Protein-Protein Interactions in Plastocyanin Function: Examination of a Divergent Plastocyanin Homolog

George S. Bullerjahn
Center for Photochemical Sciences, Department of Biological Sciences
Bowling Green State University

Introduction

Plastocyanin (PC) is a small (~10 kDa), Type 1 copper protein that functions as an electron donor to Photosystem I (PSI) from cytochrome (cyt) f in both chloroplast systems and in some strains of cyanobacteria (Gross, 1996). In some lower eukaryotes and most cyanobacteria, a small (9 kDa) c-type cytochrome serves this function (Wood, 1978), and many of these organisms can synthesize both components, replacing PC with cyt c_{6} under conditions of copper limitation (review, Raven et al., 1999). PCs from cyanobacterial and chloroplast sources can be assigned to several classes, depending on their origin and primary structure (Gross, 1996). Whereas the chloroplast PC homologs exhibit a great deal of similarity at the level of primary structure, the cyanobacterial PCs are highly variable with respect to sequence and isoelectric point. Due to such variability, only 18 amino acids are universally conserved across this family of proteins (Gross, 1996), yet all retain a common tertiary structure and coordinate a single Cu ion near a hydrophobic region at the “top” of the molecule. From structural studies as well as homology modeling of cyt c_{6} and PC, it appears that the overall distribution of nonpolar and charged residues on cyt c_{6} from a particular species closely mimics the positioning of such residues on PC from the same organism (Navarro et al., 1997).

Examining the structure of PC, the Cu binding site is defined by a cysteine, a methionine and two histidine residues that form a distorted tetrahedral cage. His87 of poplar PC is a surface-exposed residue surrounded by the hydrophobic patch; it is likely that this residue yields the electron transport pathway into the PSI acceptor. In sum, PCs are β-sheet redox proteins having a midpoint redox potential of approximately +350 mV (Gross, 1996), whereas cys c_{6} are structurally unrelated α-helical hemoproteins whose redox properties are very similar to PC (Navarro et al., 1997). As outlined below, a major objective of our work is to examine the natural variation among PC, cyt c_{6} and PSI primary and tertiary structures in order to help identify the amino acid residues responsible for mediating protein/protein interactions leading to electron transport. We are studying the structure and function of these proteins from the cyanobacterium, Prochlorothrix hollandica, as plastocyanin and PSI protein sequences have unique differences that may be functionally important in mediating protein/protein contacts.

A wealth of structural information is available on the conserved chloroplast PC homologs; indeed, several X-ray crystal structures are available: poplar (Guss and Freeman, 1983), spinach (Xue et al., 1998), Enteromorpha (Collyer et al., 1990), Chlamydomonas (Redinbo et al., 1993) and Silene (Sugawara et al., 1999). NMR solution structures have been determined for several PCs including French bean (Moore et al., 1991), parsley (Bagby et al., 1994), and Scenedesmus (Moore et al., 1988). More recently, structures have been obtained in other labs from the more variable PCs from the cyanobacteria, including: Anabaena (Badsberg, 1996), Synechocystis (Romero et al., 1998), Phormidium laminosum (Bond et al., 1999), Synechococcus (Inoue et al., 1999), and Prochlorothrix hollandica (Babu et al., 1999). Given the differences in primary structure between the chloroplast and cyanobacterial PCs, as well as the high degree of variability among the cyanobacterial PC homologs, we have focused on the structure and function of PC from the prokaryote, Prochlorothrix hollandica. This PC is the most divergent member of the protein family known; it has only 31% identity and 44%
State officials were right on the mark last week in praising a remarkable research center established and sustained at Bowling Green State University by Dr. Douglas C. Neckers.

“I really want to hold this center up as a model,” said A. Harry Andrist, director of research and graduate programs for the Ohio Board of Regents.

Frank E. Samuel, Jr., the governor’s science and technology advisor, noted that it has been a rare exception to the sorry track record of Ohio universities in turning academic science into industrial might.

Both agreed that the BGSU Center for Photochemical Sciences is exactly the kind of academic research facility that Ohio needs as it plays catch up with other states in applying academic science in the real world process of creating high-technology jobs.

Mr. Samuel and Mr. Andrist joined a distinguished group of scientists—including the likes of Columbia University’s Nicholas J. Turro and National Medal of Science winner George S. Hammond—who gathered at BGSU for the annual meeting of the center’s scientific advisory board.

When Professor Neckers established the facility in 1985, it was the first academic center in the United States devoted to research on light’s interaction with matter. That, simply put, is photochemistry. Photochemistry now is one of the hottest fields in chemistry, and similar academic centers have sprung up elsewhere. But Bowling Green’s was the earliest and still is one of the very best.

As Mr. Samuel suggested, photochemistry is an industrial force. Indeed, it is the basis of multi-billion-dollar industries and products such as solventless inks, flexographic printing (The Blade’s new presses, for example, utilize environment-friendly flexographic technology), drugs for cancer and other diseases, and manufacture of computer chips.

Dr. Neckers has participated in the development of three new businesses, including Mead Imaging, a division of Mead Corp. which marketed a revolutionary dry-imaging system.

The center also excels in its educational mandate. It is training and inspiring generations of new photochemists who will make tomorrow’s revolutionary discoveries. Some have been graduate students from Russia.

That country’s chemistry establishment just honored Dr. Neckers for his role as an educator and savior of Russian science. He received an honorary professorship in the renowned Mendeleyev University, Russia’s biggest chemistry university.

Dr. Neckers got plenty of help in fulfilling his vision, of course. It included generous financial support from local philanthropist Harold A. McMaster, for instance, and from BGSU administrations which had the wisdom to recognize the importance of his work. When your bosses like what you’re doing, it makes the job a whole lot easier.

In the final analysis, however, northwest Ohio has carved out a niche where its scientists compete internationally and make world class contributions because one person had a vision and the energy and drive to see it through.

Foresight, creativity, energy, and management skills to carry dreams to fruition make Doug Neckers a scientific treasure. State officials couldn’t have cited a better model in their admirable efforts to nurture such excellence elsewhere.

The professor may not think of himself as a role model. But it’s clear that his profession certainly does.
similarity to poplar, and 35% identity and 56% similarity to Synechocystis PC (Arudchandran et al., 1994). Overall, we argue that by comparing conserved and variable regions of the PC structures, we can identify structural features of PC and PSI involved in functionally important interactions. In this brief article I describe the work we have either completed or initiated toward this end.

