Photoswitchable Elements within a Peptide Backbone—Ultrafast Spectroscopy of Thioxylated Amides

H. Satzger,[†] C. Root,[†] P. Gilch,[†] W. Zinth,*[,†] D. Wildemann,[‡] and G. Fischer[‡]

BioMolekulare Optik, Department für Physik, Ludwig-Maximilians-Universität München, Oettingenstrasse 67, D-80538 München, Germany, and Max Planck Research Unit for Enzymology of Protein Folding, Weinbergweg 22, D-06120 Halle/Saale, Germany

Received: October 25, 2004; In Final Form: December 23, 2004

A series of thioxo compounds, thioacetamide, *N*-methylthioacetamide, a cyclic thioxoamide [(*S*)-5-thioxopyrrolidine-2-carboxylic acid ethyl ester], two thioxylated dipeptides (Ala-Ψ[CS-NH]-Ala and Phe-Ψ[CS-NH]-Ala) and a thioxylated dodecapeptide (Lys-Glu-Thr-Ala-Ala-Ala-Lys-Phe-Glu-Arg-Gln-His-Ψ[CS-NH]-Nle-Asp-Ser-Ser-Thr-Ser-Ala-Ala, or [thioxo-His^{12}]-S-peptide; Nle = norleucine) are investigated by ultrafast spectroscopy in the visible and near UV. The different molecules show very similar absorption dynamics featuring a rise of a strong visible absorption band on the subpicosecond and picosecond time scale. The decay of the visible absorption occurs within 150−600 ps. The observations are interpreted by the ultrafast formation of triplet states and their decay on the subnanosecond time scale. Comparison with published IR experiments on *N*-methylthioacetamide indicates that the cis−trans isomerization around the thioxopeptide bond is terminated within less than 1 ns.

1. Introduction

Protein folding is one of the most important still unresolved processes in the cell. Folding bridges the gap between the information held in the genetic sequence and protein structure. Its detailed understanding—the possibility to predict protein structure and function from a genetic sequence—would open unprecedented applications in biotechnology and medicine (for a recent review on the topic see ref 1). On the way to an understanding of folding processes, experiments are undertaken to study structural dynamics occurring in small proteins or oligopeptides. These experiments should yield experimental information to test and to improve simulation and prediction methods. Dynamic experiments on short time scales rely on the possibility to induce structural changes at a well-defined starting time by an external triggering event. Most interesting for investigations of the rapid processes in protein folding are optical techniques, where structural dynamics can be induced by the absorption of an ultrashort light pulse and where the structural changes are followed by optical or infrared spectroscopy. Two different molecular approaches are currently undertaken: (i) The trigger molecule, for example, an azobenzene, is attached to side chains or the termini of a peptide chain.[2] Light absorption and isomerization of the dye then induces a local strain on the amino acid chain, causing a modification of the secondary structure. (ii) Another more direct trigger process occurs when the switched moiety is part of the polypeptide backbone. In this case, a direct switching of secondary structure elements of the protein may result. It has been shown recently that the incorporation of modified azobenzene molecules in cyclic peptides allowed the structure of cyclic peptides to switch reversibly between two families of configurations.[3,4] While the incorporation of photoswitchable dye molecules in a peptide or protein chain opens up a wide range of important applications, two major difficulties result: The dye molecules impose distinct changes of the local or global protein structure. Even if their geometry is optimized, they still may deviate from the size and structure of the replaced amino acid residue. In addition, the different chemical properties may impose problems with biocompatibility. To prevent these difficulties, isosteric thioxopeptides have been used that allow predictable structural changes by photoswitching. Peptide bond thioxylation is characterized by the replacement of the carbonyl oxygen at one or more defined peptide bonds by a sulfur atom. Generally, thioxopeptides showed minimum deviation from the biological properties of the parent oxopeptide structures:[5,6] In this way the absorption spectrum of the peptide group is red-shifted from a range <230 nm to ∼260 nm for the $\pi\pi^*$ transition. Light absorption at this wavelength induces trans to cis photoisomerization without photodecomposition. In short thioxopeptides, the absorption of the cis isomer is slightly red-shifted (5 nm) as compared to the trans form. In the dark, reequilibration was characterized by a relaxation time of about 10^3 s, a time by far long enough to study the functional modifications caused by the isomer-induced backbone rearrangement.[7] Illumination around 300 nm induces the accelerated re-formation of the trans isomer.

