The molecular chaperone Brichos breaks the catalytic cycle that generates toxic Aβ oligomers

SUPPLEMENTARY NOTE

Kinetic rate laws

Following our previous analysis[19,26], the generation of fibril mass, M, when both primary and secondary nucleation events occur is described by the integrated rate law:

$$\frac{M(t)}{M(\infty)} = 1 - \left(\frac{B_+ + C_+}{B_+ + C_+ e^{\kappa t}} \frac{B_- + C_+ e^{\kappa t}}{B_- + C_+} \right)^{\frac{{k_\infty}^2}{\kappa \tilde{k}_\infty}} e^{-k_\infty t} \qquad (1)$$

where two particular combinations of the rate constants for primary nucleation (k_n), elongation (k_+), and fibril-catalysed secondary nucleation (k_2) define much of the macroscopic behaviour; these parameters are related to the rate of formation of new aggregates through primary pathways $\lambda = \sqrt{2k_+k_n m(0)^{n_c}}$ and through secondary pathways $\kappa = \sqrt{2k_+k_2 m(0)^{n_2+1}}$. Indeed, Eq. 1 depends on the rate constants through these two parameters, λ and κ, alone since $B_\pm = (k_\infty \pm \tilde{k}_\infty)/(2\kappa)$, $C_\pm = \pm\lambda^2/(2\kappa^2)$, $k_\infty = \sqrt{2\kappa^2/[n_2(n_2+1)] + 2\lambda^2/n_c}$ and $\tilde{k}_\infty = \sqrt{{k_\infty}^2 - 4C_+C_-\kappa^2}$. The initial concentration of soluble monomers is denoted *m(0)* and the exponents describing the dependencies of the primary and secondary pathways on the monomer concentration are given as n_c and n_2 respectively.

The effect of an inhibitor can in the first instance be semi-empirically captured by altering the microscopic rate constants for primary nucleation (k_n), elongation (k_+) and secondary

nucleation (k_2) in the integrated rate law in Eq. 1. Perturbing the different microscopic events results in characteristic changes in the shape of the macroscopic reaction profile that can be used to identify the mechanism of action of an inhibitor (Fig. 1a-c). This approach is exact for complete inhibition of a particular process, as shown for secondary nucleation in Fig. 1c (green dashed line). Using this approach (Fig. 1a-c and Supplementary Fig. 2), we were able to identify a Brichos domain which affects specifically the secondary nucleation rate.

In order to further quantify the inhibitory effect of the Brichos domain at chaperone concentrations where inhibition of secondary nucleation is not complete, we then used a first-principles approach by introducing into the reaction scheme the reversible binding of the chaperone along the fibril surface. Such binding decreases the surface of the fibrils available to catalyse the oligomer formation, therefore reducing the secondary nucleation rate. The number of bound monomeric units in the fibrils, M_{bound}, was evaluated by considering a Langmuir-type adsorption of the chaperone along the fibril surface:

$$\frac{\partial M_{\mathrm{bound}}}{\partial t} = k_{\mathrm{onSurf}} C_C(t)\big(M(t) - M_{\mathrm{bound}}(t)\big) - k_{\mathrm{offSurf}} M_{\mathrm{bound}}(t) \qquad (2)$$

where C_c(t) is the concentration of soluble chaperone and k_{onSurf} and k_{offSurf} are the binding and unbinding rate constants, respectively, which have been estimated independently by surface plasmon resonance (Fig. 3b). Since the characteristic time of binding is comparable to the characteristic time of incorporation of a monomeric unit into the fibril by elongation, the binding and unbinding reactions cannot be considered at equilibrium with respect to the microscopic aggregation rates. In order to predict the kinetic profiles at intermediate chaperone concentrations (thin dotted lines, Fig. 1c), Eq. 2 has been solved together with the kinetic equations[26] for the total fibrillar number, P, and mass, M, concentrations, where the

secondary nucleation process is proportional to the area of the fibril surface that does not have chaperone bound to it:

$$\frac{\partial P(t)}{\partial t} = k_n m(t)^{n_c} + k_2\big(M(t) - M_{\text{bound}}(t)\big)m(t)^{n_2} \quad (3)$$

$$\frac{\partial M(t)}{\partial t} = n_c k_n m(t)^{n_c} + n_2 k_2\big(M(t) - M_{\text{bound}}(t)\big)m(t)^{n_2} + 2k_+ m(t)P(t) \quad (4)$$

