EXPRESSION IN DROSOPHILA OF TANDEM Aβ PEPTIDES PROVIDES INSIGHTS INTO THE LINK BETWEEN AGGREGATION AND NEUROTOXICITY

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Running Title: Aggregation and Neurotoxicity of Tandem Aβ in Drosophila

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Background: Investigating the kinetics of Aβ peptide aggregation in vivo is vital to understanding Alzheimer’s Disease.

Results: Linking two Aβ40 or Aβ42 peptides together increases their aggregation rates in Drosophila, but only increases the neurotoxicity of Aβ42.

Conclusions: Increasing the rate of aggregation of Aβ increases amyloid deposition but not necessarily toxicity.

Significance: The toxicity of Aβ depends on the mechanism and not just the rate of aggregation.

SUMMARY

The generation and subsequent aggregation of Amyloid β (Aβ) peptides plays a crucial initiating role in the pathogenesis of Alzheimer’s disease (AD). The two main isoforms of these peptides have 40 (Aβ40) or 42 residues (Aβ42), the latter having a higher propensity to aggregate in vitro and being the main component of the plaques observed in vivo in AD patients. We have designed a series of tandem dimeric constructs of these Aβ peptides to probe the manner in which changes in the aggregation kinetics of Aβ affect its deposition and toxicity in a Drosophila melanogaster model system. The levels of insoluble aggregates were found to be substantially elevated in flies expressing the tandem constructs of both Aβ40 and Aβ42 as compared to the equivalent monomeric peptides, consistent with the higher effective concentration, and hence increased...
aggregation rate, of the peptides in the tandem repeat. A unique feature of the Aβ42 constructs, however, is the appearance of high levels of soluble oligomeric aggregates and a corresponding dramatic increase in their in vivo toxicity. The toxic nature of the Aβ42 peptide in vivo can therefore be attributed to the higher kinetic stability of the oligomeric intermediate states that it populates relative to those of Aβ40 rather than simply to its higher rate of aggregation.

The misfolding and subsequent aberrant aggregation of proteins into a range of potentially toxic conformers underlies many age-related neurodegenerative diseases (1). Whilst a relatively small fraction of proteins is found to be associated with such diseases, the intrinsic ability of self assembly into stable and organised amyloid aggregates is a generic feature of all such molecules (2). Whilst organisms ranging from E. coli to humans can derive functional advantage from the amyloidogenic propensity of some proteins (3), amyloid formation is predominantly associated with cytotoxicity and disease (1). The kinetics and thermodynamics of protein aggregation and amyloid formation have been extensively studied in vitro (4,5), but less is understood about the critical steps, particularly those relating to the formation of toxic species, that govern the analogous processes in vivo. A detailed molecular description of these processes is essential if we are to intervene in a rational manner to prevent or treat the many diseases that are linked to protein misfolding and aggregation.

In this paper we describe an in vivo approach for investigating the aggregation behaviour of Aβ peptides, which in their fibrillar amyloid forms are the primary constituent of the senile plaques in patients with Alzheimer's disease (AD). Such amyloid deposits are present in the brains of many elderly people, whether or not they are suffering from dementia (6), and are composed of two predominant isoforms, Aβ40 and Aβ42. The longer, Aβ42, isoform has been found to aggregate more rapidly into fibrils and, despite constituting only a small fraction of the soluble forms of Aβ peptides in the brain, is the major species found in plaques (7-9). However, the aggregation reactions for the two Aβ isoforms in vitro have been shown to differ not only in their overall rates but also in the nature of the pre-fibrillar intermediates they generate (10-12).

We have shown previously that it is possible to investigate the significance of kinetic factors in fibril formation in vitro by conjugating multiple copies of a protein to one another in a head-to-tail fashion using flexible linkers that allow the individual peptides of the tandem repeats to interact in an optimal manner, whilst substantially increasing their effective local concentration (13,14). Here we use this strategy to design tandem repeats to probe the aggregation behaviour of the Aβ peptide in vivo and its links to neurotoxicity. We demonstrate that expression of these tandem Aβ peptides in the brains of Drosophila melanogaster provides insights into the mechanisms of Aβ aggregation in vivo, the extent to which such mechanisms differ for the Aβ40 and Aβ42 peptides, and how these differences relate to their relative neurotoxicities.

