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Amyloid-Like Structures Formed by Azobenzene Peptides: Light-Triggered Disassembly

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Abstract. The light-driven disassembly process of amyloid-like structures formed by azobenzene model peptides is studied by time-resolved mid-IR spectroscopy from nanoseconds to minutes. The investigated peptide consists of two amino acid strands connected by the azobenzene switch. The peptides aggregate to amyloid-like structures when the azobenzene chromophore is in the *trans*-conformation. Illumination, resulting in a *trans*- to *cis*-isomerization of the azobenzene, leads to disaggregation of the aggregated structures. After optical excitation and isomerization of the azobenzene, one finds absorption changes which recover to a large extent on the time scale of few nanoseconds. These early absorption transients are assigned to the relaxation of vibrational excess energy (heat) or to structural rearrangements of isomerized azobenzene and the aggregated surroundings. It is only on the time scale of minutes that spectral signatures appear which are characteristic for the disassembly of the aggregated structure.

Keywords: Peptides, amyloid-disassembly, azobenzene, nanostructures, time-resolved-vibrational spectroscopy

1. Introduction

The aggregation of peptides into amyloid structures has obtained wide attention due to its relation to a variety of diseases such as Creutzfeldt Jakob, Parkinson, and Alzheimer. Many studies support aggregation of peptides, and especially oligomeric peptide structures as the cause of cell death and resulting diseases. In this context, the properties of amyloid-like peptide aggregates and their disassembly are of major interest.

The model peptide used in this study contains two amino acid strands with an azobenzene switch in the centre and was derived from the light switchable β-hairpin studied recently [1–3]. In this

compound, photoisomerization of the azobenzene (AMPP) interchanges the structure of the peptide between *trans*- and *cis*-conformations. Mid-IR spectroscopy and transmission electron microscopy (TEM) have shown that the peptides may aggregate into amyloid-like structures when the azobenzene is in *trans*-conformation. Illumination of the aggregated sample with corresponding isomerization of the azobenzene to the *cis*-form causes a disassembly of aggregates [4]. In the current work, this disassembly process and the performance of the embedded azobenzene are studied by time-resolved mid-IR spectroscopy.

2. Materials and Methods

The synthesis of the model peptide Ac-Ser-Trp-Thr-Trp-Glu-AMPP-Lys-Trp-Thr-Trp-Lys-NH_2 (AcA-zoTrpZip) has been reported previously [1]. The peptide was dissolved in deuterated methanol (Sigma Aldrich, 99.8%) at a concentration of about 3.3 mM for FTIR spectroscopy and 1.5 mM for nano- to microsecond IR spectroscopy. For the FTIR experiments, the sample was sandwiched between two CaF_2 windows (Teflon spacer 210 μm) immediately after dissolution of the lyophilized starting material and stored in an exsiccator at room temperature in the dark prior to the specific spectroscopic experiments. Stationary illumination by UV light (Xenon arc lamp, Oriel, Mod 6140 equipped with a water filter and UG 11 and WG 320 glass filters, Schott) was used for the *trans*- to *cis*-conversion. IR absorbance spectra were recorded with a Bruker IFS66 FTIR spectrometer (resolution 2 cm^{-1}). Background absorption from the solvent methanol-d_4 was subtracted.

2.1. Nano- to Microsecond IR Spectroscopy

Transient absorption spectra of the strongly aggregated sample (incubated for 17 days) were measured with a UV pump, IR probe setup. The IR probe pulse was generated by optical parametric amplification and difference frequency generation using a 110 fs pulse out of a 1 kHz Ti: sapphire regenerative amplifier system (Spectra Physics, Spitfire Pro) [5]. IR absorption changes are recorded by a spectrometer (Acton Research) and a 32 Channel MCT Array (Infrared Associates). The UV excitation pulse (355 nm) was the third harmonic of an externally triggered Q-Switched Nd: YVO_4 laser (AOT). It had a beam diameter at the sample position of about 200 μm, energy of 1 μJ, and duration of 0.6 ns and was electronically synchronized and delayed to the IR probe pulse. The jitter between pump and probe pulses was less than 1 ns. To improve the precision of the absorption experiment, every second pump pulse was blocked by a chopper wheel. For each delay time setting, 1000 single-excite/probe events were recorded, and the averaged absorbance difference was calculated. Four scans of the delay settings were averaged to obtain the transient absorption difference spectra shown in Figures 1(b) and 2(a). The sample was cycled through a CaF_2 cuvette (path length: 240 μm) at a speed to ascertain a complete sample exchange between subsequent laser shots. The exchange of the sample avoided that accumulation of disaggregated peptides did influence the measurement.

