Influence of Pressure on the Spectral Properties of Dye−DNA Supermolecules

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Received: September 19, 2002; In Final Form: November 26, 2002

We investigated a series of structurally and chemically different dye−DNA complexes via the specific behavior of spectral holes under pressure. In all samples chromophore and solvent were the same, only the DNA strands were different. The dominant interaction with the solvent is mainly electrostatic via the strongly polar BF_2 group of the chromophore. The relative change of the dipole moment upon excitation can be estimated from the experimental data. There is a significant color effect in the pressure-induced broadening of the holes, which signals a breakdown of the Gaussian approximation. The origin of this breakdown is associated with ordered structures around the chromophore due to the DNA strands but also because of the formation of ordered solvent cage structures as a consequence of the hydrophobic nature of the chromophore. The various DNA strands show characteristic features in the spectra under pressure that reflect specific structural features.

Introduction

Dye−DNA complexes are highly interesting systems because they enable the spectroscopic investigation of an optical probe with an attached chain molecule in a highly diluted solution. The DNA chain is especially interesting because there is a multitude of possible interactions, namely a series of several electrostatic interactions as well as the dispersion interaction. In addition, the DNA bases are prone to form well ordered internal structures depending on the base sequence but they can also form random structures.[1−4] As a consequence of this structural variety, it becomes possible to probe the influence of structural correlation due to the presence of the DNA strands in the vicinity of the optical probe, which is otherwise surrounded by the solvent. Apart from the scientific aspects, understanding the details of the dye−DNA interaction seems to be a necessary demand for a proper treatment of viral diseases and the respective drug design.[5]

Well suited probes for investigating dye−DNA interactions are dyes of the BODIPY type. They have a large internal flexibility, a high absorption in a spectral range that is very convenient for high-resolution laser spectroscopy, and they are efficient hole-burning systems.[6−9] They can easily be attached to DNA oligomers. For most of the DNA strands used in the present study, detailed structural information is available[1−3] so that there is a solid basis for a detailed interpretation of the spectroscopic data. Quite recently we investigated a series of dye−DNA adducts in a glycerol/water matrix with hole-burning Stark spectroscopy to shed light on the nature of the interaction between BODIPY and DNA adducts.[10] The major outcome of this investigation was that the dominating interaction is electrostatic and that the dipole moment at the dye probe changes strongly over the inhomogeneous band, in agreement with the strong color effect in the field-induced splitting of the spectral holes. The strong change in the dipole moment was attributed to a steadily decreasing degree of protonation of the BF_2 group across the inhomogeneous band.

In the present investigation we use pressure-tuning hole-burning spectroscopy to investigate the interaction of the BODIPY probe with a series of attached DNA strands and the surrounding glycerol/water solvent. Hydrostatic pressure is a very interesting parameter because it does not induce a macroscopic symmetry change in the sample like an electric field and, under some simplifying assumptions, site selective pressure experiments yield two system parameters that are not easily accessible otherwise, namely, the compressibility κ of the nearby environment of the chromophore and its so-called vacuum absorption frequency ν_{vac}.[11,12] The influence of pressure on spectral holes was first investigated by Richter et al.[13] A thorough theoretical treatment was performed by Laird and Skinner;[14] however, restricted to the case of an apolar probe in an apolar solvent. The main result of this investigation was that the so-called pressure shift is subject to a color effect; i.e., it shows a linear variation with burn frequency. The broadening, however, is independent of it as long as the so-called Gaussian approximation is valid. Later it was shown that under certain circumstances the compressibility of the solvent as well as the vacuum absorption frequency of the probe could be determined from the color effect of the pressure tuning data.[15] Because in the present series of dye−DNA adducts, dye probe and solvent are not changed, any variation observed must originate from specific features of the respective DNA strands. Such features could, for instance, be due to specific interactions, to the formation of specific hydrogen bonds, to specific elastic parameters, to specific structures, and to specific correlation effects between probe and nearby environment. The last are especially interesting because they may lead to a breakdown of the Gaussian approximation, which is widely used in current theories of inhomogeneous line broadening and probe−solvent interactions.[13,16−18] Indeed, as we will show, most of our data

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10.1021/jp0270295 CCC: $25.00
Published on Web 01/17/2003

point to a possible breakdown of this approximation in the systems investigated.

The quantity we are going to focus on is the frequency dependence of the pressure shift and the pressure broadening.

