Abstract

Recently, different aminopyrazole derivatives were examined in their activity against the aggregation of the Aβ-peptide. The inhibition experiments were often performed with an excess of ligands, while in this work, substoichiometric effects were also tested. Herein the derivatives Trimer-D3 **43**, Trimer-TEG-TEG-D1 **42** and Trimer-diamine **40** were successfully investigated.

Dose-response curves gave IC_{50} values of 2.2 µM for Trimer-D3 **43** and 6.3 µM for Trimer-TEG-TEG-D1 **42**, which are below the deployed Aβ concentration of 10 µM.

Conformational properties of the Aβ peptide with 50mol% amount of ligand were analyzed using CD spectroscopy. By adding different ligands the characteristic negative band at 218 nm of the Aβ peptide decreased in the course of time. Additionally, a positive Cotton effect was observed between 260 and 320 nm, which indicated the interaction of the aminopyrazole trimer unit with the KLVFF sequence of the Aβ peptide.

With water soluble compounds Trimer-D3 **43** and Trimer-TEG-TEG-D1 **42** PICUP experiments were performed. Trimer-D3 **43** showed no significant effect on the oligomer distribution. Trimer-TEG-TEG-D1 **42** showed the stabilization of monomers, whereas the formation of higher oligomers was not observed. TEM microscopy gave informations about morphologic changes of the aggregates with substoichiometric amounts of the ligands. Aβ(1-42) as reference showed a fibril network. The incubation with ligands led to smaller agglomerates with different morphologies. TEM pictures of the ligands without Aβ showed morphologically different associations, which could not be compared with the reference.

In the work of *März-Berberich* a non covalent binding of the trimer with a His-tagged Aβ-specific antibody via Ni^{2+} complexation could not be achieved using a mono-NTA unit. So in this work, a trimer-hybrid with a tris(NTA)-cyclam unit was successfully synthesized over 15 steps (compound **62**) to guarantee a stronger binding to the antibody. The group of *Korth* showed that the trimer-tris(NTA)-cyclam-IC16scFv complex had a thermodynamic stability in native PAGE. Furthermore, ELISA experiments determined strong affinities of the hybrid complex to the Aβ peptide with both IC16scFv and the camelide antibody VHH-E4, whereas the single compounds

showed no effect on the affinity to Aβ. Prospective studies could be focussed on inhibition experiments like ThT assays for fibril formation, CD spectroscopy for conformational changes, TEM measurements for the visualization and analytic ultracentrifugation to assign the binding constant and the interaction with Aβ oligomers to learn more about the efficiency of the hybrid complex.

The synthesis of artificial proteases for peptide cleavage plays more and more an important role in the area of pathogenic protein aggregation. A potential watersoluble protease unit is the Co^{III}-cyclen complex. This complex successfully was coupled *N*-terminally with an aminopyrazole trimer using a dodecyl spacer (compound **86**). However, the proteolytic experiments could not be managed because of non-solubility of the compound under aqueous conditions. In the future, the protease unit and the aminopyrazole trimer could be coupled over a more watersoluble spacer to ensure proteolytic experiments in water.

The influence of Trimer-D3 **43** to the secretion of the amyloid precursor protein was examined in biological tests. Western Blots established that Trimer-D3-Lys **45** could bind to APP. Therefore the change of APP secretion was investigated. Surprisingly, in the presence of Trimer-D3 **43** *C*-terminal fragments (CTFs) were formed, which were different to the fragments formed by α-, β- and γ-secretase. The input of the single compounds (aminopyrazole + D3-peptide) showed no influence on APP secretion. Single tests confirmed that Trimer-D3 **43** has no influence on the γ-secretase compared to the known inhibitor LY-411575.
For identification of the cleavage site in APP the formation of θ-CTFs (influence of BACE2) was checked. The CT15-antibody recognizing the Aβ(10-23) sequence could bind the CTF, which is induced by Trimer-D3 **43**. Therefore, in presence of the aminopyrazole derivative the processing of APP occurred closer to the *C*-terminus compared to the α-secretase cleavage site. For final clarity mass spectrometric measurements could deliver the exact cleavage position.

For the functionalization of calciumphosphate nanoparticles with fluorescence labeled aminopyrazole hybrids two derivatives could be successfully generated using automatic and manual solid phase peptide synthesis (Trimer-D3-Dansyl **88** and Trimer-D3-Coumarin **89**). Biophysical experiments showed strong inhibition efficiency in ThT-assays. UV/Vis quantifications proved successful functionalization of the

nanoparticles with the chromophores. DLS, NS and REM measurements showed consistent particle diameters of about 100 nm. MTT tests indicated no toxicity in HeLa cells. With fluorescencemicroscopic transport analysis the absorption of the fluorescence-labeled nanoparticles could not be observed.
The examination of Trimer-D3-Dansyl **88** absorption without nanoparticles showed indeed fluorescent cells, but it is supposed that the compound accumulated on the cell surface. However, toxic effects of the compound to the cells were observed. Because of the hydrophobia of the labeled compound DMSO had to be used as solvent which is probably the most critical component for the observed cell toxicity. Prospective works could use a fluorescein resin[161] to become a more watersoluble Trimer-D3 derivative so that the input of DMSO can be avoided.

Nowadays the main objective in medicine is to provide early and exact diagnostics for efficient treatments. Therefore, the focus is set on functionalization of nanoparticles.[162] Single compounds like an aminopyrazole as β-sheet breaker, a Co^{III}-cyclen complex as artificial protease and the D3-peptide as well as a His-tagged Aβ-specific antibody coordinated with Ni^{2+} to tris(NTA)-cyclam as recognition unit could be combined on nanoparticles and lead to a selective aggregation inhibitor for Aβ. The advantage is that the synthesis of smaller molecules, which are combined in the last step, is easier than the covalent composition of different drug classes.