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Native Mass Spectrometry Characterizes the Photosynthetic Reaction Center Complex from the Purple Bacterium *Rhodobacter sphaeroides*

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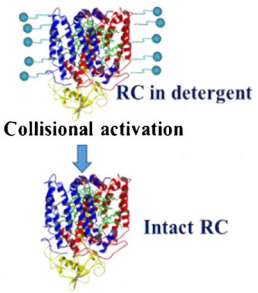

Abstract. Native mass spectrometry (MS) is an emerging approach to study protein complexes in their near-native states and to elucidate their stoichiometry and topology. Here, we report a native MS study of the membrane-embedded reaction center (RC) protein complex from the purple photosynthetic bacterium *Rhodobacter sphaeroides*. The membrane-embedded RC protein complex is stabilized by detergent micelles in aqueous solution, directly introduced into a mass spectrometer by nano-electrospray (nESI), and freed of detergents and dissociated in the gas phase by collisional activation. As the collision energy is increased, the chlorophyll pigments are gradually released from the RC complex, suggesting that native MS introduces a near-native structure that continues to bind pigments. Two bacteriochlorophyll *a* pigments remain tightly bound to the RC protein at the highest collision energy. The order of pigment release and their resistance to release by gas-phase activation indicates the strength of pigment interaction in the RC complex. This investigation sets the stage for future native MS studies of membrane-embedded photosynthetic pigment–protein and related complexes.

Keywords: Photosynthesis, Purple bacterium, Reaction center, Membrane protein complex, Native mass spectrometry, Pigment–protein interactions

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Introduction

The first stage of photosynthesis begins with light-energy harvesting by a photosynthetic antenna complex [1–3]. The collected energy is transferred into a membrane-embedded reaction center (RC), a complex of proteins, pigments, and other cofactors assembled to enable transmembrane electron transfer [4]. Noncovalent interactions hold together these subunits, pigments, and cofactors to form a functional RC unit. These interactions are essential for solar-energy capture and conversion within the photosynthetic protein complex.

Because membrane-embedded RC complexes are hydrophobic, they are difficult to study by traditional biochemical and biophysical approaches [5]. A relatively new opportunity is native mass spectrometry (MS). Here, we report a native MS and collisional activation study of an RC protein complex. Native MS enables the analysis of multi-subunit protein complexes under nondenaturing conditions [6–8]. Noncovalent interactions that hold together the complexes can be conserved in the gas phase upon native MS, allowing intact protein complexes to be interrogated by tandem MS to generate information on stoichiometry, topology, and protein–protein interactions [9, 10]. Although the ultimate goal in structural biology is a high-resolution structural model, the advantages of MS in sensitivity and fast turnaround make native MS an increasingly

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useful intermediate strategy that can yield key information prior to determination of a 3D structure [11]. Furthermore, in combination with ECD, native MS can guide protein expression for X-ray crystallography studies [12].

Most membrane-embedded protein complexes, including RC protein complexes, are not soluble in aqueous solution, and they require lipids or detergents to stabilize their native structure in solution. The Robinson group [13–16] first reported the measurement of membrane-embedded protein complexes in a detergent-micelle solution, permitting the near-native protein complexes to be observed by MS. One of the outcomes of those studies is the elucidation of the stoichiometry and protein–protein interactions of membrane-embedded protein complexes [17]. Recently, that group reported native MS studies of lipid–protein interactions within the membrane-embedded protein complex, ATPase [18, 19].

In this paper, we describe a study of the RC complex from the purple bacterium *Rhodobacter* (*Rba.*) *sphaeroides*. The RC complex from purple bacteria was the first integral membrane protein complex that had its structure determined using X-ray crystallography [20–23]. Based on the high-resolution structural model, the RC complex from *Rba. sphaeroides* is a heterotrimer with three protein subunits (H, L, and M). The assembly also contains six types of pigments and cofactors: four bacteriochlorophyll *a's* (BChl), two bacteriopheophytin *a's* (BPh), two ubiquionines (Ubi), one spheroidene carotenoid, one non-heme iron, and one cardiolipin to yield a complex of total molecular weight (MW) of 102.5 kDa (Figure 1) [24]. Because this RC complex is relatively small in size and has been well characterized by other methods, we chose it as a suitable model for native MS development and application.

We stabilized the intact RC complex by detergent micelles and introduced it to the mass spectrometer by nESI and released it from the detergent micelles by collisional activation in the gas phase. The approach successfully showed that RC complexes with different pigment binding states can be observed and that noncovalently bound subunits as well as pigments can be released by applying collisional activation. We were able to characterize in part the protein–protein and protein–pigment interactions by analyzing the dissociation pathways. Results from native MS are consistent with those from other structural studies of the same system [20, 21].

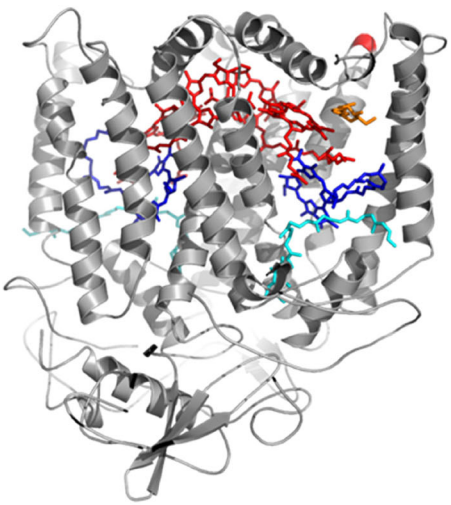
Material and Methods

Cell Culture and Membrane Preparation

Cells of *Rba. sphaeroides* were cultured anaerobically in ATCC medium 550 at 30 °C under 60 μE m^{-2} of light (~5 d). Cells were pelleted by centrifugation for 15 min at 10,000 *g*. This cell pellet was resuspended in 20 mM Tris-HCl, 1 mM EDTA (pH = 8.0) (buffer A), and the cells were broken by sonication for 15 min by using a 30% pulse cycle. Cellular debris was removed by a second centrifugation at 10,000 *g* for 15 min, and membranes were collected by ultracentrifugation of the supernatant at 200,000 *g* for 2 h.