BODIPY−DNA Cmplexes: Molecular Interactions. Just by looking at Figure 1, it is obvious that the various complexes investigated show a rich variety of molecular interactions between probe and environment: The probe itself is strongly polar due to the BF_2 group and, hence, has a strong dipole moment. The DNA strands are negatively charged. The ordered strands, in addition, are stabilized by positive charges.[19] Further, there is the electrostatic interaction as well as the hydrogen bonding between probe and solvent molecules, and there is for sure a significant dispersive interaction. Finally, despite the polar BF_2 group, the BODIPY moiety is also hydrophobic, as most aromatic π-electron systems are. All these interactions may contribute to the solvent shift and to inhomogeneous line broadening. The dipole−dipole and the ion−dipole interactions, however, do not contribute to the solvent shift as long as the orientation of the dipoles involved are random. There are two reasons why randomness is broken: First, and specific to the present systems, it is broken because of the structural correlations between DNA strand and dye probe, and second, it is broken because the solvent dipoles become partially polarized by the dipole moment of the dye. The respective deviation from isotropy can be explicitly taken into account by performing a thermal average over the orientational degrees of freedom in the solute−solvent dipole−dipole interaction. The resulting averaged interaction depends on the distance as R^{-6}. The energy scale for solvent polarization is set by the glass transition temperature T_g, because for temperatures lower than T_g the solvent molecules are frozen in and further alignment is no longer possible. For the case of a dominant dipole−dipole interaction between probe and solvent, it was shown by Loring[20] that there is a simple relation between the square of the inhomogeneous width σ_0^2 and the solvent shift s_s, namely

$$\frac{\sigma_0^2}{s_s} = kT_g \frac{|\Delta\vec{\mu}|^2}{|\vec{\mu}_0 \Delta\vec{\mu}|} \tag{1}$$

where $\Delta\vec{\mu}$ is the change of the dipole moment of the solute upon excitation and $\vec{\mu}_0$ is the respective moment in the electronic ground state. If the solvent is polarized by the solute, the respective interaction scales with the distance as R^{-6}, as has been mentioned above. The dispersion interaction is also governed by an R^{-6} dependence.

Apart from the above-mentioned attractive interactions there are also repulsive interactions. For the present study we do not consider them as very important because it seems reasonable to assume that the nearest neighbor molecules around the chromophore will adopt a position corresponding to the minimum in the Lennard-Jones potential. This seems to be true even for amorphous solids as obvious from the well structured radial correlation function reflecting local order.[21] Hence, for the nearest neighbor molecules, the first derivative of the potential with distance (and, hence, with pressure) vanishes and we may neglect them in lowest order.

Pressure Effects in Hole-Burning Spectroscopy. All the static interactions of a probe molecule with its environment are somehow reflected in the respective solvent shift s_s and in the concomitant inhomogeneous line width σ_0. Both quantities, in turn, are determined by $w(R)$, the difference in the interaction of a solvent molecule at distance R with the probe in its ground and electronic excited state, and $g(R)$, the two particle probe−solvent radial correlation function. $w(R)$ may comprise all possible interactions, as dicussed above. Note that at this stage we do not consider orientational degrees of freedom in $w(R)$. $g(R)$ describes the structural arrangement of the solvent molecules around the probe in the simplest approach, namely, neglecting any correlation among the solvent molecules. Note that, at this stage, $g(R)$ does not depend on orientational degrees of freedom, either because they do not seem to be very important (e.g., in the dispersion interaction) or because we consider thermally averaged interactions anyhow (e.g., the dipole−dipole interaction). $4\pi V^{-1} g(R) R^2\ \mathrm{d}R$ is the probability of finding a solvent molecule in a spherical shell of thickness dR and radius R around the probe. The restriction to two particle correlations is a very severe restriction for the systems considered because the building blocks of the DNA strands and even the solvent molecules may be highly correlated. Within this approach the square of the inhomogeneous width σ_0^2 and the solvent shift s_s are given by

$$\sigma_0^2 = 4\pi\left(\frac{N}{V}\right)\int w^2(R)\, g(R) R^2\, \mathrm{d}R \tag{2}$$

and

$$s_s = 4\pi\left(\frac{N}{V}\right)\int w(R)\, g(R) R^2\, \mathrm{d}R \tag{3}$$

where N is the total number of solvent molecules and V the volume.

The behavior of spectral holes under external perturbations, e.g., an electric field or pressure, may shed light on the details of the static probe−solvent interactions, for instance, on which of the various interactions dominate $w(R)$, on whether the inhomogeneous band is built from a superposition of several states, or on whether the approximations used in the models are sufficient. For instance, from our recent Stark-effect experiments[10] we found that solvent shift and inhomogeneous width of BODIPY−DNA adducts are largely determined through electrostatic interactions.

Under pressure, spectral holes experience a shift s_p, the so-called pressure shift, and a broadening σ_p, the so-called pressure broadening. Resorting again to the above-mentioned approximations, these quantities can be written as[14]

$$s_p = \left[N\langle\alpha\rangle + \left(\frac{\langle\alpha w\rangle}{\langle w^2\rangle}\right)(\nu - \nu_0)\right] \tag{4}$$

and

$$\sigma_p^2 = [N\langle\alpha^2\rangle(1 - \rho^2)]^{1/2} \tag{5}$$

ν is the wavenumber where hole-burning is performed, and ν_0 is the wavenumber at the band maximum. Note that s_p and σ_p are normalized quantities, i.e., represent frequency shift and broadening per MPa, respectively.

