Supporting Information

DNAzymes as catalysts for L-tyrosine and amyloid β oxidation

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Methods

MALDI-TOF for amyloid beta and amyloid beta dimer detection

MALDI-TOF MS was performed on a Autoflex Speed IV instrument equipped with a Nd:YAG SmartBeam laser: 355 nm, (Bruker Daltonik). Mass spectra were acquired in the linear positive ion mode using an acceleration voltage (IS1) of 19.4 kV, extraction voltage (IS2) of 18.1 kV, lens voltage of 7.5 kV, detector voltage of 3,259 V, and pulsed ion extraction delay time of 400 ns. The acquisition software was flexControl 3.4, the spectral data were checked and saved using flexAnalysis 3.4 (all by Bruker Daltonik). The reaction mixture was mixed 1:1 with a saturated solution of α-cyano-4-hydroxycinnamic acid (dissolved in a mixture of acetonitrile, MilliQ and trifluoroacetic acid in a volume ratio of 7:3:0.1). Samples were prepared using the dried droplet method to achieve a co-crystallization of both sample and matrix.

HPLC and mass spectrometry for dityrosine detection

Samples of oxidized L-tyrosine were analyzed by HPLC using a Luna C-18(2) column. The applied HPLC consisted of an Agilent 1260 Infinity setup equipped with an auto sampler, attached to a binary pump. The sample detection was achieved using a diode array detector, for the detection of absorbance, and a mass spectrometer (6200 Accurate-Mass TOF LC/MS) equipped with an electrospray ionization chamber. The fragmentation voltage was set to 175 V. A linear gradient of acetonitrile and water with 0.1% formic acid was used as eluent.

Attenuated total reflection (ATR) Fourier-Transform infrared (FTIR) spectroscopy

ATR-FTIR spectroscopy was used to characterize casted films of non-oxidized and DNAzyme-oxidized Aβ peptide. For this, 50 µL of a 125 µM Aβ peptide solution in PBS was spread on an internal
reflection element (IRE) made of zinc selenide (ZnSe), and dried in an oven at 50 °C. ATR-FTIR spectra were measured on a Tensor 27 FTIR spectrometer (Bruker Optics GmbH, Ettlingen, Germany) equipped with a dedicated ATR attachment (Optispec, Neerach, Switzerland). Spectral resolution of 2 cm\(^{-1}\) and co-adding as well as averaging 100 scans per sample was applied. FTIR measurements and data were processed using the OPUS Software (OPUS 7.0, Bruker GmH, Ettlingen, Germany). Single-channel reference intensity spectra \(I_R\) were recorded from the ZnSe IRE (50 × 20 × 2 mm\(^3\)) cleaned by a Plasma Cleaner (Harrick, Ossining USA), and single channel sample intensity spectra \(I_S\) were recorded from the ZnSe IRE coated by amyloid peptide samples. ATR-FTIR spectra were obtained calculating \(A = -\log(I_S/I_R)\).

ATR-FTIR analysis of protein secondary structure

Quantitative analysis on the secondary structure of proteins and peptides can be obtained from ATR-FTIR spectra. In the literature, there are several concepts for quantifying secondary structure based on the Amide I and II band line shape to resolve, separate, identify and assign individual Amide I band components. Firstly, curve fitting of the original Amide I band region from 1,700-1,600 cm\(^{-1}\) by the superposition of a defined number of components denoted as line shape analysis (LSA) can be used\(^{[1]}\). Second derivative spectra can be used to identify and limit Amide components, which appear as negative signals due to shoulders and unresolved maxima in the original spectrum. Secondary structure fractions are obtained by dividing the area of a given diagnostic amide component by the sum of all amide component areas. Secondly, resolution enhancement methods denoted as Fourier self-deconvolution (FSD) may be applied\(^{[2]}\). FSD is based on multiplying the originally measured interferogram by an exponentially increasing function, so that the following Fourier Transform operation results in a more structured line shape due to more narrow individual Amide I and Amide II
band components, which then are quantified by LSA (see above). However, the FSD concept requires FTIR spectra with low signal-to-noise ratio and the absence of misleading spectral features originating from atmospheric water vapor in the spectrometer. Thirdly, chemometric data treatment concepts based on factor analysis (FA)\cite{3-5} are an option. Similarly to the approach on CD spectra analysis (cf. below), FA is based on the linear combination of several factor spectra (Amide region) of proteins with known structure to represent the spectrum of the protein with unknown structure minimizing the difference by least square and other numerical methods. The error values of the found secondary fractions for all three concepts LSA, FSD, FA have never been critically assessed, but the deviation to other methods like CD, NMR and X-ray may be in the order of 5 to 10%.

