TECHNICAL NOTES

BACHEM

PIONEERING PARTNER FOR PEPTIDES

CARE AND HANDLING OF AMYLOID PEPTIDES

Bachem offers a broad range of amyloid peptides. Some of these peptides are notorious for their propensity to form scarcely soluble aggregates, which makes reconstitution difficult. As to help our customers, we have compiled a selection of literature references containing protocols for dissolving the peptides. We hope you will find some information, which could be helpful for your experimental work with our peptides.

Summary

According to the amyloid hypothesis, the deposition of amyloid β-peptides in senile plaques is a primary event in the pathological cascade for Alzheimer`s disease (AD). The amyloid β-protein consists of 39-43 amino acid residues, derived from proteolysis of a larger β-amyloid precursor protein (β-APP). With our present state of knowledge aggregation or aging of the amyloid β-protein is an essential requirement for neurotoxicity of amyloid β-protein. During this aging process, the amyloid β-protein undergoes conformational conversions, from soluble, monomeric random coil or α-helix conformation into aggregated β-sheet structures [1, 2, 3]. Several studies demonstrated that this conformational change within the aggregation process was affected by a number of factors, such as the length of the amyloid β-peptide [4], solvent hydrophobicity [5], ionic strength [6, 7], pH [8, 9, 10], peptide concentration, initial aggregation state [11, 12], buffer type, peptide counterions (e.g. CF_3COO^- vs Cl^-) [13], and the presence of partially oxidized or preaggregated forms (seeds) of the peptide [4, 7]. Given that, it is not astonishing that even the smallest modifications could drastically change the aggregation rate, respectively the solubility.

Solubilization and aggregation of lyophilized amyloid peptides

In the following section you will find some examples from literature which describe the aggregation and solubilization of amyloid β-protein fragments.

For the solubilization of amyloid β-protein (1-42) (product number H-1368) Bachem recommends the use of a 0.1 M aqueous ammonia solution (1 mg/ml, pH > 9). We advise the use of NH_4^+ ions to increase the pH-value without disturbing the peptide structure.

J. Busciglio et al. dissolved the lyophilized amyloid β-protein (1-40) (H-1194) to a stock concentration of 1.0 mg/ml in either deionized water, 35 % acetonitrile / 0.1 % TFA or 100 % DMSO and then diluted it (1 : 100) directly into tissue culture medium. Lyophilized amyloid β-protein (1-40) was poorly soluble in salt-containing buffers (e.g. PBS), but could be introduced into saline-containing solutions after being initially dissolved in one of the three vehicles described above.

The observed differential neurotoxic potency of amyloid β-protein (1-40), after dissolution in different vehicles, suggested that the conformational state of the peptide could be an important determinant of its biological activity [14].

In the article of G. Perini et al. amyloid β-protein (25-35) (H-1192) was dissolved at 1.5 mM in PBS, amyloid β-protein (1-40) (H-1194) at 1.5 mM in double-distilled water and subsequently diluted at 250 µM in PBS, and

amyloid β-protein (1- 42) (H-1368) at 500 µM in double-distilled water. Furthermore, the authors reported that fibrillogenesis by amyloid β-protein (25-35) took place within minutes at room temperature, whereas amyloid β-protein (1-40) and amyloid β-protein (1-42) required 5-6 d at 37 °C. No fibril formation could be observed for amyloid β-protein (35-25) which was dissolved in the same solvent as amyloid β-protein (25-35). When the above mentioned amyloid β-proteins were dissolved in DMSO, they didn`t form fibrils and remained in solution [15]. Contrary to the TFA salt (H-1194), the HCl salt of amyloid β-protein (1-40) (H-5568) was able to form β-structures in PBS within a few hours at 25 °C [13].

In the study of C.W. Cotman and coworkers small aliquots (1 mg or less) of amyloid β-proteins were solubilized to a concentration of 250 µM with double-deionized water. Since aggregate formation occurred over time, immediate use of newly solubilized amyloid β-protein solutions circumvented the aggregation process for most amyloid β-proteins. Repeated freeze-thawing or incubation of amyloid β-protein solutions yielded aggregated samples [16]. To overcome this obstacle, B.A. Yankner et al. [17] utilized a 35 % acetonitrile / 0.1 % TFA solvent system for amyloid β-protein solubilization. This system seemed to be an effective means to achieve initial solubility for most amyloid β-protein fragments, however, aggregation could occur over time in such solutions.

M.P. Mattson et al. reported, that stock solutions of amyloid β-protein (1-38) (H-2966) and amyloid β-protein (25-35) (H-1192) could be prepared by dissolving the peptides at a concentration of 0.5-1.0 mM in water or 1.0 mM DMSO and stored as aliquots at -20 °C until use [18].

According to S.M. Tomski & R.M. Murphy the solubility of amyloid β-protein (1-40) (H-1194) was a strong function of pH. In PBS (0.14 M NaCl, 0.01 M KH_2PO_4/K_2HPO_4, 0.02 % (w/v) sodium azide) the peptide was insoluble below pH 7.0. Below pH 3.0 or above pH 7.2 the peptide was soluble, in the latter case up to a concentration of 16 mg/ml. Furthermore, it was asserted that the solubility was not a significant function of ionic strength. As the salt concentration dropped to 75 mM at pH 7.4, the solubility limit remained virtually constant at 15 mg/ml. In deionized Milli-Q water, amyloid β-protein (1-40) was soluble up to nearly 20 mg/ml. In 0.15 M PBS, pH 7.4, at peptide concentrations below 3 mg/ml, no precipitate, turbidity, or gelation was observed, even 3 month after preparation. Above this concentration, although the peptide was originally soluble, a clear continuous gel could be formed, whereby the time for gel formation varied from about 3 weeks at 3 mg/ml to about 2.5 d at 10 mg/ml [19].

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