Calcium Ions Are Required for the Enhanced Thermal Stability of the Light-harvesting-Reaction Center Core Complex from Thermophilic Purple Sulfur Bacterium

Thermochromatium tepidum

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Thermochromatium tepidum is a thermophilic purple sulfur photosynthetic bacterium collected from the Mammoth Hot Springs, Yellowstone National Park. A previous study showed that the light-harvesting-reaction center core complex (LH1-RC) purified from this bacterium is highly stable at room temperature (Suzuki, H., Hirano, Y., Kimura, Y., Takaichi, S., Kobayashi, M., Miki, K., and Wang, Z.-Y. (2007) Biochim. Biophys. Acta 1767, 1057–1063). In this work, we demonstrate that thermal stability increased with the presence of calcium ions, and this process is fully reversible. Interchange of the thermal stability of the LH1-RC resulted in a complex with the same degree of thermal stability as that of the LH1-RCs purified from mesophilic bacteria. The enhanced thermal stability can be restored by addition of Ca$^{2+}$ to the Ca$^{2+}$-depleted LH1-RC, and this process is fully reversible. Interchange of the thermal stability between the two forms is accompanied by a shift of the LH1 protein-detergent/lipid interactions. In the case of reaction center (RC) from Tch. tepidum, the thermal stability was shown to be strongly dependent on the type and composition of detergents used (9). There are three arginine residues in the Tch. tepidum RC (10), which are not present in the RCs of other mesophiles. These basic residues are located at the membrane interface, as revealed by the high resolution crystal structure (11), and were postulated to contribute to the RC stability. However, engineering of the arginine residues into structurally homologous positions in the RC of Rhodobacter sphaeroides did not improve the thermal stability, and the native RC of R. sphaeroides showed the same degree of thermal stability to the Tch. tepidum RC under similar experimental conditions (12). Therefore, it was concluded that the Tch. tepidum RC is not inherently more stable than the R. sphaeroides RC at least when these complexes are removed from the membrane (12). We reported in a previous work that strong interaction exists between the Tch. tepidum RC and its surrounding core light-harvesting 1 (LH1) complex (13), providing evidence for a subsequent proposal that such interaction could enhance the stability of the so-called LH1-RC core complex (12). The high stability of the Tch. tepidum LH1-RC complex has been confirmed (14, 15). Reconstitution experiments using liposome revealed that the Tch. tepidum RC alone does not have pronounced stability, and it gains a remarkable stability through the interaction with LH1 complex.

Purple sulfur photosynthetic bacterium, Thermochromatium tepidum, was originally isolated from a hot springs in Yellowstone National Park and can grow anaerobically at optimum temperatures of 48–50 °C with an upper limit of 58 °C (1). This is the highest temperature of all known purple bacteria (2). A number of soluble proteins purified from this organism have been shown to be thermostable with respect to their mesophilic counterparts. Ribulose-1,5-bisphosphate carboxylase/oxygenase, a key enzyme of the Calvin cycle, from Tch. tepidum was reported to be most catalytically active at 50 °C, and it remained active at 60 °C over 20 min, whereas the same enzyme from the closely related mesophilic bacterium Allochromatium vinosum completely lost its activity over the same period (3, 4). Similar behavior was observed for the high potential iron-sulfur proteins from the two bacteria (5, 6), and was attributed to subtle differences in the amino acid sequence and structure (7).

