Amyloid hypothesis through the lens of Aβ supersaturation

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Deposition of aggregated amyloid-β (Aβ) protein in the form of amyloid plaques is a pathological hallmark of Alzheimer's disease. According to the amyloid hypothesis, Aβ aggregation initiates a pathogenic cascade, eventually leading to dementia. Being the prevailing theory for Alzheimer's disease, amyloid hypothesis has been used to guide basic research and therapeutic interventions. Supersaturation is a phenomenon that occurs when the concentration of a solute in the solution exceeds its thermodynamic solubility. In the brain, Aβ proteins are usually supersaturated. Aβ aggregation follows the principle of supersaturation (So et al., 2016). In this perspective, I discuss the biochemical implications of Aβ supersaturation in the framework of amyloid hypothesis and how this knowledge can be used to improve therapeutic development for Alzheimer's disease.

Main concepts of protein supersaturation in the context of Aβ aggregation: The framework of supersaturation has been well described in the studies of protein crystallization, which, like protein aggregation, exhibits nucleation-dependent polymerization kinetics (Coquerel, 2014). Through a series of excellent research work, Goto and colleagues (So et al., 2016) have demonstrated that the principle of supersaturation also applies to protein aggregation. **Figure 1A** depicts a protein aggregation phase diagram for Aβ. The solubility curve of Aβ divides the undersaturated region and supersaturated region. The area of supersaturation consists of a metastable zone and a nucleation zone. The boundary between metastable zone and nucleation zone corresponds to the "critical concentration" mentioned in previous studies (Hellstrand et al., 2010). In the metastable zone, Aβ does not spontaneously nucleate and aggregate. However, pre-formed Aβ fibrils can seed the aggregation in the metastable zone. In the nucleation zone, Aβ aggregation is spontaneous with its rate dependent on Aβ concentration.

Unseeded *in vitro* Aβ aggregation starts from a supersaturated solution in the nucleation zone. Once aggregation starts, it cannot be stopped before the Aβ concentration reaches its solubility limit. Therefore, supersaturated Aβ and saturated Aβ represent the beginning and end points of the sigmoidal aggregation curve (**Figure 1B**). This point is illustrated by an elegant study of Linse and colleagues (Hellstrand et al., 2010), which shows that spontaneous Aβ aggregation takes place only when the Aβ concentration is increased to approximately 200 nM. The soluble Aβ concentration at aggregation completion, which represents the solubility of Aβ, approaches around 15 nM. Therefore, under the experimental conditions of Hellstrand et al. (2010), the metastable zone of Aβ has a concentration range of 15–200 nM.

How is supersaturation achieved? *In vivo*, supersaturation can be achieved because proteins are produced one molecule at a time, allowing the protein concentration to exceed solubility limit without aggregation. In contrast, *in vitro* supersaturation is typically achieved by manipulating experimental conditions such as lowering the temperature of a saturated solution or adding additives that changes the solubility of the protein. A computational analysis of protein solubility and aggregation propensity in the whole proteome found that a substantial fraction of the proteome is supersaturated, particularly for the proteins involved in neurodegeneration-related biochemical pathways (Ciryam et al., 2013).

The presence of amyloids leads to two fundamental changes in the behavior of Aβ: First, the dominant process of Aβ aggregation changes from spontaneous to seeded aggregation (Cohen et al., 2013). Second, Aβ can no longer maintain supersaturation. Therefore, amyloid deposition has two consequences: (1) Aβ aggregation becomes a cumulative problem because existing amyloids catalyze the aggregation of newly produced Aβ on a daily basis; and (2) Aβ concentration is stuck at its solubility limit because a higher Aβ concentration than the solubility limit leads to aggregation and undersaturation by Aβ clearance leads to solubilization of Aβ from amyloid plaques. In plaque-free mice, acute inhibition of γ-secretase activity led to rapid decline of $Aβ_{42}$ concentration. In contrast, plaque-rich mice showed significantly less concentration reduction, supporting the role of amyloid plaques as a reservoir of soluble Aβ (Hong et al., 2011). After injecting isotopically-labeled Aβ into the interstitial fluid, the same study (Hong et al., 2011) found that the recovered Aβ from plaque-rich mice is only 45% of that from plaque-free mice, supporting the notion that most of the newly produced Aβ deposits to amyloid plaques. Furthermore, plaques not only permanently reduce $Aβ_{42}$ concentrations by several folds, but they also reduce the fluctuations in Aβ concentrations as a part of the circadian rhythm. Bateman and colleagues (Huang et al., 2012) studied the circadian dynamics of Aβ concentration and found that the circadian amplitude in amyloid-negative group is 15.6 pM, almost 3-fold of the circadian amplitude in amyloid-positive group (6.3 pM). Both the lowered $Aβ_{42}$ concentration and diminished circadian rhythm over a long period of time (years to decades) may be pathogenic and contribute to cognitive decline and dementia. The reduced $Aβ_{42}$ concentration and circadian fluctuation amplitude may underlie the sleep disturbances observed in amyloid-positive individuals (Wang and Holtzman, 2020).

