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Ultrafast photoisomerization of azobenzene compounds

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Abstract

The spectroscopy and dynamics of the azobenzene compound 4-(4'-aminophenylazo)benzoic acid sodium salt (APB) is described and its applicability as a reversible fast photoswitch for conformational control in small peptides is discussed. Addition of an electron withdrawing (carboxy-) and an electron donating (amino-) group on the phenyl rings of azobenzene shifts the absorption maximum of the π–π^* band of the resulting APB molecule from 320 to 420 nm. Reversible photoisomerization is detected by UV–vis, FTIR and NMR spectroscopy. Femtosecond time resolved ground and excited state dynamics were recorded, indicating that excited *trans*-APB reaches the electronic ground state within ≈ 1 ps. This ultrafast photoisomerization is followed by a wavelength dependent process in the 10 ps range which is interpreted as vibrational cooling of the product molecules. At delay times longer than 200 ps the absorption characteristics, as expected from the cw-difference spectrum, can be seen, indicating the completion of the photoprocess. © 1997 Elsevier Science S.A.

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1. Introduction

Irradiation of azobenzene and its derivatives with photon energies exciting the corresponding electronic transition causes *cis–trans* isomerization with considerable quantum yield. The photoinduced change in polarity and molecular shape has led to widespread applications of azobenzene derivatives as reversible molecular switching devices. Polymeric azobenzene liquid crystal films are suitable for optical switching or image storage devices [1]. In several cases the photoregulation of peptide conformation [2,3] or of enzyme structure, binding or activity [4] by changing the isomerization state of attached azobenzene molecules was demonstrated.

Although spectroscopic properties of azobenzene have been under extensive study for a long time, only recently the first transient absorption measurements with a femtosecond time resolution were performed. In these studies it could be shown that in electronically excited azobenzene the ground state potential energy surface is repopulated faster for the *cis*-isomer [5,6] than for the *trans*-isomer (lifetime of ≈ 0.9 ps [7]). This situation is similar to the findings in stilbene [8], where the longer lifetime of the excited *trans*-isomer was explained by the existence of a surface barrier for *trans** of $\approx 1200\ cm^{-1}$ [9], whereas the photoinduced reaction for the *cis*-side in S_1 should occur along a barrierless surface [10]. Two possible reaction pathways were proposed for $n\pi^*$ or $\pi\pi^*$ photoexcitation: an in-plane inversion at one of the two nitrogen atoms ('inversion-mechanism') or a twisting motion around the N=N double bond ('rotation mechanism') [11,12].

We investigated the ultrafast dynamics of azobenzene derivatives and azo-peptide compounds to monitor conformational changes triggered by the isomerization reaction of the photoswitch [13]. Here, we report the first characterisation of the pseudo amino acid *p*-aminophenylazobenzoic acid (APB) by analysing its photochemical properties and transient absorbance changes on a femtosecond time scale.

2. Experimental section

2.1. Materials

Solvents and reagents for synthesis and spectroscopy were of highest quality available, fully deuterated d_6– DMSO used

Abbreviations: APB: 4-(4'-aminophenylazo)benzoic acid sodium salt; DMSO: dimethylsulfoxide; FTIR: fourier transform infrared; ^{1}H-NMR: proton nuclear magnetic resonance

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for FTIR experiments was from Sigma (Germany). 4-(4'-aminophenylazo)benzoic acid sodium salt (APB) was prepared and isolated according to known procedures [17]. In principle APB was synthesised by mixing 4-nitro-benzoic acid and 1,4-diaminobenzene in 3% NaOH solution and heating to 95°C for several h. In the subsequent cooling process APB precipitates as sodium salt.

2.2. Methods

2.2.1. UV–vis spectroscopy

Reversible photochromism of APB was recorded on a two beam absorption spectrometer (Lambda 19, Perkin-Elmer) in the near UV–visible spectral region. The photoreaction was induced by illuminating the sample inside the spectrometer, the details of the set-up are described in [13]. The cw-difference spectra were recorded from 260 to 600 nm.