**Structural Characteristics of *P. hollandica* PC**

Two major structural features of PCs are the presence of two regions thought to be involved in binding the reaction partners: the aforementioned hydrophobic patch on the top of the molecule in the vicinity of the His87 (in poplar PC) Cu ligand, and a negative patch composed of acidic amino acids common to chloroplast PCs, but absent in the cyanobacterial PC homologs. Whereas PCs in general all have a hydrophobic region, *P. hollandica* PC has a hydrophobic patch that is uniquely and substantially different at the level of amino acid sequence (Arudchandran et al., 1994). For example, the conserved amino acid residues in poplar involved in forming the hydrophobic patch are Gly10, Leu12, Pro36, His87, and Ala90; indeed, Gly10 and Leu12 have been targets for site-directed mutagenesis in examining the role of this region in interactions with cyt f and PSI (Modi et al., 1992; Nordling et al., 1991; Haehnel et al., 1994; Sigfridsson, 1999). Since these two residues were initially believed to be invariant in all PCs, we were quite surprised to find that the *P. hollandica* PC has a tyrosine (Tyr12) and a proline (Pro14) residue at these respective positions (Arudchandran et al., 1994). Additionally, the *P. hollandica* protein has a unique methionine residue at position 33 corresponding to the universally conserved Asn31 (as numbered in the poplar structure). This residue lies on the border of the hydrophobic patch, and in the *P. hollandica* homolog, may extend the hydrophobic surface. To examine such differences at the structural level, we expressed and reconstituted *P. hollandica* PC to high levels in *Escherichia coli* (Babu et al., 1997), and employed high field homonuclear 1H NMR to obtain a high resolution structure (Babu et al., 1999; PDB codes 1b3i and 2b3i). Indeed, the solution structure of *P. hollandica* PC has revealed that the hydrophobic surface of this homolog adopts a distinctly different conformation than either chloroplast or cyanobacterial PCs (Figure 1). In collaboration with Miguel De la Rosa’s group (Navarro et al., 1999), functional studies of electron transport from *P. hollandica* PC to *P. hollandica* PSI reveal that the protein has a dedicated docking site on the PSI core (K_A = 8.7 x 10^3 M^-1; Table 1). Moreover, *P. hollandica* PC will not dock with chloroplast PSI, and binds *Anabaena* PSI weakly (K_A = 1.0 x 10^3 M^-1; Table 1). From these data, we propose that the surface conformation of the *P. hollandica* PC hydrophobic patch is important in yielding complex formation prior to productive electron transport. Nonetheless, since we know that Prochlorothrix PC can dock only weakly to *Anabaena* PSI merely via a Type II mechanism (Navarro et al., 1999; Navarro pers. comm.), we propose that the presence of the bulky surface-exposed Tyr12 interferes with binding heterospecific PSI. Overall, by employing a natural variant of PC as a target for future mutagenesis studies, we have eliminated any bias in determining both the positions in PC to be modified, and the precise mutations to be constructed at those relative positions.

![Figure 1. Space-filling model of the hydrophobic patches of *P. hollandica*, and *Synechocystis* PCs. The *Synechocystis* homolog has the traditional hydrophobic conserved among the PC family. Only the residues 9-14, 35-40, and 83-88 of *P. hollandica* PC and the corresponding residues in the *Synechocystis* PC are shown.](image-url)
Amino Acid Sequences of the PC/cyt c₆ Docking Site in the *P. hollandica* PSI Core

Are there compensatory amino acid changes in Prochlorothrix PSI that may accommodate the altered PC hydrophobic patch?

A component of this work is to define better the structure of the PSI donor side. Quite simply, given the PC hydrophobic patch interacts with a highly conserved domain in the PSI core, any structural variability in the patch should be reflected in a concomitant change within the docking site to adapt the altered PC surface. Further simplifying the analysis is the fact that cyanobacterial PC most likely does not interact with PSI via electrostatic interactions with the PSI PsaF polypeptide (Hippler et al., 1996); such contacts have been documented only for chloroplast systems (Hippler et al., 1997; Hippler et al., 1998). Whereas there are ongoing studies of the PsaF domain required for efficient binding of algal PC/cyt c₆ (Hippler et al., 1999), our work focuses on the structural requirements for PC/cyt c₆ docking within the PSI reaction center dimer composed of the P700-binding proteins PsaA and PsaB. Analysis of the PsaA/PsaB heterodimer comprising the PSI core has identified two symmetrical α-helices lying along the luminal side of the membrane (Schubert et al., 1997). Due to their position, and the observation that they border a lumenal cavity of the appropriate dimensions to yield a PC/cyt docking site, these so-called L and L’ helices have been proposed to interact directly with PC (Schubert et al., 1997). The sequences of such helices are also highly conserved from cyanobacteria through higher plants (Figure 2). Given the fact that *Prochlorothrix* PC can interact strongly only with *Prochlorothrix* PSI, and not *Anabaena* or chloroplast PSI (Table 1; Navarro, pers. comm.), it is likely that the binding site on PSI is uniquely adapted to accommodate the different hydrophobic surface on *Prochlorothrix* PC (Figure 1, Mychkine and Bullerjahn, in preparation). Additional evidence for this is the observation that *Anabaena* PC can only react with PSI via a collisional mechanism independent of docking (Table 1; k_{bim} = 1.1 \times 10^8 M^{-1}s^{-1}; Navarro pers. comm.). Furthermore, analysis of the deduced amino acid sequences of PsaA and PsaB from *Prochlorothrix* revealed unique differences in the regions of the docking site. Most notable is a unique arginine residue replacing a conserved tryptophan residue in the L helix (Figure 2). Such differences at the amino acid level possibly define sites of protein/protein contact between PCs and the PSI core, whose functional importance can be ascertained by subsequent site-directed mutagenesis of the *psaA/B* sequences encoding the L/L’ helices. Assaying an array of PSI mutants (future work) with PC hydrophobic patch mutants and/or cyt c₆ mutants (ongoing studies), should yield a thorough analysis of the protein-protein contacts within the docking site.

**Table 1. Kinetics of electron transport between *Prochlorothrix* PC, PSI and heterospecific PC and PSI preparations.** All assays were performed in 10mM MgCl₂ at pH 7.5. The kinetic measurements were done in collaboration with Dr. J. A. Navarro in Prof. M. A. De la Rosa’s group at the University of Seville.

<table>
<thead>
<tr>
<th>Electron transport to <em>Prochlorothrix</em> PSI (donors below)</th>
<th>K_A (M⁻¹)</th>
<th>k_et (s⁻¹)</th>
<th>k_{bim} (M⁻¹ s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Prochlorothrix</em> PC</td>
<td>8.7 X 10⁴</td>
<td>1.55 X 10³</td>
<td>————</td>
</tr>
<tr>
<td><em>Prochlorothrix</em> PC, 0.2M NaCl</td>
<td>7.2 X 10⁴</td>
<td>1.12 X 10³</td>
<td>————</td>
</tr>
<tr>
<td><em>Prochlorothrix</em> PC, 10% glycerol</td>
<td>2.6 X 10⁴</td>
<td>1.8 X 10³</td>
<td>————</td>
</tr>
<tr>
<td><em>Anabaena</em> PC</td>
<td>————</td>
<td>————</td>
<td>1.1 X 10⁸</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Electron transport from <em>Prochlorothrix</em> PC (acceptors below)</th>
<th>K_A (M⁻¹)</th>
<th>k_et (s⁻¹)</th>
<th>k_{bim} (M⁻¹ s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spinach PSI</td>
<td>No reaction</td>
<td>————</td>
<td>————</td>
</tr>
<tr>
<td><em>Anabaena</em> PSI</td>
<td>1.0 X 10⁴</td>
<td>3.2 X 10³</td>
<td>————</td>
</tr>
</tbody>
</table>

Figure 2. Deduced amino acid sequence of the L helices from the PsaA protein of the Photosystem I reaction center. The L helix borders the cavity defined as the PC docking site. Indicated in bold is a unique arginine residue.

