



Editors Choice

Spectroscopic investigation of a brightly colored psittacofulvin pigment from parrot feathers



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ABSTRACT

Octadeca-octaenal is a psittacofulvin pigment associated with the bright red coloration of parrots. It consists of a linear polyene chain terminated by an aldehyde group and therefore provides an opportunity to examine the fundamental factors controlling the excited state ordering and dynamics of polyenals. Steady-state and ultrafast time-resolved spectroscopy were performed on octadeca-octaenal and a derivative in which the aldehyde group was converted to a methylenehydroxyl group. It was found that for octadeca-octaenal, solvent proticity was more important than polarity in determining its excited state lifetime. Theoretical computations were carried out to reveal the origin of the effect.

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1. Introduction

The bright coloration of parrot feathers is due to the presence of psittacofulvin pigments, which are exclusive to the order Psittaciformes [1–3]. These unique pigments were first described in 1882 by Krukenberg [4] who recognized that their spectroscopic properties differed greatly from those of carotenoids. Later analytical experiments revealed that the red feathers of the Scarlet Macaw (*Ara macao*) contained four polyenal molecules with chromophores ranging from six to nine conjugated carbon–carbon double bonds [1]. These molecules have since been found in the plumage of several species of parrots [2], and besides providing coloration, have been reported to protect the feathers from bacterial degradation [5].

Spectroscopic studies on polyenes and carotenoids have shown that electronic transitions to and from the ground state, S_0 , to the lowest-lying excited state, S_1 , are forbidden by quantum mechanical selection rules because both states belong to the A_g^- symmetry representation in the idealized C_{2h} point group [6–8]. However, transitions to and from S_0 and the S_2 state which has B_u^+ symmetry are strongly allowed and account for the vibrant visible coloration that carotenoids [9], and apropos of the present work, psittacofulvins [2], display in nature. When these molecules have a carbonyl

group in conjugation with the π -electron system, large effects of solvent polarity on the S_1 lifetime and $S_1 \rightarrow S_n$ transient absorption (TA) spectra are observed [10]. This behavior has been attributed to an intramolecular charge transfer (ICT) state associated with S_1 [7,10,11]. Despite significant recent experimental [12–14] and computational [15] efforts, the molecular nature of the ICT state and how it controls the spectral and dynamic properties of π -electron systems of conjugated carbon–carbon double bonds remain to be fully elucidated.

A spectroscopic investigation of psittacofulvins, which are devoid of extraneous functional group substitutions and contain only a polyene backbone terminated by an aldehyde group [1,16], provide the opportunity to probe the essential factors controlling the excited state ordering and dynamics of linear π -electron conjugated molecules containing a conjugated carbonyl group. The present work investigated the photophysics of the psittacofulvin, octadeca-2,4,6,8,10,12,14,16-octaenal, abbreviated octadeca-octaenal, which is one of the most abundant pigments found in red parrot feathers. As the name implies, it has eight conjugated carbon–carbon double bonds plus an aldehyde group on one end of the molecule (Figure 1). The spectroscopic properties of octadeca-octaenal were found to be different depending on whether it was dissolved in a nonpolar solvent (*n*-hexane), a polar solvent (acetonitrile), or a protic solvent (methanol). Chemical reduction of octadeca-octaenal using sodium borohydride converted the aldehyde group to a methylenehydroxyl group and formed octadeca-2,4,6,8,10,12,14,16-octaenol [1] (Figure 1), abbreviated octadeca-octaenol, which was also investigated in this work. A

