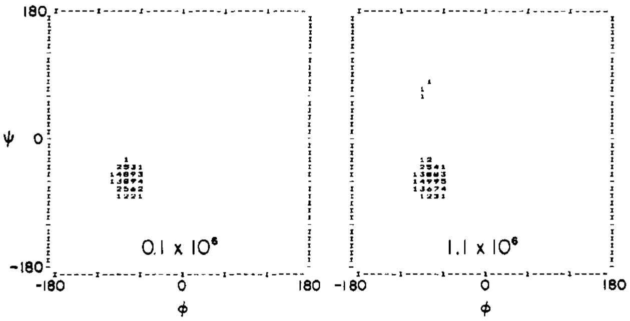

Figure 7. Chain of 12 glycines (no. 13) started and remaining in a right-handed helical state.

Monte Carlo method to simulate the arrangement of water molecules around fixed conformations of a blocked residue.

To strike a more optimistic note, the results of the simulation are encouraging. For the first time, it has been shown that an empirical potential function such as that of ECEPP contains the correct ingredients to fold polypeptides into helices. While the use of such a detailed model in simulations of protein molecules would seem to be beyond the limits of feasibility, it ought to be possible to use simulations of the kind described here to simplify the polypeptide model in ways which do not alter its structural preferences significantly. This simplified, but still physically meaningful model would then be the subject of further simulation, with appropriate solvent effects, in an attempt to emulate the protein-folding phenomenon.

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Photoresponsive Polymers. On the Dynamics of Conformational Changes of Polyamides with Backbone Azobenzene Groups

Masahiro Irie† and Wolfram Schnabel*

Hahn-Meitner-Institut für Kernforschung Berlin GmbH, Bereich Strahlenchemie, D-1000 Berlin 39, Federal Republic of Germany. Received April 6, 1981

ABSTRACT: Continuous irradiations and flash photolysis experiments have been carried out with *N,N*-dimethylacetamide and *N,N*-dimethylformamide solutions of a polyamide having azobenzene groups in the polymer backbone and with a low molecular weight model compound. The continuous irradiations revealed that trans → cis isomerization occurs upon irradiation with UV light (350–410 nm) whereas cis → trans isomerization can be induced by visible light (λ >470 nm). The kinetics of cis → trans isomerization were studied by time-resolved optical absorption measurements. It turned out that this process occurred to about 90% during the 20-ns flash (530-nm light). The remainder was completed within 100 ns after the flash, indicating the occurrence of a relatively slow relaxation of chain segments. The conformational change of the total macromolecule, subsequent to cis → trans isomerization, was studied by time-resolved light scattering measurements. Relaxation times covering the range from 0.47 (neat DMF) to 1.1 ms (DMF/ethanol, volume ratio 3:2) were found, the difference reflecting the influence of solvent quality.

Introduction

Conformational changes of linear macromolecules in solution have attracted the interest of many researchers in the past.[1] Their work was mostly devoted to polyelectrolytes, and usually equilibrium properties were measured. Comparatively little is known about the dynamics of conformational transitions. In the studies reported so far, stopped-flow or temperature jump techniques in conjunction with optical rotation,[2] circular dichroism,[3] and light scattering[4] measurements have been employed. Usually, the time resolution was not better than 10^{-3} s.

Optical absorption or emission proved to be insensitive for the observation of conformational changes.[5]

In previous papers[6] one of us (M.I.) has reported that linear polyamides with azobenzene groups in the backbone undergo conformational changes upon irradiation with

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† On leave from The Institute of Scientific and Industrial Research, Osaka University, Suita, Osaka, Japan.

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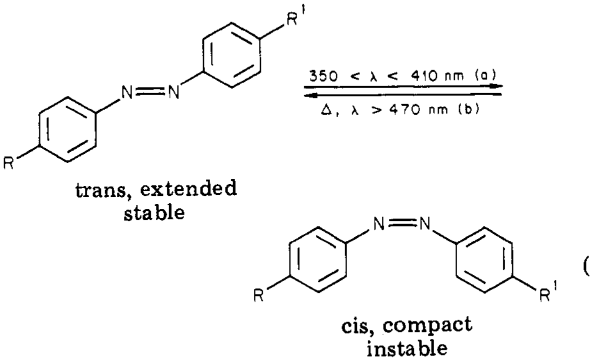
light as a consequence of cis–trans isomerization of the azobenzene groups. As illustrated by reaction 1, the trans form can be converted to the cis form upon UV light irradiation (350 < λ < 410 nm). Cis → trans isomerization, on the other hand, is achieved either by heating or by irradiation with visible light (λ >470 nm):



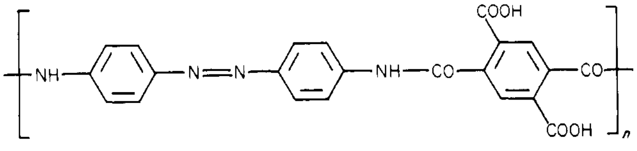
(1)

If each repeating unit of the polymer contains an azobenzene group and if a major portion of these groups is in the trans configuration, the polymer chains are extended. Vice versa, the chains form rather compact coils when a major portion of azobenzene groups is converted to the cis form. Concurrent with conformational changes are changes of various solution properties, such as intrinsic viscosity, electrical conductivity, and pH.