Purification of Reaction Center Complexes

Reaction center complexes were purified by using a method similar to that previously described [25]. Briefly, the membrane enriched pellet obtained from ultracentrifugation was resuspended in buffer A to a final concentration of OD_{850} = 50. The membranes were solubilized by the addition of lauryldimethylamine *N*-oxide (LDAO, ~30% in H_2O, Sigma Aldrich, St. Louis, MO, USA) to a concentration of 0.6% (w/v)



Components and Mass

Component	Mass (Da)	Total Mass (Da)
H Chain*	27867	27867
M Chain*	34355	62222
L Chain*	31305	93527
Bacteriochlorophyll a Special Pair	1822	95349
Bacteriochlorophyll a (Bchl)	911	96260
Bacteriochlorophyll a (Bchl)	911	97171
Bacteriopheophytins (Bph)	891	98062
Bacteriopheophytins (Bph)	891	98953
Ubiquionine	879	99832
Ubiquionine	879	100711
Spheroidene	568	101279
Iron	55	101334
Cardiolipin	1199	102533
	Total	102533

Purple Bacterium *Rhodobacter sphaeroides*

Figure 1. The high-resolution structural model of RC complexes from the purple bacterium *Rhodobacter sphaeroides*. Three protein subunits (H, M, and L) are in gray; bacteriochlorophyll *a* pigments are in red; bacteriopheophytins are in blue; and ubiquionines are in cyan. The MW of each component of RC complexes is listed (* the mass is average mass)

and stirred for 45 min at 4 °C. Solubilization was stopped by dilution of the mixture with buffer A to a final LDAO concentration of 0.1%. This mixture was ultracentrifuged again at 200,000 *g* for 1.5 h to remove insoluble debris. The supernatant was collected and loaded onto an anion exchange column (QSHP resin; GE Healthcare, Uppsala, Sweden) that had been equilibrated with 20 mM Tris-HCl (Sigma Aldrich, St. Louis, MO, USA), 1 mM EDTA (Sigma Aldrich), 0.1% (w/v) LDAO (pH = 8.0) (Buffer L) and was then washed with four column volumes of 20 mM Tris-HCl, 1 mM EDTA, 0.1% (w/v) *n*-dodecyl β-D-maltoside (DDM; Anatrace, Maumee, OH, USA) (pH = 8.0) (buffer D) to remove traces of LDAO. Reaction centers were then eluted with a linear gradient from 0 to 300 mM NaCl in buffer D. Fractions were analyzed by SDS/PAGE gel electrophoresis and absorbance spectroscopy [26], and those with OD_{280}/OD_{804} less than 1.3 were pooled.

Native MS of Reactions Center Complex

Purified reaction centers were concentrated with a 50 kDa MWCO filter (Millipore Amicon Centrifugal Filters, Billerica, MA, USA) to a concentration of ~100 μM of purified complex. The concentrated mixture was then diluted 100 times with 200 mM ammonium acetate, 0.1% DDM (pH = 7.0), and re-concentrated to a final protein concentration of 100 μM. Immediately before electrospray, this sample was diluted 1:10 with 200 mM ammonium acetate, bringing the final DDM concentration to 0.01%. Following dilution, 10 μL was loaded into an offline electrospray capillary (GlassTip 2 μm i.d.; New Objective, Woburn, MA, USA). The sample solution was admitted to a hybrid ion-mobility quadrupole time-of flight mass spectrometer (Q-IM-TOF, SYNAPT G2 HDMS; Waters Inc., Milford, MA, USA), operated in a sensitive mode ('V' optics for TOF analyzer, TOF resolution was 10,000 FWHM) under gentle ESI conditions (capillary voltage 1.5–2.3 kV, source temperature 30 °C). The source parameters (sampling cone and extraction cone) were adjusted to obtain the best signal for the protein complexes. The collision voltages at the trap and transfer region (the first and third region of the Triwave device) were adjusted from 10 to 200 V for removing the detergent and dissociating the protein complexes. The pressure of the vacuum/backing region was 5–6 mbar. Each spectrum was acquired from *m/z* 1500–7500 every 1 s for between 10 min and 5 h. The instrument was externally calibrated to 10,000 *m/z* with a NaI solution. The peak picking and data processing were performed with MassLynx (ver. 4.1, Waters Inc., Milford, MA, USA).

LC-MS

The three protein subunits were excised from SDS/PAGE gel and digested in-gel with trypsin by using a previously published protocol [27]. The protein digests were analyzed by a Waters nanoACQUITY UPLC coupled with SYNAPT G2 HDMS Q-TOF mass spectrometer. The protein digest was desalted with a trap column (UPLC trap column, 180 μm × 20 mm, 5 μm, Symmetry C18). Peptides were separated by a 60 min gradient (5%–50% acetonitrile with 0.1% formic acid) on a custom-packed nano column (75 μm × 150 mm, 5 μm, Magic C18). The mass spectrometer was operated in the MS^e mode [28]. Spectra (*m/z* 50–2000) were acquired at 1.5 s/scan for 80 min. The trap collision energy was ramped from 10 to 40 V for collision-induced dissociation. Data were analyzed by the ProteinLynx Global SERVER (PLGS, ver. 2.5; Waters, Milford, MA, USA).

Native MS Data Processing

The list of masses and peak intensities was exported and saved as txt file for re-plotting and data analysis. The spectra at different dissociation energies were analyzed by two software platforms: Massign and a MathCAD-based calculation sheet custom-built in this lab.