What does supersaturation tell us about Aβ dynamics in the brain? Based on the framework of Aβ supersaturation, the time course of Aβ concentration over an amyloid-positive person's adult lifetime can be divided into four phases: soluble phase, burst phase, reduction phase, and stationary phase (**Figure 2**). The reduction phase is when spontaneous Aβ aggregation starts. The stationary phase is when the Aβ concentration reaches a steady-state. Mild cognitive impairment and dementia appear years or decades into the stationary phase (Hadjichrysanthou et al., 2020). In an amyloid-negative person, Aβ concentration increases modestly but always remains in the metastable zone. Studies have been performed to compare the amyloid-positive group and amyloid-negative group in the stationary phase, and show that the Aβ concentration in the amyloid-positive group is markedly lower than that in the amyloid-negative group (Huang et al., 2012; Palmqvist et al., 2014). The burst phase is a prediction based on the framework of supersaturation because spontaneous Aβ aggregation requires Aβ concentration to be in the nucleation zone. This can be achieved by an accelerated increase in Aβ concentration, a "burst," so that Aβ concentration crosses the boundary of the nucleation zone (**Figure 1A**, moving up along the *Y*-axis). This can also be achieved by lowering the boundary of the nucleation zone because *in vivo* environments can modulate the boundaries between the two zones of the supersaturation and even the solubility limit of Aβ (**Figure 1A**, moving right along the *X*-axis).

What are the implications on therapeutic interventions? Anti-amyloid therapy is the most advanced therapeutic development for Alzheimer's disease. The majority of the anti-amyloid drug candidates are antibodies targeting Aβ amyloid. Aducanumab from Biogen/Eisai (Sevigny et al., 2016), a monoclonal antibody targeting Aβ fibrils, showed highly promising results in its phase 3 clinical trials as a disease-modifying treatment. In addition to the potential toxicity directly from amyloid plaques themselves, the presence of plaques can seed the aggregation of newly produced Aβ on a daily basis, which may also generate toxic species. Therefore, even though plaque load reduction can reduce the direct toxic effect of plaques, the complete elimination of Aβ aggregation can only be achieved after the vast majority of plaques have been cleared and Aβ concentration is restored to a supersaturated state.

Modulation of monomeric Aβ concentration: Lowering Aβ concentration has long been considered a therapeutic strategy. This can be achieved using inhibitors or modulators of BACE1 and γ-secretase. Alternatively, antibodies that bind soluble Aβ can also be used to lower Aβ levels. Recent development in this area has been reviewed elsewhere (Long and Holtzman, 2019). As a standalone strategy, this approach is likely most effective at the burst phase (**Figure 2**), when an increase in Aβ concentration poses the greatest risk of initiating amyloid formation. Once amyloid is formed, the mechanism of aggregation shifts from spontaneous aggregation to seeded aggregation, and Aβ concentration plays a lesser role.

Aggregation inhibitors: Due to difference between spontaneous and seeded aggregation, two types of aggregation inhibitors may be needed. One type works best to inhibit spontaneous aggregation, and another type for seeded aggregation. Spontaneous aggregation inhibitors are most important in the burst phase before a significant amount of amyloids have built up. Once the seeded aggregation becomes the dominant mechanism, inhibitors that are specifically screened for this purpose