3. Results and Discussion

Figure 1(a) shows the amide I' band of a peptide preparation after seven days of incubation. There is a strong absorption in the 1610–1630 cm^{-1} range and only weaker absorption at higher frequencies (the peak at 1678 cm^{-1} originates from residual TFA from the synthesis). Amide I absorption between

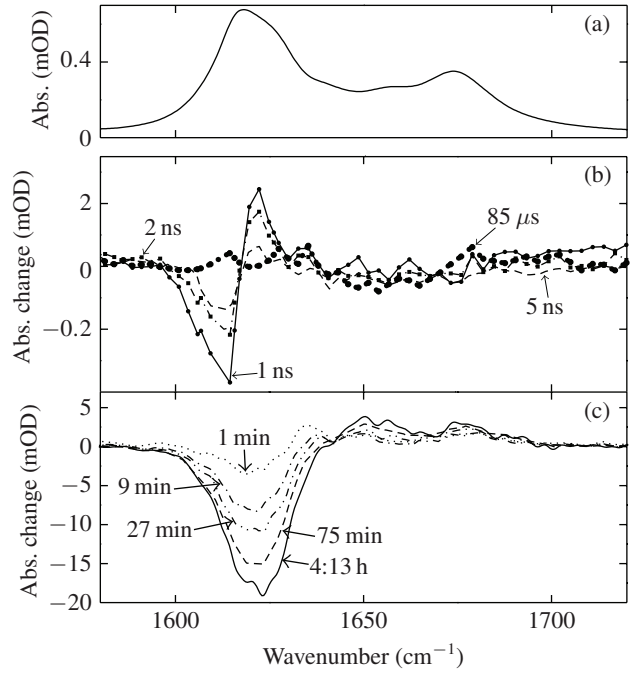

Figure 1: Absorption of the aggregated peptide (incubation time 7 days) in *trans*- (a) and transient absorption changes of this sample recorded on the time scale of minutes after 1 min UV illumination (c). Absorbance changes following UV excitation and *trans*- to *cis*-isomerization of highly aggregated peptides (incubation time 17 days) recorded on the time scale of ns (b).

1640 and 1700 cm^{-1} is well known for free amide groups or random coil peptides and for secondary structures like α-helix or β-sheet [6]. Absorption in the 1610–1630 cm^{-1} range is characteristic for aggregated amyloid-like peptides [7–9]. For the AcAzoTrpZip, an absorption at about 1625 cm^{-1} was found for weakly aggregated or oligomeric peptides, whereas an absorption at about 1615 cm^{-1} was found for strongly aggregated extended fibrils [4]. The sample after seven days in solution is composed of monomeric, weakly aggregated peptides and to a large extent of strongly aggregated amyloid-like fibrils [4].

Figure 1(b) shows the light-induced absorbance changes of a strongly aggregated sample (17 days of incubation) on the nano- to microsecond time scale. At the beginning of the observation period (1 ns after the UV excitation), a dispersive absorption change with a decreased peaking around 1612 cm^{-1} and an increased intensity at about 1622 cm^{-1} is observed. It points to changes of strongly aggregated into weakly aggregated structures. This spectral signature decays on the 5 ns time scale (Figures 1(b) and 2(a)). Later on until the end of the observation period (80 μs), no significant absorption changes were detected.