The set of Eqs. 2-4 has been integrated numerically using the microscopic reaction constants evaluated in our previous kinetic study[19] and the binding and unbinding reaction rates determined by SPR, with the latter allowed to vary in a narrow range to give the best fit to the data ($k_{\text{onSurf}} = 1 \times 10^4$ M^{-1} s^{-1} and $k_{\text{offSurf}} = 1 \times 10^{-4}$ s^{-1}). Remarkably, the integrated global kinetic profiles predict essentially perfectly the inhibitory effect at different concentrations of chaperone, as shown in Fig. 1c.

In addition to affecting the reaction profile, perturbing the different microscopic reaction rates results in dramatically different effects on the generation of low molecular weight oligomeric species. To illustrate this behaviour, we calculated the time evolution of the rate of generation of new fibrils (via on-pathway oligomers) from monomers, with this nucleation rate r_n(t) given by $r_n(t) = k_n m(t)^{n_c} + k_2 M(t) m(t)^{n_2}$. Moreover, since at the end of the reaction no oligomers are detectable in the system[19], the total number of fibrils present at the end of the process is indicative of the total number of on-pathway oligomers generated during the reaction. This value is calculated by integrating the nucleation rate r_n(t) over the reaction.

The kinetic analysis predicts that the inhibitory effect of Brichos reaches a saturation at a critical chaperone concentration corresponding to the complete saturation of the surface of

the fibrils. According to this prediction, an increase of the Brichos concentration above this critical threshold value does not have any consequences on the aggregation kinetics. We verified this hypothesis by performing kinetic experiments in the presence of increasing concentrations of Brichos (Supplementary Fig. 1).

Electrophysiology

Experiments were carried out in accordance with ethical permit granted by Norra Stockholms Djurförsöksetiska Nämnd to A.F. (N45/13). C57BL/6 mice of either sex (postnatal days 14-23, supplied from Charles River, Germany) were used in all experiments. The animals were deeply anaesthetized using isofluorane before being sacrificed by decapitation. The brain was dissected out and placed in ice-cold ACSF (artificial cerebrospinal fluid) modified for dissection. This solution contained (in mM); 80 NaCl, 24 $NaHCO_3$, 25 Glucose, 1.25 NaH_2PO_4, 1 Ascorbic acid, 3 Na Pyruvate, 2.5 KCl, 4 $MgCl_2$, 0.5 $CaCl_2$, 75 Sucrose. Horizontal sections (350 µm thick) of the ventral hippocampi of both hemispheres were prepared with a Leica VT1200S vibratome (Microsystems, Stockholm, Sweden). Immediately after slicing sections were transferred to a submerged incubation chamber containing standard ACSF (in mM): 124 NaCl, 30 $NaHCO_3$, 10 Glucose, 1.25 NaH_2PO_4, 3.5 KCl, 1.5 $MgCl_2$, 1.5 $CaCl_2$. The chamber was held at 34ºC for at least 20 minutes after dissection. It was subsequently allowed to cool to ambient room temperature (19-22ºC) for a minimum of 40 minutes. Aβ42 monomer was at 5-10 µM isolated by gel filtration in 20 mM sodium phosphate buffer pH 8.0 followed by filtration through a 200 nm sterile filter and stored on ice until use. Fibrils were prepared by incubation of a 5 µM Aβ42 solution in a non-binding plate at 37ºC for 1 h under quiescent condition. Peptides (final concentration 50 nM monomer or 50 nM monomer plus 0.3 nM fibrils) were added to the incubation solution 15

