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Minireview

Chlorophyll chemistry before and after crystals of photosynthetic reaction centers

Jack Fajer
Materials Science Department, Brookhaven National Laboratory, Building 555, Upton, NY 11973-5000, USA (e-mail: fajerj@bnl.gov; fax: +1-631-344-5815)

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Abstract

The experimental, theoretical and structural research leading to the identification and characterization of the (bacterio) chlorophyll species that mediate the primary events of solar energy transduction and dynamics is reviewed and examined from the author's perspective.

Introduction

Remarkable progress has been made in the last three decades towards unraveling the primary events of photosynthesis. This evolution derives principally from the crystal structures of the bacterial reaction centers (RCs) and, more recently, those of photosystems (PS) I and II of cyanobacteria, which have revealed the architecture used by photosynthetic organisms to convert solar energy efficiently into chemical form (Deisenhofer et al. 1995; Jordan et al. 2001; Zouni et al. 2001). Structures of light-harvesting complexes also have provided insights into the mechanisms by which incoming photons are collected and funneled into the RCs. The crystallographic results have been supplemented by elegant spectroscopic techniques, increasingly more sophisticated theoretical calculations, and molecular engineering (mutations, replacement of chromophores), all of which have helped to rationalize the mechanisms and efficiency of energy and electron transfer. [See the perspectives by Clayton (2002) on the isolation of bacterial RCs, and by Parson (2003) for the electron acceptors and donors of bacterial RCs.]

I will limit myself here to results that originated principally in my laboratory, but the reader should clearly understand that the research into the early events of photosynthesis and the subsequent development of a wide variety of biomimetic arrays that seek to duplicate the natural photodynamics and electron transport were carried out by what someone once described as 'a cast of thousands.' The historical chronology of these studies can be divided roughly into two periods. The earlier work, which predates crystal structures, basically attempted to establish what is happening at the molecular level, and the later work, stimulated by crystal structures, sought and still seeks to address why it is happening.

The optical features and the energetics of chlorophylls (Chls) and bacteriochlorophylls (BChls) *in vivo* clearly play a dominant role in solar energy conversion. I will thus begin with a brief summary of the molecular factors that determine these properties *in vitro*, before considering the more complex *in vivo* interactions.

The *in vitro* systems

The optical and redox properties of isolated chromophores can be readily explained in terms of the four-orbital model developed by Martin Gouterman

and coworkers (1963). The lowest-energy absorption bands (the Q_y transitions), which are most relevant to photosynthesis, are principally a HOMO- (highest occupied molecular orbital) to LUMO- (lowest unoccupied molecular orbital) transition. The molecular orbital (MO) scheme (Figure 1) for simple porphyrins, chlorins and bacteriochlorins indicates that within the same series the LUMOs are isoenergetic, that is, they do not change as the number of π electrons is reduced from 11 to 10 to 9 for porphyrins, chlorins and bacteriochlorins, respectively. The shrinking of the π system does, however, significantly destabilize the HOMOs, which systematically rise in the series. The net effect is that the HOMO–LUMO gaps get progressively smaller and the Q_y bands shift to the red (Chang et al. 1981). A similar effect applies to Chls and BChls and readily explains why the Q_y bands of BChls lie to the red of those of Chls (Hanson 1991). The oxidation and reduction (redox) potentials of the chromophores parallel the migration of the frontier orbitals (neglecting solvation effects) and thus, again within a homologous series, the reduction potentials of a porphyrin, chlorin and bacteriochlorin remain essentially the same, whereas the oxidation potentials become increasingly more facile with the bacteriochlorin having the lowest oxidation potential and the porphyrin the highest (Chang et al. 1981). Although this simple model readily rationalizes the optical and redox properties of the chromophores *in vitro*, the situation *in vivo* is more complex and the effects of aggregation, axial ligation, substituents and their orientation, hydrogen bonding, as well as neighboring residues became more obvious as crystal structures of RCs and antennae became available.