Brackets in the above equations mean volume averages of the respective quantities over $g(R)$. $\alpha(R)\Delta p$ describes how much $w(R)$ changes when pressure is changed by an amount Δp:

$$\alpha(R) = -\frac{\kappa}{3} R \frac{\partial w}{\partial R} \tag{6}$$

We stress that eq 6 describes the special case that all the interactions which contribute to the solvent shift and to inhomogeneous line broadening are modified by pressure. Generally

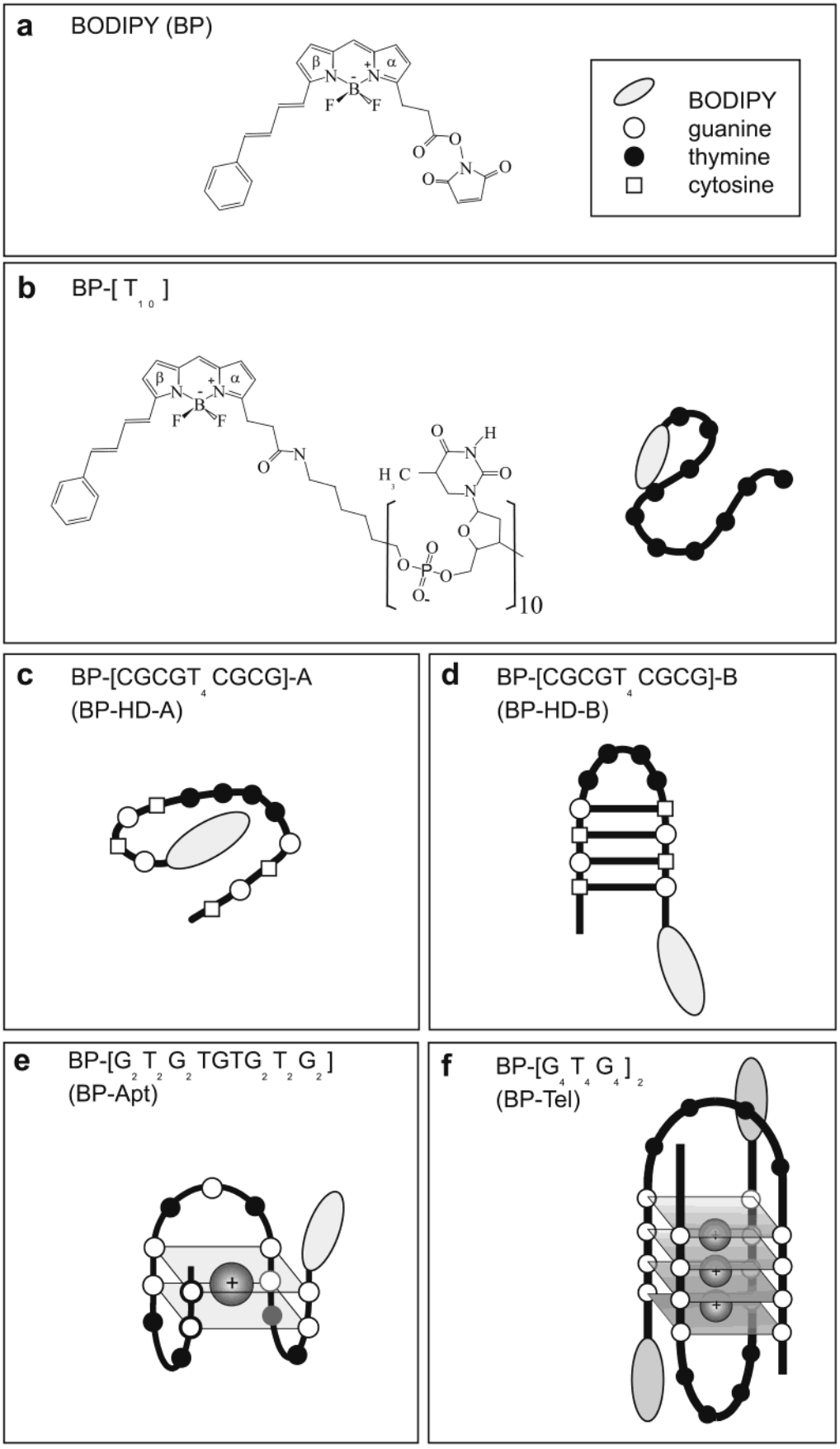

Figure 1. Chromophore and the various DNA oligomers with their proposed structures.

speaking this is not always true and, especially in the present case, where the chromophore may be surrounded by highly correlated structures with a variety of different interactions, eq 6 has to be carefully checked.