Prochlorothrix: NH₂-GRLRDFLWAQA-COOH
Universal consensus: NH₂-GWLRDFLWAQA-COOH
Mutagenesis of *Prochlorothrix* PC

To date, we have simply taken the *P. hollandica* PC hydrophobic patch and selectively changed the unique amino acid residues to the consensus residues. Thus, we are building a typical hydrophobic patch on a *P. hollandica* PC scaffold to determine whether such mutations enable the PC to bind heterospecific PSI preparations. These mutant PCs (Y12G, P14L, Y12G + P14L, and M33N) have been expressed, refolded and are currently being assayed (in collaboration with Dr. José Navarro) for docking and electron transport with both *Prochlorothrix* and *Anabaena* PSI. Our initial data set indicates that the refolded wild-type and mutant PCs can dock and are competent for electron transport to PSI, and mutants are altered in both properties. Surprisingly, the P14L mutant shows a significant increase in $k_{cat}$ (J. A. Navarro, personal communication). Further experiments will explore such interactions at varying ionic strengths and pH.

Characteristics of *Prochlorothrix* cyt $c_6$

Of some interest to us is the possibility that key structural features involved in protein/protein interactions between *P. hollandica* PC and its reaction partners may also be reiterated in the surface conformation of *P. hollandica* cyt $c_6$. If so, then the residues forming this structure are likely functionally analogous in forming a docked complex, and have coevolved with the PSI reaction center (and possibly cyt $f$) to yield a specific protein/protein contact. To date, we have purified *P. hollandica* cyt $c_6$ from copper-limited cultures, obtained a partial sequence for the protein (SwissProt accession # P81244), and characterized the protein with respect to pl (8.1, similar to the PC from this species) and midpoint redox potential (+381 mV; Wilder and Bullerjahn, in preparation). Whereas PC accumulates in whole cells only in the presence of copper in the medium, cyt $c_6$ is detectable in whole cells under all growth conditions, albeit at low levels in copper-replete, iron-limited media (Wilder and Bullerjahn, in preparation). Moreover, the protein is an effective electron donor to PSI, and like PC acts by yielding a bound complex prior to electron transfer (Navarro et al., 1999). Nonetheless, our preliminary data indicate that the docking mechanism to *P. hollandica* PSI may be different than observed between PC and PSI. Whereas PC/PSI docking was relatively insensitive to increases in ionic strength, yet inhibited by glycerol addition, cyt $c_6$/PSI docking was affected significantly by the addition of cations to 20 mM (Navarro et al., 1999). From these data we suggested that the interaction of PC with PSI involved mostly hydrophobic interactions, whereas cyt $c_6$/PSI docking occurred via electrostatics (Table 1). Thus, the docking site on the luminal side of PSI may involve different contacts depending on the electron donor. Our future work may help resolve whether cyt $c_6$/PSI and PC/PSI interactions are fundamentally different in nature.

Concluding Remarks

The studies described above are a wide range of experiments designed to provide novel insights into the structural requirements for the functional interactions of PC/cyt $c_6$ with PSI. The special structural and functional characteristics of *Prochlorothrix* PC will lead to a better understanding of the sites of protein-protein interactions leading to productive electron transfer. We believe that examining this natural variant of PC with both its native and heterospecific reaction partners provides a novel and informative perspective in understanding the molecular recognition events leading to intermolecular electron transfer, while providing additional structural information about the donor side of Photosystem I. To date, the crystal structure of *Synechococcus* sp. PSI has been determined to less than 3 angstrom resolution (Schubert et al., 1997; Klukas et al., 1999 a, b), revealing a cavity on the luminal side of the complex having the appropriate dimensions to accommodate the binding of PC or cyt $c_6$. Nonetheless, neither the precise structural requirements for docking, nor the mechanisms for discriminating between PC vs. cyt $c_6$ can be unequivocally determined from this structure. Thus, we argue that a molecular genetic approach described here provides a complementary means toward understanding the mechanisms leading to productive electron transfer to PSI.

References


About the Author

George Bullerjahn is currently professor of Biological Sciences and a principal member of the Center for Photochemical Sciences. Dr. Bullerjahn earned his A.B. degree in 1977 at Dartmouth College and in 1984 completed his Ph.D. in biology at the University of Virginia. Following an NRSA postdoctoral fellowship studying with Prof. Louis Sherman, he arrived at BGSU in 1988. His work is focused on stress responses and photosynthesis in prokaryotes. Dr. Bullerjahn’s interests also include curling, golf and home brewing. His address is Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio 43403; e-mail: bullerj@bgnet.bgsu.edu.
Chlorosomes: The Light-Harvesting Complexes of the Green Bacteria

Elena V. Vassilieva, Niels-Ulrik Frigaard, and Donald A. Bryant
Department of Biochemistry and Molecular Biology and the Center for Biomolecular Structure and Function,
The Pennsylvania State University

Introduction

The green sulfur bacteria (GSB), or Chlorobiaceae, live in light-limited environments and have a unique and powerful light-harvesting antenna, the chlorosome. The large chlorosome antennae of some species of GSB are capable of efficiently collecting the dim light that penetrates through thick water layers to depths as great as 80 m, where light transmission may be only 0.0005% of surface.1 This highly efficient light capture is made possible by the presence in chlorosomes of large amounts of highly aggregated, excitonically coupled molecules of bacteriochlorophyll (BChl).

Another family of green bacteria, the green gliding bacteria (GGB), or Chloroflexaceae, also possess chlorosomes. The excitation energy is transferred from chlorosomes to the photosynthetic reaction centers in the membrane, where charge separation takes place. The reaction centers of GSB are of the iron-sulfur type (Type I), while GGB possess quinone-acceptor-type reaction centers (Type II), like those found in purple bacteria. GSB are strictly anaerobic, photoautotrophic prokaryotes which usually oxidize reduced sulfur compounds to provide the reductant for CO₂ fixation reactions. GGB are metabolically more versatile and can grow as facultative photoautotrophs or as photoheterotrophs under anaerobic conditions or as respiring chemoorganotrophs under aerobic conditions.2 Chlorosomes are always found in the obligately phototrophic GSB, while formation of chlorosomes in the most studied gliding bacterium, *Chloroflexus aurantiacus*, is induced in the absence of oxygen.