Systematic applications of electrochemical techniques in aprotic solvents began many years ago and led to the generation of well characterized cation and anion radicals of porphyrins and (bacterio)chlorophylls. Cyclic voltammetry yielded the redox trends noted above as well as the important fact that within a given pair of metal-free and magnesium (or zinc) containing chromophores, that is, BChl versus Bpheo (bacteriopheophytin) or Chl versus Pheo, the pheophytins are easier to reduce and harder to oxidize than the metallated species. This simple difference has been extensively exploited in synthetic biomimetic models for artificial photosynthesis (see e.g., Fujita et al. 1982; Gust et al. 2001). Controlled potential electrolysis, which counts the number of electrons added or extracted per molecule at a given redox potential, allowed spectral signatures of the radicals so generated to be determined

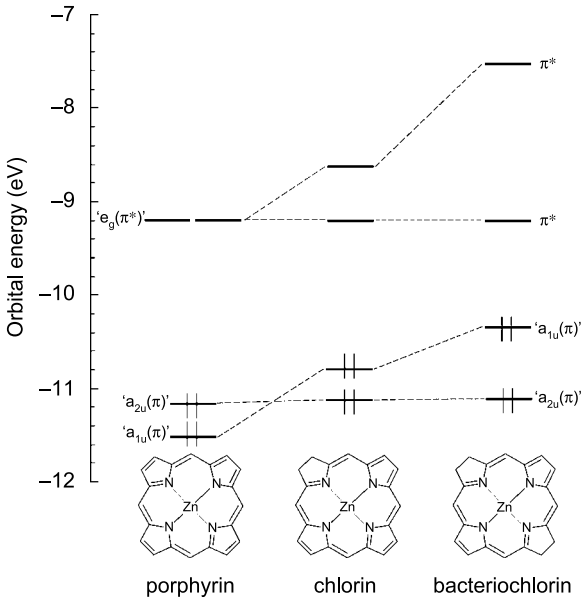

Figure 1. Energy level diagram calculated for the two highest occupied and two lowest unoccupied molecular orbitals for a porphyrin (P), a chlorin (C) and for a bacteriochlorin (BC). These determine the optical spectra of the chromophores (Gouterman et al. 1963). Note that the energy gap between the HOMO and LUMO decreases in the order P, C and BC and explains the red-shift of the lowest absorption band of the chromophores which is a HOMO to LUMO transition (adapted from Chang et al. 1981).

by optical and EPR (electron paramagnetic resonance) techniques and avoided possible side reactions induced by chemical oxidants and reductants. Chemical methods are easier to use than these electrochemical techniques and are much preferred by biophysicists. However, the electrochemical methods lent confidence to the chemical redox chemistry when the two were run in parallel.

The spectroelectrochemical methods thus yielded both optical and EPR (and later ENDOR – electron nuclear double resonance) spectra of Chl a^+, Chl a^-, Pheo a^+, Pheo a^-, $BChl^+$ and $BChl^-$ (*a* and *b*) as well as $BPheo^+$ and $BPheo^-$ (*a* and *b*) (Borg et al. 1970, 1976; Fajer et al. 1973, 1974, 1975, 1976, 1978, 1979, 1980; Davis et al. 1979a, b; Fujita et al. 1980) (Figure 2).

Comparable results were obtained for synthetic porphyrins, chlorins and bacteriochlorins. These molecules offered the significant advantage of being highly symmetrical and yielded well-resolved EPR spectra that could be readily interpreted with the aid of isotopic substitutions and semi-empirical calculations (Fajer et al. 1974; Fajer and Davis 1979). This