The thermal stability mechanism of the membrane proteins is somewhat complicated because of complex protein-protein and protein-detergent/lipid interactions (8). In the case of reaction center (RC) from Tch. tepidum, the thermal stability was shown to be strongly dependent on the type and composition of detergents used (9). There are three arginine residues in the Tch. tepidum RC (10), which are not present in the RCs of other mesophiles. These basic residues are located at the membrane interface, as revealed by the high resolution crystal structure (11), and were postulated to contribute to the RC stability. However, engineering of the arginine residues into structurally homologous positions in the RC of Rhodobacter sphaeroides did not improve the thermal stability, and the native RC of R. sphaeroides showed the same degree of thermal stability to the Tch. tepidum RC under similar experimental conditions (12). Therefore, it was concluded that the Tch. tepidum RC is not inherently more stable than the R. sphaeroides RC at least when these complexes are removed from the membrane (12). We reported in a previous work that strong interaction exists between the Tch. tepidum RC and its surrounding core light-harvesting 1 (LH1) complex (13), providing evidence for a subsequent proposal that such interaction could enhance the stability of the so-called LH1-RC core complex (12). The high stability of the Tch. tepidum LH1-RC complex has been confirmed (14, 15). Reconstitution experiments using liposome revealed that the Tch. tepidum RC alone does not have pronounced stability, and it gains a remarkable stability through the interaction with LH1 complex.

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Ca\textsuperscript{2+}-binding Enhances Thermostability of Tch. tepidum LH1-RC

the strong interaction with the LH1 complex (14). A highly purified LH1-RC core complex was shown to be stable at room temperature over 10 days (15). However, the mechanism for the enhanced thermal stability of Tch. tepidum LH1-RC has been unclear.

Another striking feature of the Tch. tepidum is that its LH1 complex exhibits an unusual Q_\text{y} absorption at 915 nm, about 35 nm red-shifted from its mesophilic homologue. In a recent study (16), we demonstrated that calcium ions are involved in this behavior through a strong interaction with LH1 polypeptides, and the Ca\textsuperscript{2+} binding to the LH1 is estimated to occur in a stoichiometric ratio of Ca\textsuperscript{2+}/LH1\textalpha subunit = 1:1. Excitation dynamics of the energy transfer have been compared in the Ca\textsuperscript{2+}-bound and Ca\textsuperscript{2+}-free forms (17). In this work, we present experimental evidence that the Ca\textsuperscript{2+} is required for the enhanced thermal stability of the Tch. tepidum LH1-RC complex. Effects of Ca\textsuperscript{2+} depletion and replacement of the Ca\textsuperscript{2+} with other metal cations on the stability were examined using absorption, CD, and differential scanning calorimetry (DSC). Based on the results, the roles of Ca\textsuperscript{2+} in the thermal stability of the Tch. tepidum LH1-RC complex are discussed in relation to several known factors responsible for the enhanced stability of proteins from thermophiles. Although protein thermal stability has been extensively investigated, to our knowledge the metal ion-induced enhancement of the thermal stability has not been reported for the proteins from photosynthetic organisms. The results of this study will provide insight into how the LH1-RC complex of this therophilic organism was adapted to the growing environment at elevated temperatures by utilizing natural resources.

MATERIALS AND METHODS

Sample Preparation—LH1-RC complex from Tch. tepidum was isolated and purified as described previously (15) with minor modification. Briefly, the chromatophores were treated with 0.35% (w/v) lauryldimethylamine N-oxide at 25 °C for 60 min. After centrifugation, the pellet was treated with 1.0% (w/v) n-decylphosphocholine (DPC) to obtain the LH1-RC-rich components. The extract was purified by a DEAE anion-exchange column (Toyopearl 650S, TOSOH) equilibrated at 4 °C with a buffer containing 20 mM Tris-HCl (pH 7.5) and 0.8% (w/v) DPC. The LH1-RC fractions eluted by a linear gradient of CaCl\textsubscript{2} from 10 to 25 mM were collected. The LH1-RC complexes from A. vinosum and Rhodospirillum rubrum were used as references for a comparison with that of Tch. tepidum. Chromatophores from A. vinosum were treated with 1.5% (w/v) n-octyl β-D-glucopyranoside (OG) in 20 mM Tris-HCl (pH 8.0) at 25 °C for 60 min, followed by centrifugation at 4 °C and 150,000 × g for 90 min. The supernatant was purified with a sucrose density gradient centrifugation at 150,000 × g for 12 h in a 10–40% (w/v) continuous sucrose gradient solution containing 20 mM Tris-HCl (pH 7.5) and 0.7% (w/v) OG to isolate the LH1-RC complexes. The Rsp. rubrum chromatophores were treated with 1.0% (w/v) OG in 20 mM Tris-HCl buffer (pH 8.5) at 25 °C for 60 min, followed by centrifugation at 4 °C and 150,000 × g for 90 min. The supernatant was loaded onto the DEAE anion-exchange column (Toyopearl 650S, TOSOH) equilibrated at 4 °C with a buffer containing 20 mM Tris-HCl (pH 7.5) and 0.1% (w/v) n-dodecyl maltoside. The LH1-RC fractions eluted with a linear gradient of NaCl from 75 to 160 mM were collected. Detergents in the LH1-RC samples from A. vinosum and Rsp. rubrum were replaced with DPC by repeatedly washing the samples with a buffer containing 20 mM Tris-HCl (pH 7.5) and 0.8% (w/v) DPC.