Under the electron microscope chlorosomes appear as ellipsoidal bodies appressed to the cytoplasmic side of the inner membrane of the bacterial cell. Chlorosomes of *Chlorobium tepidum* have an average length of 140-150 nm and a width of about 60 nm. The fully developed chlorosomes of *Cf. aurantiacus* are smaller and typically have a length around 100 nm, a width of 20-40 nm and a height of 10-20 nm. The chlorosome core is composed of rod-like elements that are probably formed from highly aggregated BChls. Each chlorosome can contain from 10 to 30 of these rod elements, and several models for the organization of the chlorophylls in such aggregates have been proposed.3,4 Chlorosomes are surrounded by a galactolipid/protein monolayer envelope. Lipids, wax esters, proteins, carotenoids and quinones are also components of these structures. Chlorosomes contain a small amount of BChl a that is thought to occur in the baseplate region of the chlorosome. Such a localization follows from the energetically downhill direction of the transfer of excitation energy toward the pigments with greatest red-shifted absorption/fluorescence peaks as shown schematically in Figure 1; i.e. from carotenoids to BChl c, d or e (713 - 750 nm, see also Figure 3) to BChl a (795 nm) in chlorosomes and further to the membrane-bound antenna complexes and reaction centers. A review explaining the principles of pigment oligomerization and the energy transfer in chlorosomes with a general description of chlorosome structure was published in *The Spectrum* by Dr. R. E. Blankenship.5 A comprehensive review by Blankenship and co-workers³ should be consulted for the references to the literature prior to 1995.

Chlorosome Proteins

The polypeptides present in the chlorosome are quite unusual in a number of aspects. Firstly, the protein-to-pigment ratio in chlorosomes is the lowest known for all types of photosynthetic antennas.4 In all other antenna systems proteins bind the light-harvesting pigments and thus provide the structural matrix necessary for effective transfer of excitation energy. However, convincing evidence indicates that in chlorosomes pigment-pigment interactions, but not pigment-protein interactions, predominate and play important roles in light-harvesting. The roles of the chlorosome proteins in the structure, function, and biogenesis of this unusual antenna complex have produced highly contentious debates among various workers in this area.³ Chlorosome chlorophylls are able to self-aggregate in solutions and the aggregates are capable of ultrafast energy transfer. Under the electron microscope, these artificial complexes may also resemble the rod-shaped elements seen in the chlorosome cores (see³ for references). These observations provide the basis for the assumption that chlorosome proteins are probably not of paramount importance for the light-harvesting function.
Chung and co-workers showed by SDS-PAGE and N-terminal amino acid sequence analyses that highly purified chlorosomes of the moderately thermophilic green sulfur bacterium *Cb. tepidum* contain ten different polypeptides, denoted CsmA through CsmJ. Before that work, only two proteins of Chlorobium-type chlorosomes, CsmA and CsmB had been characterized. Within the last few years the genes encoding all major chlorosomal proteins of *Cb. tepidum* were cloned, over-produced in *E. coli*, purified, and used to raise antibodies. On the electrophoretogram shown in Figure 2 the ten chlorosome proteins of *Cb. tepidum* (Figure 2, lane 1) are identified by the letters corresponding to their gene designations. For comparison the polypeptide composition of chlorosomes isolated from *Cf. aurantiacus* is shown on the same gel (Figure 2, lane 3).
It was first shown by immunogold labeling that chlorosome proteins of *Cf. aurantiacus* are components of the chlorosomal envelope.\textsuperscript{11} Similar results, but for the chlorosome proteins of *Cb. tepidum*, were later obtained in our group by immunoprecipitation and protease susceptibility studies.\textsuperscript{9, Vassilieva et al., manuscript in preparation} Additionally, low concentrations of detergents (e.g., SDS) can selectively extract nearly all chlorosome proteins while only extracting trace amounts of BChl c.\textsuperscript{12}

Treatment of the isolated chlorosomes with SDS in the presence of hexanol caused the complete extraction of all proteins while less than 4% of the BChl c was extracted.\textsuperscript{Vassilieva et al., manuscript in preparation} Moreover, the spectroscopic properties of the protein-free chlorosomes were similar to those of intact chlorosomes. These data confirm the localization of all chlorosomal proteins to the surface, envelope-layer of the chlorosome and further indicate that proteins are not associated with the rod-shaped BChl aggregates in the chlorosome interior.

**Figure 3.** Absorption spectra of chlorosomes isolated from *Cb. tepidum* ATTC49652 (green trace), *Cb. vibrioforme* NCIMB8327 (black trace), and *Cb. phaeobacteroides* 1549 (brown trace). The spectra have been normalized at the Qy absorption maxima which are indicated in nanometers.

Figure 3 shows the absorption spectra of chlorosomes isolated from three closely related organisms that share identical CsmA proteins\textsuperscript{6, S. Persson, personal communication}. BChl c - containing strain *Cb. tepidum*, BChl d - containing strain *Cb. vibrioforme*, and BChl e - containing strain *Cb. phaeobacteroides*. It is generally believed that different antenna BChls, absorbing at different light wavelength ranges, can facilitate the occupation of specific ecological niches. However, it seems that CsmA is not used “to tune” to the absorption properties of the BChls in GSB as is typically observed for the protein components of antenna complexes in plants and other bacteria. CsmC is 91% identical in *Cb. tepidum* and *Cb. vibrioforme*, and CsmB is 84% identical.\textsuperscript{8} The overall similarity between all other chlorosome proteins of *Chlorobium* species should be equivalently high, because antibodies produced against recombinant proteins from *Cb. tepidum* recognized chlorosome proteins from *Cb. vibrioforme* and *Cb. phaeobacteroides*.\textsuperscript{Vassilieva and Bryant, unpublished results} BChl c found in chlorosomes of *Cf. aurantiacus* has stearol as the major esterifying alcohol instead of farnesol as in BChl c of *Cb. tepidum* and also differs in methylation at the C8 and C12 positions. In *Cb. tepidum* and *Cf. aurantiacus*, the CsmA polypeptides are about 30% identical. An alignment of the CsmA proteins from these two bacterial groups suggests a functional similarity and evolutionary relationship for this most abundant chlorosome protein.\textsuperscript{I3} It has been suggested that histidine in the conserved region sequence motif GHW might bind to the central Mg atom of BChl c.\textsuperscript{13} However, because of the low protein-to-BChl c ratio, it is obvious that only in a fraction of pigment molecules could such an interaction occur. It was recently proposed that in *Cf. aurantiacus* BChl a might bind to CsmA at this position.\textsuperscript{14}

**Structural Motifs / Sequence Similarity of the Chlorosome Proteins**

CsmA and CsmE are 49% identical, and both are synthesized as precursor proteins that are C-terminally processed to produce the respective mature polypeptides.\textsuperscript{8} Upon closer inspection it becomes clear that there are in fact three families of chlorosome proteins: CsmA/CsmE, CsmB/CsmF, and CsmC/CsmD. The CsmB and CsmF proteins are 29% identical and 63% similar in sequence. Moreover, these two proteins share substantial sequence similarity to the N-terminal domain of CsmH. The CsmC and CsmD proteins are also clearly related in sequence and exhibit 26% identity and 45% similarity. At the same time the C-terminus of CsmH shares sequence similarity with CsmC and CsmD.