Oligopeptide derivatives of thioamides are small molecules containing the thioxylated peptide bond and allow one to study the basic photophysical properties of a biologically relevant moiety. The photochemistry of one of the simplest thioacetamides that can undergo photoisomerization—*N*-methylthioacetamide, or NMTAA—has recently been studied by Helbing et al.,[4] employing femtosecond transient absorption and IR spectroscopy. In these experiments the spectroscopic changes occurring upon $\pi\pi^*$ excitation of *trans*-NMTAA have been monitored. Transient IR spectra indicate that the formation of the cis isomer and the recovery of the ground-state trans form has biphasic kinetics. Time constants of 8.5 and 250 ps have been determinded for the two phases. The ratio between cis

* Corresponding author: tel +49 89 21809201; fax +49 89 21809202; e-mail zinth@physik.uni-muenchen.de.
[†] Ludwig-Maximilians-Universität München.
[‡] Max Planck Research Unit for Enzymology of Protein Folding.

10.1021/jp045151t CCC: $30.25
Published on Web 02/17/2005

formation and trans recovery is about 30% for either phase. Surprisingly, only the 250-ps component recurs in the transient absorption experiment in the visible region. This experiment on the other hand features kinetics with a time constant of ca. 1 ps, which might have been missed in the IR experiment due to a lower time resolution. Setting these details of the photokinetics aside, these results suggest that the incorporation of a thioxoamide group in a peptide should allow for the photoinduction of conformational changes on the 100 ps time scale.

Here, we will present femtosecond transient absorption studies on three linear peptides where one peptide bond has been replaced by its thioxo congener. These experiments will show that the trans−cis isomerization of thioxopeptides proceeds in a similar fashion as in NMTAA. Furthermore, we looked at other thioxoamides in an attempt to shine light on the mechanism of the isomerization. Of particular interest is a cyclic thioxoamide where the isomerization pathway is blocked.

2. Materials and Methods

Spectroscopy. Stationary ultraviolet and visible absorption spectra were recorded on a Lambda 19 (Perkin-Elmer) spectrophotometer. For the femtosecond absorption experiments a homemade Ti-sapphire laser chirped pulse amplifier (CPA) system was used, which delivers pulses at a wavelength of 800 nm and a repetition rate of 1 kHz. The excitation pulses at $\lambda =$ 265 nm (energy $\sim 0.5\ \mu J$) are generated by frequency tripling (frequency doubling to 400 nm and subsequent mixing with the 800 nm fundamental pulse) of the laser output or by frequency doubling of light pulses at 530 nm generated by a non-collinear optical parametric generator (NOPA).[8] The excitation pulses were focused onto the sample cell at a diameter of ca. 0.2 mm. The absorption changes generated by the excitation process are recorded by a femtosecond white light continuum generated from a small part of the fundamental at 800 nm focused onto a CaF_2 plate.[9] The relative (linear) polarization of the white light probing and the excitation pulses was at the magic angle. In most experiments probing was performed between 340 and 750 nm. The temporal resolution of the pump−probe experiment was deduced from the transient absorption changes of the dye *p*-terphenyl. A fit of this rise, performed under the assumption that *p*-terphenyl responds instantaneously, yields an instrumental response time in the range of 200 fs. The optical density of the sample solutions was adjusted to be 1 OD at the excitation wavelength of 265 nm, so that the excitation energy is almost completely absorbed halfway through the optical path length. After the transient absorption of the sample solution was measured, the experiment was repeated with the neat solvent. In the wavelength region above 500 nm, signal contributions of the solvated electron could be observed both in ethanol and in water. Scaling the transient absorption of the solvent by a factor of 0.5 and subtracting this signal from the transient absorption of the sample solution could nicely remove the signal contributions of the solvent. Delay time zero for the individual probe wavelengths was determined by a Sellmeier fit to the (chirped) delay time zeroes of the solvent signal. The data shown have been corrected for the chirp of the probe light and signal contributions from the solvent. The complete set of data was recorded over a longer period of time. Therefore, the excitation conditions and accordingly the signal magnitudes for different samples varied and should not be compared.

Samples were dissolved in absolute ethanol except for the [thioxoHis^{12}]-S-peptide, which was dissolved in aqueous phosphate buffer (2.8 mM, pH 7). The concentrations were adjusted to arrive at an optical density of ∼1 OD at the excitation wavelength of 265 nm (this corresponds to concentrations of 1−3 mM). The sample was exchanged between subsequent laser shots by pumping through a flow cell (fused silica windows, optical path length 0.5 mm) by a peristaltic pump. Care was taken to avoid accumulation of the cis product by utilizing sufficient sample amounts (∼5 mL).

Data Analysis. Qualitative information on the number and the time scales of the involved kinetic processes was obtained by a data visualization technique based on the logarithmic differentiation of absorption changes (LDAC).[10] In addition, by singular value decomposition, the number of involved kinetic components was also estimated. The thereby obtained information was used to derive starting values for a global least-squares fitting algorithm (Levenberg−Marquart) with sums of exponentials (convoluted with the instrument response function, which is assumed to have Gaussian shape; for additional information on global data analysis see, for example, refs 11 and 12).