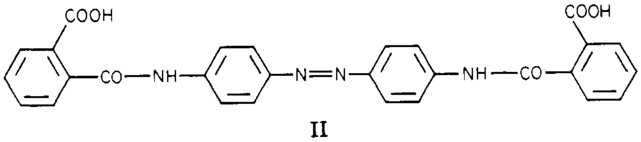
This paper reports results of laser flash photolysis studies aiming at measuring the rate of chain unfolding. For this purpose the light scattering detection method appeared appropriate because of its capability of recording changes of molecular size. The subject of this investigation was the cis → trans isomerization according to reaction 1b. The polyamide was irradiated with single 20-ns flashes of 530-nm light and the rate of the light scattering intensity change at 514 nm was measured. Moreover, the kinetics of the isomerization reaction were followed by recording optical absorption changes during and after the flash.

Experimental Section

A. Materials. The polyamide I and the low molecular weight model compound II were used throughout this work. The polyamide I was synthesized by polycondensation of p,p'-diaminoazobenzene and pyromellitic dianhydride.[6b] p,p'-Diaminoazobenzene was prepared according to Witt and Kopitschin.[8] Pyromellitic dianhydride was purified by vacuum sublimation. Further details about the synthesis of I and II have been reported before.[6b] The average molecular weight of I has been determined as $\bar{M}_w = 8.3 \times 10^3$ ($dn/dc = 0.262$ mL/g in N,N-dimethylformamide with the aid of a Chromatix low-angle light scattering instrument (KMX-6).



I



II

N,N-Dimethylacetamide was purified as described before.[6b] N,N-Dimethylformamide (Uvasol, E. Merck) was used without further purification.

B. Irradiations. For continuous irradiations a high-pressure mercury lamp (HBO 500 W) was used. Appropriate wavelengths were selected with the aid of Toshiba cutoff filters. Ground-state absorption spectra were recorded with a Perkin-Elmer spectrophotometer (Model 550S).

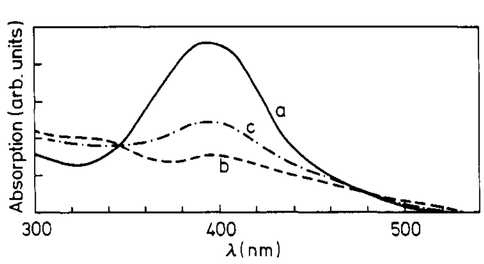


Figure 1. Absorption spectra of polyamide I obtained with N,N-dimethylacetamide solutions: (a) polymer in thermal equilibrium at 22 °C, predominantly trans form; (b) polymer in the photostationary state under UV light (410 > λ > 350 nm), predominantly cis form; (c) polymer after irradiation with several flashes of 530-nm light, initially possessing spectrum b.

For flash photolysis studies a Nd/YAG laser was used (λ 530 nm after frequency doubling).[9] The light scattering (LS) detection system was the same as that described before.[10] Due to a strong emission the time resolution of the LS detection system was limited to about 30 μs. This did not interfere with our measurements because the lifetime of the observed relaxation processes was much longer.

Results and Discussion

A. Cis–Trans Isomerization As Studied by Optical Absorption Measurements. Figure 1 shows absorption spectra obtained with N,N-dimethylacetamide solutions of polymer I. Spectrum a was recorded with the nonirradiated polyamide I. The cis–trans ratio corresponds in this case to the thermal equilibrium achieved at 22 °C, where the trans content dominates over the cis content. Therefore spectrum a is mainly due to the trans form and the peak at 390 nm is ascribed to the trans form. Continuous irradiation with UV light (350–410 nm) leads to spectrum b as a consequence of trans → cis isomerization. When the polymer solution was irradiated with several laser flashes at 530 nm immediately after attaining the photostationary state by irradiation with UV light, spectrum b converted to spectrum c. The significant spectral changes of cis → trans isomerization induced by the 530-nm flashes are *decreasing absorptions* at 320 and 510 nm and an *increasing absorption band at* 390 nm. Isosbestic points are located at 346 and 480 nm. Quite similar observations were made with the model compound II. The content of *cis*-azobenzene groups in the stationary state under UV irradiation was estimated as 0.65 for the polyamide and as 0.75 for the model compound. The lower value observed in the case of the polymer indicates that trans → cis isomerization is impeded to some extent by steric hindrance.