The experimental set-up used for the femtosecond time resolved pump-and-probe measurements is described in detail elsewhere [13,14]. The transient absorption changes of 0.5 mM *trans*-APB in ethanol were induced by 1 μJ excitation pulses at 435 nm and analysed between 350 and 540 nm with an instrumental response function around 200 fs. The pump and probe pulses were focused to diameters of 220 and 100 μm, respectively, in the sample cuvette, parallel polarisation was used in all experiments. The time zero was determined independently in every experiment by recording the two photon absorption process in appropriate glass filters (BG-filters, Schott, Germany). The transients shown are an average of typically 500 single shots. For delay times up to 1 ps linear scaling is used, for longer times the scaling is logarithmic. Experimental data were fit by a sum of exponential functions convoluted with the instrumental response function.

2.2.2. IR spectroscopy

Stationary mid-IR absorption spectra and light induced difference spectra of *trans*-APB were recorded on a Bruker IFS 66/CS FTIR spectrometer. The sample was dissolved in d_6-DMSO to a final concentration of 20 mM, the optical pathlength was approx. 20 μm. The solvent spectrum was subtracted before and after irradiation and water contribution was minimised by continuously purging the sample compartment with dried air.

2.2.3. NMR spectroscopy

^{1}H-NMR spectra (500.13 MHz) of APB prior and after irradiation (30 min at 420 nm with a xenon high pressure lamp (19 μW cm^{-2}) in NMR tubes (∅ 5 mm)) were recorded in d_6-DMSO (34 mM) on a Bruker AMX 500. For the 1D spectra the following parameters were used: size 16 K, sweep width 7575.7 Hz. The NMR spectra were transformed with XWINNMR 1.0 on a Silicon Graphics IRIS Indigo R4000. Nomenclature of the APB protons in Table 2 and Fig. 3 is according to Fig. 1.

2.2.4. Chromatography

HPLC was carried out with Waters equipment (Eschborn, Germany) on Nucleosil 300/C8 (Machery & Nagel, Düren, Germany) using a linear gradient of acetonitrile/2% H_3PO_4 from 5:95 to 80:20 in 30 min (UV monitoring at 214 nm).

3. Results and discussion

3.1. Absorption spectra

As a first step in the analysis of photoinduced conformational changes in small linear or cyclic peptides, we synthesised *p*-aminophenylazobenzoic acid (APB), which can be inserted as a photoactive 'amino acid' in a peptide chain.

Substituting the two phenyl rings of azobenzene with an amino and a carboxy group, respectively has a drastic effect on the absorption properties: compared to *trans*-azobenzene, where the main feature in the absorption spectrum lies at 320 nm, the strong $\pi \rightarrow \pi^*$ transition exhibits a strong red shift

Table 1
Band assignments of the FTIR spectra of APB and observed absorption changes after illumination (see Fig. 3)

Absorption band (cm^{-1})	Tentative assignments	Absorption change after irradiation	Remarks
1653	ν(C=O)	Not affected	
1602	$\delta(NH_3^+)$ ν(phenyl ring) $\nu_{as}(COO^-)$	Strong decrease	[a]
1561	$\delta(NH_3^+)$	Band disappears	
1508	ν(ring) $\delta(NH_3^+)$	Absorption increase, shift to lower frequencies	[b]
1420	$\nu_{as}(COO^-)$ ν(N=N)	Band disappears	[c]
1400 to 1100	ν(C–N)	Mainly absorption decrease	
1233	δ_{ip}(C–H) ν(C–N)	Band disappears	
1140	δ_{ip}(C–H) ν(C–N)	Band disappears	

[a] Since this band is also present in samples lacking the COO^- group (APB-Pro-OtBu; APB (free acid)), a C–C stretching mode or a contribution from the NH_3^+-group (which should be present in DMSO) seems likely.

[b] Two in-plane phenyl ring modes are observed at somewhat lower frequencies in azobenzene [18]; both modes exhibit a shift to lower frequencies upon *trans* to *cis* isomerization.

[c] The N=N stretching vibration in *cis*-azobenzene is observed at 1512 cm^1. The corresponding infrared transition for the *trans*-isomer is symmetry forbidden, but in the Raman spectrum a signal is found at 1440 cm^{-1}, which was interpreted as N=N stretch [18].