EXPERIMENTAL PROCEDURES
Generation of transgenic Drosophila

Tandem Aβ transgenes were synthesised (Eurofins MWG Operon, Munich, Germany) using insect optimised codons. Aβ peptides within tandem constructs are linked either directly, or by a 12 (GGGSGGGGGSGG) or a 22 (GGGSGGGGGGSGGSGGSGG) amino acid linker, as indicated in the text. These linker regions were chosen on the basis of previous work (13) and of Monte Carlo simulations designed to estimate the degree of conformational flexibility associated with the various linkers. Each construct was subcloned into the pUASTattB (Genbank accession number: EF362409) plasmid downstream of a secretory signal peptide derived from the Drosophila necrotic gene (28). Site specific transgenesis to the 51D locus (yM{int.Dm}ZH2Aw*;M{3xP3RFP.attP}ZH-51D) was achieved using the φC31 system (15). The transgenic lines were backcrossed for 6 generations into w1118 flies to obtain isogenic lines. To analyse the effect of Aβ expression, transgenic UAS-Aβ lines were subsequently crossed to GAL4-driver lines, allowing a tissue specific expression. Double transgenic flies were generated to express two identical transgenes (51D/51D) using the same driver. Importantly, a 51D line without insert but crossed to the GAL4-driver line was used as a negative control for all crosses to account for genetic effects and the possible consequences of GAL4 protein expression in specific tissues.
Biochemical analysis - SDS PAGE

Twenty fly heads were homogenised in 2% (w/v) SDS in water, sonicated for 480 s and centrifuged at 18,000 g for 20 min at 4°C. The supernatant was collected as the “SDS soluble fraction”. The remaining pellet (“the SDS insoluble fraction”) was washed in PBS, before being resuspended in 5 µL of a 80% (v/v) DMSO, 20% (v/v) water solution and incubated for 1 hr at room temperature before the addition of 15 µL of 50 mM Tris-HCl, pH 8.8. The samples were again sonicated for 480 s in a water bath, and a brief centrifugation was performed to eliminate any visible debris. The supernatant was collected as the “SDS insoluble fraction”. These fractions were then separated by SDS-PAGE and probed for ß-actin using a mouse monoclonal anti ß-actin antibody (6E10; Covance, USA) as described previously (17).

Biochemical analysis – filter retardation assay

Protein aggregates in brain extracts from flies reared at 25 °C and collected 24 hrs post-eclosion were detected using a filter retardation assay. Fifteen fly heads were homogenised in 60 µL of a 2% (w/v) SDS solution and sonicated for 8 min on ice. Samples were briefly centrifuged to pull down debris and total protein concentration was measured by BCA assay (Thermo Scientific Pierce, USA). Equal amounts of protein for each sample were then centrifuged at 18,000 g for 20 min at 4°C. The supernatant was removed and loaded on a SDS-PAGE gel, transferred on a membrane and probed with a ß-actin antibody. The pellet was washed with PBS and resuspended in 20 mM Tris-HCl, pH 8.0, 15 mM MgCl2, 0.5 mg/ml DNase I and incubated for 1 hr at 37°C. The samples were then loaded onto a nitrocellulose membrane (0.11 µm pore size) using a 96-well dot blotting apparatus (Bio- Rad Laboratories, Hemel Hempsted, UK). Once the samples had passed through the membrane under vacuum the membranes were removed from the apparatus and boiled for 5 min in PBS and incubated in blocking buffer containing 5% (w/v) milk in PBS with 0.05% (w/v) Triton. The immunodetection of ß-actin was then performed as described previously (17). The signal intensity of each spot was quantified by densitometry using ImageJ software (distributed by National Institutes of Health, USA) and normalised to the intensity of the corresponding ß-actin band from the immunoblotted SDS PAGE gel. Statistical comparisons between groups were made using ANOVA followed by Dunnett’s Multiple Comparison Test. All the statistical analysis was performed using GraphPad Prism (GraphPad Software, Inc.)

