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Potentiation of beta-folding of \( \beta \)-amyloid peptide 25–35 by aluminum salts

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Abstract

The formation of the \( \beta \) pleated configuration of the amyloid peptide fragment 25–35 in aqueous solution, has been studied using thioflavin-T fluorescence as an indicator of such folding. Both phosphate and adenosine triphosphate (ATP) enhance the formation of aggregated \( \beta \)-sheets. This phosphate-induced aggregation is greater in the presence of aluminum sulfate in a dose dependent manner. In the absence of ATP or phosphate, aluminum salts do not promote aggregation. It is proposed that a particulate aluminum phosphate complex may form critical nuclei upon whose surface the amyloid peptide can change its configuration. This capacity for seeding may be a relevant factor in the formation of insoluble proteinaceous materials such as amyloid plaques and neurofibrillary tangles found in Alzheimer’s disease.

The ability of proteins to abandon their native alpha helical configuration and to form pleated sheets in the beta configuration has been posited to be an important event in various neurological diseases such as Alzheimer’s disease and bovine spongy encephalomyelitis. Such a transition of key proteins to the \( \beta \) structure may facilitate the formation of stable interchain complexes and the consequent appearance of aggregated proteins. The presence of an insoluble proteinaceous matrix not readily degraded by intracellular proteases has been proposed to bear a causal relation to some manifestations of neurological disease [12,13]. Thus, such a conformational change in \( \beta \)-amyloid may contribute to the peptide’s neurotoxicity [21].

The role of aluminum in neurological disease remains unresolved. Claims of elevated levels of aluminum in brains of Alzheimer’s disease patients are controversial [19]. While most reports in this area have been equivocal, there is no doubt that excess plasma levels of aluminum following renal dialysis have been associated with neuropathy in humans [23]. Furthermore, aluminum neurotoxicity has been demonstrated in experimental animals [10,28]. The ubiquitous presence of aluminum in the environment and its tentative association with some neurological disorders, warrant further examination of potential mechanisms by which aluminum salts may affect the CNS in a deleterious way.

In the current study, we have examined the manner in which aluminum may affect the transition between the soluble form of an amyloid peptide and its aggregated form. While aluminum salts did not directly influence this process, they were able to potentiate the tendency of phosphates to bring about the \( \beta \)-pleating of this peptide.

Amyloid (25–35) peptide (18.9 \( \mu \)M, 20 \( \mu \)g/ml) and other peptides were incubated together with 10 \( \mu \)M thioflavin-T. Buffers used were 100 mM potassium phosphate or 50 mM Tris–HCl, pH 7.4 Fluorescence was determined with a Perkin–Elmer spectrofluorometer using an excitation wavelength of 440 nm and emission of 482 nm [16]. Incubations were performed at 21°C for 1 h in the presence of varying concentrations of aluminum sulfate or Al acetylacetonate. The time used was selected since this period gave maximal values and more prolonged incubations did not further elevate fluorescence.

Protein aggregation was also assayed in the absence of thioflavin-T, turbidimetrically by spectrophotometric measurement at 405 nm, and by fluorometric determination of light scattering, both excitation and emission being at 405 nm [32].

Each determination was derived from four to six separate assays. Differences between samples were assessed by one-
responsive to aluminum.

was greatly enhanced but the reverse peptide was unre-

cducible anomalies in the dose-response curve, suggesting

Table 1
Formation of β-sheets following incubation of peptides with thioldavin-T probe

<table>
<thead>
<tr>
<th>Addition</th>
<th>20 μM Al$_2$(SO$_4$)$_3$ present</th>
<th>Fluorescent emission (482 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>–</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>None</td>
<td>+</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>β-amyloid (25–35) (20 μg/ml)</td>
<td>–</td>
<td>20.7 ± 4.3</td>
</tr>
<tr>
<td>β-amyloid (25–35) (20 μg/ml)</td>
<td>+</td>
<td>61.7 ± 3.9*</td>
</tr>
<tr>
<td>β-amyloid reverse peptide, (35–25) (20 μg/ml)</td>
<td>–</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>β-amyloid reverse peptide, (35–25) (20 μg/ml)</td>
<td>+</td>
<td>0.4 ± 0.2</td>
</tr>
</tbody>
</table>

* Incubation was for 1 h at 21°C in 100 mM phosphate buffer, pH 7.4.

way Analysis of Variance followed by Fisher’s Least Significant Difference Test. The acceptance level of significance was $P < 0.05$ using a two-tailed distribution.

Aluminum acetylacetonate was from Aldrich, Milwaukee, WI and all other chemicals were purchased from Sigma (St. Louis, MO).

Analysis of the fluorescent signal from the thiolavin-T probe, showed that a significant proportion of the βA (25–35) peptide in phosphate buffer was present in the β-conformation. This was in marked contrast to the corresponding peptide with the reverse amino acid sequence (βA 35–25) where no fluorescence was detectable (Table 1). In the presence of 20 μM Al$_2$(SO$_4$)$_3$, aggregation of βA (25–35) was greatly enhanced but the reverse peptide was unresponsive to aluminum.

The aluminum-effected increase in β-folding of β-A (25–35) was concentration-dependent and could be detected at levels of Al$_2$(SO$_4$)$_3$ below 1 μM (Fig. 1). There were reproducible anomalies in the dose-response curve, suggesting the formation of aluminum phosphate complexes specifically potent in enabling pleating. The more lipophilic acetylacetonate salt of aluminum was also able to induce fluorescence of the βA-thiolavin-T mixture (Fig. 1) and expressed a more linear dose-response characteristic.

