Blue Fluorescent Exciplexes Consisting of *trans*-Stilbene and Antibodies

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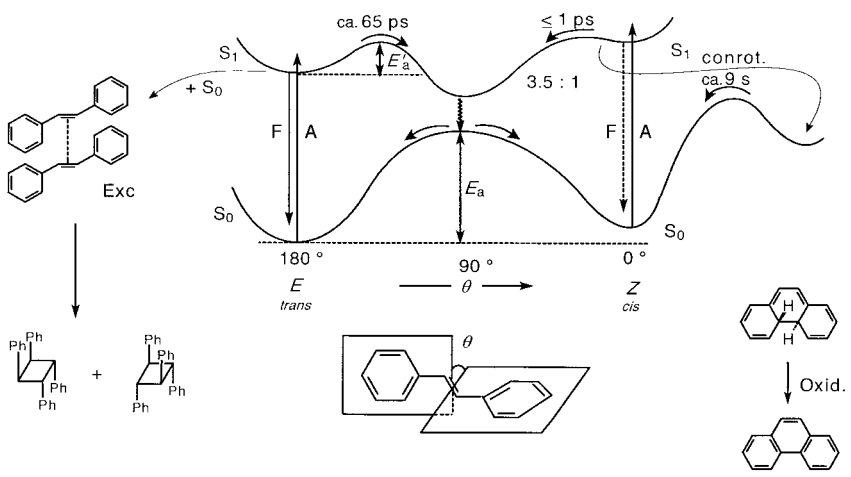
Stilbene and its derivatives belong to the best investigated molecules in organic photochemistry and photophysics.[1–4] Besides the variety of deactivation processes that follow an electronic excitation, it is mainly aspects of materials science [2] that have awakened interest in stilbenoid systems over the last two decades. Recently a completely new area of application was conceived by Simeonov et al.[5] They studied the influence of protein–ligand interactions on electronically excited states with monoclonal antibodies against stilbene haptens.[6]

Scheme 1 shows a survey of the photochemistry of stilbenes. The *trans/cis* isomerization (*E*/*Z* isomerization) has, in the ground state S_0, a high activation barrier ($E_a = 180 \pm 20\ kJ\,mol^{-1}$), which can be strongly reduced by a variety of catalysts. Much easier is the decoupling of the olefinic π bond in the electronically excited singlet state S_1. The first detailed reaction model was proposed by Saltiel[7] more than 30 years ago; all later models[1–4] have positioned the cross-section through the potential energy hypersurface S_1 in an analogous way, so that the torsion around the C–C double bond represents the decisive parameter on the reaction coordinate.



Scheme 1. An overview of the *E*/*Z* isomerization of stilbene and the competitive processes.[1–3] F = fluorescence, A = absorption.

The S_1 state has, on the side of the *E* configuration, an activation barrier E'_a, which can be rationalized by an avoided crossing with a higher excited singlet state. This barrier provokes temperature-dependent competition with the fluorescence. The fluorescence quantum yield Φ_F increases from 4–8% at room temperature to almost 100% at very low temperatures.[1, 2] A very small barrier exists on the side of the *Z* configuration; moreover, the deactivation by fluorescence plays an inferior role: $\Phi_F \approx 10^{-4}$ at room temperature in *n*-hexane.[8] The reaction coordinate on the S_1 potential energy hypersurface leads from both starting configurations to a state that has a $\theta = 90°$ angle of twist and whose average lifetime is in the femtosecond range. In the nonadiabatic photoreaction, the ground state S_0 is reached through a funnel,[9] whereby the rotation relaxation to the *E* or the *Z* configuration has about the same probability.[1, 2] As a competing reaction to the isomerization, a stereoselective dimerization via nonfluorescent excimers Exc has to be considered on the *trans* side; this process becomes the dominating product channel, especially at concentrations above 10^{-2} M. The conrotatory electrocyclic reaction that yields 4a,4b-dihydrophenanthrene represents a monomolecular competitive reaction on the *cis* side. The cyclization is thermally and photochemically reversible in the absence of oxidants. Internal conversion ($S_1 \rightarrow S_0$) and intersystem crossing ($S_1 \rightarrow T_1$) are not important, either for (*E*)- or (*Z*)-stilbene.[10, 11] The critical question to ask here is now: How do the properties, described above and summarized in Scheme 1, change when stilbene molecules are complexed in monoclonal antibodies?

To increase the solubility in water and to insure a better binding to the carrier protein, Simeonov et al.[5] used the glutaric acid monoamide derivative **1** instead of the unsubstituted stilbene; the electronic nature of **1** differs only slightly from that of the parent compound.