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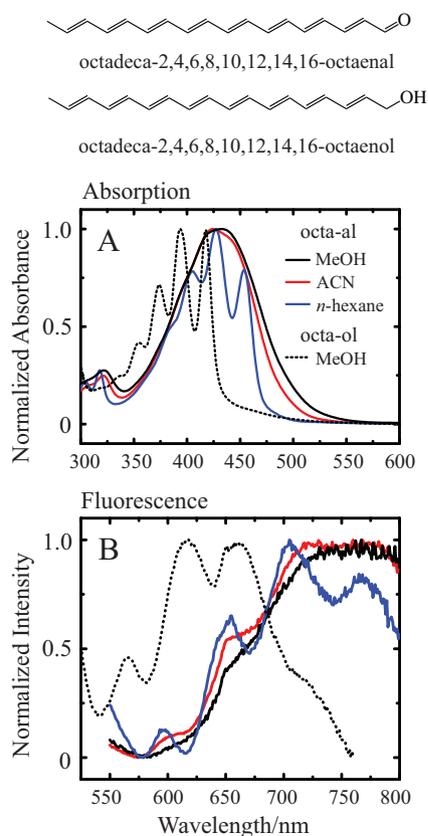


Figure 1. Molecular structures and IUPAC names of the psittacofulvin, octadeca-octaenal, and its chemically-reduced product, octadeca-octaenol (top). (A) Normalized absorption spectra, and (B) normalized fluorescence emission spectra of octadeca-octaenal (octa-al) in MeOH (black lines), ACN (red lines) and *n*-hexane (blue lines) overlaid with that of octadeca-octaenol (octa-ol) in MeOH (dotted lines). The excitation wavelengths for the fluorescence spectra were 435 nm for octa-al in ACN, 432 nm for octa-al in MeOH, 427 nm for octa-al in *n*-hexane and 400 nm for octa-ol in MeOH.

comparison of the excited state energies and dynamics of the two molecules elucidated the role of the carbonyl moiety in the formation of the ICT state. In addition, quantum computations were carried out to investigate the effect of solvent environment on the photophysical properties of octadeca-octaenal.

2. Materials and methods

2.1. Isolation and purification of octadeca-octaenal

Red feathers from domestic parrots of the species Timneh African Gray (*Psittacus timneh*), African Gray (*Psittacus erithacus erithacus*), and Scarlet Macaw (*Ara macao*) were collected during molt and provided by members of the Connecticut Parrot Society. Feathers from the latter two species have been reported to contain psittacofulvin pigments [2]. The pigments were extracted from the feathers according to the methods described in [17] and purified in a manner similar to that published in [1] whereby the dried extracts were dissolved in 6:4 (v/v) acetonitrile (ACN):methanol (MeOH) and purified by high-performance liquid chromatography (HPLC). The chromatographic protocol employed a Waters Atlantis T3 analytical column (5 μ m, 4.6 mm \times 250 mm) and was programmed for an isocratic delivery of 6:4 (v/v) ACN:MeOH at a rate of 1.5 mL/min. Identification of the chromatographic peaks was based on a comparison of the absorption spectra from the photodiode array detector with those previously reported for psittacofulvins [1]. It was determined that a small amount of the pigment,

hexadeca-2,4,6,8,10,12,14-heptaenal, eluted close to the retention time of the most prominent pigment, octadeca-octaenal. Therefore, in order to achieve high sample purity of octadeca-octaenal, its peak was collected and re-chromatographed two additional times. All solvents were HPLC-grade purchased from Sigma–Aldrich.

2.2. Spectroscopy

Absorption spectra were measured using a Varian Cary 50 UV/Vis absorption spectrometer on samples dissolved in either MeOH, ACN or *n*-hexane. Fluorescence spectroscopy was conducted using an Horiba Jobin-Yvon Fluorolog-3 on samples held in a 4 mm cuvette. The excitation wavelength was tuned to the absorption maximum (λ_{\max}) value in the absorption spectra. Transient absorption spectra of the molecules were measured using the laser spectrometer described in [18]. All spectroscopic measurements were carried out at room temperature.