In Fig. S6A, ATR-FTIR spectra of casted films from aqueous solutions (125 μm in PBS buffer) of untreated Aβ peptide and the Dz-00-oxidized Aβ peptide are presented. In both spectra, a main peak position of the Amide I band (80% ν(C=O) vibration) at approximately 1,630 cm$^{-1}$ and the Amide II band (40% δ(NH), 60% ν(C-N) vibration) at approximately 1,530 cm$^{-1}$ show up. Both signals can be unambiguously assigned to the typical spectral features of the β-sheet structure of peptides and proteins based on the concept of transition dipole moment coupling between neighbored peptide units in the parallel and antiparallel β-sheet structure\cite{6-8}. Additionally, in the Amide I band of both samples, a shoulder at around 1,690 cm$^{-1}$ is present, which is also indicative for β-sheet structures. There has been earlier\cite{9} and more recent debate\cite{6} that the presence of this high wavenumber band at 1,690 cm$^{-1}$ together with the low wavenumber band at 1,630 cm$^{-1}$ is indicative for both antiparallel and parallel β-sheet. Moreover, the absence of this high wavenumber component at 1,690 cm$^{-1}$ would be diagnostic for pure parallel β-sheet. Hence, for both unmodified and Dz-00-oxidized Aβ peptide both parallel and antiparallel β-sheet structures are claimed.
Quantification of the β-sheet content in the Aβ peptide via the sensitive Amide I band was performed by LSA following a concept reported therein\cite{10}, which was applied to a small peptide. Deviating to this reference, a Gaussian/Lorentzian (50/50\%) line shape was used. At first, ATR-FTIR spectra on the two Aβ peptide samples were baseline-corrected between 1,710 and 1,590 cm\(^{-1}\) and the second derivative spectra (Fig. S6A) were computed using the OPUS Software (OPUS 7.0, Bruker Optics GmbH, Ettlingen). Five negative peaks at the wavenumber positions of 1,630, 1,654, 1,663, 1,675 and 1,695 cm\(^{-1}\) were identified in the spectral region 1,710-1,590 cm\(^{-1}\) of the Amide I band, where the components at 1,654, 1,663 and 1,675 cm\(^{-1}\) were of lower significance as compared to the 1,630 and 1,695 cm\(^{-1}\) component. ATR-FTIR spectra of the Aβ peptide samples in the spectral region from 1,710 to 1,590 cm\(^{-1}\) were represented by the sum of these five Lorentzian/Gaussian (50/50\%) components, each with their respective wavenumber position, and integrated using the curve fit tool of the OPUS software (OPUS 7.0, Bruker Optics GmbH, Ettlingen). During the fitting procedure, the wavenumber positions were fixed at the values given above, and also the half widths were fixed at an arbitrary value of 25 cm\(^{-1}\). Only the integrated areas were allowed to be optimized by a least-squares approach to minimize the differences between measured and calculated curves. The relative β-sheet content was obtained by the ratio of the 1,630 cm\(^{-1}\) component area and the sum of all component areas, multiplying by 100. The 1,695 cm\(^{-1}\) component also assigned to antiparallel β-sheet structure was not considered, since it is part of the related typical 1,630/1,695 doublet band. Due to lacking significance of the other components at 1,675, 1,663 and 1,654 cm\(^{-1}\), no quantification of the related secondary structure fractions assignable to turn-, random- and α-helix, respectively, was performed.

The obtained β-sheet percentages of both samples are given in the Table S1. A β-sheet content of approximately 40\% and 43\% was found for unmodified and Dz-00-modified Aβ peptide, respectively,
which can be seen as invariant considering the error values approximately 5 % (Table S1). Errors of secondary structure portions were calculated as standard deviation (n=3).

Table S1: β-sheet content of non-oxidized and Dz-00-oxidized Aβ peptide.

| Sample                     | β-sheet content / [%] FTIR | β-sheet content / [%] CD |
|----------------------------|---------------------------|-------------------------|
| Original Aβ peptide        | 39.9 ± 5.7                | 37.3 ± 4.1              |
| Dz-00-oxidized Aβ peptide | 42.9 ± 5.4                | 36.3 ± 5.4              |