Results and Discussion

RC Protein Complexes in DDM Micelles

Success with native MS requires appropriate sample preparation. We stabilized the RC protein complexes with the detergent LDAO during purification [25], and then replaced LDAO with DDM by an ion exchange-based LC. We took advantage of the spectral properties of this pigment–protein complex and its rich absorption spectrum to establish its structural integrity prior to MS analysis. For example, the Q_y absorption bands of BChl in RC complexes were red-shifted in comparison to isolated BChl in organic solvent. The absorption spectrum of RC complex from *Rba. sphaeroides* showed transitions at 540, 600, 760, 802, and 865 nm at room temperature [26]. We monitored the quality and quantity of the RC samples by their characteristic absorption spectra.

Although membrane proteins are notoriously difficult to study by MS, the measurement of integral membrane proteins under denaturing condition has been established by Whitelegge et al. [29, 30]. Herein, we describe the analysis of an RC, an integral membrane protein complex, in its native state, by native MS. Following the use of optical spectroscopy, we analyzed the RC sample with native MS and found strong signals from the DDM micelles and clusters (data not shown). We adjusted the DDM concentration in the final native MS solution and found that low DDM concentrations (<CMC) in the sample solution caused aggregation and precipitation of the RC complex and blockage of the nESI-tip opening. Adjusting the DDM concentration during sample preparation is crucial for obtaining high-quality mass spectra of the RC complexes. Here, the RC complex concentration in the sample stock was at least five times higher than that required for native MS. The RC sample stock was further diluted with DDM-free ammonium acetate solution to adjust the DDM concentration to approximately 0.01% (w/w, CMC for DDM) before native MS analysis.

Release of the RC Complex from DDM Micelles

We set up the mass spectrometer with parameters that were optimized for analysis of soluble protein complexes [31]. No peaks that could be assigned to multiply charged RC proteins were seen under such MS conditions (Figure 2, bottom spectrum). The most abundant ion in the spectrum is that of DDM of *m/z* 511. We observed a broad peak between *m/z* 4500 and 6500, likely corresponding to DDM micelles in a broad distribution. Although signals from the RC complexes are buried under this broad peak, we used collisional activation to break the DDM micelle clusters and remove extra DDM molecules attached to the RC complexes.

Increasing the collisional energy not only breaks up the DDM clusters but also shifts the DDM peaks to a lower *m/z* region (<2000 *m/z*). Collisional activation also initiates release of DDM molecules from the RC complexes. We observed peaks corresponding to multiply charged ions (still approximately 300 *m/z* wide) by applying higher collisional voltage at the trap region (>100 V, a fuller description of the collisional energy effects is given later) (Figure 2). The center *m/z* values of the near-Gaussian broad peaks give an approximate mass for the multiply charged species of 107 kDa, which is larger than that predicted for the intact complex (calculated MW 102.5 kDa, Figure 1). This larger experimental MW and peak broadening are likely caused by an abundance of nonspecific adducts comprised of water, salts, and DDM still associated with the gas-phase RC complex. In native MS, large protein complexes usually have a number of water molecules that bridge the complexes, and some lipids and detergents can be tightly bound when the complex is membrane-embedded [32].

These noncovalent adducts are dissociated by applying collisional activation. Weakly bound adducts, those with water or detergents (usually nonspecific adducts), dissociate at low collisional energy level (<100 V for collisional voltage). The noncovalently attached pigments, which are key components of RC complexes, require high collision energy (achieved at >100 V of collision voltage) to release. That the complex retains the pigments in native MS is strong evidence that it is native or nearly so upon introduction. If it were denatured, the pigments would release. At the highest collision energy level (200 V for collisional voltage), the peaks representing multiply-charged complex ions shift to a higher *m/z,* lower charge (Figure 2 top spectrum), indicating that charged sub-adducts are removed in addition to neutral molecules. Simultaneously, the peaks become narrower as the corresponding RC complexes release water and detergent adducts (Figure 2 top spectrum).