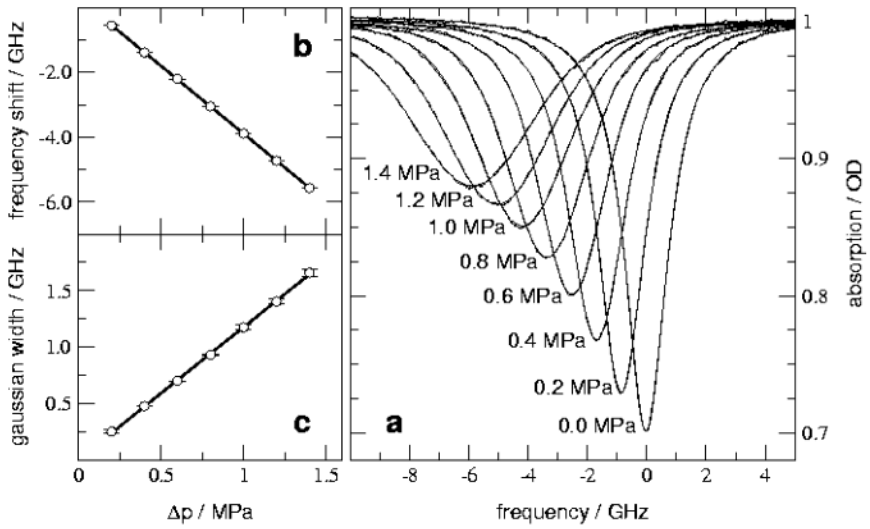

Figure 2. Behavior of a hole under pressure. Pressure changes with respect to atmospheric pressure are indicated. The shift of the central frequency of the hole as well as the respective broadening are perfectly linear with pressure. Sample: BP-[T_{10}] at 594 nm. Temperature: 2 K.

κ is the isothermal compressibility. Strictly speaking it is a local compressibility because the elastic response of the system is measured through the interaction of the chromophore with its environment, which is of short range.

ρ measures the degree of correlation between the configurations with and without pressure and is given by

$$\rho^2 = \frac{\langle \alpha w \rangle}{\langle w^2 \rangle} \tag{7}$$

ρ^2 varies between 0 and 1.

Equation 4 attains a very simple and useful form in case $w(R)$ goes such as R^{-n}. In this case it is straightforward to show that

$$s_p = \frac{n}{3}\kappa[\nu - \nu_{vac}] \tag{8}$$

ν_{vac} is the vacuum absorption wavenumber of the dye probe, i.e., the wavenumber where the solvent shift and, hence, also the pressure shift vanishes. Accordingly $\nu - \nu_{vac}$ is the wavenumber-selected solvent shift, i.e., the solvent shift of those molecules that absorb at wavenumber ν. Because the most important solute−solvent interactions (dispersion interaction, dipole−induced dipole interaction, dipole−dipole interaction with solvent polarization) scale with R^{-6}, we evaluate the parameters in eq 8 with $n = 6$.

Summarizing this section, we stress again that the above results are valid within a hierarchy of approximations. Most important is the Gaussian approximation. Within the frame of the latter, the inhomogeneous line shape and the pressure-induced broadening kernel of the hole are Gaussian. The pressure-induced width σ_p is independent of the frequency within the inhomogeneous band whereas the pressure-induced shift s_p depends in a linear fashion on it.

Experimental Section

Sample Preparation. The various samples BP, BP-[T_{10}], BP-HD-A, BP-HD-B, BP-Apt, and BP-Tel (see Figure 1 for definition of abbreviations) were purified by HPLC, dried, and dissolved in 0.01 M Tris buffer at pH 7.5. Glycerol was added so that the final solution was a mixture of four parts buffer and five parts glycerol (v/v). In the case of BP-Apt and BP-Tel, ions are needed to stabilize the structure; hence, we added 0.15 M NaCl to 100 mL of buffer. The respective solution was again mixed with glycerol in a volume ratio of 4:5. The optical densities (ODs) of the final solution in the 2 mm cuvettes used were between 0.4 and 1. All holes were detected in the transmission mode.

The BODIPY chromophore and the respective complexes with the various DNA strands are shown in Figure 1. All these complexes have already been investigated by hole-burning Stark-spectroscopy[10] in the same solvent as used in the present investigation. The respective data (except BP-Tel) are published elsewhere.

Spectroscopy. The experiments were performed at a temperature of 2 K. The holes were burnt with a dye ring laser in rhodamine 6G, which was pumped by an argon ion laser. Typical power levels for burning and reading were of the order of μW and nW, respectively. The relative depth of the holes varied between 30 and 60%, depending on sample and burn wavenumbers.

Pressure was transmitted via He gas. The respective level was varied up to 1.5 MPa. The pressure-induced shift as well as the respective broadening were perfectly linear with pressure (Figure 2b,c). The samples were sealed in plastic bags to prevent them from sticking to the walls of the pressure chamber.

Data Evaluation. The initial hole was fitted to a Lorentzian profile which was, in all cases, a very good fit. For the pressurized holes, the initial Lorentzian was convoluted with a Gaussian (the so-called pressure kernel) to fit the experimental data. The pressure broadening σ_p is the width of this kernel at its inflection point.

Results

In Figure 2 we show how a spectral hole changes under pressure. The data are for BP-[T_{10}] at 594 nm. Figure 2a shows the change of the shape of the hole as a function of pressure: The hole shifts to the red by about 4 GHz/MPa and it broadens by about 1 GHz/MPa. Shift as well as broadening are perfectly linear with pressure (Figure 2b,c). Pressure shifts to the red were observed for all samples. The respective magnitude $s_p(\nu_0)$, as determined at the center of the inhomogeneous band, were remarkably constant (Table 1).