minutes before transferring slices to the interface-style recording chamber. While incubating slices were continuously supplied with carbogen gas (5% CO_2, 95% O_2) bubbled into the ACSF. Recordings were carried out in hippocampal area CA3 with borosilicate glass microelectrodes, pulled to a resistance of 3-7 MΩ. Local field potentials (LFP) were recorded at 34ºC using microelectrodes filled with ACSF placed in stratum pyramidale. LFP oscillations were elicited by applying kainic acid (100 nM) to the extracellular bath. The oscillations were allowed to stabilize for 20 minutes before any recordings were carried out. LFP recordings were performed with a 4 channel amplifier / signal conditioner M102-amplifier (Electronics Lab, Faculty of Mathematics and Natural Sciences, University of Cologne, Cologne, Germany). The signals were sampled at 10 kHz, conditioned using a Hum Bug 50 Hz noise eliminator (Quest Scientific, North Vancouver, BC, Canada), software low-pass filtered at 1 kHz, digitized and stored using a Digidata 1322A and Clampex 9.6 software (Molecular Devices, CA, USA). Power spectral density plots (from 60 s long LFP recordings) were calculated in averaged Fourier-segments of 8192 points using Axograph X (Kagi, Berkeley, CA, USA). Oscillation power was calculated by integrating the power spectral density between 20 and 80 Hz. Data in the text is reported as means ± standard errors of the means. For statistical analysis the Mann-Whitney U-test was used.

LFP recordings in area CA3 revealed control gamma oscillations of $5.58 \times 10^{-9} \pm 3.98 \times 10^{-10}$ V^2 power (n=16; Fig. 5). When slices where incubated for 15 min with 50 nM monomeric Aβ42 prior to kainate superfusion the resultant gamma oscillation power was modestly but significantly decreased ($3.71 \times 10^{-9} \pm 5.59 \times 10^{-10}$ V^2; n=14; U=167.0, n_1=16, n_2=14, p=0.022 two-tailed, Fig. 5). When adding a seeding solution of 0.3 nM fibrillar Aβ42 to the 15 min incubation with 50 nM monomeric Aβ the subsequent kainate-induced gamma oscillations were severely decreased in power ($0.66 \times 10^{-9} \pm 1.52 \times 10^{-10}$ V^2; n=8; U=128.0,

n_1=16, n_2=8, p<0.0001 two-tailed, Fig. 5), supporting the notion that seeding with minute concentrations of fibrillar Aβ42 greatly increases the cytotoxicity of monomeric Aβ42. The seeding solution of 0.3 nM fibrillar Aβ was tested in the absence of monomeric Aβ42 and under these conditions did not result in a significant change to gamma oscillation power ($4.83 \times 10^{-9} \pm 2.36 \times 10^{-10}$ V^2; n=8; U=80.5, n_1=16, n_2=8, p=0.32 two-tailed, Fig. 5). In order to test the ability of Brichos to protect against both the moderate cytotoxic effect of monomeric Aβ42 as well as the severe cytotoxic effect of seeded monomeric Aβ42 we repeated both experiments with 1 µM Brichos present in the 15 min incubation with 50 nM monomeric Aβ42. The Brichos concentration was chosen at 25 KD to ensure near saturation (96%) of the Brichos-binding sites on the fibrils. Under these conditions neither monomeric Aβ42 ($5.06 \times 10^{-9} \pm 3.23 \times 10^{-10}$ V^2; n=15; U=144.5, n_1=16, n_2=15, p=0.34 two-tailed, Fig. 5) nor monomeric Aβ seeded with 0.3 nM fibrillar Aβ42 significantly altered the power of kainate-induced gamma oscillations ($6.06 \times 10^{-9} \pm 7.99 \times 10^{-10}$ V^2; n=8; U=87.5, n_1=16, n_2=8, p=0.15 two-tailed, Fig. 5). The Brichos solution of 1 µM was tested in the absence of monomeric Aβ42 and under these conditions did not result in a significant change to gamma oscillation power ($5.22 \times 10^{-9} \pm 1.07 \times 10^{-9}$ V^2; n=8; U=77.0, n_1=16, n_2=8, p=0.45 two-tailed, Fig. 5).