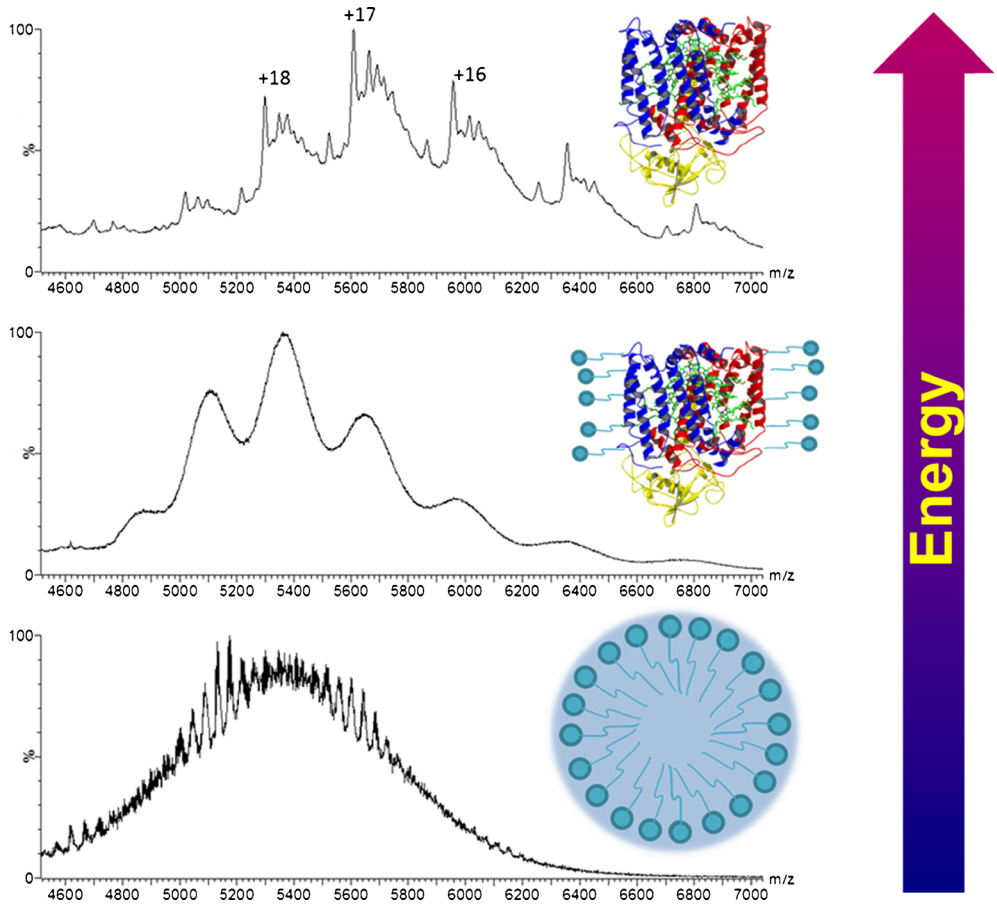


Figure 2. Release of the RC complex from detergent micelles by collisional activation. At low collisional energy (bottom spectrum), the broad peak represents the micelle ion. The detergent micelles and extra bound detergent were removed by increasing the collisional energy at the trap region of a Synapt G2 mass spectrometer

Peak Assignment of Native MS Spectrum of Reaction Center

At high collision energy levels (achieved with 150–200 V of collisional voltage), the mass spectrum of RC complexes divides into three regions: low (*m/z* 100 to 2000), middle (*m/z* 2000 to 4000), and high (*m/z* 5000 to 6500) regions (Figure 3). We used two algorithms, Massign software package [32] and a MathCAD-based calculation sheet, to assign peaks in these mass spectra. For the MathCAD calculation sheet, each set with a series of charge states (at least three states) was identified as a group with a near Gaussian distribution. The MW was calculated based on the charge state series (the development of algorithms for peak assignment will be described in a separate report). For Massign, the peak assignment process used the previously published software package (http://massign.chem.ox.ac.uk) [32].

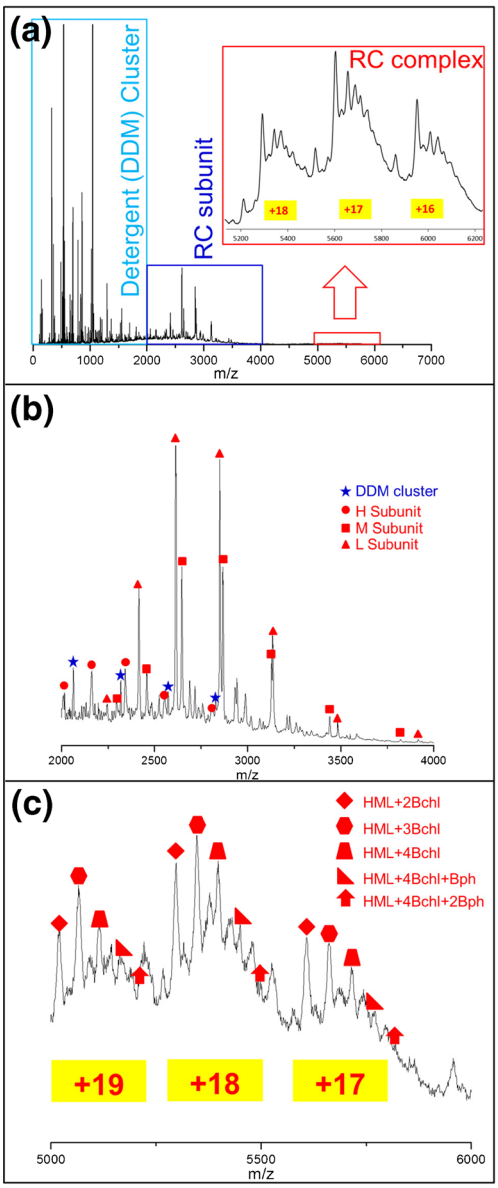


Figure 3. Mass spectra of RC complex sample under native MS conditions. **(a)** Full spectrum, **(b)** middle *m/z* region (*m/z* 2000–4000), and **(c)** high *m/z* region (*m/z* 5000–6000)

In the low *m/z* region, the base peak represents a DDM cluster. The separation between peaks is 510 Da, corresponding to a DDM species (Figure 3a). Not surprisingly, the large DDM clusters undergo dissociation as a consequence of collisional activation to release DDM neutrals.

In the middle *m/z* region, we found signals representing the RC protein subunits carrying multiple charges. Based on the MW calculated from the RC protein primary structure, we assigned peaks to the three individual subunits, H, M, and L (Figure 3b). The dissociated single subunit carries much more charge than when it is part of the entire RC. The highest charge state for each subunit is as follows: +14 for H subunit (calculated MW 27,867 Da, 1990 Da per charge); +15 for M subunit (calculated MW 34,355 Da, 2290 Da per charge); +14 for L subunit (calculated MW 31,305 Da, 2236 Da per charge), whereas only up to 20 charges were observed for the intact RC complexes (5126 Da per charge). We suggest, as have others [33–37], that when the RC complex ions are activated by collision (with argon in this case), the outer regions of the subunit start to unfold, initiating charge migration to those regions of the complex. As more charges move to the unfolded region, the Coulombic repulsion in the core is reduced further, and the RC complex is stabilized. The partially unfolded subunit continues to unfold, driven by the internal energy increase from the collisions, and eventually dissociates to be lost from the RC complex ion. Although it is common to observe asymmetric dissociation in native MS experiments, the dissociated subunit carrying such a large portion of charges (14–15 of 20 charges) is rare for conventional asymmetric dissociation. We could not observe, however, the remaining stripped complex under the current experiment conditions. Because reaction center complexes have a special conformation compared with other reported membrane embedded proteins, this special conformation may afford this asymmetric dissociation.