The N-terminal domains of CsmI and CsmJ, and by analogy CsmX, have been shown to be closely related in sequence to adrenodoxin-type ferredoxins and to bind 2Fe-2S clusters.\textsuperscript{18} Since most ferredoxins are water-soluble proteins, a relevant question is how do these proteins interact with the chlorosome envelope. Although the overall sequence identity is low, the C-terminal domains of CsmI, CsmJ and CsmX appear to be related to the precursor forms of CsmA and CsmE. The multiple alignment suggests that the C-terminal domains of CsmI, CsmJ and CsmX contain an insertion of 28 to 33 amino acids between two conserved sequence motifs. These sequence alignments also suggest that the C-terminus of CsmJ may be endoproteolytically processed in a manner similar to CsmA and CsmE, since a sequence (...L-P-G-S ↓ T-C...) that closely resembles the processing site (...L-K/R-G-S ↓ S-P...) for CsmA and CsmE\textsuperscript{6,9} is
found in this protein. Figure 1 above shows a schematic representation of the domain structures for the chlorosome proteins of Cb. tepidum. Although ten proteins are found in the chlorosome envelope, these proteins contain only four structural motifs, each represented by a different shape in the diagram. Most of the apparent protein structural diversity has arisen by gene duplication and divergence or by gene fusion events to develop new proteins from existing structural motifs (e.g., CsmA/E + an adrenodoxin domain or the combination of CsmC/D and CsmB/F motifs in CsmH).

**Quenching of Energy Transfer in Chlorosome Under Aerobic Conditions**

GSB cannot grow in the presence of O$_2$. One important reason for this is that O$_2$ and the strong reductants generated by the reaction center can spontaneously react to form reactive oxygen species that are toxic to the cells. The GSB have a unique mechanism that can help to alleviate this problem: a redox-dependent quenching of energy transfer in the chlorosomes. This quenching is inactive under reducing conditions and active under oxidizing conditions, and it appears to involve direct redox titration of a chemical species within the chlorosomes. When activated, the quenching mechanism prevents energy transfer in the chlorosome and thus photosynthetic activity in the reaction center. Experimentally this reversible quenching phenomenon is observed both in isolated chlorosomes and intact cells as a 10- to 50-fold drop in steady-state BChl c fluorescence, a decreased excited-state lifetime for BChl c from 50-100 ps to 10-15 ps, and a decreased photosynthetic activity of the reaction center.15

The redox midpoint potential ($E_{m7}$) of the fluorescence quenching in isolated chlorosomes is in the -100 to -150 mV range.16,17 The quenching species is readily activated by O$_2$ both in isolated chlorosomes and intact cells and readily inactivated by dithionite. It seems likely that the necessary reductants in vivo to reverse such quenching will originate in the reaction center or from soluble components in the cytoplasm. One route of delivery of such electrons could be through the iron-sulfur proteins in the chlorosome envelope.$^{10}$ CsmJ ($E_{m7}$ -201 mV) could be capable of reducing the quenching species. CsmI or CsmX (or both) has a rather high midpoint potential ($E_{m7}$ +92 mV) and one of these proteins could mediate oxidation of the quenching species by oxidants such as O$_2$. In support of this suggestion, the iron-sulfur clusters of CsmI, CsmJ, and CsmX are unusually stable in the presence of O$_2$.10

Chlorosomes from Cb. tepidum contain a unique, and rather oxidizing ($E_{m7}$ +36 mV), isoprenoid quinone called chlorobiumquinone (CK, 1’-oxomenaquinone-7).18,17 Quinones are good quenchers of chlorophylls and become increasingly better as their midpoint potential becomes more positive.19 CK could thus be responsible for, or at least involved in, the redox-dependent quenching in chlorosomes. Cf. aurantiacus does not exhibit redox-dependent quenching and also does not contain CK. Interestingly, however, chlorosomes from both Chlorobium and Chloroflexus species contain menaquinone$^{17}$ but this quinone has a much lower midpoint potential ($E_{m7}$ -81 mV$^{19}$) than CK and may not be involved in quenching. Although Cf. aurantiacus does not naturally possess redox-dependent quenching, addition of hydrophobic and rather oxidizing quinones can induce such quenching which affects both fluorescence$^{20}$ and energy transfer to the reaction center.21 These observations suggest that, with respect to redox-regulation, a major difference between the chlorosomes of the two types of green bacteria could be largely due to the indigenous quinones that are found in the chlorosomes of the GSB.

The midpoint potential of fluorescence quenching in isolated chlorosomes correlates poorly with the midpoint potential of CK in aqueous solution at neutral pH. However, the exact form of the reduced CK in the chlorosomes is not clear. CK may be reduced by a one-electron or by a two-electron reaction, and the midpoint potential may be significantly shifted in the unusual environment inside the chlorosome. Neither the pH nor the hydrophobicity of the chlorosome interior is known. Future research should determine the exact species of CK that is present and should identify other components present in the chlorosomes under various redox conditions.

Several of the questions raised above are currently being investigated in our lab. We are trying to modify or eliminate completely the iron-sulfur proteins CsmI, CsmJ, and CsmX, as well as CK, by targeted mutagenesis of Cb. tepidum.22 These experiments should provide a much better understanding of the mechanism and physiological function(s) of the quenching process.

**Comparison of Chlorosome Proteins in Cb. tepidum and Cf. aurantiacus**

It has been shown that the csmC and csmA genes are co-transcribed,$^6$ and the organization of the csmCA operon, including ORFZ and ORFX upstream and downstream, is similar in Cb. tepidum and Cb. vibrioforme. A similar arrangement is found in Cf. aurantiacus except that the csmC gene is missing.$^6,23$ Chlorosomes of Cf. aurantiacus have been reported to contain only three or four polypeptides$^{24}$ (see Figure 2), and these chlorosomes do not appear to contain
Fe-S clusters. However, one of the four proteins is an obvious homolog of CsmA and is even C-terminally processed in a similar manner to CsmA in *Cb. tepidum*.23 Lehmann and co-workers13 reported a 29-residue long N-terminal amino acid sequence for a protein, found in *Cf. aurantiacus* chlorosomes which we designate CsmO on Figure 2. CsmO is clearly related in sequence to the CsmB/CsmF family (Figure 4, B). Finally, *Cf. aurantiacus* chlorosomes also contain proteins with apparent molecular masses of 11 and 18 kDa, which are the products of the *csmM* and *csmN* genes.25 These two proteins are distantly related in sequence to the CsmC and CsmD proteins of *Cb. tepidum* (Figure 4, A). Hence, each protein found in the chlorosomes of *Cf. aurantiacus* is related in sequence to a protein found in the chlorosome envelopes of the GSB.