Figure 3 shows the inhomogeneous absorption spectra of the various dye−DNA complexes measured at 2 K, and how pressure shift (a, c, e, g, i, l) and pressure broadening (b, d, f, h, k, m) change across the inhomogeneous band. There are a

TABLE 1: Inhomogeneous Width σ_0, Average Solvent Shift $s_0 = |\nu_0 - \nu_{vac}|$, Average Pressure Shift $s_p(\nu_0)$, and Ratio σ_0^2/s_0 for the Various Dye−DNA Complexes

sample	σ_0/ cm^{-1}	s_0/ cm^{-1}	$s_p(\nu_0)$/(GHz/MPa)	σ_0^2/s_0
BODIPY	370	865	−4.75	158
BP-[T_{10}]	409	1081	−4.32	155
BP-HD-A	366	909	−4.55	147
BP-HD-B	378	957	−4.88	149
BP-aptamer	520	1425	−5.18	190
BP−telomer	514	1509	−5.05	175

couple of noteworthy features: First, in all cases, the normalized pressure shift decreases in a linear fashion with decreasing wavenumber as predicted by the Laird−Skinner theory. The slope of the respective straight line (i.e., the apparent compressibility, eq 8), however, shows characteristic variations with the various complexes. Second, the normalized pressure broadening, contrary to the prediction of the Laird−Skinner theory, does show a significant dependence on frequency. For some samples this dependence is quite strong, e.g., for BP-HD-B. For others, e.g., the BP-Apt and the BP-Tel, it is quite weak. This frequency dependence of the pressure broadening points to a breakdown of the Gaussian approximation. In all cases, the pressure broadening increases toward the red. The respective data show a tendency toward a superlinear behavior.

Due to the perfect linearity of the pressure shift with frequency we could in all cases determine the vacuum frequency (or wavenumber) ν_{vac} as the frequency where the pressure shift vanishes. From this frequency and the maximum of the inhomogeneous band we determined the average solvent shift $s_0 = \nu_0 - \nu_{vac}$. For the BP-Apt and the BP-Tel, s_0 is of the order of 1500 cm^{-1}, for the other complexes of the order of 1000 cm^{-1}. The respective numbers together with σ_0, $s_p(\nu_0)$, and σ_0^2/s_0 (eq 9) are summarized in Table 1. Table 2 summarizes the apparent compressibilities and the vacuum wavenumbers.

Discussion

Dominant Interaction in BODIPY. In a recent paper Bergström et al. reported on the exceptional light spectroscopic properties of tetramethyl BODIPY by performing electroabsorption experiments and quantum chemistry calculations.[22] The outcome of their study had an interesting result: The stability of the spectrum against pH as well as polarity changes of the solvent is based on the fact that the changes of the relevant dipole moments and polarizabilities upon electronic excitation is very small and, in addition, the $\Delta\vec{\mu}$ vector is orthogonal to the relevant transition dipole moment.

The BODIPY probe in our experiments differs from the tetramethyl-BODIPY used in the Bergström et al. investigation in several respects: It has no obvious symmetry, and it has an aromatic substituent that is attached to the BODIPY moiety via a conjugated bridge. One of the consequences of this structural difference is a significant red shift of the spectrum by about 100 nm. However, also all the other properties on which, according to Bergström et al., the exceptional spectral features are based, change dramatically: They become pH-dependent, they depend in a characteristic fashion on the DNA adduct, the dipole moments change drastically upon excitation, and $\Delta\vec{\mu}$ is parallel to the transition as well as to the ground state dipole moment. The experimental basis for these results is provided by our recent hole-burning Stark-effect experiments.[10] Because the respective results are important for the interpretation of the present pressure experiments, we briefly summarize the essentials: First, we found a rather strong splitting of the holes in an external field, if the field was parallel to the polarization direction of the burning laser. From this it follows unequivocally that $\Delta\vec{\mu}$ is preferentially parallel to the transition dipole moment. In addition, a large splitting of the hole in the external field implies not only that $\Delta\vec{\mu}$ is large but also that the dipole moments involved are large as well, in agreement with the very polar BF_2 group. The conclusion is that the leading interaction between chromophore and environment is the dipole−induced dipole interaction, as was also corroborated by the strong color effect in the splitting of the hole. Note that the strength of this interaction depends in an inverse fashion on the glass transition temperature via the Boltzmann factor and, hence, is strong for low freezing temperatures but may be rather insignificant for room-temperature glasses and polymers.

This reasoning has two implications: First, we argued above that we base our interpretation of the pressure shift phenomena on the reasoning that the repulsive potential does not seem to play a leading role because it affects the nearest neighbor molecules around the chromophore, which we assume to have settled in a local energy minimum so that only second-order derivative terms in the respective pressure perturbation may play a role, which we neglect. If so, the influence of pressure on the shift of the hole occurs via the attractive part of the potential, and in the uncomplexed dye, we have identified the leading term as the dipole−induced dipole interaction, which falls off with distance from the probe as R^{-6}. An additional contribution from the dispersion interaction does not change this distance dependence. In this case we can resort to eq 8 and determine κ from the slope in Figure 3a. The respective number is about 0.1 GPa^{-1}. Note that κ is a local quantity. The obtained value fits into the order of magnitude range known from organic glasses, polymers, and biopolymers.[11,12] We call κ an "apparent compressibility" because it depends on the details of the interaction and on the model used (see eq 8).