From the crystal structure of the RC complex [20, 38, 39], the M and L subunits constitute the majority of the transmembrane region, each with five transmembrane α helices (Figure 1). Strong interaction between M and L subunits may prevent their dissociation during the native MS experiment. The third subunit, H, resides largely on the cytoplasmic side of the complex and contains only one trans-membrane α helix. The H subunit contains a larger soluble region and has more residues carrying charges than the other RC subunits. In the native MS experiment, the dissociated H subunit carries more charges than the other RC subunits (1990 Da per charge versus 2290/2236 Da per charge). These observations suggest that the conformation of the RC complex ion is close to the native state shown in the crystal structure. The soluble region of the H subunit may unfold first and carry more charges during dissociation.

There are several small peaks associated with the major peaks of M and L subunits at each charge state (Supplementary Figure S1). Our peak assignments indicate that those small peaks represent M and L subunits with two or less attached

BChl pigments. From the high-resolution X-ray crystallography structure [20, 38–40], the core region of the RC complex contains a bundle of four α helices from M and L subunits, two from each. Two BChl pigments form a dimer in which their terra-pyrrole rings overlap at the core of RC complexes. In our experiment, up two BChl pigments remain attached to the dissociated M and L subunits at the highest collisional energy level (achieved with 200 V). This strongly suggests that two BChl pigments, the BChl dimer, strongly interact with M and L subunits at the core of the RC complex.

Over the high *m/z* region, the multiple peaks constituting one charge state are spaced by ~900 Da, which is close to the MWs of BChl or BPh molecules (Figure 3c). The mass resolving power is insufficient to be more precise than within 100 Da. The first peak series on the left represents the RC protein (M, H, and L subunits) plus two BChl pigments. The RC protein constituents (M, H, and L subunits) remain attached to as many as four BChl pigments. The ~900 Da spacing of peaks continues over the mass spectral pattern. The species observed on the far right in the spectrum (Figure 3c) suggest the

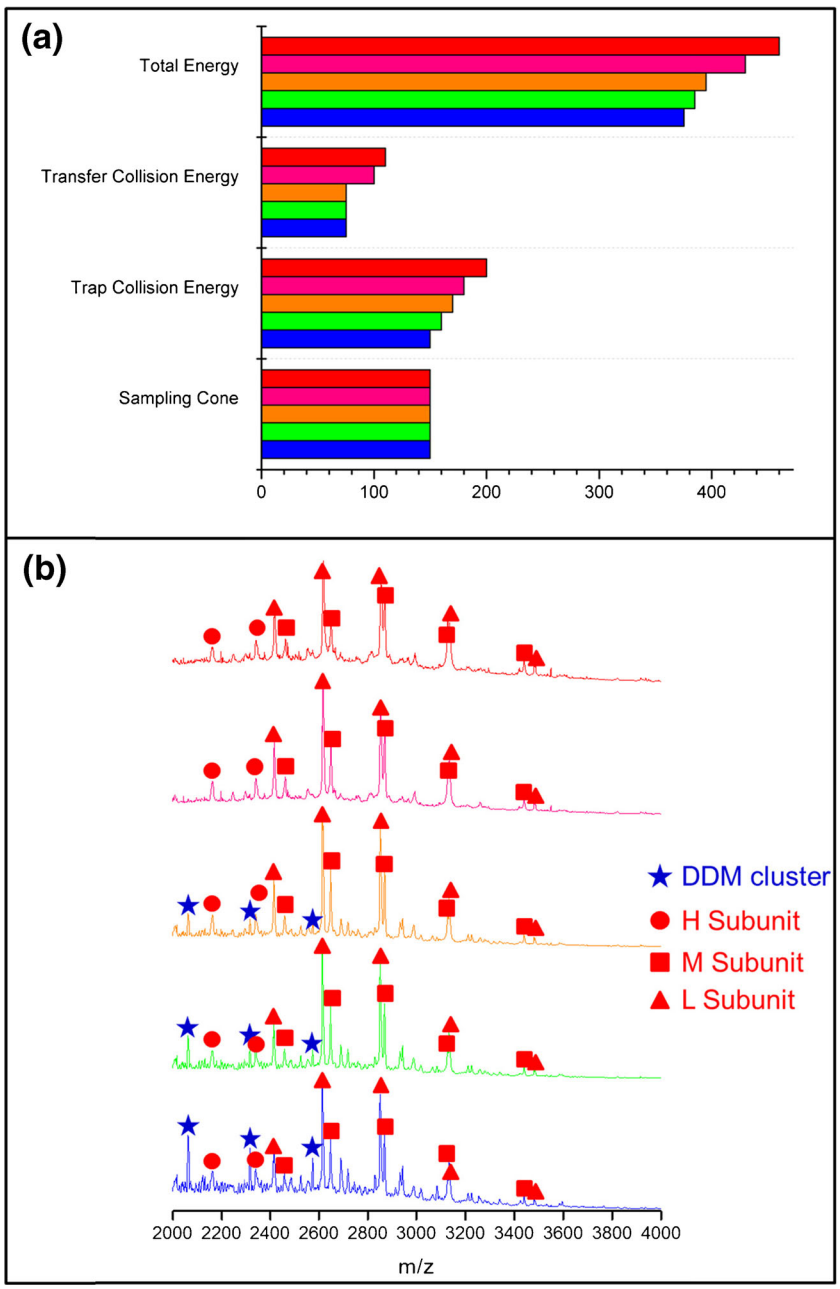


Figure 4. The mass spectra of RC complexes as a function of collision energy. **(a)** The energy inputs (proportional to collision voltage, unit V) as applied to the sampling Cone, trap CE, and transfer CE. **(b)** The collision-energy-dependent spectra of the middle *m/z* region. The color of each spectrum represents the collisional energy level as applied in **(a)**. The DDM cluster disappeared at higher collisional energies

existence of RC protein complexes (M, H, and L subunits) with all the chlorophyll-type molecules (both BChl and BPh) attached.