**Conclusions**

Until very recently, the existence of chlorosomes in both the Chlorobiaceae and Chloroflexaceae was considered confusing.4 Major differences between these groups include their reaction center types as well as their metabolic and physiological diversity. However, recent phylogenetic analyses of the Mg-tetrapyrrole biosynthesis genes and enzymes have identified the GSB and GGB as closest relatives.26 The possibility of a reaction center displacement or other lateral gene transfer event(s) could explain some of the differences between the two groups.

The common structural motifs found in all chlorosome proteins further confirm the point of view that these two groups of green bacteria are related. It also appears that the structural complexity of chlorosome proteins of the GSB and GGB are more similar than previously imagined. Chlorosome protein heterogeneity appears to have arisen by gene duplication and divergence among a small number of protein types. Elucidation of the structural features of these protein types should provide much additional information about role of proteins in the chlorosome envelope. Since the recombinant CsmH protein is produced in high yield from *E. coli* in water-soluble form, future structural
studies by either multi-dimensional NMR analysis or X-ray crystallography will focus on this protein. Experiments with a csmC knock-out mutant has already shown that the absence of this protein affects energy transfer. It can be anticipated that further genetic analyses will reveal a wealth of new information concerning the roles of chlorosome proteins in the functioning of these unique antenna structures.

Finally, the complete genomic sequence of a representative member of the GSB, *Cb. tepidum*, has recently been determined by The Institute for Genomic Research (TIGR\textsuperscript{10} Eisen et al., manuscript in preparation). Moreover, methods for the insertional inactivation of genes through natural transformation have been developed for this organism. It can be anticipated that much new information concerning structure, function, biogenesis, and physiology will result from these new tools for the study of photosynthesis in these unusual organisms.

**Acknowledgment**

This work was supported by grant DE-FG02-97ER20137 from the U. S. Department of Energy to D. A. B.

**References**

10. Vassilieva, E. V.; Antonkine, M. L.; Zybalov, B. L.; Yang, F.; Jakobs, C.; Golbeck, G. H.; Bryant, D. A. *Biochemistry*, accepted for publication.

**About the Authors**

Dr. Vassilieva received a Ph.D. in biochemistry from the Russian Academy of Medical Sciences in 1994. She was a postdoctoral fellow with Dr. M. Meagher at the University of Nebraska-Lincoln and is currently a postdoctoral fellow with Dr. Bryant.
Dr. Frigaard received a Ph.D. in biochemistry in 1997 from Odense University, Denmark. He was a postdoctoral fellow at Tokyo Metropolitan University and since 1999 has been a postdoctoral fellow with Dr. Bryant.

Dr. Bryant received his B.Sc. in chemistry from the Massachusetts Institute of Technology in 1972 and his Ph.D. in Molecular Biology from the University of California at Los Angeles in 1977. After postdoctoral work at the Institut Pasteur, Paris, with Dr. Roger Stanier and at Cornell University with Dr. R. K. Clayton, in 1981 he joined the faculty of The Pennsylvania State University, where he is currently the Ernest C. Pollard Professor of Biotechnology and Professor of Biochemistry and Molecular Biology. He may be contacted at the Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, Pennsylvania 16802. Dr. Bryant’s e-mail address: dab14@psu.edu

Advertising Special in The Spectrum

The Spectrum is a quarterly publication of the Center for Photochemical Sciences, Bowling Green State University, Bowling Green, OH 43403.
Phone 419-372-2033 Fax 419-372-0366
Email photochemical@listproc.bgsu.edu
WWW http://www.bgsu.edu/departments/photochem/

Executive Director: D. C. Neckers
The Spectrum Editor: Pat Green
Production Editor: Alita Frater

COPYRIGHT PERMISSION

A person may make a single copy of any or all articles in this issue for personal use. Copying beyond that permitted by the U.S. Copyright law is allowed provided that the appropriate per copy fee is paid through the Copyright Clearance Center, Inc., 27 Congress St., Salem, MA 01970. For reprint permission, please write to the Center for Photochemical Sciences.

EDITORIAL POLICY

The Spectrum reserves the right to review and edit all submissions. The Spectrum is not responsible for contents of articles.

Articles submitted to The Spectrum will appear at the discretion of the editorial staff as space is available.
Fluorescent Radical Cation in Solution as a Probe for Ion Pair Dynamics

Nobuyuki Ichinose and Tetsuro Majima
The Institute of Scientific and Industrial Research (ISIR), Osaka University

Introduction

Fluorescence spectroscopy has a sensitivity that allows detection at the single-molecule level. However, fluorescence detection of radical ions has been unsuccessful because of their extremely low fluorescence quantum yields in solution even if their parent molecules are highly fluorescent. This is due to the low excitation energy that favors nonradiative deactivation transition and the high chemical reactivity of radical ions in the excited state.1 For this reason, only a few examples of fluorescent radical ions have been reported and some of these are quite doubtful. Nevertheless, recent reports on the efficient fluorescence emissions from the excited radical ions with fluorescence quantum yields ($\Phi_f \approx 10^{-3}$) have opened the door to detecting electron transfer phenomena by fluorescence spectroscopy.3-5 Undoubtedly, radical ions are important intermediates in electron transfer reactions in photochemical, radiation chemical, and electrochemical processes. Generation of radical ions in solution includes one-electron transfer and should be accompanied by ion pair formation, e.g. radical cation-radical anion, radical cation-anion, etc. Absorption spectroscopy of radical ions is not sensitive enough to distinguish ion pairs from free ions, while fluorescence spectroscopy is sensitive enough. Here we demonstrate the fluorescence from excited 1,3,5-trimethoxybenzene radical cation (TMB$^{•+}$*) as an example of fluorescent radical ions and apply it to the monitoring of the dissociation and formation of radical ion pairs generated from photoinduced electron transfer and pulse radiolysis.6,7 The fluorescent nature of radical cations of the TMB family studied by the pulse radiolysis-laser flash photolysis combined method8 is also described.9-11

Fluorescent Nature of TMB$^{•+*}$ in Solution

We observed the fluorescence of TMB$^{•+*}$ by the two-laser two-step excitation technique.5 TMB$^{•+*}$ was generated by photoinduced electron transfer reaction in an aerated acetonitrile (MeCN) solution containing 1,4-dicyanonaphthalene (DCN) as an electron-accepting sensitizer, biphenyl (BP) as a co-sensitizer, and TMB (Chart 1). Excitation with a XeCl excimer laser (308 nm, 8 ns) initiated electron transfer between DCN$^*$ and BP giving radical ions, DCN$^{•-}$ and BP$^{•+}$, which led to the secondary electron transfer with O$_2$ and TMB, respectively. TMB$^{•+}$ showed an absorption spectrum between 400 and 600 nm with a peak at 590 nm. Excitation of TMB$^{•+}$ at 532 nm with a second harmonic pulse (6 ns) of a Nd:YAG laser afforded a fluorescence spectrum around 620 nm having mirror symmetry to the absorption spectrum (Figure 1). The fluorescence intensity increased with the increase of the delay time of the second laser pulse relative to the first 308-nm laser pulse. This corresponded to the growth of the TMB$^{•+}$ concentration and to the decay of the BP$^{•+}$ concentration monitored by the absorbance at 670 nm. These results ruled out a possibility of the fluorescence from fluorescent side products. $\Phi_f$ was measured to be $2 \times 10^{-3}$ which allowed an estimation of its lifetime of 210 ps using the Strickler-Berg relationship. This gave a natural radiative rate constant of $9.5 \times 10^6$ s$^{-1}$.