Second, from the present pressure experiment we can determine the average solvent shift s_0 because the vacuum frequency ν_{vac} is known (see Table 2). s_0 is to the red and is large, of the order 1000 cm^{-1}. If the probe solvent interaction is electrostatic in nature, a red shift can occur only if the dipole moment in the excited state $\vec{\mu}_1$ is preferentially parallel to the dipole moment in the ground state $\vec{\mu}_0$. In all other cases a blue shift would result.[23,24] From eq 1 it is then possible to estimate the change of the dipole moment upon electronic excitation: Because $\vec{\mu}_0$ and $\Delta\vec{\mu}_0$ are mainly parallel, eq 1 simplifies to

$$\frac{\sigma_0^{\,2}}{s_0} = kT_g\left[\frac{\Delta\mu}{\mu_0}\right] \tag{9}$$

Inserting the numbers for the uncomplexed BODIPY, namely, $\sigma_0 = 355$ cm^{-1}, $s_0 = 850$ cm^{-1} (see Figure 3a,b), and $kT_g = 140$ cm^{-1} (assuming a glass transition temperature for glycerol water of about 200 K), we obtain for $\Delta\mu/\mu_0$ a value of about 1. The conclusion is that, on average, the dipole moment in the excited state is about twice as large as the respective one in the ground state.

A large change of the dipole moment upon optical excitation is quite often accompanied by a large electron phonon coupling, meaning that excitation occurs preferentially to a highly excited phonon state of the lattice. If so, the relative intensity in the zero phonon line (the Debye−Waller factor) may become very small and narrow bandwidth hole-burning may become impossible.[25] Although, in our case, hole burning is quite efficient, there is no contradiction to a large change of the dipole moment upon electronic excitation as long as $\Delta\vec{\mu}$ and $\vec{\mu}_0$ are parallel

