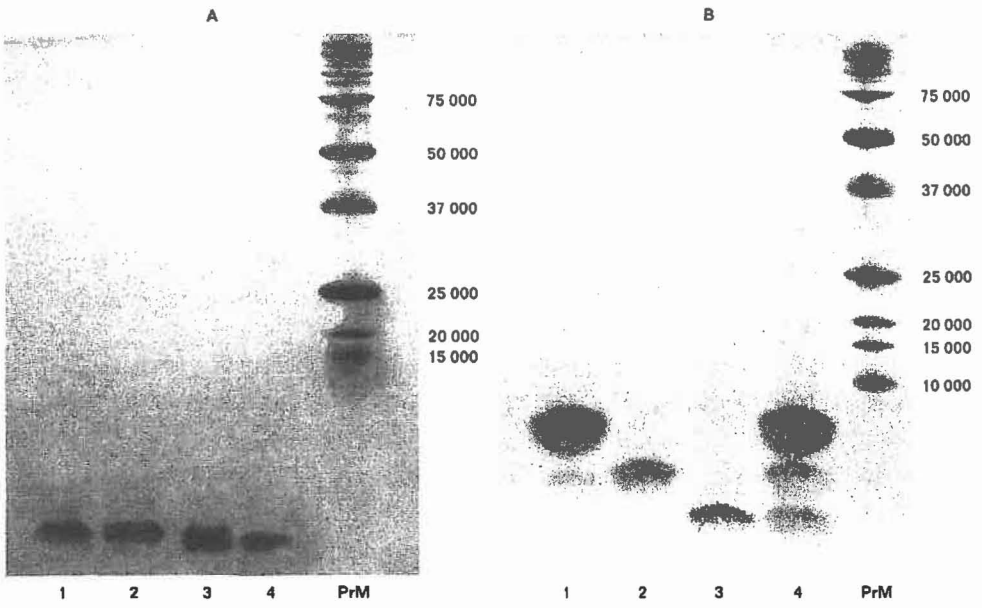

Fig. 2 Silver staining analysis of amyloid-β peptides separation (16.5%T 3%C Tris-Tricin-SDS-PAGE). PrM indicate the protein molecular weight standard; lane 1 - 3 represent single forms of Aβ peptides, $A\beta_{1-38}$ (M_r 4131), $A\beta_{1-40}$ (M_r 4329), and $A\beta_{1-42}$ (M_r 4514); lane 4 represents mixture of the three peptides. A – no urea included in gels; B – the stacking and separation gel contained 5M and 8M urea, respectively

PAGE by most proteins (including Aβ peptides) [24]. The longer fragment ($A\beta_{1-42}$) migrated faster than the shorter ones, which was in agreement with previously described findings [10,24].

In conclusion, these results indicate that the Tris-Tricine resolving gels containing 8 M urea allow separation of Aβ peptides differing only by not more than a single hydrophobic amino acid. The resolution is satisfactory even for minigels. The simplicity of this technique could make it ideal for the routine study. By the described procedure we would like to report alternative method for analysis of Aβ isoforms related to Alzheimer's disease.

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