Longevity assays

Flies expressing ß variants were crossed with the elav-c155-GAL4 driver line. From the progeny, 100 mated female flies were collected on the day of eclosion and their longevity was analysed as described previously (29). Differences in survival were analysed using the Kaplan-Meier survival plots and log-rank analysis (GraphPad Prism, GraphPad Software Inc., USA). Statistical significance was set at p<0.05.

Immunohistochemistry

Fly brains were dissected from adult flies expressing tandem ß peptides under the control of GMR-GAL4 24 hrs post-eclosion and immunostained with the mouse anti-ß antibody 6E10 as described previously (17). Confocal scanning images were collected using identical acquisition parameters at intervals of 5µm using a Nikon Eclipse C1si on Nikon E90i upright stand (Nikon Corporation) with a 20x objective. Images were projected and processed using ImageJ software (distributed by National Institutes of Health, USA).

RESULTS

We designed tandem ß constructs in which two copies of the ß monomer were linked together by a 12 amino acid linker (T12ß; Fig. 1A, ii & vi). The length and glycine rich composition of this linker peptide were designed to provide sufficient flexibility to allow the individual ß peptides to adopt a wide range of conformations. This objective is achieved whilst still significantly reducing the entropic barrier associated with aggregate formation for the two ß sequences within the tandem construct as compared to two freely-diffusing monomeric ß molecules. Identical sets of tandem constructs were made for ß40 and ß42 peptides (Fig. 1A, T12ß40 and T12ß42).

To determine the effects on aggregation in vivo of linking pairs of ß peptides together, we generated transgenic Drosophila in which either the tandem ß40 or the tandem ß42 construct was inserted into an identical genomic locus; controls were also generated in which single copies of the monomeric ß or ß42 peptides were inserted into the same genomic locus. The use of a single defined genomic locus for insertion of all transgenes ensures that levels of transcription of each transgene are comparable.
by minimising variability in expression arising from the different genomic context of transgenes inserted in different locations. We tested the efficacy of this approach by comparing the transcript levels, measured by quantitative RT-PCR, for flies expressing either \( \alpha \beta _{40} \) or \( \alpha \beta _{42} \) inserted in the same genomic locus. There was no significant difference in their levels of expression. (Supplementary Fig. 1) (15,16).

Retinal expression of a single tandem \( \alpha \beta \) peptide transgene, or of two transgenes of the monomeric counterparts, was achieved using the GMR-Gal4 driver, and total head homogenates were prepared to analyse the extent of \( \alpha \beta \) aggregation in each case. \( \alpha \beta \) aggregates were quantified by a filter retardation assay. Both T\(_{12}\)A\(_{40}\) and T\(_{12}\)A\(_{42}\) formed significant quantities of large, insoluble deposits in the fly head (Fig. 1B) as measured by this assay. By contrast, non-transgenic flies (Fig. 1B, “51D”) did not contain detectable levels of such mature SDS insoluble aggregates, although a very low level of non specific binding of the antibody to the insoluble material from these flies was observed. Immunohistochemical staining of the fly brains confirmed the presence of discrete aggregates for both tandem peptides, although their appearances differed somewhat, being more clearly punctate in flies expressing T\(_{12}\)A\(_{42}\) as compared to T\(_{12}\)A\(_{40}\) (Fig. 1C).

Dissolution of SDS-insoluble material in 80% (v/v) DMSO followed by SDS-PAGE and western blotting confirmed that aggregation of the tandem peptides is increased very significantly in comparison to the corresponding monomeric peptides. Both T\(_{12}\)A\(_{40}\) and T\(_{12}\)A\(_{42}\) generated abundant SDS-insoluble aggregates (Fig. 1D, “T\(_{12}\)A\(_{40}\)” and “T\(_{12}\)A\(_{42}\)” while the negative control (i.e. Fig. 1D, “51D”) along with the corresponding monomeric \( \alpha \beta \) peptides, whether expressed from one (Fig. 1D, “\( \alpha \beta _{40}\)”, “\( \alpha \beta _{42}\)”) or two (Fig. 1D, “\( \alpha \beta _{40/40}\)”, “\( \alpha \beta _{42/42}\)” copies of the respective transgenes, failed to generate a signal on the western blot. Whilst the single dominant band on the western blot for both tandem species indicates that the SDS insoluble aggregates were largely dissociated to monomeric peptides by treatment with 80% (v/v) DMSO, interestingly T\(_{12}\)A\(_{42}\) was found to have retained some residual supramolecular structure.