Synthesis of Thioxylated Compounds. Thioacetamide was obtained from Fluka (Germany). It was used without further purification. Synthesis of thioxylated peptides was done according to Wildemann et al.[13] *N*-Methylthioacetamide and (*S*)-5-thioxopyrrolidine-2-carboxylic acid ethyl ester were synthesized according to the following procedure (all chemicals were purchased from Fluka, Germany): 500 mg (6.8 mmol) of *N*-methylacetamide and 200 mg (1.3 mmol) of (*S*)-5-oxopyrrolidine-2-carboxylic acid ethyl ester were each dissolved in 100 mL of tetrahydrofuran. After addition of 2 equiv of Lawesson reagent, the mixture was stirred for 2 h at 0 °C. The solvent was evaporated and the crude products were purified by reverse-phase high-performance liquid chromatography (RP-HPLC). Product identity was verified by electrospray ionization (ESI) mass spectrometry. *N*-Methylthioacetamide and (*S*)-5-thioxopyrrolidine-2-carboxylic acid ethyl ester were obtained in yields of 78% and 65%.

3. Results

The common motif of all investigated molecules is the thioxoamide group (see structures in Figure 1a). Three of them contain amino acid residues. Thioactamide (TAA) is the smallest of the investigated set. Due to the symmetric substitution of the nitrogen, no cis−trans isomers exist. In the cyclic thioxoamide CTA the five-membered ring restricts the thioxoamide bond angle ω to about 0°, thus preventing photoisomerization. The linear thioxo compounds of this study are known to show reversible photoisomerization about the C−N bond.[7,14] The stationary UV/vis absorption spectra of the investigated samples are similar. The exact band positions are given in Figure 1b. The thioxo group has a pronounced UV absorption band around 270 nm. In NMTAA this transition is slightly blue-shifted (260 nm) as compared to the other compounds (∼268 nm). The thioxylated dodecapeptide ([thioxoHis^{12}]-S-peptide) mimics a polypeptide chain where the thioxopeptide moiety is the linker between two long peptide segments, because the peptide bond between the amino acid residues His^{12} and Nle^{13} (norleucine) was thioxylated. When the UV absorption bands of NMTAA and the thioxo peptides are excited, pronounced and long-lasting changes of the absorption spectrum occur.[7,14] The original absorption band decreases and a new absorption band centered around 280 nm grows in. The difference absorption spectrum for Phe-Ψ[CS-NH]-Ala is shown in Figure 1c. The absorption change is caused by the trans to cis photoisomerization around the thioxo peptide bond of Phe-Ψ[CS-NH]-Ala resulting in 19%