2.3. Reduction of octadeca-octaenal to octadeca-octaenol using sodium borohydride

A purified sample of octadeca-octaenal was dissolved in \sim 2 mL MeOH and the absorption spectrum was recorded to have an optical density of \sim 0.5 a.u. in a 1 cm cuvette. A few granules of sodium borohydride (NaBH_4 , Acros Organics) were added directly to the cuvette and the absorption spectrum was measured again. Reduction was considered complete once the λ_{\max} had shifted to \sim 395 nm as described in [1]. Octadeca-octaenol was found to be less stable than octadeca-octaenal, and therefore care was taken to minimize exposure of both samples to light and air.

2.4. Computational methods

The computational methods used in this investigation are identical to those used to describe the ICT state of peridinin in [15]. We direct the reader to this work for a description of and justification for using these procedures in studying linear polyene systems.

3. Results and discussion

Figure 1A shows the room temperature spectra of octadeca-octaenal (denoted octa-al in the figure) in MeOH, ACN and *n*-hexane overlaid with the spectrum of the reduced molecule, octadeca-octaenol (denoted octa-ol in the figure) in MeOH. The spectra correspond to the strongly-allowed $S_0 \rightarrow S_2$ transition typical of polyenes. In *n*-hexane, octadeca-octaenal exhibits clear vibrational structure with its (0-0) band appearing at 454 nm. The λ_{\max} of the spectrum corresponds to the (0-1) vibronic band and appears at 427 nm. In the polar solvents, MeOH and ACN, the spectra display features typical of carbonyl-containing carotenoids whereby the vibrational structure is reduced relative to that observed in nonpolar solvents, and the spectra are broadened asymmetrically toward longer wavelengths [19]. The broadening is more substantial in the protic polar solvent MeOH, than in the aprotic polar solvent, ACN. The (0-0) band in the spectra of octadeca-octaenal is not resolved in MeOH or ACN where the λ_{\max} values are 435 and 424 nm, respectively. There is also a distinct *cis*-peak in the spectra indicating the presence of some amount of *cis*-isomers in the samples which were also evident from the HPLC chromatographic analysis.

Chemical reduction of octadeca-octaenal to octadeca-octaenol results in an \sim 40 nm blue-shift of the absorption spectrum caused by a decrease in the effective π -electron conjugation chain length upon conversion of the conjugated aldehyde group of octadeca-octaenal to an unconjugated methylenehydroxyl group in octadeca-octaenol [1] (Figure 1). The absorption spectrum of octadeca-octaenol exhibits highly-resolved vibrational structure even in

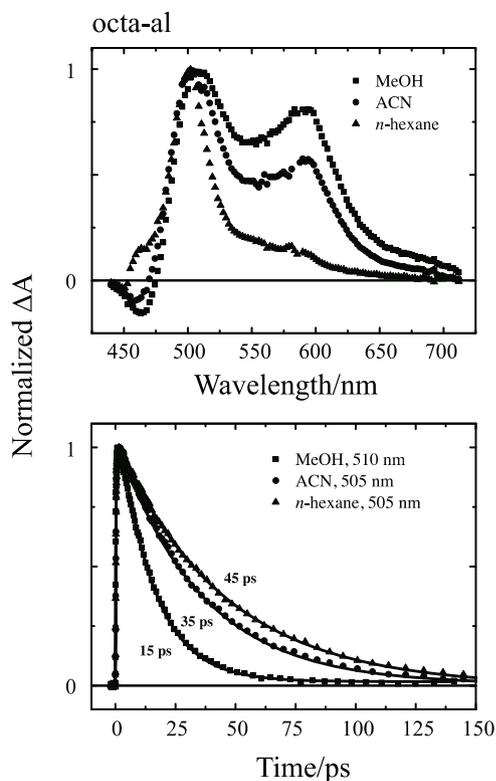


Figure 2. Normalized TA spectra (top) and kinetic traces (bottom) of octadecaocetaenal in MeOH, ACN and *n*-hexane. The spectra were obtained at a 3 ps delay time following excitation at 460 nm. The kinetic traces were detected at the λ_{\max} of the $S_1 \rightarrow S_n$ band. Solid lines are computer generated fits that yielded the lifetime of the lowest excited state.

the polar solvent MeOH in contrast to the featureless spectrum of octadecaocetaenal in that same solvent (Figure 1).