An intense fluorescence appeared under UV light during the stoichiometric addition of (*E*)-**1** to a number of mono-

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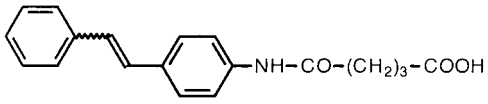


(*E*)- and (*Z*)-**1**

clonal antibodies (EP2 mAb: **19G2**, **20F2**, **21C6**, **16H10**, etc.). The color varies from powder blue to faint purple according to the complex mAb–(*E*)-**1**. From amongst the investigations with a total of 15 antibodies,[5, 12] two examples shall be selected here for comparison with the uncomplexed stilbene **1**: the blue fluorescent complex **19G2**–(*E*)-**1** and the purple fluorescent complex **16H10**–(*E*)-**1** (Table 1).

Table 1. Comparison of selected thermodynamic and spectroscopic data for **1** and its complexes with the antibodies **19G2** and **16H10** in an aqueous buffer solution[a] at 20–21 °C.[5, 12]

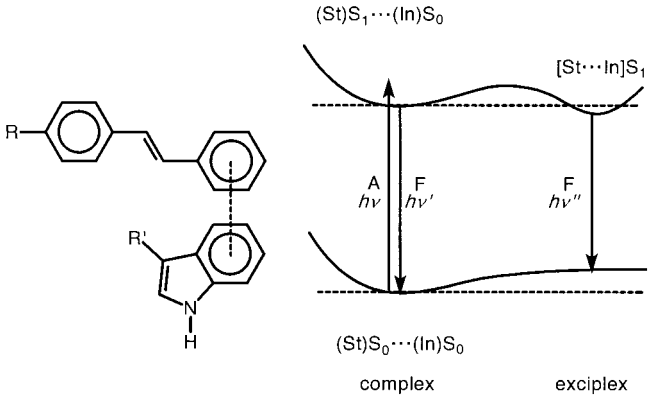
	(*E*)-**1**	**19G2**–(*E*)-**1**	**19H10**–(*E*)-**1**
dissociation constants:			
K_d ((*E*)-**1**) [μM]	–	0.16	0.31
K_d ((*Z*)-**1**) [μM]	–	1.7	2.3
UV absorption:			
λ_{max} [nm]	320	325	320
$10^{-4}\varepsilon_{max}$ [$cm^{-1}M^{-1}$]	3.32	3.15	2.82
fluorescence:			
λ_{max} [nm]	388	410	380
quantum yield Φ_F	0.02	0.78	0.28
fluorescence lifetime τ_F [ns]	0.071	22.9, 7.6, 1.1, 0.086	0.864, 0.375
intrinsic lifetime[b] τ [ns]	3.6	31	4.3
photostationary state:[c]			
E/*Z*	< 1	32.3	2.2

[a] Buffer solution: 10 mM Na_3PO_4, 150 mM NaCl (pH 7.4) with 5% *N*,*N*-dimethylformamide as cosolvent. [b] $\tau = \tau_F/\Phi_F$. [c] $\lambda \geq 300$ nm.

The dissociation constants of the complexes mAb–(*E*)-**1** have an average value of 0.37 μM at room temperature in the applied buffer solution; those of the corresponding complexes mAb–(*Z*)-**1** have a value of about 2.16 μM. With one exception, the *E* configuration of **1** is always bound a bit more strongly than the *Z* configuration. There is no significant difference between **19G2**–**1** and **16H10**–**1**. The X-ray measurements of **19G2**–(*E*)-**1** show that, at 4 °C, the hapten that is not covalently bound represents a planar (*E*)-stilbene, whose longitudinal axis points towards the center of the monoclonal antibody. The phenyl group, which is distal to the glutaric acid chain, lies parallel to the benzene ring of the indole belonging to tryptophan 103 on the H chain of the antibody. Therefore, the π,π interaction of the two benzene rings has a significant meaning.[13]

The UV and the fluorescence excitation spectrum of **19G2**–(*E*)-**1** exhibit a red-shift respective to free (*E*)-**1** (Table 1) and a modified vibrational fine structure at room temperature. Analogous effects can be found for the fluorescence band. However, much more dramatic is the increase of the fluorescence quantum yield Φ_F from 2% for (*E*)-**1** to 78% for **19G2**–(*E*)-**1**. On cooling, the fluorescence band of the complex **19G2**–(*E*)-**1** is hypsochromically shifted and becomes more structured. The main change occurs in the temperature interval between −13 and −33 °C where the intensity of the blue fluorescence decreases; the fluorescence has completely disappeared at −60 °C, but reappears on warming. This result is surprising and is contrary to the behavior of free (*E*)-stilbene. As shown in Scheme 1, cooling favors the fluorescence, because the activation barrier of the isomerization route is less easily overcome. The photostationary states of stilbene and the stilbene derivative **1** are characterized by *E*/*Z* ratios smaller than one for longwave irradiations ($\lambda \geq 300$ nm).[14] The $E \rightarrow Z$ photoisomerization[15] of **19G2**–(E)-**1** hardly appears at room temperature (Table 1). Hence, the stilbene **1** complexed in the antibody fundamentally modifies its photophysical and photochemical behavior.