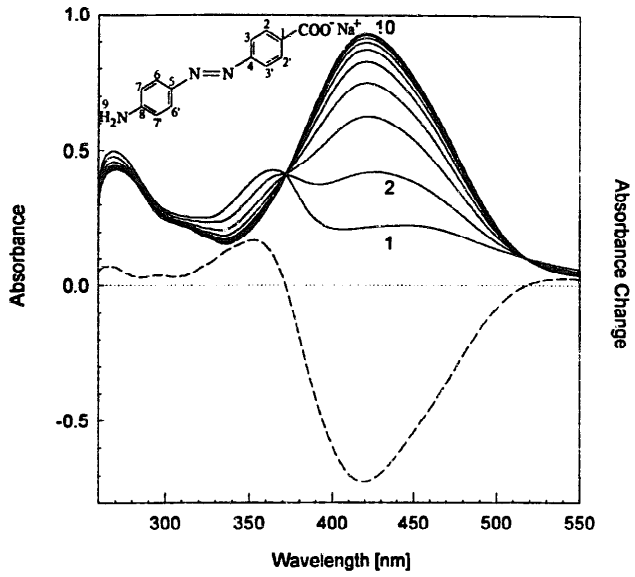

Fig. 1. UV–vis absorption spectrum of 40 μM APB (sodium salt, see inset) in DMSO, recorded in a 1 cm cuvette. Illumination of *trans*-APB at 415 nm (1.8 mW) leads to the formation of a photostationary equilibrium within 2 min (curve 1), the light-minus-dark difference spectrum is displayed as dashed line. The *trans* $\pi \rightarrow \pi^*$ band recovers in the dark, the curves 2–10 are recorded in 1 min intervals, the two stable isosbestic points are found at 372 and 518 nm.

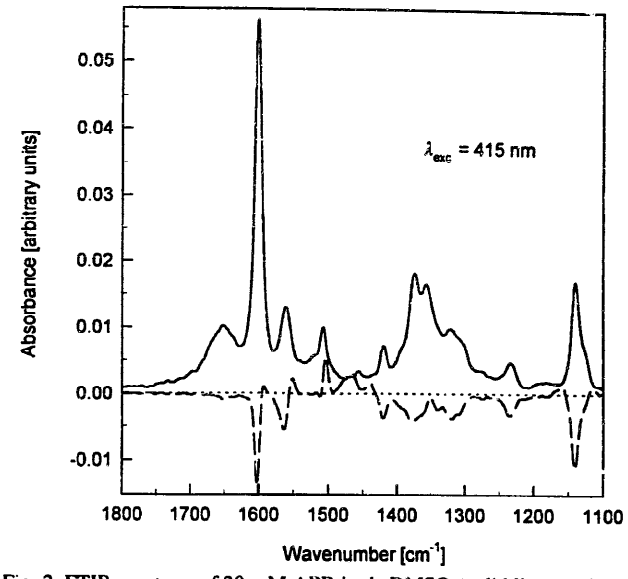

Fig. 2. FTIR spectrum of 20 mM APB in d_6-DMSO (solid line) and light-minus-dark difference spectrum (dashed line); the solvent contributions are small in the spectral range shown and were subtracted. The difference spectrum is an average of 40 individual measurements, the sample was continuously illuminated at 415 nm (50 μW). For an assignment of the absorption lines see text and Table 1.

to 420 nm in *trans*-APB, which allows direct excitation of this isomer of APB with the 435 nm pump pulse. The position of the low lying n $\rightarrow \pi^*$ transition, which should be at similar wavelengths for both isomers is located at 440 nm for the *cis*-isomer (see Fig. 1, curve 1). In *trans*-APB, it is expected in the long wavelength wing of the strong $\pi \rightarrow \pi^*$ band (see Fig. 1, curve 10). This effect is in accordance with the results in other modified *trans*-azobenzene compounds, where a close energetic proximity of the n $\rightarrow \pi^*$ and the $\pi \rightarrow \pi^*$ states in molecules of the aminoazobenzene type were recorded [15,16]. Illumination of *trans*-APB near the maximum of the $\pi \rightarrow \pi^*$ transition ($\lambda_{exc} = 415$ nm) leads to the formation of a photostationary equilibrium within 2 min (Fig. 1, curve 1), also the formation of the $\pi \rightarrow \pi^*$ states for the *cis*-isomer with an absorption maximum at 360 nm is clearly visible in the spectrum of the illuminated sample and in the light-minus-dark difference spectrum (Fig. 1, dashed line).