The same conclusions were arrived at from the fact that the quantum yield for cis → trans isomerization in the case of the polymer is about 25% lower than that for the model compound. The respective measurements were carried out with N,N-dimethylformamide solutions of I and II after UV-induced trans → cis isomerization yielded an absorbance of 0.24 at 530 nm. The solutions were then irradiated with single 20-ns flashes of 530-nm light and the changes of the optical absorption (at 510 nm) 200 μs after the flash were compared.

Results of detailed flash photolysis studies concerning absorption changes of UV-pretreated solutions are presented in Figure 2, where difference spectra recorded 30 ns after a 20-ns flash of 530-nm light are shown. The difference spectra correspond exactly to spectral changes expected on grounds of the occurrence of cis → trans isomerizations as illustrated in Figure 1.

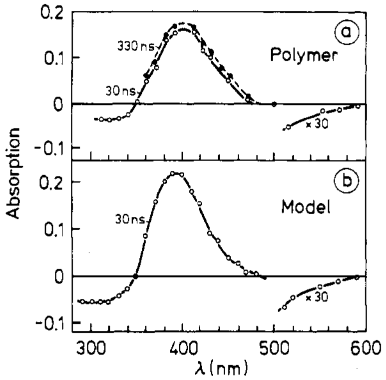


Figure 2. Transient difference spectra obtained upon irradiation of *N,N*-dimethylacetamide solutions of I (a) and II (b) with a 20-ns flash of 530-nm light. The concentration in both cases was 1.1 × 10^{-2} g/L.

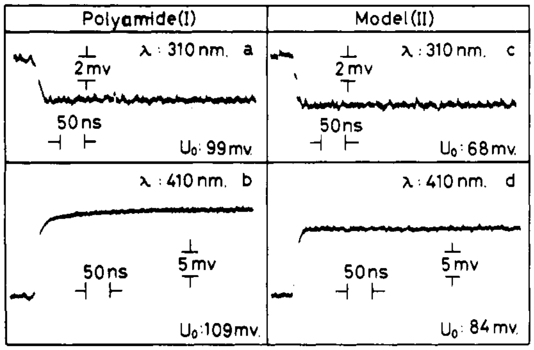


Figure 3. Cis → trans isomerization of polyamide I and model compound II at 22 °C in *N,N*-dimethylacetamide (1.1 × 10^{-2} g/L). The oscillograms illustrate the decrease and increase of optical absorption at 310 (cis form) and 410 nm (trans form) during and after irradiation with a 20-ns flash of 530-nm light.

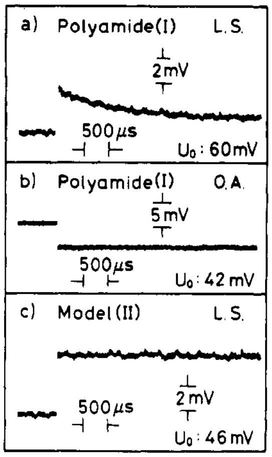


Figure 4. Chain unfolding and cis → trans isomerization of polyamide I in *N,N*-dimethylacetamide solution (0.31 g/L). The oscillograms illustrate changes of light scattering intensity (a, c) at 514 nm and optical absorption at 514 nm (b) during and after a 20-ns flash of 530-nm light. Traces a and b, polyamide I; trace c, model compound II.

It is interesting to note that in the case of the polymer the rapid spectral change during the flash is followed by a somewhat slower process, as shown in Figure 2a. This behavior is also illustrated in Figure 3, where oscillograms demonstrating the decrease and increase of the optical absorption during and after the flash at 310 and 410 nm, respectively, are shown. While the total change of the absorption occurs during the flash in the case of II, about 10% of the total change is completed—in the case of the polyamide—only during a period about 100 ns after the flash, corresponding to a relaxation time of 10^{-8} to 10^{-7} s. Obviously a small fraction of the electronically excited azobenzene groups cannot convert to the trans configuration without restraint, which might be caused by relatively slow motions of chain segments. In this connection, it is worth mentioning that from ultrasonic absorption measurements on solutions of synthetic polymers the occurrence of two relaxations in the range 10^{-8}–10^{-6} s has been inferred,[11] which was attributed to local cooperative conformational changes in the chains. Moreover, a relaxation time of about 10^{-8} s was observed for the intramolecular self-solvation in poly(methyl methacrylate) chains with pendant spirobenzopyran groups after photochemical conversion of the latter to merocyanine groups.[7]