After incubation of βA (25–35) in Tris–HCl buffer at the same pH (7.4), basal thiolavin-T fluorescence was much more pronounced than when phosphate buffer was used (Fig. 2). However, in this case the addition of 20 μM aluminum sulfate to the peptide solution did not alter the extent of fluorescence (Fig. 2). In phosphate buffer, the aggregation of βA was greatly enhanced following the addition of 20 μM adenosine triphosphate (ATP) and a lesser stimulation occurred when Tris buffer was used (Fig. 2). While 20 μM Al inhibited the aggregation-inducing effect of ATP in phosphate buffer, it markedly potentiated this effect in the presence of Tris buffer.

In the absence of the thiolavin-T probe, no fluorescence was detectable in any peptide samples, and when thiolavin was incubated with Al$_2$(SO$_4$)$_3$ alone, there was also no perceptible fluorescence. Two other means of evaluating the formation of β-pleating and subsequent reduction in protein solubility were tested, namely the quantitation of turbidity and of light scattering of preparations. However, these were found to be less sensitive and less reproducible (data not shown).

Several different procedures have been used to study conformational changes in proteins and their consequent aggregation, and this can complicate comparison of results. For example, using either a turbidometric assay [9] or Congo Red [32], a lower pH has been found to promote aggregation of β-amyloid and other peptides, while the opposite has been reported using CD [28] or after centrifugal precipitation of aggregates [18]. We have chosen to perform the current study at neutral pH on the grounds that this is most physiologically relevant, and that aggregates formed at lower pH are not capable of seeding fibril growth [32]. The thiolavin-T assay was found to be more reproducible than other assays of physical properties of aggregates such as increased light scattering or turbidity. This may have been in part due to the colloidal properties of aluminum salts. The thiazole dye, thiolavin-T, when selectively excited to yield a fluorescent signal [15]. The increase in thiolavin-T fluorescence may precede the formation of precipitable complexes [31] and is more sensitive than the use of Congo Red [16].

Since the relation of aluminum to neurodegenerative disease is unresolved, the possibility of aluminum-induced modulation of protein tertiary configuration has been considered previously. Aluminum salts are able to associate with high affinity to amyloid peptides [30] and have been reported to promote aggregation of β-amyloid [5,8,18], Tau [17,24] and a variety of other proteins including neurofibrillary proteins [25,26]. However, there are also reports that aluminum salts are able to reverse the formation of amyloid fibrils [3,20].

Fig. 1. Aggregation of βA (25–35) peptide in the presence of varying concentrations of aluminum sulfate or aluminum acetylacetonate. Incubation was at 21°C for 1 h, in 100 mM phosphate buffer, pH 7.4. Data represent the mean ± SE of three to four individual determinations.
In most of the studies cited above, the concentrations of aluminum were between 0.4 and 35 mM and thus unlikely to reflect cerebral aluminum content which has been reported to be around 30–150 μM [2,11]. Aluminum nitrate (10 μM) was found to promote aggregation of β-amyloid 25–35 peptide [6] and our results are in strong agreement with this.

It has been proposed that the acceleration of amyloid aggregation occurring in the presence of negatively charged liposomes may relate to the attraction of cationic peptides to the negatively charged surfaces of such vesicles [4,29]. Since aluminum at neutral pH exists largely as a highly hydrated polynuclear negatively charged aluminate, it may attract peptides in a similar manner.

The effects of aluminum and ATP upon β-pleating were synergistic in Tris–HCl but not in phosphate buffer. This may be because the prior sequestration of aluminum by high levels of phosphate prevented any interaction between aluminum and ATP. However, in the absence of ATP, aluminum promoted peptide aggregation in phosphate but not in Tris buffer. Thus an interaction between aluminum and either monophosphate or ATP seems essential to enable enhanced β-pleating of amyloid peptide. The metal-binding ability of tri-phosphates is much more powerful than that of the monophosphate moiety owing to the formation of six membered chelates, and these are strongly cationic at neutral pH [14]. ATP is able to trigger β-folding of a variety of proteins [1,33]. The strong synergistic interaction of triphosphates with aluminum can lead to the formation of further anionic complexes [14]. The colloidal nature and negative charge of such complexes may attract cationic peptides and facilitate their transition to a higher entropic state, as implied by destruction of the α-helix. Using PIPES, a nitrogenous buffer, aluminum was found to potentiate ATP-promoted aggregation of the 25–35 amyloid peptide [7]. In the present study, using a similar buffer (Tris), we were able to corroborate this effect.

Aluminum acetylacetonate promoted β-pleating with a more linear dose-response profile than Al₂(SO₄)₃. This may be related to the finding that the organic aluminum salt is more lipophilic (having a chloroform/water partition coefficient of 72 ± 5%), and as such is less likely to form ratiometrically critical aluminum phosphate complexes. Al acetylacetonate may create a more hydrophobic environment where AB aggregation can be enhanced [27]. Aluminum acetylacetonate is an analog of neutral aluminum tris-chelate complex which can be formed in the gastrointestinal tract with isomaltol [22]. Thus the acetylacetonate may also be relevant to the study of aluminum in biological systems.

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