We examined several compounds belonging to the TMB family, i.e. those bearing the 1,3,5-trioxylbenzene structure, whose pseudo-D$_3h$ symmetry causes the degeneracy of the SOMO and SHOMOs. Therefore, radical cations of these compounds have low D$_{0-D1}$ and high D$_{0-D2}$ energy separations. This makes the D$_{2-D0}$ transition sufficiently radiative to compete with the D$_2$-D$_1$ internal conversion (Figure 2). We observed fluorescence from excited radical cations of 1,3,5-trioxylbenzene derivatives such as 3,5-dimethoxyphenol,9 1,3-dihydroxy-5-methoxybenzene...
These results indicated that complete symmetry of the substitution on the oxygen atoms is not necessary to observe fluorescence from excited radical cations, and that considerable variations in the parent molecules could be introduced. We also observed fluorescence from the excited radical cation of hexamethoxybenzene (HMB •+) as an example of pseudo-D_{6h} molecules. The discussion of the symmetry has been described in the reports on the fluorescence from excited radical cation of fluorobenzenes in the vapor-phase or noble gas matrices. Although there has been several reports on the fluorescence from excited radical cations in solution, no attempt has been made to design the parent molecules.

The fluorescence quantum yield of the 1,3,5-trioxylbenzene radical cations generated by pulse radiolysis in 1,2-dichloroethane (DCE) decreased with the introduction of alkyl or acetyl substituents at the oxygen atoms, suggesting that they act as quenchers. For example, Φ_f of the 1,3,5-trihydroxybenzene radical cation (THB•+) is 2.0 x 10^{-3}, 1.1 x 10^{-3} for TMB•+, and ≈ 0 for the 1,3,5-triacetoxybenzene radical cation (TAB•+). However, the mechanisms involved in the quenching of the excited 1,3,5-trioxylbenzene radical cation moiety are not the same. Internal conversion promoted by the C-H vibration would operate in TMB •+*, while intramolecular electron transfer would take place in TAB •+*. Excitation of TMB•+ in benzonitrile or in DCE in the presence of benzonitrile gave no or weak fluorescence owing to quenching by the intermolecular electron transfer. This process has an exothermicity of close to -0.57 eV as estimated from the ionization potentials (Ip) of TMB and benzonitrile (Ip = 8.11 eV and 9.62 eV, respectively), and the excitation energy of TMB•+* (2.03 eV). Similarly, fluorescence from TMB•+* was not observed in acetone (Ip = 9.67 eV) and cyclohexane (Ip = 10.32 eV). On the other hand, fluorescence of TMB•+* was observed in MeCN, DCE, 1,1,1,3,3,3-hexafluoro-2-propanol, 2,2,2-trifluoroethanol, and other chlorinated solvents (Ip > 10.4 eV). The absence of the fluorescence from TAB •+* is reasonably explained by exothermic intramolecular electron transfer quenching of the radical cation moiety in the excited state by the acetyl group (Ip = 10 eV).

**Formation Dynamics of [TMB•+/Cl-] in DCE**

Pulse radiolysis of TMB in chlorinated solvents gives TMB•+ as a transient species. Although the absorption spectrum of TMB•+ in MeCN showed a sharp peak at 590 nm during laser flash photolysis, the broad spectrum peaked around 500 nm and was observed in a highly chlorinated solvent such as CCl_4 or CH_2Cl_2 during pulse radiolysis (Figure 3). The transient absorption spectrum was time-dependent in DCE showing a spectrum similar to that in MeCN immediately after the pulse but similar to that in CCl_4 at >200 ns. This spectral change was attributed to the ion pair [TMB•+ / Cl-] formation in DCE. This was
also observed by increasing the TMB concentration. Two mechanisms for the ion pair formation are considered: one is a collision of TMB•+ and Cl-, and another is trapping of Cl• by TMB. Radiation chemical action induced by the electron pulse causes ionization and excitation of DCE. Subsequent fragmentation leads to the generation of DCE•+ and Cl• as chemically active species. Bimolecular electron transfer between these species and TMB gives free TMB •+ and [TMB•+/Cl-], respectively. The transient species showing a peak around 500 nm was assigned to [TMB•+/Cl-].

Collision between TMB•+ and Cl- proceeded with the rate constant of 3.0 x 1011 M-1 s-1 which is much larger than the diffusion-controlled rate constant in DCE (7.6 x 109 M-1 s-1) owing to their strong Coulombic interaction in the low dielectric medium (ε = 10). The Φf of TMB•+ as a function of the delay time decreased rapidly as shown in Figure 4. The addition of 0.01 M of tetrabutylammonium chloride as a source of Cl- drastically accelerated the decrease of Φf as well as causing a blue shift of the transient absorption. The Φf of TMB•+ fell to (2-3) x 10-5 within 20 ns after the electron pulse under this condition. This value would represent Φf of [TMB•+*/Cl-] and the low Φf was attributed to the intra-cage electron transfer quenching by Cl-. On the other hand, free TMB•+ in DCE was generated by a hole-transfer reaction between BP•+ and TMB during the pulse radiolysis of a TMB solution containing a large amount of BP. Although free TMB•+ emits a fluorescence with Φf ≈ 1 x 10-3, Φf decreased rapidly due to the ion pair formation. The free ion fraction could be estimated from the observed Φf. Pulse radiolysis of 0.01 M TMB in DCE gave TMB•+, whose ion pair fraction was 50% immediately after the electron pulse irradiation and 75% at 1 µs. The fast ion pair formation could be attributed to the reaction of Cl• with TMB.

**Diffusion Dynamics of [TMB•+ / DCN•-] in MeCN**

The chemistry of radical ions is of importance in photoinduced electron transfer phenomena. The free ion yield is often discussed in synthetic...
applications, carrier generation, initiation of polymerization, etc. Back electron transfer within the geminate radical ion pair plays an important role in the generation of free radical ions. This subject has been intensively studied by means of transient absorption spectroscopy in the range of ps to ns. However, there is no distinct difference in the absorption spectra of radical ions between those in the ion pair and free ion except for some sharpening of the spectrum of free radical ions. It has been widely accepted that the species derived from electron donor (D) and acceptor (A) are separated by 0.3-0.5 nm in exciplexes or contact ion pairs and by 0.7 nm in solvated ion pairs. These dissipate by diffusion to give free radical ions in polar solvents such as MeCN (Scheme 1). However, there has been no direct observation of the distance. It is also of interest to elucidate how the attractive Coulombic interaction between radical ions in the ion pair competes with the dissociative diffusional motion.