We also observed a series of peaks corresponding to species of ~600 Da higher MW than the single M or L subunits (Supplementary Figure S1) in the middle *m/z* region. Similarly, we observed additional peaks situated between those representing attachment of BChl or BPh to the protein (Supplementary Figure S2) in the high *m/z* region. Those peaks are broader than the base peak in the same charge state, and they are separated by ~600 Da. This mass difference is smaller than the MW of any chlorophyll molecule identified in RC complexes. Previous native MS studies of the FMO antenna protein complexes showed that collisional activation initiates fragmentation of BChl molecules to lose the phytol "tail," leaving the head group of MW 631 Da still attached (as labeled in Figure 3b) [41]. In lieu of a BChl head group, we cannot rule out the possibility that tightly bound lipids or detergents contribute to the mass shift (~600 Da).

Collisional Dissociation Pathway of the RC Complexes

In the Synapt G2 Q-IM-TOF system, collisional activation can be applied and varied in three different sections: source (sampling-cone voltage), trap (trap-collisional voltage), and transfer (transfer-collision voltage). In studies of the RC complexes, increasing the collisional energies in the trap and transfer regions has more impact on the RC complex than those in the source region. We examined the effect of varying energy at both the trap and transfer regions (Figure 4a) by obtaining mass spectra over five different collisional energies (Figures 4b and 5).

The loss of pigments or cofactors during dissociation is sequential, resulting from different interactions within the RC complex. Those pigments or cofactors on the outer region weakly interact with the protein and are released first (<100 V collisional voltage for trap region). Chlorophyll molecules (BChl and BPh) that attach to the core of RC complexes remain intact under such conditions. The biggest change in the high *m/z* region (Figure 5) occurs when the collision voltage is above 150 V for the trap region: ions with four attached BChl molecules are dominant at 160 V (trap collisional voltage, green spectrum in Figure 5). Increasing the collision voltage causes more pigments to release until the RC protein complex (H, M, and L subunits) containing only two BChl pigments remains (trap collisional voltage of 180 and 200 V, purple and red spectra in Figure 5). These tightly bound BChl molecules are likely a special BChl dimer (often called the "special pair" [42]) that is located in the core of the RC complexes. This indicates the strong interaction between BChl dimer and the core of RC complex (M and L subunits).

This strong interactions of two pigments with the protein have been known for more than two decades when pigment modifications complementing site-directed mutagenesis were made in studies of structure and function of RC complexes

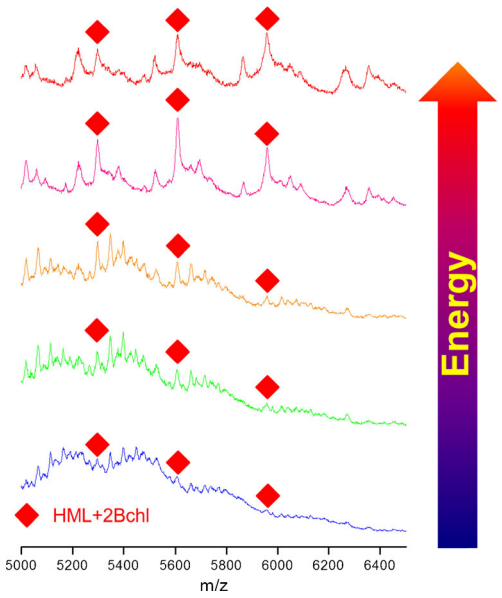


Figure 5. Native mass spectra of RC complexes in the high *m/z* region (*m/z* 5000–6500) as a function of collisional energy. The major peaks (three charge states from +19 to +17) with three RC protein subunits (HML) plus two BChl *a* pigments (BChl dimer) are highlighted by red diamonds. The color of each spectrum represents the collisional energy levels as described in Figure 4a

from *Rba. sphaeroides* [43–45]. Although various chemical and enzymatic methods [46, 47] showed great success for exchanging the monomeric BChl and BPh pigments in RC complex, replacing BChl dimer in the core of RC complex has not been achieved [42, 48]. The non-exchangeable feature of the BChl dimer indicates its stronger interaction with protein than any other pigment and cofactor inside the RC complexes. Remarkably, such information can be elucidated by a native MS experiment with small sample consumption and quick turnaround.

Conclusion

The RC complex from *Rba. sphaeroides* is an important model not only for complexes in photosynthesis but also for development of native MS. The complexes are built of protein scaffolding that holds several pigments and cofactors in a specific orientation for light energy conversion [39]. That these intact RC complexes, more than most other assemblies studied thus far, can be introduced into the gas phase and interrogated by collisional activation testify to the ability of native MS to introduce protein assemblies in a native or near-native state. The release of pigments follows the sequence from the outer region to the core of the RC complex, from loose to tight binding. Native MS shows that the BChl dimer at the core of RC complexes is the tightest bound pigment, consistent with other observations. It is interesting to speculate whether the fragmentation order is the same as that for assembly of pigments onto the protein framework.

Native MS gives insights into the stability and the assembly of the RC complex from purple bacteria with quicker turnaround and less sample consumption than traditional biophysical and biochemical approaches. This study is likely to be a precedent for the systematic native MS studies of membrane-embedded pigment–protein complexes from other photosynthetic organisms. The outcome opens the door for native MS studies of other membrane-embedded photosynthetic pigment–protein complexes, which can be elucidated with less sample consumption compared with traditional approaches. Our future studies will include complementary approaches of accurate mass [49, 50] and ion mobility [10, 51] to generate additional information about key functional units important in this reaction center and in photosynthesis in general.