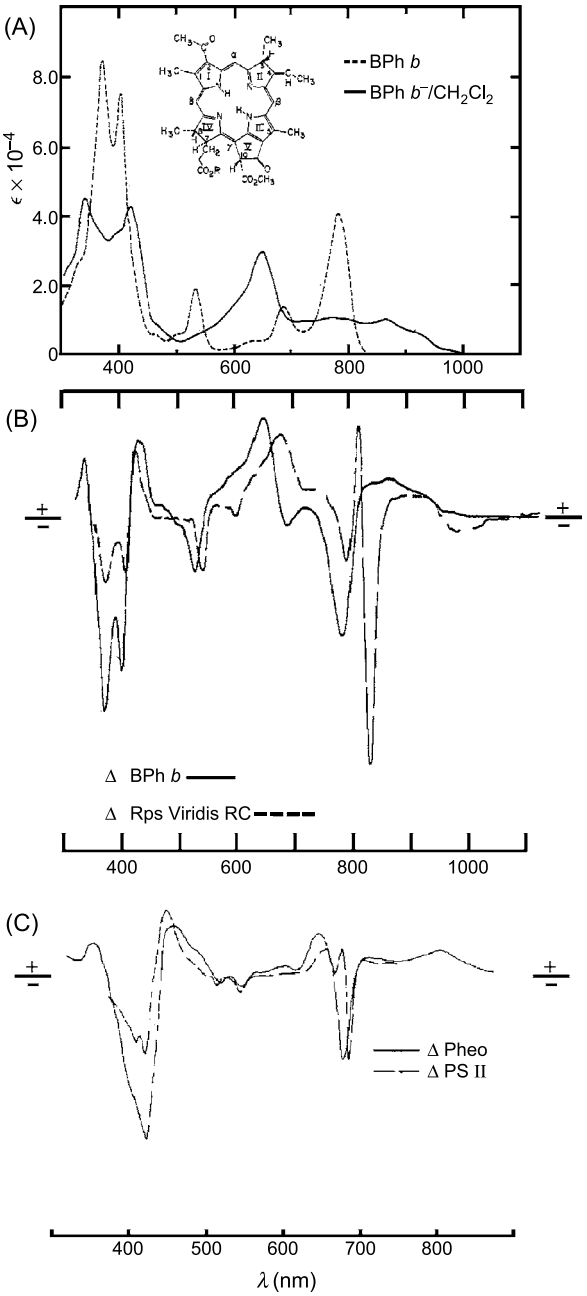

Figure 2. (A) Optical spectra of BPheo *b* and of its anion radical generated electrochemically. (B) Comparison of the difference spectra caused by reduction of BPheo *b* (Δ BPheo *b*) versus the changes observed on photoreduction of Rps viridis RC. The sharp peak at ~800 nm is attributed to an electrochromic shift of the adjacent BChl *b* (adapted from Davis et al. 1979b). (C) Optical changes observed on electrochemical reduction of Pheo *a* to its anion radical (Δ Pheo) versus those observed on photoreduction of PS II (adapted from Fajer et al. 1980).

work provided the first insights into the electronic profiles of the various cation and anion radicals and lent confidence to calculations and experimental results for the highly asymmetric Chl and BChl radicals. Subsequent developments and improvements in both ENDOR [originally invented by George Feher (see Feher 1998)] and semi-empirical calculations by Klaus Möbius, Wolfgang Lubitz and Martin Plato yielded detailed mappings for the spin profiles of Chl and BChl radicals (for reviews, see Lubitz 1991 and Plato et al. 1991).

The idea of the special pairs for the reaction center chlorophylls

It became obvious early on that, although the spectral features of Chl a^+ and BChl a^+ resembled those of $P700^+$ and $P870^+$, they differed in significant respects. These differences prompted Joe Katz, Jim Norris, George Feher and their coworkers (Norris et al. 1971; Feher et al. 1975) to propose the existence of 'special pairs' or dimers of Chls and BChls as primary electron donors of PS I and bacterial RCs. Photoexitation and electron ejection resulted in dimeric cation radicals in which the residual unpaired electron (or hole) is shared approximately equally by the two molecules, that is, $(Chl_2)^+$ and $(BChl_2)^+$.

The picosecond spectroscopy: primary charge separation *in vivo*

The development of picosecond spectroscopy by Peter Rentzepis and Maurice Windsor and its application to bacterial photosynthesis by their coworkers (Dutton et al. 1975; Rockley et al. 1975) unveiled an entirely new time domain in photosynthesis: the primary charge separation. The photochemical production of chemical cation and anion radicals occurred on a picosecond time scale. At the time resolution then available (~6 ps), this charge separation involved the generation of $P870^+$ in *Rhodobacter sphaeroides* or $P960^+$ in *Rhodopseudomanas viridis* and BPheo a^- or BPheo b^- (Fajer et al. 1975, 1976, 1978; Davis et al. 1979). Remarkably, the BPheo anion radicals that are photogenerated in a few picoseconds and exist for only a few hundred picoseconds could be readily identified by the spectral signatures obtained *in vitro* with lifetimes of days. Similar comparisons of spectral features and energetic considerations subsequently suggested that Chl *a* is the likely primary acceptor in PS I and Pheo *a* in PS II (Fujita et al. 1978; Fajer et al. 1980; see also Klimov 2003; Seibert and Wasielewski 2003), which is consonant with the recent