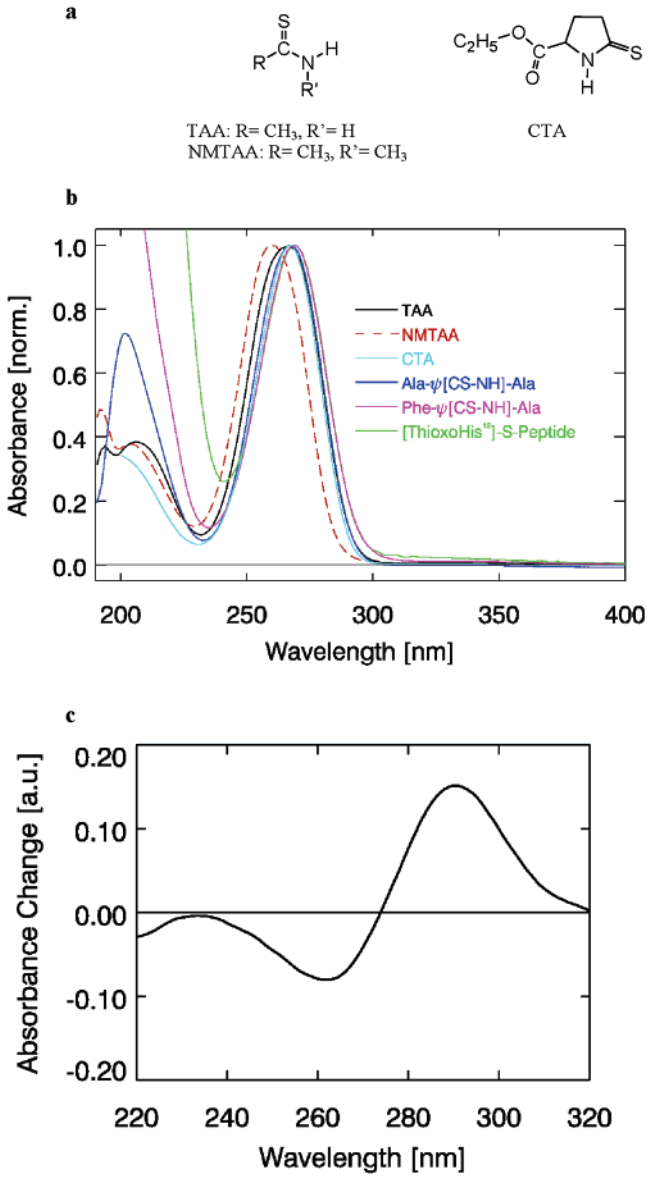

Figure 1. (a) Molecular structures of thioacetamide (TAA), *N*-methylthioacetamide (NMTAA), and the cyclic thioxoamide (*S*)-5-thioxopyrrolidine-2-carboxylic acid ethyl ester (CTA). (b) Absorption spectra of TAA, CTA, NMTAA, and the investigated peptides Ala-Ψ[CS-NH]-Ala, Phe-Ψ[CS-NH]-Ala, and [thioxoHis^{12}]-S-peptide. (c) Absorption change induced by cw illumination of the peptide Phe-Ψ-[CS-NH]-Ala.

cis isomer in the photoequilibrium.[7] In the dark the original absorption spectrum of Phe-Ψ[CS-NH]-Ala is recovered with a time constant of ~2000 s (sodium phosphate buffer, pH 7.0, 283 K).[7] The long lifetime of the cis isomer at this condition is equivalent to a large rotational barrier of the potential energy surface in the electronic ground state.

Excitation of the thioxo compounds with ~200 fs laser pulses centered at 265 nm results in spectrotemporal behavior that is qualitatively alike for all compounds. Exemplary results depicted in Figure 2 for *N*-methylthioacetamide NMTAA and Phe-Ψ-[CS-NH]-Ala illustrate this similarity. Immediately after excitation there is a spectrally broad absorption increase. As can be seen from the difference spectra recorded at distinct delay times t_D (Figure 2, second row), this absorption increase is large in the UV at 350 nm, but it extends throughout the whole visible region. Apparently this absorption represents the initially populated $\pi\pi^*$ state. Subsequently there is a pronounced change in the absorption spectrum on the time scale of ~0.5−5 ps, which leads to a spectrum with a pronounced absorption band centered in the visible region. This band peaks around 540 nm for all samples except for TAA and CTA, for which it is centered around 470 nm. These spectral features decay with time constants of some 100 ps. Numerical values were retrieved by a global fitting procedure that employs a sum of exponentials convoluted with the experimental response. The retrieved decay-associated spectra and time constants support the qualitative picture of the kinetic behavior (data are compiled in Table 1).

TABLE 1: Compilation of Time Constants and Absorbance Maxima[a] Obtained in the Global Analysis of Transient Absorption Experiments on the Thioxo Compounds

compound	τ_1, ps	τ_2, ps	τ_3, ps
TAA	0.4 (600, −)	3.9 (470, −)	500 (470, +)
NMTAA	0.8 (580, −)	3.8 (500, −)	280 (540, +)
CTA	0.3 (500, −)	2.4 (460, −)	670 (470, +)
Ala-Ψ[CS-NH]-Ala	0.3 (530, −)		180 (540, +)
Phe-Ψ[CS-NH]-Ala	0.2 (530, −)		120 (530, +)
[thioxoHis^{12}]-S-peptide	0.8 (540, −)		350 (530, +)

[a] Absorbance maxima in nanometers are given in parentheses; − indicates a rise, + indicates a decay.

For all compounds studied, a delayed rise of the transient absorption in the visible is retrieved. For TAA, NMTAA, and CTA this rise is noticeably biphasic with time constants of 0.3−0.8 ps (~0.5 ps in the following) and 2.4−3.9 ps (~4 ps in the following). The spectra associated with the two time constants have approximately equal amplitude but differ in shape. The ~0.5 ps spectra represent an absorption rise that peaks at longer wavelength (500−600 nm) than the ~4 ps spectra (460−500 nm). For the peptides the rise can be described single-exponentially with one time constant ranging from 0.2 to 0.8 ps. Notably, the decay-associated spectrum of this *one* rise component has its peak around 550 nm, that is, in between the peaks of the spectra associated with the *two* time constants retrieved for TAA, NMTAA, and CTA. The decay of the transient absorption in the visible can be described single-exponentially for all compounds. The time constants of this decay range from 120 ps (Phe-Ψ[CS-NH]-Ala) to 670 ps (CTA) (see Table 1). The differences between the compounds are clearly visible, when the transient data are spectrally integrated over the transient absorption band in the visible region (Figure 3). There is no obvious trend in the decay behavior. The decay is faster for the peptide Phe-Ψ[CS-NH]-Ala (120 ps) than for NMTAA (280 ps); the cyclic compound CTA (670 ps) shows a very similar time constant as TAA (500 ps).

The experimental results on NMTAA presented here are in line with the UV/vis femtosecond transients reported in ref 4. In both studies a rather intense excited-state absorption peaking around 350 nm is observed immediately after photoexcitation. With the decay of this peak, a new species with an absorption maximum at ~540 nm grows in. While we have determined two time constants of 0.8 and 3.8 ps for this transient, ref 4 gives one constant of 1.1 ps. Treating the rise in our data with just one exponential yields a time constant of 2 ps, in rather good agreement with ref 4. We note, however, that we find a pronounced biphasic character of the rise for NMTAA. In both studies, the decay of the 540 nm feature is found to occur with very similar time constants of 260 ps (ref 4) and 280 ps (this study).