In contrast to azobenzene, where both isomers are stable on a timescale of several hours at room temperature, the *trans*-isomer is strongly favoured in APB: in the dark the 420 nm band recovers (Fig. 1, curves 2–9) and after 10 min the spectrum is identical to the initial APB -curve, indicating a thermally induced *cis* to *trans* isomerization on that timescale.

The stationary mid-IR absorption spectrum for *trans*-APB is shown in Fig. 2 (solid line). The dominant absorption features show up in the wavenumber range between 1100 and 1700 cm^{-1} with two prominent bands at 1140 and 1602 cm^{-1}. A reliable band assignment to the experimental data is difficult, due to the coupling of the azo vibrations with the phenyl modes, but a comparison to the detailed vibrational analysis of *trans*-azobenzene that has been carried out recently [18] is helpful. The most intense band at 1602 cm^{-1} is also found in compounds without the COO^- group (like APB with an attached amino acid; data not shown) and therefore an interpretation of this band as a high frequency C–C stretch is favoured. This phenyl ring mode is found at 1609 cm^{-1} in azobenzene and related to a fundamental ring mode ($\nu_{8a/b}$) of benzene. The photoinduced formation of *cis*-APB leads to an absorption decrease of this band (see the light induced difference spectrum, Fig. 2, dashed line). For another phenyl ring mode of azobenzene, a frequency shift of several wavenumbers is observed upon isomerization (from 1484 to 1480 cm^{-1}) leading to a differential feature in the difference spectrum [6]. A likely candidate for this mode in the FTIR spectrum of APB is the band at 1508 cm^{-1}, since illumination leads to an absorption increase and to a shift of this band to lower frequencies. The 1140 cm^{-1} band, which should contain some C–N stretching contributions, disappears upon illumination.

3.2. NMR spectroscopy

The ^{1}H-NMR spectra of APB before and after 30 min irradiation at 420 nm are shown in Fig. 3. It can be seen that upon illumination a complete new set of resonances appears, which can be assigned to *cis*-APB. The chemical shifts of the *trans* and *cis* isomer of APB are listed in Table 2. All signals of the *cis*-isomer are shifted to higher field thus allowing the quantification of the two isomers before and after irradiation. In the initial state no *cis*-isomer could be detected. Upon irradiation under the conditions chosen in the NMR experiments, only 10% *cis*-isomer is present. This may be due to the high absorbance of the *trans*-APB solution at the wave-