Whilst joining two copies of the \( \alpha \beta \) peptide by a linker sequence increases the effective concentration of the \( \alpha \beta \) peptides, too short a linker may create steric barriers to the formation of optimally stable aggregates and, conversely, too long a linker will reduce the favourable entropic factors promoting faster aggregation (14). To investigate further the interplay between these different factors, we proceeded to generate tandem dimeric \( \alpha \beta \) peptides in which the linker between the two \( \alpha \beta \) sequences was either removed (T\(_{11}\)A\(_{40}\)), or extended to 22 amino acids (T\(_{22}\)A\(_{42}\)). When analysed by SDS-PAGE and western blotting we found that all the dimer constructs, when expressed using the GMR-Gal4 driver, generated abundant SDS insoluble aggregates (Fig. 2). This finding indicates that in all cases the tethering of two copies of \( \alpha \beta \) peptides increases the rate of formation of insoluble aggregates. Material from flies expressing T\(_{22}\)A\(_{42}\), was not completely disaggregated to monomeric peptides but generated a ladder of bands on the western blot; similar bands were not detectable for any tandem \( \alpha \beta _{40}\) constructs.

Aggregation-prone \( \alpha \beta \) peptides may cause developmental neurotoxicity in the Drosophila eye when their expression is driven by GMR-Gal4. We therefore compared the severity of the rough eye phenotype elicited by expression of the tandem \( \alpha \beta \) peptides with the results from the corresponding monomeric \( \alpha \beta \) peptides. The retinal expression of two copies of the monomeric \( \alpha \beta _{40}\) or \( \alpha \beta _{42}\) transgenes yields flies with eyes that are normal and comparable to non-transgenic controls (Fig. 3A, vii-ix). However T\(_{12}\)A\(_{42}\) expression in the retina causes severe eye malformation with loss of the ommatidial arrays and the appearance of melanised patches of necrotic tissue (Fig. 3A, v). This severe rough eye phenotype is also observed upon expression of T\(_{22}\)A\(_{42}\) (Fig. 3A, vi), but is notably less severe upon expression of T\(_{11}\)A\(_{42}\) (Fig. 3A, iv). By contrast, expression of tandem \( \alpha \beta _{40}\) in the retina does not result in a detectable rough eye phenotype regardless of linker length (Fig. 3A, i-iii) and despite the accumulation of insoluble material (Fig. 2).

A quantitative measure of the toxic effects of tandem \( \alpha \beta \) peptides in the adult CNS was provided by determining the longevity of flies expressing transgenes driven by the pan-neuronal elav-GAL4 construct. Concordant with the eye phenotypes described above, flies expressing a single copy of the T\(_{12}\)A\(_{42}\) construct (Fig. 4A red line) have a significantly shorter lifespan when compared to controls expressing two transgenes (Fig. 4A blue line) of the unlinked \( \alpha \beta _{42}\) (median survival 6 days vs. 19
days, n=100 for both genotypes, p<0.0001). Likewise T22Aβ42 expression in the brain causes a further reduction in longevity (Fig. 4A green line). Moreover, the longevity assay revealed significant neurotoxicity for the TnLAβ42 (Fig. 4A yellow line, median survival 23 days vs. 35 days for the unlinked Aβ42/Aβ42 control, n=100 for both genotypes, p<0.0001). Despite the deposition of insoluble aggregates, however, the expression of all the tandem Aβ42 constructs was compatible with a normal lifespan, and equivalent, within experimental error, to that of control flies (Fig. 4B). Thus, longevity assays show that while the existence of both 12 or 22 residue linker peptides accelerates the formation of insoluble aggregates for both Aβ42 and Aβ40, high levels of neurotoxicity are evident only for the Aβ42 peptides.