Room temperature fluorescence spectra of octadecaocetaenal in MeOH, ACN and *n*-hexane overlaid with the spectrum of the reduced molecule, octadecaocetaenol in MeOH, are shown in Figure 1B. The spectra from both molecules are significantly red-shifted from their corresponding absorption profiles indicating that the emission is associated with an $S_1 \rightarrow S_0$ transition. Fluorescence excitation spectra recorded by monitoring these emissions are in good agreement with their corresponding absorption spectra. The emission profiles from octadecaocetaenal in *n*-hexane and octadecaocetaenol in MeOH show pronounced vibronic structure which is lessened in the spectra from octadecaocetaenal in the polar solvents, MeOH and ACN.

Transient absorption (TA) spectra of octadecaocetaenal in MeOH, ACN and *n*-hexane recorded 3 ps after excitation at 460 nm are given in Figure 2 (top panel). The TA spectrum in *n*-hexane shows a prominent $S_1 \rightarrow S_n$ absorption band peaking at 502 nm accompanied by a weaker absorption profile extending out to ~ 650 nm. In polar solvents, this weaker excited-state absorption feature emerges as a distinct band having a maximum at 595 nm. The wavelength position and strong dependence of this band on solvent polarity indicates that it is due to an ICT $\rightarrow S_n$ transition, which is well-documented in the TA spectra of carbonyl-containing carotenoids [11,20]. The degree of charge transfer character in the excited state of the molecule can be quantified by the ratio of the amplitudes of the $S_1 \rightarrow S_n$ and ICT $\rightarrow S_n$ transitions which is 0.8 in MeOH and 0.6 in ACN. These different values indicate that in the protic solvent, MeOH, the charge transfer character is enhanced relative to that in the aprotic solvent, ACN.

Figure 2 (bottom panel) shows kinetic traces associated with the buildup and decay of the TA signal of octadecaocetaenal in MeOH,

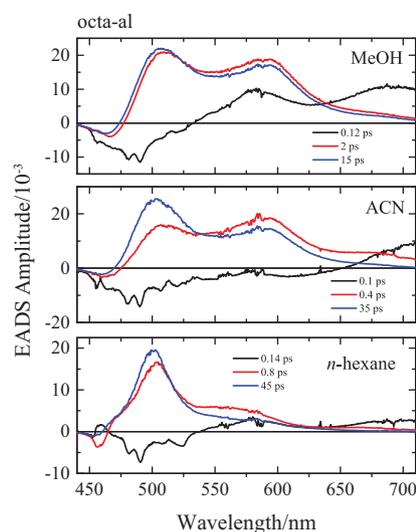


Figure 3. EADS obtained from global fitting the spectral and temporal datasets obtained from octadecaocetaenal in MeOH, ACN and *n*-hexane. The two distinct negative peaks seen in the initial EADS in all solvents are Raman bands corresponding to C–C and C=C stretches of the molecule.

ACN and *n*-hexane. The spectra were recorded at the λ_{\max} positions of the $S_1 \rightarrow S_n$ spectra. The solid lines in the figure represent computer-generated fits of the kinetic profiles that provide the lifetime of the lowest excited state of the molecule in the different solvents. The response of the excited state kinetics of octadecaocetaenal to solvent polarity is very similar to that reported for carotenoids containing carbonyl groups [10,11,20]. A significant decrease in the lifetime of the excited state occurs as the solvent polarity is increased. However, in contrast to that reported for all other carbonyl-containing carotenoids, octadecaocetaenal displays a significant change in lifetime in the polar protic solvent, MeOH, compared to in the polar, aprotic solvent, ACN. Even though ACN is slightly more polar than MeOH, the excited state lifetime in ACN is markedly longer (35 ps) than in MeOH (15 ps). This suggests that for this molecule, solvent proticity is more important in determining the excited state lifetime than solvent polarity.