4. Discussion

The experimental results may be summarized as follows: After the population of the excited electronic state, there is a

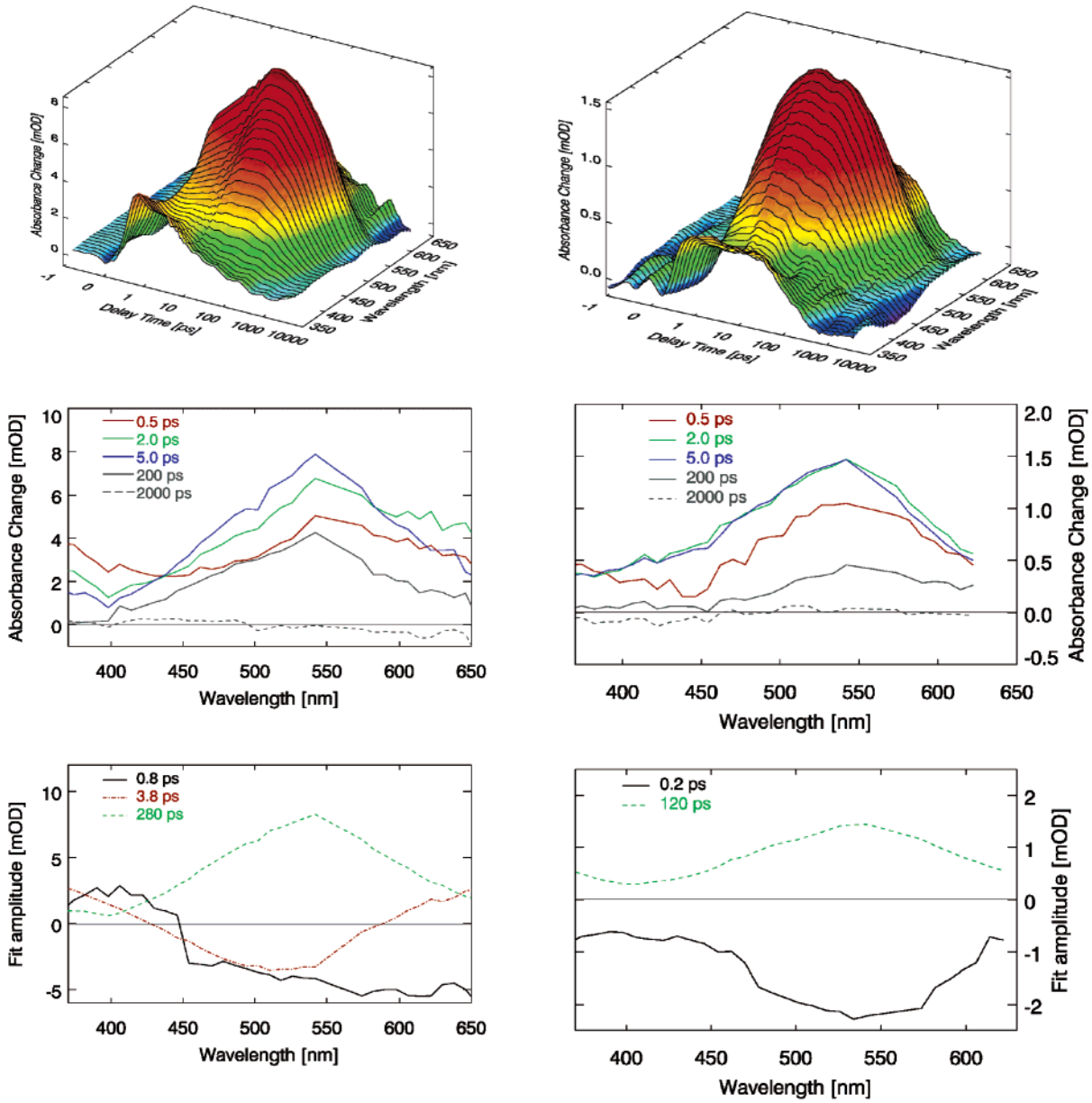

Figure 2. Femtosecond transient absorption data for NMTAA (left) and Phe-Ψ[CS-NH]-Ala (right). The first row gives an overview, the second row shows transient spectra at selected delay times, and the third row depicts decay-associated spectra obtained from a global analysis. Note that in the first row the time scale is linear from −1 to 1 ps and logarithmic thereafter.