Acknowledgments

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References

1. Jones, M.R.: Photosynthesis: a new twist to biological solar power. Curr. Biol. **7**, R541–R543 (1997)
2. Barber, J.: Photosynthetic energy conversion: natural and artificial. Chem. Soc. Rev. **38**, 185–196 (2009)
3. Hammarstrom, L.: Overview: capturing the sun for energy production. Ambio **41**(Suppl 2), 103–107 (2012)
4. Blankenship, R.: Molecular mechanism of photosynthesis, 2nd edn. Wiley-Blackwell, New York (2014)
5. Rees, D.C., DeAntonio, L., Eisenberg, D.: Hydrophobic organization of membrane proteins. Science (New York, NY) **245**, 510–513 (1989)
6. Sharon, M., Robinson, C.V.: The role of mass spectrometry in structure elucidation of dynamic protein complexes. Annu. Rev. Biochem. **76**, 167–193 (2007)
7. Benesch, J.L., Ruotolo, B.T., Simmons, D.A., Robinson, C.V.: Protein complexes in the gas phase: technology for structural genomics and proteomics. Chem. Rev. **107**, 3544–3567 (2007)
8. Heck, A.J., Van Den Heuvel, R.H.: Investigation of intact protein complexes by mass spectrometry. Mass Spectrom. Rev. **23**, 368–389 (2004)
9. Benesch, J.L., Ruotolo, B.T.: Mass spectrometry: come of age for structural and dynamical biology. Curr. Opin. Struct. Biol. **21**, 641–649 (2011)
10. Zhong, Y., Hyung, S.J., Ruotolo, B.T.: Ion mobility-mass spectrometry for structural proteomics. Expert Rev. Proteomics. **9**, 47–58 (2012)
11. Hyung, S.J., Ruotolo, B.T.: Integrating mass spectrometry of intact protein complexes into structural proteomics. Proteomics **12**, 1547–1564 (2012)
12. Zhang, Y., Cui, W., Wecksler, A.T., Zhang, H., Molina, P., Deperalta, G., Gross, M.L.: Native MS and ECD characterization of a fab–antigen complex may facilitate crystallization for X-ray diffraction. J. Am. Soc. Mass Spectrom **27**, 1139–1142 (2016)
13. Barrera, N.P., Di Bartolo, N., Booth, P.J., Robinson, C.V.: Micelles protect membrane complexes from solution to vacuum. Science (New York, NY) **321**, 243–246 (2008)
14. Barrera, N.P., Isaacson, S.C., Zhou, M., Bavro, V.N., Welch, A., Schaedler, T.A., Seeger, M.A., Miguel, R.N., Korkhov, V.M., van Veen, H.W., Venter, H., Walmsley, A.R., Tate, C.G., Robinson, C.V.: Mass spectrometry of membrane transporters reveals subunit stoichiometry and interactions. Nat. Methods **6**, 585–587 (2009)
15. Laganowsky, A., Reading, E., Hopper, J.T., Robinson, C.V.: Mass spectrometry of intact membrane protein complexes. Nat. Protoc. **8**, 639–651 (2013)
16. Borysik, A.J., Hewitt, D.J., Robinson, C.V.: Detergent release prolongs the lifetime of native-like membrane protein conformations in the gas phase. J. Am. Chem. Soc. **135**, 6078–6083 (2013)
17. Marcoux, J., Robinson, C.V.: Twenty years of gas-phase structural biology. Structure (London, Engl.1993) **21**, 1541–1550 (2013)
18. Zhou, M., Morgner, N., Barrera, N.P., Politis, A., Isaacson, S.C., Matak-Vinkovic, D., Murata, T., Bernal, R.A., Stock, D., Robinson, C.V.: Mass spectrometry of intact v-type atpases reveals bound lipids and the effects of nucleotide binding. Science (New York, NY) **334**, 380–385 (2011)
19. Barrera, N.P., Zhou, M., Robinson, C.V.: The role of lipids in defining membrane protein interactions: insights from mass spectrometry. Trends Cell Biol. **23**, 1–8 (2013)
20. Deisenhofer, J., Epp, O., Miki, K., Huber, R., Michel, H.: Structure of the protein subunits in the photosynthetic reaction centre of rhodopseudomonas viridis at 3a resolution. Nature **318**, 618–624 (1985)
21. Allen, J.P., Feher, G., Yeates, T.O., Komiya, H., Rees, D.C.: Structure of the reaction center from rhodobacter sphaeroides r-26: The cofactors. Proc. Natl. Acad. Sci. **84**, 5730–5734 (1987)
22. Nogi, T., Fathir, I., Kobayashi, M., Nozawa, T., Miki, K.: Crystal structures of photosynthetic reaction center and high-potential iron-sulfur protein from thermochromatium tepidum: Thermostability and electron transfer. Proc. Natl. Acad. Sci. **97**, 13561–13566 (2000)
23. Arnoux, B., Gaucher, J.F., Ducruix, A., Reiss-Husson, F.: Structure of the photochemical reaction centre of a spheroidene-containing purple-bacterium, *Rhodobacter sphaeroides* y, at 3 a resolution. Acta Crystallogr. D **51**, 368–379 (1995)
24. Leonova, M.M., Fufina, T.Y., Vasilieva, L.G., Shuvalov, V.A.: Structure-function investigations of bacterial photosynthetic reaction centers. Biochemistry (Biokhimiia) **76**, 1465–1483 (2011)
25. Tiede, D.M., Vashishta, A.C., Gunner, M.R.: Electron-transfer kinetics and electrostatic properties of the *Rhodobacter sphaeroides* reaction center and soluble *c*-cytochromes. Biochemistry **32**, 4515–4531 (1993)
26. Woodbury, N.W., Allen, J.P.: The pathway, kinetics, and thermodynamics of electron transfer in wild type and mutant reaction centers of purple nonsulfur bacteria. In: Blankenship, R.E., Madigan, M.T., Bauer, C.E. (eds.) Anoxygenic photosynthetic bacteria, pp. 527–557. Springer Netherlands, Dordrecht (1995)
27. Shevchenko, A., Tomas, H., Havlis, J., Olsen, J.V., Mann, M.: In-gel digestion for mass spectrometric characterization of proteins and proteomes. Nat. Protoc. **1**, 2856–2860 (2006)
28. Bateman, R.H., Carruthers, R., Hoyes, J.B., Jones, C., Langridge, J.I., Millar, A., Vissers, J.P.: A novel precursor ion discovery method on a hybrid quadrupole orthogonal acceleration time-of-flight (Q-TOF) mass spectrometer for studying protein phosphorylation. J. Am. Soc. Mass Spectrom. **13**, 792–803 (2002)
29. Whitelegge, J.P.: Integral membrane proteins and bilayer proteomics. Anal. Chem. **85**, 2558–2568 (2013)
30. Whitelegge, J.P., Gundersen, C.B., Faull, K.F.: Electrospray-ionization mass spectrometry of intact intrinsic membrane proteins. Protein Sci. **7**, 1423–1430 (1998)
31. Zhang, H., Liu, H., Niedzwiedzki, D.M., Prado, M., Jiang, J., Gross, M.L., Blankenship, R.E.: Molecular mechanism of photoactivation and structural location of the cyanobacterial orange carotenoid protein. Biochemistry **53**, 13–19 (2013)
32. Morgner, N., Robinson, C.V.: Massign: An assignment strategy for maximizing information from the mass spectra of heterogeneous protein assemblies. Anal. Chem. **84**, 2939–2948 (2012)
33. Felitsyn, N., Kitova, E.N., Klassen, J.S.: Thermal decomposition of a gaseous multiprotein complex studied by blackbody infrared radiative dissociation. Investigating the origin of the asymmetric dissociation behavior. Anal. Chem. **73**, 4647–4661 (2001)
34. Jurchen, J.C., Williams, E.R.: Origin of asymmetric charge partitioning in the dissociation of gas-phase protein homodimers. J. Am. Chem. Soc. **125**, 2817–2826 (2003)
35. Boeri Erba, E., Ruotolo, B.T., Barsky, D., Robinson, C.V.: Ion mobility-mass spectrometry reveals the influence of subunit packing and charge on the dissociation of multiprotein complexes. Anal. Chem. **82**, 9702–9710 (2010)