In order to examine in more detail the origin and dynamics of the excited states associated with the transient spectra, global fitting according to a sequential decay model was carried out on the spectral and temporal datasets from octadecaocetaenal. The resulting amplitude traces are termed evolution associated decay spectra (EADS) [21] and are shown in Figure 3 for the molecule in MeOH, ACN and *n*-hexane. The overall dynamics of octadecaocetaenal are very similar in the three solvents. The S_2 state depopulates within 100–150 fs to form a hot S_1 state that relaxes in 2 ps or less. The S_1 state then decays with a lifetime dependent on the solvent polarity and proticity as noted above.

An overlay of the TA spectra and kinetic responses for octadecaocetaenal and the reduction product, octadecaocetaenol, in MeOH are given in Figure 4. The presence of a strong additional, red-shifted band in the TA spectrum (top trace) and the pronounced shortening of the excited state lifetime (bottom trace) for octadecaocetaenal clearly indicate that the conversion of the aldehyde group in octadecaocetaenal to a methylenehydroxyl group in octadecaocetaenol inhibits the characteristic behavior associated with charge transfer character in the excited state. Indeed, the fact that the TA spectrum of octadecaocetaenal in *n*-hexane is so similar to that of octadecaocetaenol in MeOH, except for the red-shift due to longer effective conjugation of octadecaocetaenal, argues that essentially no ICT character is present in the lowest excited state of octadecaocetaenal.

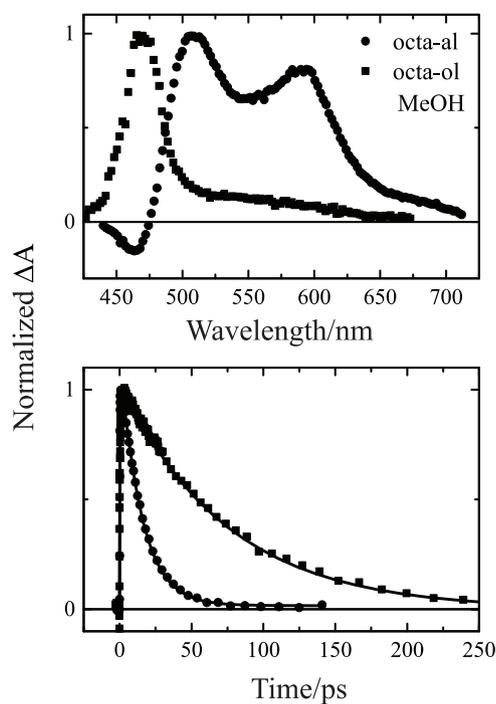


Figure 4. Overlay of the TA spectral profiles (top) and excited state kinetic traces (bottom) obtained for octadecaocetaenal and octadecaocetaenol in MeOH. The TA spectra were obtained at a 3 ps delay time following excitation at 460 nm (octadecaocetaenal) and 420 nm (octadecaocetaenol). The kinetics traces were detected at the λ_{\max} of the $S_1 \rightarrow S_n$ band. The data in both plots were normalized to their maximum values.

Global fitting of the datasets recorded from octadecaocetaenal in MeOH (Figure 5) reveals values of the kinetic components comparable to those found for octadecaocetaenal in *n*-hexane (Figure 3) except that the S_1 lifetime is substantially longer for octadecaocetaenol at 74 ps compared to 45 ps for octadecaocetaenal. This is to be expected based on the shorter π -electron conjugation length of octadecaocetaenol compared to octadecaocetaenal (Figure 1), which leads to a higher S_1 state energy for octadecaocetaenol. In fact, the 74 ps S_1 lifetime of octadecaocetaenol is very similar to the 68 ps S_1 lifetime reported for octaene which is a synthetic, open-chain C_{30} carotenoid having an effective π -electron conjugation length of $N=8$ [22]. In *n*-hexane, the lifetime of 45 ps for octadecaocetaenal matches that of a carotenoid having an effective conjugation length of 8.5 [18]. Also,