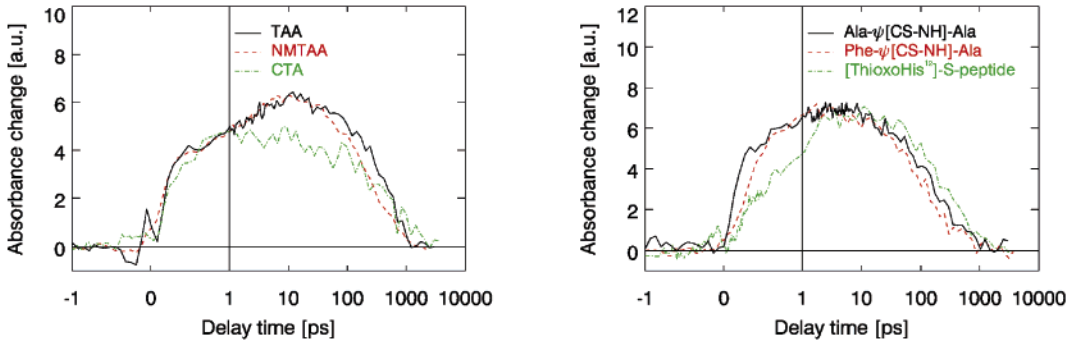

Figure 3. Temporal behavior of the spectral integral of the transient absorption peaking around 500 nm. Note that the time axis is linear from −1 ps to 1 ps and logarithmic thereafter. Data for TAA, NMTAA, and CTA are compiled in the graph on the left; those for the thioxylated peptides on the right.

rise of a strong visible absorption band centered around 550 nm on the (sub)picosecond time scale. While for TAA, NMTAA, and CTA this rise is distinctly biphasic, a single exponential suffices to describe this rise for the linear thioxopeptides. The spectra associated with the single-exponential rise resemble the "average" of the two rise spectra of the

thioxoamides. The decay of this absorption band occurs on the 100 ps−1 ns time domain. Aside from differences in the observed time constants, the different samples show very similar behavior. It can safely be stated that the spectrotemporal response of the investigated compounds is determined by their common "chromophore", namely, the thioxoamide group. The interpretation of the experimental findings therefore has to focus on the (electronic) properties of this group.

Helbing et al.[4] have performed quantum chemical calculations on the CASSCF level addressing the character of the excited states. In line with spectroscopic results, they have identified four excited states in the energetic range of interest. Two of these states have singlet multiplicity and nπ^* and $\pi\pi^*$ orbital character. The other two carry triplet multiplicity. As only the singlet $^1\pi\pi^*$ state shares a substantial transition moment with the ground state, it is the state initially populated. In their calculations Helbing et al. focus on structural distortions of the thioxoamide, which bring the different electronic states in energetic vicinity so that transitions between them can take place. Accordingly, important coordinates are the C═S bond elongation and the pyramidalization of the thiocarbonyl carbon. The torsion of the central thioxopeptide bond—the coordinate that connects the trans and the cis isomer—has virtually no influence on the energies of the excited states. This is in line with our finding that the "locked" thioxoamide CTA has very similar photokinetics as those undergoing unperturbed cis−trans isomerization. The data from the locked molecule CTA support the interpretation that the isomerization occurs during the deactivation of the electronically excited states but does not strongly promote this decay. The calculations of Helbing et al. also point to the fact that very similar deactivation patterns are expected if the reaction proceeds in states with triplet multiplicity. It seems very likely that indeed most of the observed kinetics are due to processes associated with triplet states.

It is well-known that, in molecules with close-lying $\pi\pi^*$ and nπ^* states, intersystem crossing can be very efficient since the spin flip can be accompanied by a change of the orbital character of the state (El-Sayed's rule[15]).[16] For example, in aromatic ketones, intersystem crossing times of well below 10 ps have been measured.[17,18] Recently, some of the present authors have reported an intersystem crossing time of 1 ps for xanthone, which is an aromatic ketone with a very rigid structure.[19] This 1 ps time constant has been assigned to a transition from a $^1\pi\pi^*$ to a ^{3}nπ^* state. That process is followed by a slower internal conversion within the triplet manifold populating the lowest triplet state, the $^3\pi\pi^*$ state. This process proceeds with a time constant of 10 ps. The behavior of the visible absorption of the thioxoamide compounds parallels that of xanthone. For the thioxoamides (TAA, NMTAA, and CTAA), biphasic rise behavior was observed. The spectral signatures of the rise of the peptides indicate that for them the two time constants are so close that they are not distinguished. Thus, by analogy we assign the time constants of a few hundred femtoseconds to an intersystem crossing (ISC) that populates the ^{3}nπ^* state (cf. Figure 4). This is even faster than the value reported for xanthone. The fast ISC process in xanthone is assumed to be due to the oxygen in the carbonyl group. For the thioxoamides the sulfur is responsible for the fast ISC. Thus the acceleration of ISC in the thioxoamides is not surprising since spin−orbit coupling, which mediates the ISC, is higher by a factor of 2 in sulfur as compared to oxygen.[20] This ISC process should then be followed by an internal conversion (IC) process in the triplet