Ca\textsuperscript{2+}-depleted Tch. tepidum LH1-RC complex was prepared as described previously (16). The native LH1-RC complex, which has an LH1 Q_\text{y} absorption of ~915 nm and is therefore designated as B915, was first passed through a size-exclusion column (Sephadex G25M PD10, GE Healthcare) to remove excess of salts in solution. The filtrate was then incubated at 0 °C for 15 min in darkness with 0.5 mM EDTA to remove the bound Ca\textsuperscript{2+}, followed by extensive washing with a buffer containing 20 mM Tris-HCl (pH 7.5) and 0.8% (w/v) DPC to remove the residual EDTA. The resulting LH1-RC complex has an LH1 Q_\text{y} absorption ~880 nm and is therefore designated as B880. For metal cation-substitution experiments, 40 mM Ca\textsuperscript{2+}, Mg\textsuperscript{2+}, Sr\textsuperscript{2+}, Ba\textsuperscript{2+}, and Cd\textsuperscript{2+} was added to the B880 sample as chloride, and the mixture was incubated at 0 °C overnight.

Thermal Degradation—Thermal degradation of the LH1-RC complexes was monitored via the LH1 Q_\text{y} band intensities after incubation at a given temperature for 0–96 min. For the Tch. tepidum LH1-RC complexes, sample concentrations were normalized with respect to the carotenoid band at 514 nm because this band has been shown to be unaffected by Ca\textsuperscript{2+} depletion and metal substitution (16). In the CD measurements, concentrations of B915 and B880 were adjusted to an absorbance of 0.10 at 280 nm, and the thermal denaturations were examined by monitoring both the CD intensity at 222 nm and the LH1 Q_\text{y} absorption intensity after 5 min of incubation at a given temperature. Thermal stabilities of the metal-substituted LH1-RC complexes were assessed using the relative LH1 Q_\text{y} peak intensities monitored at 50 °C as a function of the incubation time.

Differential Scanning Calorimetry—DSC measurements of the LH1-RC complexes from Tch. tepidum were conducted using a nanoDSC II calorimeter (model 6100, Calorimetry Science Corp.). Sample concentrations were adjusted to 3.1 and 2.6 mg/ml for B915 and B880, respectively, in a buffer containing 20 mM Tris-HCl (pH 7.5) and 0.8% (w/v) DPC. Thermal degradation of the samples was monitored at a range of 10–100 °C at a heating rate of 1 °C/min. The DSC measurement for the B880 was carried out in the absence of Ca\textsuperscript{2+}. For the measurement of B915, 20 mM CaCl\textsubscript{2} was added to the sample solution. The reference buffer for each measurement was the same as that used for each sample preparation.

Spectroscopic Measurements—Absorption and CD spectra were recorded at room temperature on a Beckman DU-640 spectrophotometer and a Jasco J-720w spectropolarimeter, respectively (16). The CD spectra were recorded with 20 nm/min scan speed, 1.0-nm bandwidth, and 2-s response time.