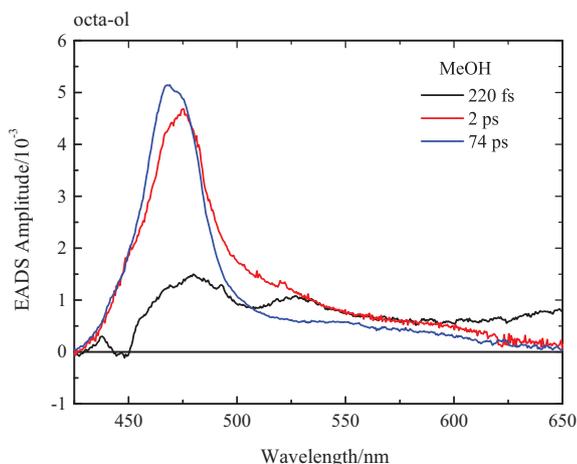


Figure 5. EADS obtained from global fitting the spectral and temporal datasets obtained from octadecaocetaenol in MeOH.

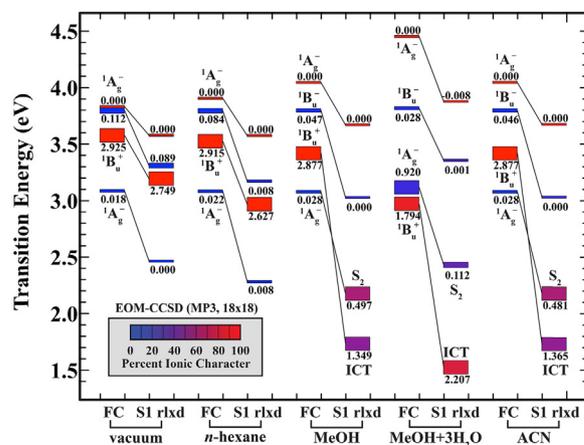


Figure 6. Effect of solvent environment on the lowest excited Franck-Condon (FC) and first excited relaxed (S_1 rlx) singlet states of the octadecaocetaenal based on EOM-CCSD procedures. The Franck-Condon geometry is that generated by minimization of the ground state by using B3LYP/6-31G(d) density functional methods and PCM solvation theory. The S_1 relaxed geometry is that generated by using RHF/D95 CIS methods with an 8×8 window and PCM solvation theory. Transition energies are relative to the MP3 ground state, and an active space of 18×18 was used for the EOM-CCSD calculations. The use of the MP3 ground state as the reference state is discussed in Reference [15]. Solvent polarity increases from left to right, but the left most pair is for vacuum conditions. The calculations involving MeOH + 3H₂O include three discrete water molecules minimized near the carbonyl group.

the difference in the S_1 lifetime of octadecaocetaenal in *n*-hexane (45 ps) compared to the value measured for octadecaocetaenol (74 ps) corresponds well to the change expected from shortening the effective π -electron conjugation from $N=8.5$ (octadecaocetaenal) to $N=8$ (octadecaocetaenol) [22].