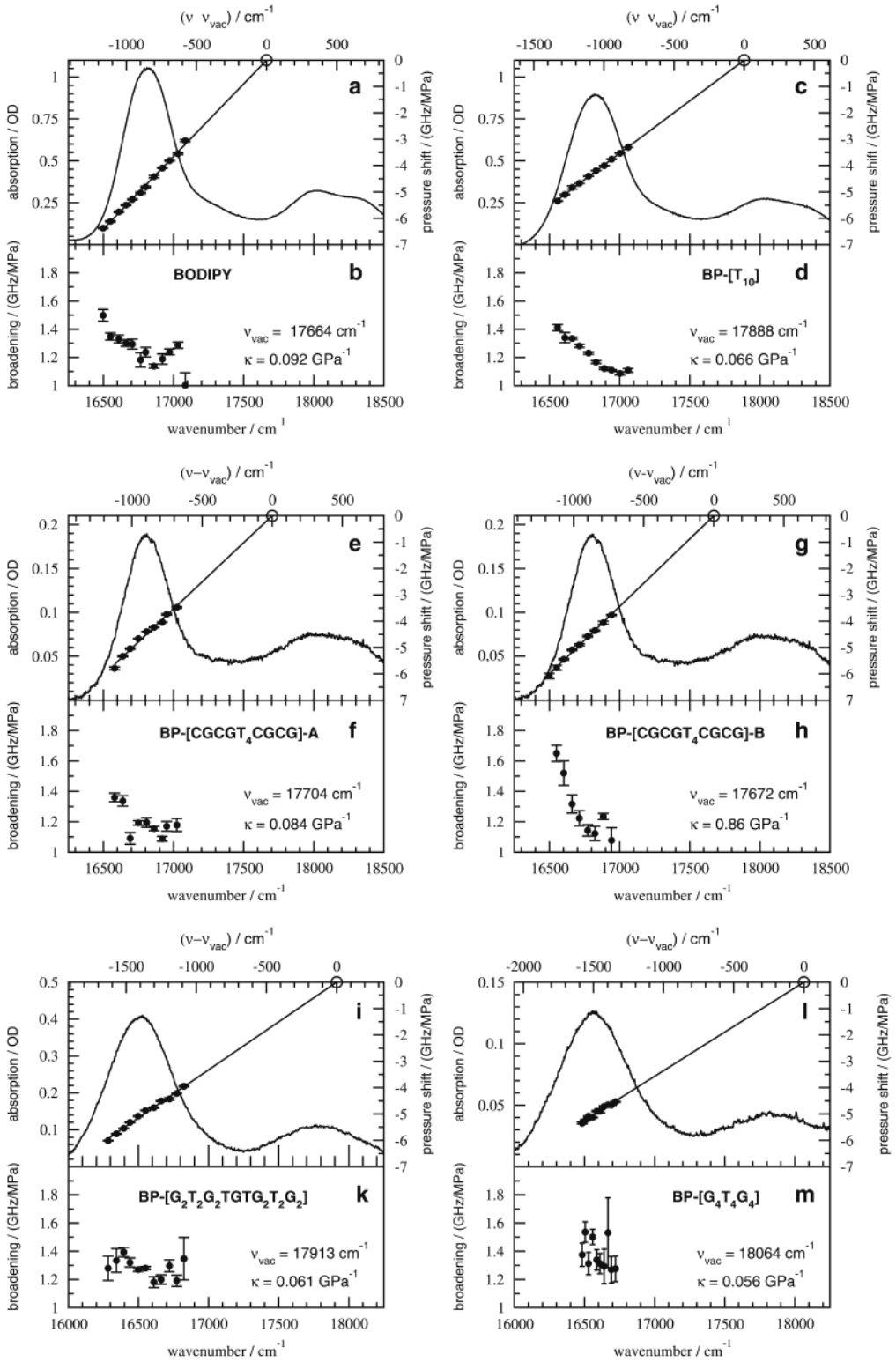

Figure 3. Absorption spectrum and pressure shift s_p as a function of burn wavenumber and the width σ_p of the Gaussian pressure kernel as a function of burn wavenumber. All experiments are done at 2 K.

and $\vec{\mu}_0$ is sufficiently large. If so, the change of the equilibrium position of the solvent molecules in the excited state of the probe may be rather moderate because they are already highly polarized and the respective orientation does not change. In addition, the larger the chromophore the smaller the change in the equilibrium position of the lattice coordinates.

DNA Complexes under Pressure. Although the expressions for pressure shift and broadening in the Laird−Skinner model were derived for an apolar molecule in an apolar solvent, we can certainly take them as guidelines for discussing more complex interaction patterns as they occur for instance in the present series of supermolecules.

As we stressed in the Introduction, all the samples that we investigated have the same probe and the same solvent. The data for the various complexes show similarities and differences. As to similarities, the data are remarkably linear in Δp and ν.

TABLE 2: Investigated Dye−DNA Complexes, Compressibilties κ, and Vaccuum Wavenumbers ν_{vac} As Determinded from the Hole-Burning Experiments (Eq 8 with $n = 6$)

sample	κ/GPa^{-1}	ν_{vac}/cm^{-1}
BODIPY	0.092	17664
BP-[T_{10}]	0.066	17888
BP-HD-A	0.084	17704
BP-HD-B	0.085	17672
BP-aptamer	0.061	17913
BP-telomer	0.056	18064

From this linearity and the fact that the holes do not split under pressure, we conclude that the respective optical bands are not built from a superposition of a few structurally different complexes. Also, the average pressure shift $s_p(\nu_0) = 2\kappa|\nu_0 - \nu_{vac}|$ is remarkably similar for the various samples (Table 1) and just varies within some ±10%. The same is true for the ratio σ_0^2/s_0 (eq 9), whose maximum variation is within ±17%. From this observation we conclude that the main interaction that causes the pressure shift is roughly the same for the whole series.

As to the differences, it is the slopes of the pressure data over burn wavenumber that change. The respective variation covers more than 60%. Because probe and solvent are not changed, these differences in the spectral properties must solely stem from the attached DNA strands.

There are several possibilities to introduce individual features of the dye−DNA complexes into the pressure dependence of the holes:

(i) The apparent compressibility κ could change. Note that κ is a local property reflecting the elastic properties of an environment around the probe whose scale is determined by the range of the relevant interaction. This nearby environment may even be modified under the influence of the probe. Larger parts of the DNA strands are certainly within this range and, because some of the strands have a floppy structure, the complex itself could be compressed and, hence, could influence the compressibility as measured in a hole-burning experiment.

(ii) The pressure sensitive interaction could change.

And, finally, (iii) the correlation between $\alpha(R)$ and $w(R)$ as expressed in the correlation factor $\langle \alpha w \rangle$ could change. This would, for instance, be the case, if pressure shift and solvent shift were partly independent.

Note that in the present investigation it is not the absolute value of κ in which we are interested but rather the relative change within the series investigated. As can be seen from Table 2, the slope of the straight lines (Figure 3) decreases steadily from the value of the uncomplexed dye to the very rigid aptamer or telomer structures. We argue that, in these complexes, a significant contribution to $w(R)$ comes from the local electric fields generated by the charges of the metal ions that stabilize the respective highly ordered structures. They mainly contribute to the solvent shift, as is obvious from the large red shift as compared to the other dye−DNA complexes. Also, they contribute significantly to the inhomogeneous line width. However, this interaction is likely to be much less modified by the small pressure levels of our experiments than the probe solvent interaction because the ions reside inside the very rigid quadruplex structures (see Figure 1). The probe solvent interaction, however, does not significantly change among the various samples as obvious from the average pressure shift that is roughly constant throughout the series. So for the BP-Apt and the BP-Tel, we have a situation where the pressure sensitive line broadening, which we attribute to the dipole-induced dipole interaction, is convoluted with a less pressure sensitive line broadening. This stretches the total color effect over a larger spectral range, thus decreasing the respective slope. Strictly speaking, the linearity of the data should suffer under such conditions. Indeed, the data in Figure 3i (BP-Apt) show an onset of a nonlinear behavior. The data range in Figure 3l (BP-Tel) is too small for definite statements, although even there a nonlinear tendency is present. In terms of the Laird−Skinner theory, this means that $\alpha(R)$ and $w(R)$ shows a rather low correlation or, in other words, solvent shift and pressure shift have different origins. It seems that this is the most likely reason for the smaller color effect in the pressure behavior of the BP-aptamer and the BP-telomer. It is clear that, in this case, the slope in the frequency dependent pressure shift cannot be related anymore to the compressibility of the solvent.