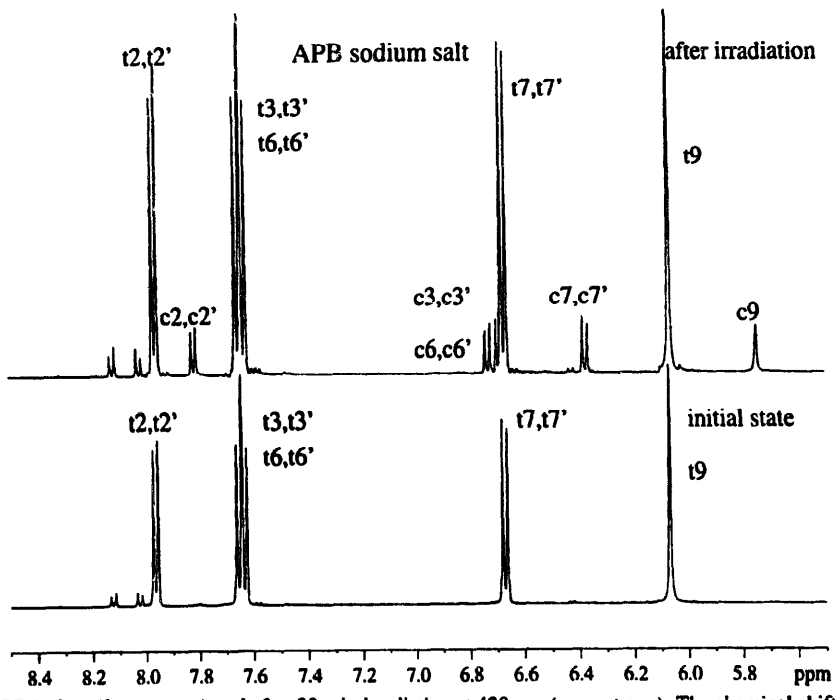

Fig. 3. ^{1}H-NMR spectra of APB before (lower trace) and after 30 min irradiation at 420 nm (upper trace). The chemical shifts of the two isomers are given in Table 2.

Table 2
Chemical shifts of APB before and after a 30 min irradiation at 420 nm

	δ (ppm) before irradiation		δ (ppm) after irradiation [a]
t2, t2'	7.95 (d)	c2, c2'	7.8 (d)
t3, t3'	7.6 (d)	c3, c3'	6.7 (d)
t6, t6'	7.6 (d)	c6, c6'	6.7 (d)
t7, t7'	6.65 (d)	c7, c7'	6.38 (d)
t9, t9'	6.05 (s)	c9, c9'	5.75 (s)

[a] Molar ratio of *trans*/*cis* isomers of 90:10; (t = *trans*; c = *cis*) was determined by peak integration of the ^{1}H-NMR spectra.

length of irradiation combined with the large optical density of the NMR sample and the reisomerization of the sample during the experiment. The absence of *cis*-isomer in the non-irradiated state was further confirmed by reverse phase HPLC where the elution pattern of the synthetic compound shows one single peak.

3.3. Femtosecond absorption experiments

The dynamics of the isomerization processes were investigated using femtosecond time resolved absorption measurements. In Fig. 4, the transient absorption changes are plotted for different wavelengths of the probing light pulses. These wavelengths are indicated by arrows in the spectrum of APB shown as an inset in Fig. 4d. The photoisomerization was initiated by excitation of the π–π^* transition of *trans*-APB in ethanol at 435 nm. The transient absorbance changes exhibit a weak component in the 100 fs regime, strong absorption changes occur with ≈0.9 ps. Additional kinetic components are found with time constants of ≈ 12 and 280 ps.

The electronically excited APB can be readily monitored in the long-wavelength wing of the *trans*-absorption band where neither *trans*-APB nor *cis*-APB absorb in their electronic ground state (see absorption spectrum, inset of Fig. 4d). The related time resolved experiment (Fig. 4d, λ_{pr} 540 nm) shows at time zero a strong initial absorption increase, indicative of a strong excited state absorption. Most of this induced absorption decays with $\tau_1 = 0.9$ ps. There is also a weak (rel. amplitude < 10%) additional component decaying within several picoseconds. The best fit is obtained with a 3 ps kinetic component.

Probing APB at 400 nm (in the short wavelength wing of the *trans*-APB band) reveals an initial absorption decrease due to the bleaching of the ground state absorption. After an initial ≈ 100 fs transient, a weak 0.9 ps component is seen at this spectral position. Apparently, excited state absorption and ground state recovery, that are expected to show the same temporal behaviour, but contribute with different signs to the transient signal, cancel to a large extent at 400 nm. At longer delay times, the absorption recovers partially with lifetimes