36. Benesch, J.L., Robinson, C.V.: Mass spectrometry of macromolecular assemblies: Preservation and dissociation. Curr. Opin. Struct. Biol. **16**, 245–251 (2006)
37. Rostom, A.A., Robinson, C.V.: Disassembly of intact multiprotein complexes in the gas phase. Curr. Opin. Struct. Biol. **9**, 135–141 (1999)
38. Deisenhofer, J., Epp, O., Miki, K., Huber, R., Michel, H.: X-ray structure analysis of a membrane protein complex. Electron density map at 3 a resolution and a model of the chromophores of the photosynthetic reaction center from rhodopseudomonas viridis. J. Mol. Biol. **180**, 385–398 (1984)
39. Feher, G., Allen, J.P., Okamura, M.Y., Rees, D.C.: Structure and function of bacterial photosynthetic reaction centres. Nature **339**, 111–116 (1989)
40. Roy, C., Lancaster, D., Ermler, U., Michel, H.: The structures of photosynthetic reaction centers from purple bacteria as revealed by X-ray crystallography. In: Blankenship, R.E., Madigan, M.T., Bauer, C.E. (eds.) Anoxygenic photosynthetic bacteria, pp. 503–526. Springer Netherlands, Dordrecht (1995)
41. Wen, J., Zhang, H., Gross, M.L., Blankenship, R.E.: Native electrospray mass spectrometry reveals the nature and stoichiometry of pigments in the fmo photosynthetic antenna protein. Biochemistry **50**, 3502–3511 (2011)
42. Goldsmith, J.O., King, B., Boxer, S.G.: Mg coordination by amino acid side chains is not required for assembly and function of the special pair in bacterial photosynthetic reaction centers. Biochemistry **35**, 2421–2428 (1996)
43. Kirmaier, C., Gaul, D., DeBey, R., Holten, D., Schenck, C.C.: Charge separation in a reaction center incorporating bacteriochlorophyll for photoactive bacteriopheophytin. Science (New York, NY) **251**, 922–927 (1991)
44. Shkuropatov, A., Shuvalov, V.A.: Electron transfer in pheophytin a-modified reaction centers from *Rhodobacter sphaeroides* (r-26). FEBS Lett. **322**, 168–172 (1993)
45. Kirmaier, C., Weems, D., Holten, D.: M-side electron transfer in reaction center mutants with a lysine near the nonphotoactive bacteriochlorophyll. Biochemistry **38**, 11516–11530 (1999)
46. Struck, A., Müller, A., Scheer, H.: Modified bacterial reaction centers. 4. The borohydride treatment reinvestigated: comparison with selective exchange experiments at binding sites ba, b and ha, b. Biochim. Biophys. Acta **1060**, 262–270 (1991)
47. Scheer, H., Meyer, M., Katheder, I.: Bacterial reaction centers with plant-type pheophytins. In: Breton, J., Verméglio, A. (eds.) The photosynthetic bacterial reaction center ii: Structure, spectroscopy, and dynamics, pp. 49–57. Springer US, Boston (1992)
48. Scheer, H., Hartwich, G.: Bacterial reaction centers with modified tetrapyrrole chromophores. In: Blankenship, R.E., Madigan, M.T., Bauer, C.E. (eds.) Anoxygenic photosynthetic bacteria, pp. 649–663. Springer Netherlands, Dordrecht (1995)
49. Thompson, N.J., Rosati, S., Rose, R.J., Heck, A.J.: The impact of mass spectrometry on the study of intact antibodies: from post-translational modifications to structural analysis. Chem. Commun. (Cambridge, Engl.) **49**, 538–548 (2013)
50. Lossl, P., Snijder, J., Heck, A.J.: Boundaries of mass resolution in native mass spectrometry. J. Am. Soc. Mass Spectrom. **25**, 906–917 (2014)
51. Ruotolo, B.T., Benesch, J.L., Sandercock, A.M., Hyung, S.J., Robinson, C.V.: Ion mobility-mass spectrometry analysis of large protein complexes. Nat. Protoc. **3**, 1139–1152 (2008)