Cell viability and cytotoxicity

Assays were performed on SHSY-5Y human neuroblastoma cells cultured under standard conditions at 37ºC in a humidified incubator with 5% CO2. Cells were seeded at a density of 25,000 per well in a white walled, clear bottomed 96 well plate and cultured for 24 hours in DMEM/10% FBS. The culture media was then replaced with pre-warmed phenol red free DMEM without serum into which the peptide samples or NaH_2PO_4 buffer were diluted 1:4.

Peptide monomer was isolated by gel filtration in 20 mM sodium phosphate buffer at pH 8.0 followed by filtration through a 200 nm sterile filter and stored on ice until added to the cells. Seeds were prepared by placing a monomer solution in a 96-well non-binding plate (Corning 3881) at 37ºC for 1 h. Completion of fibril formation was confirmed by ThT fluorescence on a withdrawn aliquot. Seeds were then diluted 1:100 into fresh monomer. The experiments for monomer and monomer plus seeds were repeated in the presence of Brichos. Buffer and Brichos were sterile filtrated (200 nm). The cells were cultured in the presence of the peptides or buffer (five-fold diluted in medium) for a further 24 hours before the cytotoxicity and viability assays were performed. Caspase-3/7 activity was measured using the Apo-ONE Homogeneous Caspase-3/7 assay (Promega, Southampton, UK). The fluorogenic caspase-3/7 substrate was diluted 1:100 in the lysis buffer provided and added to the cell medium at a 1:1 ratio. The reagent/cell mix was then incubated for 1 hour before measuring the fluorescence at ex. 480 nm/em. 520 nm in an Optima FluoStar plate reader. Cell viability was measured using the Cell Titer 96 Aqueous One MTS reagent from Promega. The MTS reagent was added to the cell culture medium and incubated with the cells at 37ºC in a humidified incubator with 5% CO_2 before the absorbance at 495 nm was measured in an Optima Fluostar plate reader. All values given for both assays are normalized relative to the buffer treated cells.

Cell viability as measured by the MTS assay is drastically reduced in the presence of 1 μM Aβ42 without or with 10 nM seeds (Supplementary Fig. 4a), while the addition of 1 μM Brichos leads to essentially full recovery of cell viability. An equivalent trend is observed for the caspase 3/7 assay, although the analysis is less straightforward since the assay suffers from the limitation that the single colorimetric readout represents the global effect of many possible cellular processes. An increase of caspase signal is commonly associated with an

increase of apoptosis activity of the cells, although caspase activation has been observed in the absence of apoptosis[63]. Critically, a rapid decrease of cell viability (as observed on addition of Aβ42) results in a decrease in the number of cells able to generate the caspase signal, therefore potentially inducing a decrease of caspase fluorescence rather than an increase. As a consequence, the result of the assay is significantly affected by the time of the readout with respect to the characteristic time of cell death. To account for this behaviour, we used the same assay for two separate clones of the SHSY-5Y cells with widely different sensitivity to perturbations of the growing medium. In Supplementary Fig. 4b we show one example of the caspase activation assay for the more sensitive cells (the same cells used for the MTS assay in Supplementary Fig. 4a) with 1 μM Aβ42 without or with 10 nM seeds. In this case, the caspase activity decreases on addition of Aβ42 relative to untreated cells, likely reflecting that cells die quickly, therefore reducing the number of cells able to generate signal after 24 hours. The effect of Aβ42 on the caspase signal is, however, abolished by 1 μM Brichos. In Supplementary Fig. 4c we show an alternative example of the caspase activation assay for the less sensitive cells with 10 μM Aβ42 without or with 100 nM seeds. In this case the caspase activity is increased by Aβ42 compared to untreated cells, indicating that the cell viability is still high but several cells have initiated the apoptosis processes. Again, the effect of Aβ42 on the caspase signal is abolished in the presence of Brichos. Importantly, despite the two cell lines having different sensitivities to toxic species and exhibiting different relative changes in caspase activity with respect to the control, in both cases the results clearly show that the toxic effects of Aβ42 aggregation are removed by the presence of Brichos. The results of the viability and cytotoxicity assays (Supplementary Fig. 4) show that the addition of Brichos decreases the toxicity of reactions with or without pre-formed fibrils. These results are in excellent agreement with the electrophysiology data shown in Fig. 5.