Further information is provided by time-resolved emission spectroscopy in the picosecond range. The fluorescence of (*E*)-**1** at room temperature in the buffer solution shows a monoexponential decay with an average lifetime τ_F of 71 ps, which is equivalent to the value for the unsubstituted (*E*)-stilbene (Scheme 1). On the contrary, the decay function of **19G2**–(*E*)-**1** consists of four components, whereby the long-lived component, with $\tau_F = 22\,900$ ps, is the most important one. The, in principal, very problematic fit of the decay curve with four exponential functions is supported by four different emission spectra, which relate to the different time regimes: The weak emissions with maxima between 380 and 400 nm correspond more or less to the emission of free (*E*)-**1** and can be probably explained by complexes that have small differences in the surroundings of the stilbene chromophore. The long-lived component with an intense and bathochromically shifted emission ($\lambda_{max} = 420$ nm) is due to an exciplex which is created and degraded in the nanosecond range. The π,π interaction between the stilbene and the indole building blocks (St···In) seems to be a decisive factor for this effect (Scheme 2).



Scheme 2. Exciplex formation and deactivation by fluorescence.

The exciplex corresponds to a dissociative term in the ground state, that is, a spontaneous dissociation occurs on the energy hypersurface of the ground state. How much the ground-state complexes and the exciplexes differ in their

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geometrical parameters or activated vibrational modes, remains a speculation.[5] The temperature-dependent dynamics of the systems, which do not permit a reorganization to the exciplex below about $-30\,^{\circ}C$, are an important feature.

The difference between the purple (violet) fluorescent systems, for example, **16H10**–(*E*)-**1**, and the blue fluorescent complexes, like **19G2**–(*E*)-**1**, still remains to be explained. The data shown in Table 1 reveal *one* difference in particular: **16H10**–(*E*)-**1** is not capable of generating a long-lived exciplex—the intrinsic radiation lifetime is nearly unchanged compared to that for free (*E*)-**1**. The reduced quantum yield Φ_F relative to **19G2**–(*E*)-**1** is due to an increased amount of *Z* configuration in the photostationary state.

Photodimerization is not relevant in the monoclonal antibody as a competitive process to fluorescence and isomerization (Scheme 1), but the electrocyclic ring-closure reaction with subsequent oxidation by the oxygen present (from air) can be observed. Simeonov et al. explain the total absence of fluorescence after 60 minutes of UV illumination by the irreversible production of phenanthrene.[5]

How do the photophysics and photochemistry of stilbene chromophores in antibodies differ from other host systems with stilbene guests, like cyclodextrins,[16] clathrates,[17] inclusion compounds in deoxycholic acid,[18] or liquid crystals?[19] Steric constraints, symmetry of the cavity, local polarity, local viscosity, and energy transfer between guest and host induce a variety of modified characteristics compared to stilbene in solution. The "fine-tuning" that is based on the molecular dynamics of the antibody–stilbene complexes is new. Photochemical sensors of the type described here promise interesting applications, for example, in immunochemistry, histological investigations, and genomic studies.

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[9] An inferior adiabatic way also exists for the *cis*→*trans* isomerization.[1, 2]

[10] The quantum yield for the intersystem crossing $S_1 \rightarrow T_1$ has a value of about 10^{-3}.[1]

[11] With an energy transfer from triplet sensitizers it is possible to populate T_1 and so to induce an *E*/*Z* isomerization.

[12] Details about the preparation and characterization are available from *Science Online* at the website: http://www.sciencemag.org/feature/data/1052306.shl.

[13] The crystallographic analysis at $-173\,^{\circ}C$ revealed the same arrangement for the active region of the antibody when it was complexed with stilbene (*E*)-**1**.

[14] The *Z* configuration can predominate up to values of more than 80%; the exact *E*/*Z* ratio depends on the irradiation wavelength: $E/Z = (\varepsilon(Z)\,\phi(Z \rightarrow E))/(\varepsilon(E)\,\phi(E \rightarrow Z))$.

[15] The thermal isomerization (see also Scheme 1) and an isomerization catalyzed by the antibody can be excluded.

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