Whilst we have shown previously that the systematic introduction of single amino acid substitutions reveals correlations between the intrinsic aggregation rate and toxicity of the Aβ sequence, an increase in the overall aggregation propensity does not always result in a corresponding increase in in vivo neurotoxicity (17). Rather, such toxicity has been found to correlate more strongly with the formation of prefibrillar oligomeric aggregates leading to the conclusion, in accord with other studies, that it is these species that are likely to be particularly damaging to neuronal cells (18-22). To investigate the levels of soluble, oligomeric aggregates of Aβ we prepared extracts of SDS soluble proteins from fly heads and visualised the species present using SDS-PAGE and western blotting. When we expressed peptides in the neuronal tissue of the retina we observed abundant SDS-soluble oligomeric Aβ aggregates for tandem Aβ42 constructs containing linker peptides (Fig. 5A, “T22Aβ42” and “T12Aβ42”). Importantly, we observed a correlation between the abundance of such SDS soluble aggregates and the severity of the rough eye phenotypes when the constructs were expressed in the retina. Likewise quantification of these retinal SDS-soluble aggregates correlated with the reduction in median survival when the same constructs were expressed throughout the brain (Fig. 5B). No such SDS-soluble aggregates were detected in flies expressing any of the Aβ40 constructs (Fig. 5A “T22Aβ40” and “T12Aβ40”) and were likewise absent for both TnLAβ peptides (Fig. 5A, “TnLAβ40” and “TnLAβ42”). Quantification of the SDS-soluble Aβ aggregates demonstrated a remarkably close logarithmic correlation (R²=0.98) between the abundance of these oligomeric Aβ42 aggregates in the retinal tissue and the relative decrease in median survival of flies with these constructs expressed in the brain (Fig. 5B, data representative of three repeats). These data strongly suggest that the observed neurotoxic effect of Aβ in Drosophila is caused by soluble oligomeric species, rather than by insoluble aggregates.

**DISCUSSION**

In this study we have created tandem dimers of the most common forms of the Alzheimer’s disease related Aβ peptide, Aβ40 and Aβ42, in which two copies of the peptide are conjoined head-to-tail by a flexible linker of varying length, and have expressed them in neuronal tissues of Drosophila melanogaster. We find that the deposition of insoluble aggregates of these dimeric peptides in neuronal tissue is substantially increased when compared to equivalent concentrations of their monomeric counterparts. This finding is consistent with in vitro studies undertaken previously on two model protein aggregation systems, the SH3 domain of PI3 Kinase and an immunoglobulin domain of cardiac titin (13,14) in which such repeat sequences were found to aggregate much more rapidly than the monomeric species; such a result can be attributed to an increase of approximately an order of magnitude in the effective concentration that is engendered by the covalent linkage of two peptides (13). Strikingly, the propensity of Aβ42 dimers to populate soluble oligomeric species is significantly greater than that of Aβ40 dimers, indicating that their mechanisms of aggregation may differ significantly in vivo, or alternatively that the peptides have different intrinsic stabilities and thus are present in the cell at differing concentrations. Furthermore it is the appearance of these soluble oligomeric aggregates, rather than the level of insoluble aggregates, that is associated with high levels of neurotoxicity in our Drosophila model.

The detection of stable soluble oligomers of dimeric Aβ42 but not dimeric Aβ40 is consistent with recent observations from another in vitro study in which these two peptides, when constrained by an intramolecular disulfide bond (Aβ40cc peptides), were found to aggregate via different pathways resulting in very different propensities to populate stable oligomers and protofibrils (23). In this case Aβ42cc was observed to be more prone to form oligomers and larger protofibrils rich in β- sheet
content than Aβ40cc, and to be more neurotoxic than its more disordered counterparts. Two other recent studies that have linked Aβ40 peptides either by cysteine substitution at position S26, or at the N or C termini have also reached similar conclusions (24,25). Thus we conclude that the differential ability of Aβ40 and Aβ42 dimers to populate stable oligomeric states observed in vivo in the present study is likely to reflect underlying differences in the kinetics of specific steps in their aggregation mechanisms.