crystal structures of PS I and PS II (Jordan et al. 2001; Zouni et al. 2001). As the time resolution of the laser spectroscopy improved to better than 1 ps, a role for BChls *a* or *b* evolved either as real or virtual primary electron acceptors (two-step vs. superexchange mechanisms). However, the early sequence of electron transfer events seems to be clearly determined by the differences in reduction potentials of the BChls and Bpheos. The concepts of 'special pairs' as donors and BChls and BPheos as acceptors were confirmed by the several bacterial RC structures that were reported within a short time span by Hans Deisenhofer, Robert Huber, Hartmut Michel, George Feher, Doug Rees, Jim Norris and Marianne Schiffer. Without slighting the importance of these pioneering papers, I will refer here only the later, higher-precision structures (Ermler et al. 1994; Diesenhofer et al. 1995).

Theoretical calculations

Photosynthetic bacteria

The bacterial RC structures inspired numerous theoretical calculations of various levels of sophistication. The net conclusions were that the dimeric donors were supermolecules whose properties derived mainly from excitonic and charge resonance interactions (see, e.g., Thompson et al. 1991). One of the interesting results to evolve from the calculations done by Mark Thompson, Mike Zerner and co-workers is that the difference between P870 and P960 does not arise from the different peripheral substituents of BChls *a* and *b* but rather is dictated by the vertical spacing between the two overlapping rings of the chromophores, a proposal supported by the higher-precision structures of *Rhodobacter sphaeroides* and *Rhodopseudomonas viridis* RCs cited above. The same calculations were extended to predict the configurations of the donor BChls *g* in Heliobacteria (Thompson and Fajer 1992). Although BChl *g* differs from BChl *b* only by the replacement of the acetyl group on ring I by a vinyl group, the lowest-energy absorption band shifts from 960 nm in *Rps. viridis* to 800 nm in Heliobacteria. Here again, the calculations predict that the spacing between the monomers that comprise the (putative) dimer increases by 0.3–0.4 Å with some small reorientation of the vinyl group of BChl *g* compared to that of the acetyl group of BChl *b in vivo*.

Oxygenic photosynthesizers: Photosystems I and II

The variable spacing in the constructs of the primary donors is also evident in the recent structures of Photosystem (PS) I (Jordan et al. 2001) and PS II (Zouni et al. 2001). (For historical perspectives on PS I and PS II, see Petra Fromme and Paul Mathis, and Horst Witt, respectively, this issue.) In PS II, the two chlorophylls believed to form P680 are considerably farther apart than those attributed to P700. In fact, Zouni et al. suggest that P680 is comprised of monomeric Chls with only weak excitonic interactions. The concept that P680 might be a monomer was raised long ago by Davis et al. (1979a, b) who showed that a synthetic Mg chlorin as well as Chl *a* itself exhibited significant differences in unpaired spin density profiles with concomitant changes in oxidation potentials as a function of ligand and solvent. The oxidation potential of ligated monomeric Chl *a* in some media (0.93V vs. NHE) approached that of P680, ~1.1 V.

I would venture the guess that the Chls in P680 are planar (*vide infra*) so as to maximize the electron-withdrawing effect of the 9-keto group, that this group is hydrogen-bonded, and that the vinyl group on ring I is oriented perpendicular to the chlorin plane. In addition, the oxidation potential of P680 may be further raised by partial positive charges among the residues that form its microenvironment (Gudowska-Nowak et al. 1990; Fajer et al. 1992).

Although the early EPR and ENDOR studies sought to deduce the composition of the primary donors (P) by examining the oxidized P^+ radicals, it may be worthwhile to distinguish between the properties of P and P^+. One of the obvious functions of P is to act as a phototrap that is usually (but not always) red-shifted relative to the other chromophores in the RCs and antennae. The use of dimers, with their red-shifted maxima, clearly fulfills this function, and dimers are easier to oxidize than the other monomeric chromophores in the RCs. *The need for a high oxidation potential in PS II thus seems to override the merits of the dimeric forms used in PS I and bacterial RCs.* The electronic properties of the dimers also differ significantly from those of the monomers (Thompson et al. 1990, 1991) and may thus play an important role in the vectorial electron transfer along the functional branch, particularly in the bacterial RCs. Intriguingly, a conformational asymmetry seems to be built-in in the dimeric donors that would further enhance the directionality of electron transfer.