Similarly, in the case of several interactions with a different sensitivity to pressure, the extrapolated frequency where the apparent color effect vanishes loses its physical interpretation as the vacuum absorption frequency.

Interestingly, the color effect in the spectra of the BP-HD-A and BP-HD-B differs only in a marginal fashion from the respective one in the free BODIPY (Table 2). The conclusion is that the leading pressure sensitive interaction in these complexes is the same. Obviously, the DNA strands in these complexes protect the chromophore only a little from the interaction with the solvent. The situation is quite different for BP-[$T]_{10}$. The slope, i.e., the apparent compressibility, is significantly smaller. This may have structural reasons: As has already been surmised on the basis of a vibrational analysis, the chromophore is likely to be covalently bound to the DNA chain, in this case.[7] From the low apparent compressibility we infer that the DNA strand may protect to some degree the chromophore from the solvent.

Comparison of the Frequency Dependence of the Stark Effect and the Pressure Effect. It is interesting to compare the frequency dependence of the Stark effect with the respective one of the pressure effect. First of all, both effects show a parallel overall behavior: They are roughly linear with frequency and increase toward the red edge of the inhomogeneous band. In addition, both effects show characteristic differences with the attached DNA strands. However, characteristic changes of the slopes with the DNA adducts do generally not parallel each other. For instance, as compared to the free BODIPY, the color effect for BP-[$T]_{10}$ is stronger in the electric field than with pressure, whereas it is just the other way around for BP-HD-A und BP-HD-B. In the BP-Apt and the BP-Tel the color effect in both parameters are lowest.

What do we learn from this behavior? First, the color effect in the electric field shift reflects only the increase of the permanent dipole moment of the chromophore, as we go from the blue to the red edge of the inhomogeneous band. Hence, it is sensitive to a property of the probe. On the other hand, the color effect in the pressure shift reflects, in the simples case, a property of the solvent (although a local one), namely κ. However, there is no cogent reason that both properties are strictly correlated; hence, we observe the above-mentioned variations. Although there is no need for a strict correlation, the overall parallelism in the color effect of both parameters is easy to understand: In the simplest version (eq 8), the pressure shift scales with the solvent shift. For constant molecular coupling parameters, a larger solvent shift and, hence a larger pressure shift means a shorter probe solvent distance. So we learn from the parallel behavior of Stark shift and pressure shift that the shorter the distance of the solvent molecules from the probe is, the larger is the dipole moment of the probe, a

reasonable result because a larger dipole moment of the probe attracts the solvent dipoles.

Color Effect in the Pressure Broadening. Within the Gaussian approximation there should not be any color effect in the pressure broadening of spectral holes. The fact that it does occur signals a breakdown of this approximation. Note that the Gaussian approximation is based on the assumption that the dispersion of the solvent shift is solely due to fluctuations in the (large) number of solvent molecules around the probe. A breakdown of this approximation was discussed in detail by Kador.[17] According to his calculations, the width increased toward the red edge in an almost linear fashion and the absolute magnitude of the change was quite small of the order of 10%. For the samples, which we consider here, the situation is quite different: For some of the samples the change in the width is rather large, e.g., Figure 3h, and, in most cases, it shows a nonlinear tendency. A breakdown is likely to occur if the probe solvent interaction changes as a function of frequency, and/or if ordered structures are formed around the probe. In this latter case the nearby environments of the probe molecules are not anymore determined by the random fluctuations of the large numbers of interacting solvent molecules. In our case either one of these situations seems to play a role. From the Stark experiments we know that the dipole moment increases toward the red edge of the band. Partially ordered structures may occur for hydrophobic molecules in water containing solvents. Despite the polar group, the BODIPY most probably falls into this class of molecules. The loss in entropy is compensated for by strong hydrogen bonds through the formation of partially ordered structures. The respective network of hydrogen bonds may not as easily be compressed as random glassy structures; hence, the apparent compressibilities are at the lower edge of what is known of organic solvents. Further, the DNA strands may interfere with the formation of ordered solvent cages; hence, they induce characteristic modifications in the respective microenvironments as it is reflected in the respective behavior of the spectra under pressure.

Summarizing Conclusions

Complexes of BODIPY-type chromophores with DNA-oligonucleotide strands show a complex interaction scenario determined by the polar as well as hydrophobic nature of the chromophore, the water/glycerol solvent, which may possibly form ordered solvent cages and, last but not least, by the individual structures of the DNA strands. This complex scenario manifests itself in specific site dependent features (color effects) of spectral holes in electric and pressure fields, most noticeable in a breakdown of the Gaussian approximation.

Acknowledgment. The authors acknowlege the support of this joint research project through the DAAD (***Nr. D/9922928***). J.F. acknowlegdes further support from the DFG (FOR358/1) and from the Fonds der Chemischen Industrie.

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