RESULTS

Fig. 1 shows changes in the absorption spectra of the Tch. tepidum B915 and B880 at 50 °C as functions of time. The native B915 complex exhibits a largely red-shifted LH1 Q_\text{y} absorption band at 914.5 nm (15, 16). Overall spectral character, including LH1 Q_\text{y}, (914.5 nm), Q_\text{x}, (592 nm), and Soret bands...
The above measurements were made by monitoring the $Q_y$ absorption bands of BChl $a$ molecules, which sensitively reflect structures higher than 50 °C (Fig. 2B). The marked difference obviously indicates that calcium ions are required for the thermal stability of the Tch. tepidum LH1-RC complex.

The thermal stabilities of the Tch. tepidum B915 and B880 were compared with those of native LH1-RC complexes from mesophilic purple bacteria. The LH1 $Q_y$ bands were at 884 nm for A. vinosum (B884) and 875 nm for Rsp. rubrum (B875). There was no essential difference in the thermal stability between the native and EDTA-treated mesophilic LH1-RC samples (see supplemental material). This indicates that the Ca$^{2+}$ concentration has no clear effect on the stability of LH1-RC from mesophilic species, in contrast to the effect on Tch. tepidum LH1-RC. Degradation profiles of the A. vinosum and Rsp. rubrum LH1-RC complexes were similar to that of the Tch. tepidum B880. The temperature dependences of the LH1 $Q_y$ peak intensities at different time intervals are shown in Fig. 3 for the four LH1-RC complexes. The Tch. tepidum B880, A. vinosum B884, and Rsp. rubrum B875 revealed almost identical behavior, whereas the Tch. tepidum B915 showed an enhanced thermal resistance under the same experimental conditions. These results indicate that removing Ca$^{2+}$ reduced the thermal stability of the Tch. tepidum LH1-RC to a level similar to that of its mesophilic counterparts.
the configuration of the chromophores in the LH1 complex. To gain information on the protein stability, we examined temperature dependence of the secondary structure of the LH1-RC complex using far-UV CD spectroscopy. Fig. 4, A and B, shows the CD spectra of Tch. tepidum B915 and B880, respectively, at a variety of temperatures between 30 and 90 °C. Both the B915 and B880 exhibited a similar spectral shape at 30 °C (16), suggesting that there was no change in the secondary structure of the polypeptides upon Ca<sup>2+</sup>/H<sub>11001</sub> depletion. However, the CD spectrum of B880 significantly changed with increasing temperature, whereas that of the B915 was almost completely retained up to 70 °C. The relative CD intensities at 222 nm are plotted in Fig. 4C as a function of the temperature. Above 60 °C, the CD intensity of B880 rapidly decreased with elevation of the temperature and reached about 30% at 90 °C. In contrast, the relative CD intensity of the B915 remained at 85% even after incubation at 90 °C. The results indicate that the thermal stability of the secondary structure also decreased with the removal of Ca<sup>2+</sup> from the LH1-RC complex. It was noted that the CD intensity of the B880 remained almost unchanged with incubation at 30–50 °C where a significant decrease had already occurred for the corresponding LH1 Q<sub>y</sub> absorption. When the CD intensity of the B880 began to diminish rapidly at temperatures higher than 60 °C, the LH1 Q<sub>y</sub> absorption band was completely eliminated. A similar tendency was also observed for the B915, indicating that thermal degradation first occurred as decomposition of the LH1 complex into monomeric forms of the BChl<sub>a</sub>-bound /H<sub>9251</sub>- and /H<sub>9252</sub>-polypeptides, and was followed by denaturation of the secondary structures of the LH1 polypeptides.