P700 is obviously a heterodimer comprised of Chl *a* and Chl *a*′ (Jordan et al. 2001). The Chl *a* molecule is also bent and the rings III/V fragment appear to be out-of-plane. This would minimize the electron-withdrawing effect of the 9-keto group by taking it out of conjugation, making the molecule easier to oxidize. In the bacterial RCs as well, the conformations of the two BChls *a* or *b* that comprise the dimers are different (Ermler et al. 1994; Deisenhofer et al. 1995). MO calculations on each monomer subunit indicate that the different conformations would result in different oxidation potentials as well as shifted optical transitions (Barkigia et al. 1988). Indeed, contrary to the earlier conclusions that the unpaired electron is equally delocalized over the two Chls or BChls in the oxidized dimers, recent ENDOR results indicate that the electron is mainly localized on one Chl in $P700^+$ (Webber and Lubitz 2001), and that the spin distribution varies among different bacterial RCs to as much as 2:1. This asymmetry can be further modulated by mutations around the BChls that affect the oxidation potentials of the dimers but may also not be structurally innocent (for a recent review, see Lubitz et al. 2002).

Consequences of conformational variations

Lastly, I would point to some additional consequences of conformational variations, a subject that we have focused on for several years now. As the crystallographic resolution of high molecular weight protein complexes improved with the use of synchrotron radiation, it became increasingly clear that the porphyrinoid cofactors in photosynthetic and heme proteins are flexible and can adopt multiple nonplanar conformations. Besides the RC results mentioned above, a multiplicity of conformations has been noted in the crystal structures of the light-harvesting antenna complexes of *Rps. acidophila* (Prince et al. 1997; McLuskey et al. 2001) as well as in the Fenna–Mathews–Olson ('FMO') proteins of *Prosthecochloris aestuarii* (Tronrud et al. 1986) and *Chlorobium tepidum* (Li et al. 1997). (See John Olson, this issue, for a historical account of the discovery of the FMO protein.) The FMO complex is particularly striking: the seven BChls *a* associated with a subunit of the complex all exhibit different conformations and have a variety of axial ligands. The axial ligands, hydrogen bonds and nearby residues that constitute the microenvironment of photosynthetic chromophores and heme prosthetic groups may thus define the protein scaffolding that controls the deformations of the cofactors.

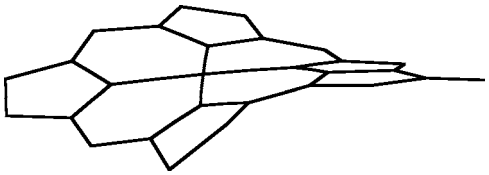

Figure 3. Edge-on-view of a nonplanar chlorophyll derivative which illustrates a macrocycle distortion often seen in structures of protein complexes comprised of (bacterio)chlorophylls and hemes. Peripheral substituents are omitted for clarity.

The obvious question arises as to the consequences of these structural distortions on the optical spectra, redox properties, excited state lifetimes, and rates of energy and electron transfer. Moreover, if the molecules are indeed as malleable as the crystallographic data indicate, further conformational changes could accompany oxidations and reductions and thereby modulate electronic coupling between donors and acceptors. Such changes also could propagate to the surrounding protein as gating or signaling mechanisms. It is not feasible to discuss these fundamental issues thoroughly in a few pages (besides the fact that some of the pertinent questions have not yet been answered). I will simply summarize recent results (Figure 3).

Calculations based on the crystal coordinates of the BChls in proteins certainly suggest that both optical and redox properties would be affected. For example, calculations based on the crystal coordinates of the FMO complex predict that the different conformers and neighboring residues give the seven BChls different spectral properties (Gudowska-Nowak et al. 1990).