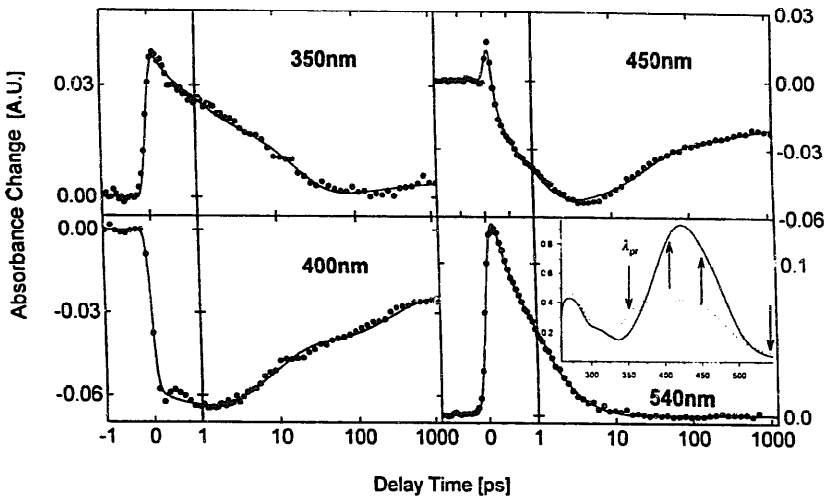

Fig. 4. Transient absorption changes of APB in ethanol at four selected spectral positions (see inset). All experiments are performed with parallel polarisation of pump and probe pulses. Data points are plotted as dots, best fit curves as lines. Note that the timescale is linear between −1 and 1 ps and logarithmic for longer delay times.

of ≈12 and 280 ps. Anisotropy experiments clearly show that the 280 ps process is due to the reorientational motion of the APB molecule. The absorption decrease remaining at the very late delay times is of special interest. This decrease can be related to the loss of strongly absorbing *trans*-APB molecules and the formation of weaker absorbing *cis*-APB molecules. At 450 nm, on the long-wavelength side of the *trans*-APB absorption band a similar bleaching, due to the *trans* to *cis* reaction, is found at late delay times. At short delay times we find contributions of the 0.9 and 20 ps component with opposite signs. Of further interest are results recorded at 350 nm, i.e., in the spectral region, where *cis*-APB absorbs stronger than *trans*-APB. Here, one finds at late delay times a weak absorption increase due to the *cis*-APB molecules formed during the reaction. Earlier signals are dominated by the 0.9 and 12 ps processes.

The values of the intermediate ps-time constants should only be taken as a rough description of the related process. A decay time that varies with the spectral position would be expected from the vibrational cooling of hot molecules, analogous to the situation reported for stilbene [21]. Correspondingly, in APB the time constant is shorter for wavelengths further away from the absorption maximum of the steady state spectrum. In addition, the spectral dependence of the amplitude of this component further supports the idea of molecular cooling.

A very rough estimate of the quantum yield can be obtained by considering the energy of the excitation pulse (1 μJ), the absorption cross section of the APB molecule and the long lasting absorbance changes at 400 and 450 nm. A value of (20±10)% was obtained for the *trans* to *cis* isomerization, which compares well to the quantum yield found for azobenzene [12].

3.4. Interpretation of the femtosecond absorption data

The time-resolved experiments exhibit three different kinetic processes: A weak, ultrafast, $\tau \leq 100$ fs, process points to an initial reactive motion out of the initially populated Franck–Condon regime. Similar kinetic processes have been observed in a number of isomerization reactions [19,20]. The 0.9 ps reaction is linked to the decay of an intense excited state absorption. We presume, that this kinetic process represents the decay of the excited electronic state to the ground state and should be indicative of the isomerisational motion. The 3–15 ps component is connected with an absorption increase in spectral regions of high *trans*-APB absorption while it is related with absorption decrease in the wings of the *trans*-APB absorption band. We tentatively assign this component, because of the spectral signature, the observed wavelength dependence and the wavelength dependent time constants, to a cooling of APB molecules in the electronic ground state, where a considerable amount of the photon energy is used to heat the molecules. Although we cannot rule out the possibility that a short lived (picosecond) intermediate state as proposed for azobenzene in [7] causes the absorbance changes: The dispersive character of the ps kinetics and the spectral dependence argue against the assumption of an intermediate as the only cause of this component.

In conclusion, we have shown that APB undergoes extremely rapid photoisomerization from the *trans* to the *cis* form after excitation of the π–π^* transition at 435 nm. The observed absorption changes strongly indicate that isomerization proceeds within ≈1 ps.

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