Theoretical calculations were carried out to investigate the origin of the observed photophysical properties of octadecaocetaenal in polar and protic solvents. The methods and procedures are identical to those used in [15]. The effect of solvent environment on the excited state level ordering and photophysical properties of octadecaocetaenal based on EOM-CCSD methods is shown in Figure 6. In vacuum and the non-polar solvent, *n*-hexane, the calculations predict a lowest excited $^1A_g^-$ state and the relaxed first excited singlet state retains $^1A_g^-$ -like character. These results are fully consistent with the experimental observations. However, the highly polar solvents MeOH and ACN are predicted to have a similar effect on the level ordering, with the creation of an ICT state upon relaxation in the excited singlet state manifold (Figure 6). These results are reflected qualitatively, but not quantitatively, with respect to experiment. In particular, the protic solvent, MeOH, is observed to have a much larger impact on the excited state lifetime than the more polar solvent, ACN. This observation is attributed to the protic properties of MeOH, and the calculations provide a perspective on the origin of this effect. Because the Polarizable Continuum Model (PCM) procedures do not include discrete hydrogen bonding, protic effects were simulated by carrying out calculations in MeOH in which one, two or three water molecules are added near the carbonyl group. We used water rather than explicit MeOH to minimize the number of atoms involved to maintain tractability in the theoretical calculations. Preliminary excited state calculations with one discrete MeOH molecule (not shown) yielded virtually identical results to those with one discrete water molecule, which suggest that the use of water to simulate the desired protic effects is indeed an appropriate substitution. The calculations predict that hydrogen bonding to the carbonyl group affects both the absorption and emission properties of octadecaocetaenal as shown in Figure 6. The effect of a single water molecule near the carbonyl group is demonstrated in Figure 7 in which the dipole moment of

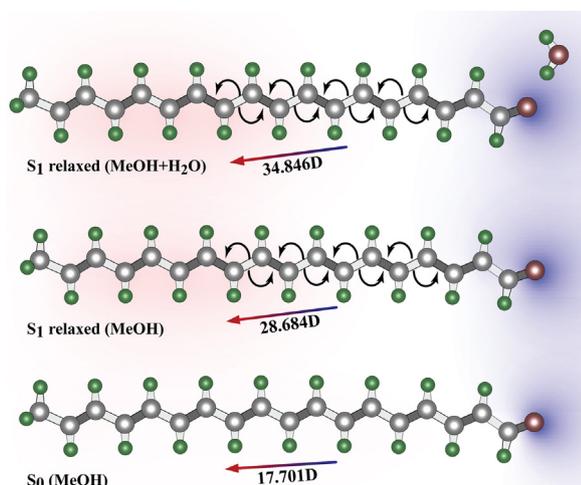


Figure 7. The effect of solvent polarity and discrete hydrogen bonding on the nature of the ICT state in octadecaocaenal in MeOH. The intensity of the background color is proportional to the charge density, with excess positive charge shown in red and excess negative charge shown in blue. Although an ICT state is formed in all strongly polar solvents, a protic solvent enhances the charge transfer character of the ICT state by stabilizing negative charge at the carbonyl oxygen.

the relaxed excited singlet state is enhanced by 20%. Three water molecules (not shown) enhance the S_1 relaxed dipole moment to 39 D, comparable to the large dipole moment observed in peridinin in polar solvents [15]. Protic solvents also have a significant impact on the allowedness and the energy of the lowest singlet state as shown in Figure 6, both for the Franck–Condon and the S_1 relaxed geometries. The combination will decrease the lifetime of the excited state by increasing both radiative and non-radiative coupling of the lowest singlet state to the ground state. This observation provides an explanation of the protic effects observed in Figures 2 and 3.

The nature of the ICT state in octadecaocaenal in MeOH is explored in Figure 7. The ICT state plays an important role in the enhancement of energy transfer in peridinin containing proteins, but it is not proposed that the formation of an ICT state in octadecaocaenal serves a similar purpose in parrot feathers. Nevertheless, the fact that this simple polyenal generates an ICT state is interesting from a photophysical perspective. Although the ground state of octadecaocaenal displays the standard alternation of single and double bonds, relaxation in the excited singlet state manifold generates significant bond order reversal within the conjugated π -electron system. When this bond order reversal is compared to that observed in peridinin [15], the magnitude of the bond order reversal is comparable, but does not result in as large a dipole moment change unless a protic solvent is involved. In contrast, the ICT state in peridinin is not influenced by protic solvents, only solvent polarity.

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