Circular dichroism (CD) spectroscopy

CD spectroscopy was used to characterize unmodified and DZ-00-oxidized Aβ peptide solutions at the concentration of 125 μM related to amino acid residues. CD spectra in the UV range (190 to 280 nm) were measured on a J810 spectral polarimeter (Jasco GmbH, Groß-Umstadt, Germany). Dedicated CD-compatible quartz cuvettes with 1 mm path length were used. CD spectra were corrected by subtracting respective buffer (PBS) spectra. For the examination of the secondary structure of amyloid peptide, CD spectra were analyzed in the range of 190 to 280 nm using the tool CD-PRO-Analysis applying the analysis option CONTIN of Jasco Software Spectra Manager Version 2 (Version 2.06.00 [Build 6]) from JASCO GmbH (Groß-Umstadt, Germany). As output, the relative (percentage) fractions of α-helix, β-sheet, random coil and turn can be obtained, based on CD data processing\textsuperscript{[11-12]}. Errors of secondary structure portions were calculated as standard deviation (n=3).
**CD analysis of protein secondary structure**

Quantitative information on the secondary structure of protein and peptides from CD spectra can be obtained by a chemometric methodology similarly to FA at FTIR spectra. This methodology is based on work of using up to 17 proteins with known conformations from X-ray, and computing basic CD spectra for the α-helix, β-sheet, β-turn and random coil, which are used to fit CD spectra of unknown structure applying a linear regression[13-14]. More current concepts include a larger pool of CD spectra of known protein structures[15] and apply single value decomposition, convex constraint analysis, ridge regression, self-consistent method[12] up to neural networks[16] to replace linear regression-based concepts.

The CD spectra of unmodified and Dz-00-modified Aβ peptide samples in the UV range (190 to 280 nm, Fig. S6B), which is sensitive to n-π* or π-π* transitions of the Amide chromophores, did not reveal significant spectral differences. Negative CD signals at approximately 195 nm indicative for random coil and weaker signals at approximately 220 nm indicative for β-sheet were observed. We measured a minimum of 198 nm in our CD-spectra for Aβ peptide, consistently with others[17]. For quantitative analysis, the CD spectra of both samples were analyzed, as described above. The obtained β-sheet percentages of both samples are given in the Tab. 2. β-sheet contents of approximately 37% and 36% were found for non-oxidized and Dz-00-oxidized Aβ peptide, respectively. These values are slightly lower compared to those found by ATR-FTIR, and the difference between both samples is also not significant considering the error values. Errors of secondary structure portions were calculated as standard deviation (n=3).

In conclusion, both methods only show marginal spectral differences between the non-oxidized and the Dz-00-oxidized Aβ peptide samples. Obviously, the significant modification and macroscopic aggregation of the Dz-00-oxidized Aβ peptide does not necessarily result in a change of the secondary
structural level at that low yields. To further investigate the differences in oxidized and untreated Aβ beta peptide, we then employed HPLC to overcome the undistinguishable structural analysis.

**Electron paramagnetic resonance spectroscopy (EPR)**

Continuous wave (CW) EPR experiments were performed on an EMX-plus spectrometer (Bruker BioSpin) equipped with the resonator ER 4119 HS-W1 and the variable temperature unit ER4141VT. All measurements were done at room temperature and at X-Band frequency (microwave frequency: ~9.4 GHz). For the detection of the hydroxyl radical we prepared a reaction mixture with 100 nM Dz-00, 250 μM H₂O₂ and 2mM 5,5-dimethyl-pyrrolidine N-oxide (DMPO). The mixture was loaded into a glass capillary (Blaurand®, intraMark, 50 μL) and sealed with wax. The spectra were recorded with a center field of 3360 G, sweep width 150 G, microwave power of 20 mW, receiver gain of 2x10⁵, modulation frequency of 100 kHz, modulation amplitude of 1 G, time constant of 164 ms, conversion time of 164 ms, 4 scans and 1024 data points. The spectra of the buffer solution was also recorded and subtracted from the DMPO spectra to eliminate any background signals.

**EPR analysis**

Radicals formed during the oxidation of L-tyrosine catalyzed by DNAzymes were investigated with EPR spectroscopy. The trapping agent DMPO was used to react with the formed hydroxyl radicals to stable DMPO-OH radicals leading in a four-line EPR spectrum. But the obtained spectrum in Figure S11 showed a spectrum which indicates the formation of the oxidized form of the DMPO-OH radical (DMPO-Ox). DMPO-Ox was formed due to oxidation of the DMPO-OH radical with remaining H₂O₂ in the solution. The formation of the DMPO-Ox with their characteristic spectra was shown
previously\textsuperscript{18-19}. The splitting of the hyperfine coupling is due to coupling of the free radical with the nitrogen and the two equivalent protons on the number 2 carbon\textsuperscript{20}.