Laser flash photolysis of an argon-saturated MeCN solution containing TMB and DCN with the XeCl laser (308 nm, 8 ns) generated radical ions as revealed by the absorption of TMB•+.7 Fluorescence generated by the subsequent excitation of TMB•+ (532-nm, 6 ns, delay time of -50-400 ns relative to the first excitation) showed no increase corresponding to the formation of TMB•+ (Figure 5a). In the case of an aerated solution, on the other hand, the initial increase was clearly observed. Although this could be due to fluorescence quenching by DCN•-, no depletion of TMB•+ absorption was observed by the second 532-nm laser pulse. Therefore, this quenching was not attributed to electron transfer from DCN•- to TMB•+. To Förster type energy transfer. From the calculation based on the spectroscopic data of TMB•+ fluorescence and DCN•- absorption, a critical energy transfer distance ($R_0$) of 1.3 nm was estimated.

We analyzed both fluorescence intensity and absorption according to Förster theory. Fluorescence intensity ($I(t)$) at a delay time ($t$) of >200 ns decayed in a similar manner to the transient absorption monitored at 590 nm. For this reason, the fluorescence from TMB•+ was considered to be unquenched. Then, $I_q(t)$ as unquenched $I(t)$ at $t$ was introduced as a variable parameter which was obtained by the fitting of $I(t)$ at $t > 200$ ns to the absorption decay curve. The quenching efficiency $E(t) = 1 - I(t)/I_q(t)$ calculated from the experimental value of $I(t)$ was transformed as a simple function of distance between energy donor and acceptor ($E(t) = R_0^4/(R(t)^6 + R_0^4)$). Thus we obtained the time dependence of the distance between the radical ions at $t < 200$ ns (Figure 5b).
Curve fitting of the plot in Figure 5 with the simple Fickian diffusion equation \( R(t) = (2D t)^{1/2} \) gave a diffusion constant of \( 8.5 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1} \), which is much smaller than that expected for MeCN (2.0 \( \times 10^{-5} \text{ cm}^2 \text{ s}^{-1} \)). This may be due to the Coulombic interaction between the radical ions in the pair [TMB•+/DCN•−]. Furthermore, we obtained similar results from an experiment with 9,10-dicyanoanthracene (DCA, \( R_0 = 1.4 \text{ nm} \)). The addition of LiClO₄ to the solution accelerated the dissociation of the radical ions, thereby supporting the present results. The unquenched-to-quenched ratio of the fluorescence intensity \( (1 - E(t))/E(t) \) will be equal to \( R_0^6/(8D t^3) \) assuming Fickian diffusion. A double logarithmic plot of the experimental value of \( (1 - E(t))/E(t) \) vs \( t \) showed a linear relationship with a slope of -3. This is consistent with operation of Förster mechanism in the quenching of TMB••* fluorescence by the radical anion in its vicinity (Figure 5c). Moreover, the interaction between oppositely charged radical ions remains for 200 ns even in a polar solvent. These conclusions are in accordance with those reported from recent electron spin resonance studies.15,16 This also suggests the Coulombic interaction based on a point-charge model is invalid for radical ions.17

Conclusions

We have demonstrated by a two-color two-step laser excitation method and a pulse radiolysis-laser flash photolysis combined method that radical cations of 1,3,5-trioxylbenzene derivatives in solution fluoresce with moderately high \( \Phi_f \approx 10^{-3} \) in the range of 580-800 nm. The fluorescence has been readily observed with a conventional photomultiplier detector. These types of compounds are thus useful as electron-transfer probes for homogeneous and heterogeneous systems such as photocatalysts, biological oxidation, and so forth. As an example, ion pair dynamics have been described here where \( \Phi_f \) sensitively changes via ion pair-free ion interconversion. The mechanisms involved depend on the nature of counter anion species and the solvents. We are currently seeking fluorescent radical anions as well as highly fluorescent radical cations.

This article is dedicated to the late Prof. Setsuo Takamuku, ISIR, Osaka University.

Acknowledgments

We sincerely thank our collaborators Dr. K. Endo, Kanazawa University; Dr. T. Tanaka and Dr. S. Kawanishi, Japan Atomic Energy Research Institute; and Dr. K. Isagawa and Ms. S. Tojo, ISIR. We are also grateful to Prof. M. A. J. Rodgers, Bowling Green State University, for helpful discussions. Our work has benefited greatly by the LINAC system in the Radiation Laboratory of ISIR, Osaka University and has been partly supported by a Grant-in-Aid for Scientific Research (Nos. 09226223, 10132237, 09450319, and 12650811) from Ministry of Education, Science, Sport and Culture of Japan.

References

About the Authors

Nobuyuki Ichinose received his Ph.D. from Osaka Prefectural University in 1989 and worked on the Microphotoconversion Project, ERATO, Hokkaido University, and the Japan Atomic Energy Research Institute. In 1998 he joined the research group of Prof. Tetsuro Majima as a research associate at ISIR, Osaka University; e-mail: ichinose@sanken.osaka-u.ac.jp.

Tetsuro Majima received his Ph.D. from Osaka University in 1980 and worked as a research associate with Richard A. Caldwell at the University of Texas at Dallas for two years before moving to the Institute of Physical and Chemical Research (RIKEN, Japan). In 1994 he became an associate professor at Osaka University. He is currently a professor and the winner of the 2000 Japan Photochemistry Association Award. His address is ISIR (SANKEN), Osaka University, Ibaraki, Osaka 567-0047, Japan; e-mail: majima@sanken.osaka-u.ac.jp; web: http://www.sanken.osaka-u.ac.jp/labs/mec/index2.html.

Symposium - Photochemistry Becomes More Complex*

ACS National Meeting

Organic Division

San Diego, California

Monday and Tuesday afternoons, April 2-3, 2001

Invited speakers:
David F. Eaton, DuPont
Harry B. Gray, Caltech
George Hammond
Jay K. Kochi, University of Houston
Nathan S. Lewis, Caltech
Eve Menger
Douglas C. Neckers, Bowling Green State University
Thomas L. Penner, Kodak
Nicholas J. Turro, Columbia University
Richard G. Weiss, Georgetown University
James T. Yardley, Allied Chemical

For more information, please consult the website
http://chem.pdx.edu/~wamserc/Hammond/
or contact Carl C. Wamser, Portland State University
WamserC@pdx.edu

* Organic divisional rules prohibit dedicating a symposium to an individual except in specific circumstances. This symposium is intended to be a joint celebration of the progress of photochemistry as influenced by George Hammond on the event of his 80th birthday. There will be some informal gatherings as well.


For reprints of any of these publications, please write or e-mail the Center for Photochemical Sciences and refer to the reprint by number. Reprints of articles in press will be provided upon publication of the article.