In attempts to further assess the physicochemical consequences of the nonplanar distortions observed *in vivo*, we have turned to conformationally designed porphyrins in which introduction of multiple or bulky peripheral substituents enforces nonplanarity that is retained in solution because of steric constraints, allowing the (photo)physical and chemical effects of distortions to be documented. Although the synthetic chromophores are obviously not exact models of the *in vivo* molecules, these studies have revealed instructive trends and have established that nonplanarity can significantly alter optical, redox, radical, magnetic and excited state properties (for a review, see Fajer 2000). Briefly, distortions destabilize the HOMO more than the LUMO and the smaller HOMO–LUMO

gap causes optical red shifts. In addition, because the redox potentials track these orbitals, the molecules become easier to oxidize with comparatively little effect on reductions. Crystal structures of oxidized nonplanar porphyrins do show additional distortions. Perhaps the most striking effects of nonplanarity are observed in the lifetimes of the excited singlet states: these can be shortened by as much as three orders of magnitude (from nanoseconds to picoseconds). The short lifetimes observed at room temperature return to normal at low temperatures. We have attributed these dramatic lifetime changes to the fact that nonplanar chromophores can traverse multiple conformational surfaces in the excited state that are separated by only small energy barriers (Gentemann et al. 1997). Additional evidence for this flexibility is seen even in the ground state: multiple conformers of the same molecule are found crystallographically, sometimes even in the same crystals (Barkigia et al. 1988, 1998). Clearly, conformational variations provide an attractive and simple mechanism for modulating a wide range of physical and chemical properties of porphyrin derivatives *in vitro* and *in vivo*. Such conformational effects can be introduced by synthetic manipulations *in vitro*, and by a combination of axial ligation, hydrogen bonding, and nearby residues *in vivo*. Note also that if nearby residues of cofactors help to define a structural scaffolding *in vivo*, site-directed mutations may alter the protein pocket and indirectly affect the conformations and hence the properties of the chromophores (McLuskey et al. 2001).

I end this perspective with a photograph of myself taken in 1977 (Figure 4).

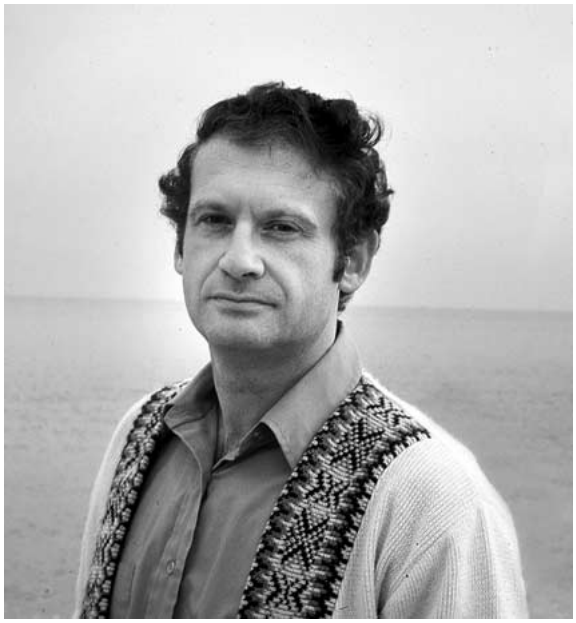

Figure 4. A photograph of Jack Fajer in 1977.

Acknowledgments

It was not feasible, in these few pages, to credit properly the researchers in photosynthesis who have made the field one of the exciting areas of biology, biophysics and chemistry. Nonetheless, it is a pleasure to acknowledge some of them, and the many friends, collaborators and coworkers acquired through three decades: K.M. Barkigia, D.C. Borg, D.C. Brune, C.K. Chang, L. Chantranupong, M.S. Davis, D. Dolphin, R.H. Felton, A. Forman, E. Fujita, I. Fujita, L.R. Furenlid, E. Gudowska-Nowak, L.K. Hanson, D. Holten, B. Ke, H. Levanon, D. Melamed, K. Mobius, M.D. Newton, D.J. Nurco, M.W. Renner, K.M. Smith, L.D. Spaulding, M.A. Thompson, J.P. Thornber, and M.C. Zerner. I am also indebted to Govindjee and William Parson for their help and thoughtfulness in the editing of this paper. (Given the present intense interest in nanoparticles, molecular wires and photonic devices, I note with some satisfaction that the photosynthetic community has been there and done that.) The work at Brookhaven has been generously supported by the Chemical Sciences Division of the US Department of Energy under contract DE-AC-02-98CH10886.

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