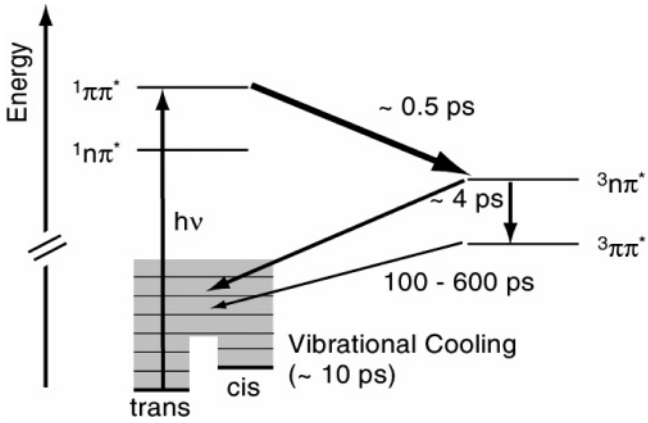

Figure 4. Scheme illustrating the relative energetic ordering of the excited states of the thioxo compounds and possible deactivation paths following the excitation of the $^1\pi\pi^*$ state. The ordering of the singlet states is derived from the spectroscopic results.[25] In analogy to aromatic thiocarbonyls, an inversion of the nπ^* and $\pi\pi^*$ states in the triplet manifold is assumed.

manifold, that is, a ^{3}nπ^* to $^3\pi\pi^*$ transition. For the thioxoamides TAA, NMTAA, and CTA we associate the 3−4 ps component with this transition. For the linear thioxopeptides within this reasoning this IC process is somewhat faster so that kinetically it cannot be distinguished from the ISC. The final recovery of the electronic ground state, either in trans or in cis form, occurs then by another ISC process on the 100 ps−1 ns time scale. This above assignment requires an inversion of the triplet states, that is, the $^3\pi\pi^*$ has to lie below the ^{3}nπ^* state. To our knowledge there are no spectroscopic data on the energetics of the triplet state of thioxoamides. Yet there are results on aromatic thiocarbonyls[21] stating that in polar solvents such an inversion occurs. An inversion for the thioxoamides is thus at least not unlikely.

On the basis of the assumption that triplet states play an essential role in the excited-state deactivation, one can speculate on the role of the spin state for the recovery of the ground state and the biphasic formation of the cis isomer reported by Helbing et al.[4] (cf. Figure 4). According to El-Sayed's rules, a transition from the ^{3}nπ^* state to the ground state should be faster than from $^3\pi\pi^*$. Thus, one might envision a branching during the decay of the ^{3}nπ^* state: both the $^3\pi\pi^*$ state would be populated and the ground state would be recovered. Upon this ISC process a large amount of energy (2−3 eV) has to be transferred into nuclear degrees of freedom, leading to a strong vibrational excitation of the ground state. Such a vibrational excitation is known to relax in the 10 ps time domain (see, for example, refs 22−24). This reaction scheme can be used to explain the fast component in the isomerization process observed in the IR experiments: Isomerization may occur either directly during the ISC process or from the vibrationally excited ground state. In the first case, the manifestation of the cis ground-state spectrum could be delayed by the vibrational cooling time. In the second case, isomerization proceeds in parallel with the 10 ps cooling process. Both interpretations could explain why the 10 ps component found in the time-resolved IR-experiments is not detected by UV/vis spectroscopy. The part of the population that has arrived in the $^3\pi\pi^*$ state is "trapped", since from here ISC to the ground state is not El-Sayed-allowed. In our considerations we focused on spin effects in order to obtain a consistent picture of the observed transients in the thioxoamides. This does not imply that nuclear motions such as those

pointedout by Helbing et al.[4] do not leave a kinetic imprint and may be important to explain the observed transients. At the present level of experimental information, no definite decision on the molecular mechanisms can be made.

Finally, we turn to the isomerization of the linear thioxopeptides. As we have stated above, their kinetics are—besides the less pronounced picosecond transient—essentially identical to those observed in NMTAA. We could not directly detect the formation of the cis isomer of the peptides, since its spectroscopic signature lies too far in the UV for our setup. From steady-state experiments it is evident that the cis form of the peptide is formed.[7] For NMTAA the IR experiments have shown[4] that the cis formation is finished within 250 ps, a value that is also present in the experiments with visible detection. These kinetics support the standard picture of photoisomerization: Since the barrier for isomerization in the electronic ground state potential surface of the thioxoamides is too high to allow rapid ground-state isomerization of thermalized molecules at room temperature, the isomerization has to occur in the excited electronic state, during the transition to the ground state or immediately afterward, in the vibrationally hot ground state. Thus, it is very likely that the processes on the 100 ps time scale observed here for the peptides terminate the formation of the cis isomer. This shows that the incorporation of the thioxoamide group in oligopeptides of 20 amino acid in length, at least, indeed allows us to induce and to study peptide dynamics on time scales of >100 ps.