The thermal stability of the Tch. tepidum LH1-RC complexes was studied quantitatively by DSC measurement. Fig. 5 shows the endotherms of B915 and B880. In each case, a
single transition was observed in the temperature range of the scan. The B915 exhibited a sharp peak centered at 75.0 °C, indicating a high degree of structural integrity. For the Ca\(^{2+}\)-depleted B880, a much broader transition was detected with the maximum shifted downward to 59.9 °C, and there were two small shoulders around 40 and 68 °C. Taking into account the temperature dependencies of absorption and CD spectra, the DSC main peaks are corresponded to the temperatures at which the LH1 complexes are dissociated into monomeric \(\alpha\)- and \(\beta\)-polypeptides. If this is the case, the results indicate that Ca\(^{2+}\) binding to the LH1 enhances the thermal stability of the LH1-RC complex by 15 °C. Enthalpy changes \(\Delta H\) of these transitions were calculated to be 6690 and 5210 kJ/mol for the B915 and B880, respectively. The difference could be interpreted as evidence of destabilization by removal of the Ca\(^{2+}\) from B915.

To further examine the effects on the thermal stability, reconstitution experiments were carried out using the Ca\(^{2+}\)-depleted B880 and various divalent cations. Beside the alkali earth metal cations, Cd\(^{2+}\) was chosen as a probe because it has the same valence and similar ionic radius as Ca\(^{2+}\). Fig. 6 shows the time changes of relative LH1 \(Q_\text{y}\) intensities at 50 °C for the native and reconstituted LH1-RC complexes. Of these cations, only the Ca\(^{2+}\)-substituted LH1-RC displayed essentially the same stability as the native B915. Other metal ion-substituted LH1-RC complexes also exhibited enhanced stability relative to the B880, but the extent was strongly dependent on the cation species. The results can be rationalized in terms of stability order: native B915(914.5) \(\approx\) Ca\(^{2+}\)(914.5) > Ba\(^{2+}\)(890) \(\approx\) Sr\(^{2+}\)(890) > Mg\(^{2+}\)(887.5) > Cd\(^{2+}\)(887.5) \(>\) B880(880.5), where the LH1 \(Q_\text{y}\) peak positions in nanometer are indicated in parentheses. No correlation was observed between the thermal stability and the ionic radius of the cations. However, the thermal stability seems to be correlated with the LH1 \(Q_\text{y}\) peak position between the metal ion-substituted complexes; the greater the red shift, the more stable the LH1-RC complex.

**DISCUSSION**

This study demonstrates that Ca\(^{2+}\) ions are required for the enhanced thermal stability of the LH1-RC core complex from *Tch. tepidum*. Removal of the Ca\(^{2+}\) from the native complex B915 resulted in the form B880 with thermal stability reduced to that of its mesophilic counterparts. The enhanced thermal stability of B915 can be completely restored by addition of Ca\(^{2+}\) to B880. This reversible process is also accompanied by an interchangeable shift of the LH1 \(Q_\text{y}\) transition between 915 and 880 nm, as reported previously (16). The Ca\(^{2+}\)-induced thermal stability of the *Tch. tepidum* LH1-RC may be closely related to the living environment of this organism, as the bacterium was collected from a reddish mat embedded in the carbonate sinter of a sulfide thermal spring (~45 °C) located in the Mammoth Hot Springs, Yellowstone National Park (1), which is known to contain rich mineral calcium carbonate. In this regard, the results of this study provide useful information on the adaptive strategy utilized by this photosynthetic organism to survive in an environment of elevated temperatures using the natural resources.

Metal ion-induced enhancement of thermal stability has been reported for water-soluble proteins. *Nereis* sarcoplasmic Ca\(^{2+}\)-binding protein was shown to undergo large conformational and stability changes upon ion binding (18). The structural change from a molten globule (apo-state) to the ion-bound native form was accompanied by increases in both chemical and thermal stabilities in the order Ca\(^{2+}\) > Mg\(^{2+}\) > apo. Similar characteristics were observed for parvalbumins, a group of small vertebrate-specific Ca\(^{2+}\)-binding proteins (19). The Ca\(^{2+}\)-binding domain exhibited higher thermal stability and strong tendency to self-associate in the presence of Ca\(^{2+}\). The results of this study add a new example that large membrane proteins can also acquire thermal resistance by utilizing metal ions. Unlike the water-soluble proteins mentioned above, the *Tch. tepidum* LH1-RC undergoes very small changes in conformation upon Ca\(^{2+}\) binding, which can only be detected by the LH1 \(Q_\text{y}\) transition (16). The far UV-CD spectra did not show any marked change between the B915 and B880 forms.
suggesting that the conformational change may occur mainly in the side chains and/or tertiary structure (16).