A particularly clear finding of the present study is that, while Aβ aggregation is required for neurotoxicity, it is clearly not sufficient. This conclusion is evident from the finding that the retinal expression of all the dimeric Aβ40 constructs failed to generate any finding that the retinal expression of all the tandem Aβ40 peptides which are ubiquitously expressed in the nervous system. All Aβ40 constructs were found to have negligible effects on longevity whilst all the tandem Aβ42 peptides significantly reduced median survival. Moreover, as determined by the rough eye and longevity phenotypes, the degree of neurotoxicity observed is dependent on the length of the linker peptides; the tandem Aβ42 constructs containing linker peptides are clearly more toxic than those without a linker sequence and longer linkers were found to result in more severe phenotypes than shorter linkers. Moreover the fractional reduction in fly longevity was observed to be proportional to the abundance of oligomeric aggregates for each of the tandem Aβ42 constructs. Whilst the most neurotoxic conformations are denied to tandem repeats of Aβ42 in the absence of a linker peptide, presumably because of steric factors, our data indicate that longer linker peptides allow the greater flexibility required to generate stable, neurotoxic, oligomeric aggregates.

In the present study the distinction between Aβ deposition in vivo and toxicity is consistent with the observation that many elderly humans are able to accumulate large numbers of β-amyloid plaques in their brains without suffering significant clinical consequences (26). The comparable flies are those expressing tandem Aβ40, where abundant Aβ deposition may occur in the brain without neurotoxic consequences. Our data are consistent with the view that genetic or environmental factors that destabilize oligomeric prefibrillar aggregates, relative to either the monomeric or fibrillar forms of the peptide, could protect the brain in elderly individuals despite ongoing plaque formation. The detailed characterisation of the variety and proportions of aggregates formed in vivo will, therefore, be essential to understand their specific proteotoxicity.

Taken together our results demonstrate that the aggregation process for Aβ42, as the peptide undergoes transitions in vivo that convert it from a soluble monomeric state to insoluble amyloid deposits, involves the relatively high population of a variety of misfolded oligomeric species that are particularly toxic. This accumulation of prefibrillar intermediates may be attributable to the magnitude of the energy barriers that trap such species in local energy minima on the energy landscape that describes the aggregation process, or to the other differences in the relative importance of the various microscopic processes that contribute to the overall aggregation reaction (4). Our results suggest that the aggregation process for Aβ40, however, appears to progress more rapidly to mature forms of aggregate without populating such intermediate states to an extent detectable in vivo. This observation is consistent with recent studies of Aβ40 in vitro that show clearly that oligomeric species formed during aggregation are rapidly sequestered by molecular chaperones (27).

It is likely, therefore, that the toxic effects of the lower levels Aβ40 oligomers relative to those of Aβ42 can be more effectively suppressed by the inherent protective mechanisms present in vivo. In any case, the results of this paper suggest that therapeutic advantage could be gained not only by increasing the barrier to initial peptide aggregation but also by facilitating the progression of Aβ42 oligomers towards the more inert fibrillar state.