In conclusion, we have investigated a series of thioxo compounds by visible and near-UV absorption spectroscopy. We have shown that peptides containing a thioxo peptide bond show very similar absorption kinetics as thioacetamide and *N*-methylthioacetamide. After excitation of the $\pi\pi^*$ transition, we observe absorption transients on the 1 ps and ca. 300 ps time scale. The initially excited electronic state decays on the subpicosecond time scale into an intermediate state, most probably a triplet state. The visible absorption changes recover with time constants of 120−670 ps. Apparently the cis−trans isomerization of the investigated thioxo compounds is terminated within this time.

References and Notes

(1) Dobson, C. M. *Nature* **2003**, *426*, 884.
(2) Kumita, J. R.; Smart, O. S.; Woolley, G. A. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 3803.
(3) Spörlein, S.; Carstens, H.; Satzger, H.; Renner, C.; Behrendt, R.; Moroder, L.; Tavan, P.; Zinth, W.; Wachtveitl, J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 7998.
(4) Helbing, J.; Bregy, H.; Bredenbeck, J.; Pfister, R.; Hamm, P.; Huber, R.; Wachtveitl, J.; De Vico, L.; Olivucci, M. *J. Am. Chem. Soc* **2004**, *126*, 8823.
(5) Sifferlen, T.; Rueping, M.; Gademann, K.; Jaun, B.; Seebach, D. *Helv. Chim. Acta* **1999**, *82*, 2067.
(6) Frank, R.; Jakob, M.; Thunecke, F.; Fischer, G.; Schutkowski, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 1120.
(7) Zhao, J. Z.; Wildemann, D.; Jakob, M.; Vargas, C.; Schiene-Fischer, C. *Chem. Commun.* **2003**, 2810.
(8) Piel, J.; Beutter, M.; Riedle, E. *Opt. Lett.* **2000**, *25*, 180.
(9) Huber, R.; Satzger, H.; Zinth, W.; Wachtveitl, J. *Opt. Commun.* **2001**, *194*, 443.
(10) Satzger, H.; Zinth, W. *Chem. Phys.* **2003**, *295*, 287.
(11) Beechem, J. M.; Ameloot, M.; Brand, L. *Anal. Instrum.* **1985**, *14*, 379.
(12) Holzwarth, A. R. Data analysis of time-resolved measurements. In *Biophysical Techniques in Photosynthesis*; Amesz, J., Hoff, A. J., Eds.; Kluwer: Dordrecht, The Netherlands, 1996.
(13) Wildemann, D.; Drewello, M.; Fischer, G.; Schutkowski, M. *Chem. Commun.* **1999**, 1809.
(14) Ataka, S.; Takeuchi, H.; Harada, I.; Tasumi, M. *J. Phys. Chem.* **1984**, *88*, 449.
(15) El-Sayed, M. A. *J. Chem. Phys.* **1963**, *38*, 2834.
(16) Turro, N. J. *Modern Molecular Photochemistry*; The Benjamin/Cummings Publishing Co., Inc.: Menlo Park, CA, 1978.
(17) Damschen, D. E.; Merritt, C. D.; Perry, D. L.; Scott, G. W.; Talley, L. D. *J. Phys. Chem.* **1978**, *82*, 2268.
(18) Cavaleri, J. J.; Prater, K.; Bowman, R. M. *Chem. Phys. Lett.* **1996**, *259*, 495.
(19) Satzger, H.; Schmidt, B.; Root, C.; Zinth, W.; Fierz, B.; Krieger, F.; Kiefhaber, T.; Gilch, P. *J. Phys. Chem. A* **2004**, *108*, 10072.
(20) Murov, S. L.; Carmichael, I.; Hug, G. L. *Handbook of Photochemistry*; Marcel Dekker: New York, Basel, and Hong Kong, 1993.
(21) Maciejewski, A.; Szymanski, M.; Steer, R. P. *Chem. Phys. Lett.* **1988**, *143*, 559.
(22) Seilmeier, A.; Kaiser, W. Ultrashort Intramolecular and Intermolecular Vibrational Energy Transfer of Polyatomic Molecules in Liquids. In *Topics in Applied Physics*; Kaiser, W., Ed.; Elsevier: Amsterdem, 1993; Vol. 60.
(23) Kovalenko, S. A.; Schanz, R.; Hennig, H.; Ernsting, N. P. *J. Chem. Phys.* **2001**, *115*, 3256.
(24) Schrader, T.; Sieg, A.; Koller, F.; Schreier, W.; An, Q.; Zinth, W.; Gilch, P. *Chem. Phys. Lett.* **2004**, *392*, 358.
(25) Maciejewski, A.; Steer, R. P. *Chem. Rev.* **1993**, *93*, 67.