B. Conformational Changes As Studied by Light Scattering Measurements. As will be shown below the rather rapid cis → trans isomerization of the azobenzene groups in the polymer caused a decrease of the light scattering intensity. Actually chain unfolding leads to an increase in the mean-square radius of gyration, $\langle s^2\rangle$, and might also cause a variation of the second virial coefficient, A_2. Prerequisite for the applicability of this consideration is that the molecular weight $\bar{M}_w$ remain unchanged. This can be seen from the light scattering equation that holds for coiled linear polymers in dilute solution:[12]

$$\frac{Kc}{R_\vartheta} = \frac{1}{\bar{M}_w} + \frac{16\pi^2\langle s^2\rangle}{3\lambda^2\bar{M}_w}\sin^2(\vartheta/2) + 2A_2c$$

Here $K = (2\pi^2 n_0^2/N_A\lambda_0^4)(dn/dc)^2$, c is the polymer concentration, R_ϑ is the Rayleigh ratio, n_0 is the refractive index of the solvent, dn/dc is the specific refractive index increment, λ_0 is the wavelength of incident light ($\lambda = \lambda_0/n_0$), and N_A is Avogadro's number.

Figure 4a shows a typical oscilloscope trace illustrating the change of the light scattering intensity during and after the flash. The experiment was carried out with a polyamide sample pretreated by UV irradiation for trans → cis isomerization. The trace in Figure 4a affords a careful analysis because it contains information on both cis → trans isomerization and the subsequently occurring conformational change. The rapid increase of the light scattering intensity reflects the concurrent decrease of the optical absorption at 514 nm, as depicted by the oscillogram in Figure 4b, and a change in the specific refractive index increment. The decrease of the light scattering intensity following the rapid increase in Figure 4a reflects the conformational change involving a decrease of $\langle s^2\rangle$ and possibly a change in A_2. The fact that the trace does not go down to the original base line is caused by a change in the specific refractive index increment. This base line shift occurs simultaneously with the change of the optical absorption, i.e., in less than 100 ns. It therefore does not interfere with the conformational relaxation occurring with a half-life of about 0.5 ms. For comparison, Figure 4c shows an oscillogram illustrating the change of the light scattering intensity observed in the case of the model compound II. This trace only reflects changes of the optical absorption and the specific refractive index increment.

Conjectures concerning the decrease of the light scattering intensity, in the case of the polymer, as being caused by main-chain scissions can be discarded because it has been shown previously that cis–trans isomerizations can be performed in both directions many times without detectable molecular weight changes.[6]

It is interesting to compare conformational relaxation rates measured with other systems by other techniques with the relaxation rate found in the present investigation.

For example, much slower conformational transitions have been observed with a copolymer of maleic acid and styrene in aqueous solution.[5] Also the unfolding of globular proteins, such as lysozyme,[13] proceeds relatively slowly. On the other hand, the helix-coil transition of charged poly(α-amino acids) in aqueous solution occurs much faster.[2]

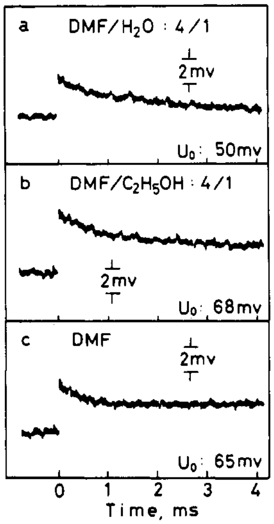


Figure 5. Chain unfolding of polyamide I in various solvent mixtures at 22 °C. The oscillograms illustrate changes of the light scattering intensity during and after the flash in (a) 4:1 (v/v) DMF/water, (b) 4:1 (v/v) DMF/ethanol, and (c) neat DMF. The polymer concentration was 0.29 g/L.

Table I
Conformation Relaxation of Polyamide Chains Subsequent to Cis → Trans Isomerization of Azobenzene Groups in the Backbone

solvent	$\tau_{1/2}$,[a] s
N,*N*-dimethylacetamide	5.8×10^{-4}
N,*N*-dimethylformamide (DMF)	4.7×10^{-4}
4:1[b] DMF-ethanol	7.2×10^{-4}
3:2[b] DMF-ethanol	11.0×10^{-4}
4:1[b] DMF-water	8.8×10^{-4}

[a] Half-lives of the decrease of the light scattering intensity at 514 nm and 22 °C. [b] Volume ratio.

Our studies were also devoted to another aspect, namely, the dependence of the rate of unfolding of polyamide I on the nature of the solvent. Typical oscillograms are shown in Figure 5, where one can see that in 4:1 (v/v) DMF/water and 4:1 (v/v) DMF/ethanol mixtures the rate of conformational change is lower than in neat DMF. The results can be interpreted in terms of a worsening of solvent quality, which causes a retardation of the rate of unfolding. All values obtained are compiled in Table I.

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