This observation is in line with the results from illumination experiments on the time scale of minutes presented in Figure 1(c). Illumination of the sample from Figure 1(a) by UV light for 1 min switches a fraction of the azobenzene chromophores from *trans* to *cis* (confirmed by UV/VIS spectroscopy, data not shown). However, immediately after illumination, the IR spectrum in the amide I range shows only weak changes. It is only on the time scale of 10 min after illumination that a decrease

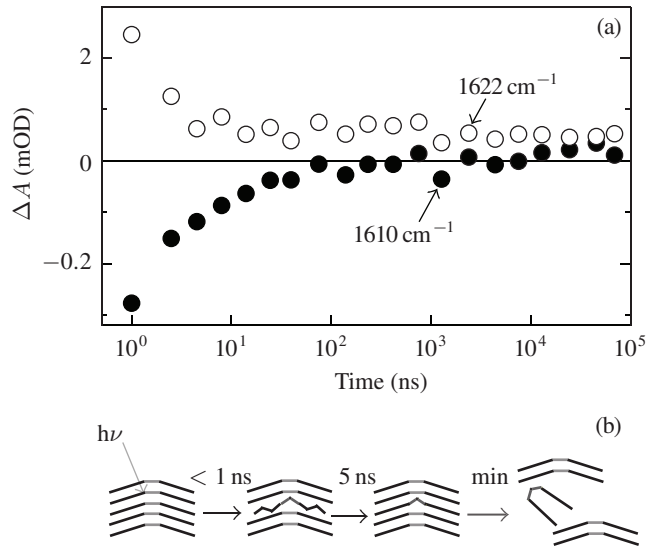

Figure 2: Absorption transients at 1610 and 1622 cm^{-1} on the time scale of ns to μs (a) connected to the change in peptide aggregation. Model for the light-induced disassembly (b).

in IR absorption in the 1610–1630 cm^{-1} range (i.e., over the whole band of aggregated structures) and an absorption increase at higher frequencies (where nonaggregated peptides absorb) take place.

Another observation should be added which may facilitate the interpretation of the transient IR spectra. It has been shown by UV/VIS spectroscopy that the *trans*- to *cis*- isomerization of azobenzene AMPP in the nonaggregated β-hairpin peptide occurs with a quantum yield of 20%. When the peptides are aggregated, stationary experiments point to a strongly reduced quantum yield of ca. 2% (see supplementary materials of [4]).

In the following, we present a simplified model (visualized in Figure 2(b)) of the isomerization/disassembly process which is consistent with the different observations for aggregated and nonaggregated azobenzene hairpin peptides. Absorption of a photon by *trans*-azobenzene leads to motions on the excited state potential energy surface towards the interaction region with the *cis*-state. For azobenzene in solution, this motion is not hindered, and the molecules complete the motion and reach the *cis*- state with high efficiency (20%). When this process is hindered, for example, by constraints deriving from an aggregated peptide, the isomerization yield may be strongly reduced. Apparently, the close packing of the peptide in the aggregates has this effect.

The observed absorption transients on the ns time scale can be explained by two molecular models. (i) After the isomerization of the azobenzene to the *cis*- form, the isomerized chromophore does not fit into the aggregated surroundings, and a considerable strain is built up between the *cis*-azobenzene and the surrounding aggregate. This interaction may be visible as the dispersive absorption changes observed in Figure 1(b). On the 5 ns time scale, the strain is partially released by a rearrangement of the system, and the absorption changes disappear. (ii) A second explanation is based on the transient heating of the azobenzene and its surroundings by the excess energy released upon isomerization and internal conversion. This heating of the peptide may change the equilibrium, with a reduction of the number of highly aggregated molecules and related increase of weakly aggregated molecules. When this excess

heat is transferred away from the aggregated structures to the surrounding solvent (on the few ns time scale), the heat-related population change and the corresponding absorption difference signal decay.

In both models, the excitation of the azobenzene chromophore causes a significant disturbance of the aggregated peptide which decays within a few nanoseconds. Finally the isomerized *cis* azobenzene molecules act as local defects which induce the slow disassembly of the aggregated structures. This disassembly process leads to the broad absorption decrease found in the 10 min time range in the band of aggregated peptides. The time dependence of the absorption clearly shows that the isomerization of the azobenzene switch does not directly disrupt the aggregates. The isomerization leads to distortions of the structures which induce disassembly via thermally driven steps on much longer time scales. The use of azobenzene as a switching element for aggregated structures has also been demonstrated very recently in the context of the Alzheimer related Amyloid β peptide [10]. Apparently the azobenzene switch is well suited for the manipulation of amyloidlike aggregates.

Acknowledgments

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