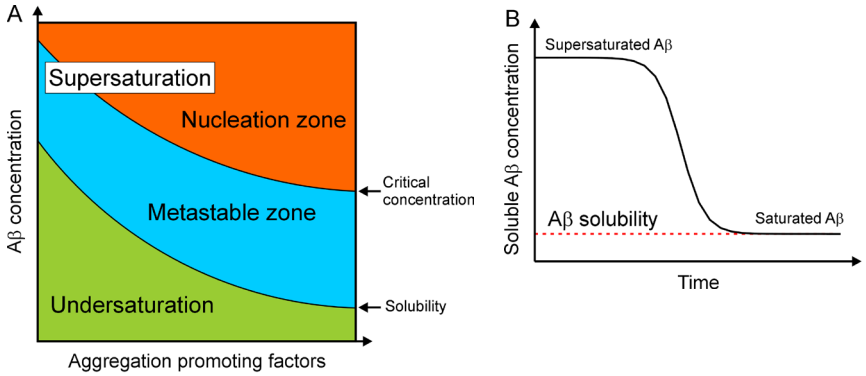

Figure 1 | **Aβ supersaturation and aggregation.**
(A) A phase diagram of Aβ supersaturation. Both Aβ concentration and environmental factors affect the phase diagram. The area of supersaturation consists of a metastable zone and a nucleation zone. In the metastable zone, Aβ does not spontaneously aggregate but can aggregate in the presence of aggregate seeds. In the nucleation zone, Aβ can aggregate spontaneously with its rates correlated with Aβ concentrations. (B) Changes in soluble Aβ concentration as a result of aggregation. Aggregation starts from supersaturated Aβ and ends with the concentration at solubility limit that is determined by solution conditions and environmental factors. Aβ: Amyloid-β protein.

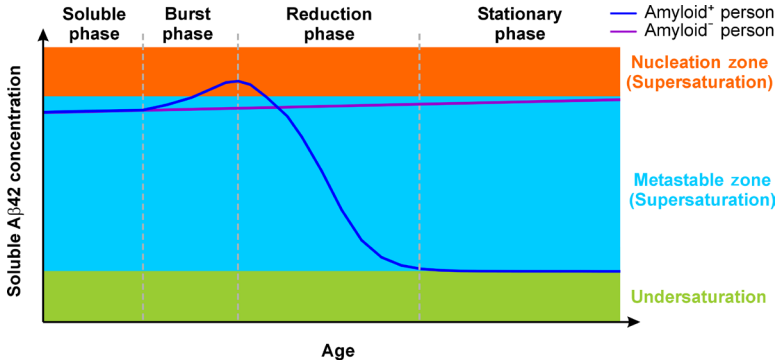

Figure 2 | **Changes of Aβ concentration over a person's adult lifetime in the framework of supersaturation.**
Two hypothetical persons are considered here: one eventually becomes amyloid-positive (blue line) and the other one remains amyloid-negative (purple line). The Aβ concentration in the amyloid-negative person has a slow linear increase but never goes into the nucleation zone. For the amyloid-positive person, Aβ concentration can be divided into four phases. In soluble phase, Aβ concentrations are in the metastable zone and there are no differences between the amyloid-positive and amyloid-negative persons. In the burst phase, there is an acceleration of Aβ concentration increase. As a result, the Aβ concentration crosses into nucleation zone, which leads to spontaneous aggregation. In the reduction phase, Aβ gradually loses ability to maintain supersaturation as amyloid plaques spread through the extracellular space in the brain. In the stationary phase, Aβ concentration reaches its solubility limit in the extracellular space of the brain, and it will remain largely unchanged for the remainder of this person's life time. Cognitive impairment and dementia take place in the stationary phase. Aβ: Amyloid-β protein.

should be used.

Toxicity blockers: Proteins or small molecules that can bind to toxic species directly can serve as toxicity blockers. This class of therapeutic molecules would be effective throughout the course of Alzheimer's disease. It may be particularly helpful in combination with anti-amyloid therapy, which by itself does not eliminate the toxicity of soluble Aβ. However, this type of potential drugs are also the most elusive due to a lack of understanding of both the mechanism of toxicity and the structures of the toxic Aβ species.

Personalized Aβ biomarkers: Measurements of $A\beta_{42}$ in human cerebrospinal fluid show a wide range of concentrations. Although the amyloid-positive and amyloid-negative groups can be distinguished using a cutoff of $A\beta_{42}$ concentration, a large number of individuals, for example, 8% of cases in Palmqvist et al. (2014), do not show agreement between $A\beta_{42}$ concentration and amyloid imaging. This is likely due to large inter-individual differences in $A\beta_{42}$ concentrations. One solution to this problem is to establish Aβ concentration as a personalized biomarker. Then changes in Aβ concentration can be compared to the past levels of the same individual. It has been shown that the $A\beta_{42}$ concentrations in amyloid-positive and amyloid-negative cohorts differ by 2–3 fold (Huang et al., 2012; Palmqvist et al., 2014). A change of this magnitude would be readily detected using the same individual's history of Aβ concentration. The personal history of Aβ concentration will be particularly useful for detecting if Aβ supersaturation is restored after a therapeutic intervention that has removed the amyloid aggregates.

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