REFERENCES

Aggregation and Neurotoxicity of Tandem Aβ in Drosophila

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Figure 1. Tandem Aβ peptides deposit as insoluble aggregates in neuronal tissue. (A) A series of tandem Aβ40 or Aβ42 peptides were designed and introduced transgenically into Drosophila melanogaster. (B) (i) Filter trap assays demonstrate that expression of both T12Aβ40 and T12Aβ42 at 25 °C results in the accumulation of large (>0.11 μm diameter), SDS insoluble aggregates. Fibrillar aggregates of synthetic Aβ were used as positive controls. In contrast extracts from non-transgenic flies (“51D”) contain no aggregates. ii) Densitometric analysis of the filter retardation assay shows significant differences among groups: T12Aβ40 vs 51D p ≤ 0.01**, T12Aβ42 vs 51D p ≤ 0.001***. These values were compared after normalization of total protein loaded using the β-actin staining (iii). (C) Anti-Aβ immunofluorescence detects large deposits in T12Aβ40 and T12Aβ42 expressing fly brains but not in control (“51D”) tissue from non-transgenic flies. (D) Flies expressing one or two Aβ transgenes (Aβ40, Aβ40/Aβ40, Aβ42, Aβ42/Aβ42) do not accumulate significant amounts of SDS insoluble Aβ peptide as compared to non-transgenic flies (“51D”). In contrast the T12Aβ40 peptide and the T12Aβ42 peptides form abundant insoluble aggregates. Equal protein loading was confirmed by β-actin staining (*).

Figure 2. Western blotting reveals the presence of SDS-insoluble Aβ42 and Aβ40 aggregates for flies expressing all the tandem constructs regardless of linker length. Non-transgenic control flies (“51D”) show no insoluble Aβ.

Figure 3. (A) Tandem constructs of Aβ40 do not cause developmental deficits when expressed in the retina using GMR-Gal4 (i-iii). In contrast all the tandem constructs of Aβ42 (iv-vi) except TNL Aβ42 (iv) cause in a severe rough eye phenotype at 25°C. For comparison, when the untethered Aβ peptides were driven from two transgenes both Aβ40 (viii) or Aβ42 (ix) appeared identical to controls (vii). Survival analysis of dimeric Aβ constructs. Longevity assays confirm that longer linker peptides increased the toxicity for Aβ42 (B) but not Aβ40 (C). Flies were reared at 18°C and transferred to 29°C post-eclosion. The median survival time is given in brackets.

Figure 4. Neurotoxicity correlates with the levels of soluble oligomeric aggregates of Aβ. (A) Retinal expression of Aβ constructs, followed by western blotting using the 6E10 monoclonal antibody, reveals SDS-soluble Aβ aggregates in flies expressing T22Aβ42 and T12Aβ42. In contrast TNL Aβ42 generates very few soluble aggregates while all tandem constructs based on Aβ40 generate no detectable soluble oligomers. (B) There is a correlation (R²=0.98) between the logarithm of the levels of SDS-soluble Aβ aggregates resulting from expression in the eye and the reduction in the median survival in flies expressing the corresponding Aβ constructs in their brain (see Fig. 4A). The degree of increase in aggregate levels was determined for T22Aβ42 (1), T12Aβ42 (2) and TNL Aβ42 (3) by densitometry taking control flies (4) as the baseline.
Aggregation and Neurotoxicity of Tandem Ap in Drosophila

Fig. 1

A
i) $\text{A\beta}_{40}$ $\text{A\beta}_{40}$
ii) $\text{A\beta}_{40}$ 12h hikor $\text{A\beta}_{40}$
iii) $\text{A\beta}_{40}$ 22.6 hikor $\text{A\beta}_{40}$
iv) $\text{A\beta}_{40}$ $\text{A\beta}_{32}$
v) $\text{A\beta}_{42}$ 22.6 hikor $\text{A\beta}_{32}$

B
i) 51D $\text{A\beta}_{40}$ AB40
ii) $\text{T}_{1/2}\text{A\beta}_{40}$ $\text{T}_{1/2}\text{A\beta}_{40}$
iii) $\text{T}_{1/2}\text{A\beta}_{40}$ $\text{T}_{1/2}\text{A\beta}_{40}$

C
51D $\text{T}_{1/2}\text{A\beta}_{40}$ $\text{T}_{1/2}\text{A\beta}_{40}$

D
IB: A\beta
IB: \beta-actin

Normalized Median Intensity

$\text{kDa}$ $\text{A\beta}_{40}$ $\text{A\beta}_{40}$ $\text{A\beta}_{32}$ $\text{A\beta}_{32}$

IB: A\beta
IB: \beta-actin
Fig. 2
Fig. 4

A

B

Log relative intensity of oligomeric aggregates

% reduction median survival