The resulting $g$ value for the DMPO-Ox radical is 2.0072 with the hyperfine coupling constants $A_N = 7.3$ G and $A_H = 4.1$ G. The values can be compared with other groups who obtained similar values\textsuperscript{19-20}.

When L-tyrosine was additional employed to the reaction mixture DMPO-Ox could not be detected. This is due to the following reaction of the hydroxyl radicals with the added L-tyrosine and furthermore affirms the formation of tyrosyl radicals (Figure S11A). Furthermore we depicted a proposed mechanism (Figure S11B) as suggested earlier\textsuperscript{21}.

**Figures**

![CD-spectra of G-quadruplexes](image)

**Figure S1**: CD-spectra of G-quadruplexes. 20 µM of the ssDNA were measured in the range of 195 nm to 335 nm. The data of Dz-00 (A) as well as ln1-sp (B) showed a negative peak at 240 nm indicating a parallel conformation. ln1-sp also shared a positive peak at 265 nm (criteria of a parallel conformation). After heating up ln1-sp or Dz-00, the peak was shifted to 254 nm, and a positive peak at 295 nm appeared, indicating a mixed form of G-quadruplexes of parallel and anti-parallel conformations.
**Figure S2:** Kinetic behavior of DNAzyme formation of Dz-00 by varying the stoichiometry of DNA to hemin measured at 404 nm. For evaluating the time point of active DNAzyme formation, different molar ratios of DNA to hemin were applied, and their absorbance was measured time-dependent.

**Figure S3:** HPSEC chromatograms of exemplary sample of L-tyrosine (black) oxidized to dityrosine (red) by hemin. For the detection of L-tyrosine and dityrosine, HPSEC was applied using fluorescence as a detection method instead of absorbance, as previously described. The peak at 24.3 min is representative for L-tyrosine, which was exited at 250 nm and analyzed by 305 nm (black). The same sample, when analyzed at 405 nm (red), showed a second prominent peak at 23.0 min, which corresponds to dityrosine.
Figure S4: Calibration of dityrosine in PBS measured by fluorescence detection.

Figure S5: Calibration of dityrosine in PBS measured by HPLC.
Figure S6A: Mass spectrum of L-tyrosine. B: Mass spectrum of dityrosine.

Figure S7: DNAzyme stoichiometry test comparing stoichiometries of 10:1 (1 µM DNA: 100 nM hemin), 1:1 (100 nM DNA: 100 nM hemin), and 100 nM free hemin. Dz-00 was tested at a stoichiometry of 10:1 with an excess of DNA (final concentration of 1 µM) to investigate its influence on catalytic activity. The concentration of L-tyrosine and H₂O₂ was 250 µM. The data indicate that there was no significant influence on L-tyrosine oxidation when DNA was employed in excess.
**Figure S8A:** ATR-FTIR spectra of casted films of unmodified Aβ peptide (black) and Dz-00-modified Aβ peptide (red). Films were prepared from 125 µM solutions. Original (top) and second derivative (bottom) spectra are shown in the Amide I and Amide II range.

**B:** CD spectra of 125 µM solutions of unmodified Aβ peptide (black) and Dz-00-modified amyloid peptide (red).
Figure S9: MALDI-TOF MS analysis of amyloid beta (blue) and amyloid beta dimer (red). The presence of amyloid beta is indicated by a mass signal at 4,345.5. A mass signal at 8,727.3 indicates the presence of the amyloid beta dimer.

Figure S10: DNAzyme activity test in the presence 5% and 10% FBS. The concentrations of hemin and DNA were increased to 5 µM and 500 nM, respectively. The underlying trend shows that the activity of DNAzymes was reduced in the presence of serum proteins, but the DNAzymes were still active. Furthermore, the background fluorescence of the serum itself masked the catalytic reaction within the first minutes after initiation leading to apparently lower activity, which was only determined when the measured fluorescence was higher than the background signal.
Figure S11A: The EPR spectrum obtained when 2 mM DMPO was added to a mixture of 100 nM DNAzyme (Dz-00) and 250 µM H₂O₂ in PBS buffer. The formed hydroxyl radicals react with the DMPO to the DMPO-OH radical which was further oxidized to DMPO-Ox. The g value of the radical is 2.0072 with the hyperfine coupling constants $A_N = 7.3 \text{ G}$ and $A_H = 4.1 \text{ G}$. B: The proposed mechanism of dityrosine formation based on oxidation of L-tyrosine by as-formed hydroxyl radicals.

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