This work clarifies several speculations derived from previous studies on the stability of Tch. tepidum RC. From the high resolution crystal structure of this complex (11), three basic residues on the periplasmic side of the membrane, which are not present in the RCs of other mesophiles, were highlighted as candidates for specific interactions that may contribute to an enhanced thermal stability if it occurs. In an attempt to isolate pure, RC-free LH1 complexes from this bacterium, we found that interaction between the LH1 and RC was so strong that separation of the two complexes was not successful (13). Subsequent studies showed that these basic residues mentioned above do not actually enhance the thermal stability of the RC (12), and the Tch. tepidum RC alone does not have pronounced stability (14). These results, together with modeling analysis, led to a proposal that the RC is stabilized by the surrounding LH1 complex through strong ionic interactions between the basic residues in RC and acidic residues at the C-terminal end of the LH1 α-polypeptide (12, 14). This study provides evidence that interaction alone between Tch. tepidum RC and LH1 is not enough to bring about an enhanced thermal stability because the B880 revealed a stability at the same levels as those of the mesophilic counterparts (Fig. 3), and the Ca$^{2+}$ is an indispensable cofactor acting as a trigger to induce a more stabilized structure of the LH1-RC complex.

The possible interaction site identified previously coincides with the Ca$^{2+}$-binding site proposed in our recent work (16). There are three Asp residues in the C terminus of Tch. tepidum LH1 α-polypeptide, which are considered to serve as potential ligands to the Ca$^{2+}$. A deletion unique to the α-polypeptide in this region is also thought to play an essential role in forming the Ca$^{2+}$-binding site. The unusual red shift of the Tch. tepidum LH1 Q$_y$ transition at 915 nm was demonstrated to be related to Ca$^{2+}$ binding, and the Q$_y$ transition is strongly dependent on metal cations (16). We have shown in this study that the thermal stability of the divalent cation-reconstituted LH1-RC seems to be related to the LH1 Q$_y$ transition. The complex with a greater LH1 red shift tends to have a higher thermal stability (Fig. 6). However, it is not clear whether there exists a general correlation between the LH1 Q$_y$ transition and thermal stability. Two other LH1 complexes from purple bacteria strain R. parvum 930I were reported to exhibit Q$_y$ absorptions at 963 nm (20) and 909 nm (21, 22), respectively. The large red shifts were explained in terms of enhanced exciton interaction among the BCHl $\alpha$ molecules and specific interactions between BCHl $\alpha$ and LH1 polypeptides, but thermal stability of these complexes has not been examined, although the optimum growth temperature for the R. parvum 930I was reported to be 30°C. As the Ca$^{2+}$ is involved in the marked changes of the Tch. tepidum LH1-RC in both spectroscopic and thermal properties, it is intriguing to speculate on the details of the specific interactions at molecular level between the Ca$^{2+}$, LH1, and RC. Although a number of high resolution structures have been available for RC and LH2 complexes (11, 23–26), structures reported for the LH1 remain at low resolution (27–31). A crystal structure at 4.8 Å has been determined for the LH1-RC complex from Rhodopseudomonas palustris (32). To gain more detailed information on the side-chain conformation of LH1 complex, structures at higher resolutions are required, and to this end the Tch. tepidum LH1-RC has recently been crystallized (15).

Under the experimental conditions of this study, both the Tch. tepidum B915 and B880 revealed a similar pattern of thermal degradation to those of the LH1-RC complexes from mesophilic bacteria, i.e. dissociation of the LH1 complexes into monomeric forms of the BCHl $\alpha$-bound α- and β-polypeptides, followed by a denaturation of the polypeptides. The B915, however, displayed much higher stability than the B880 complex. The DSC measurements provided quantitative evidence that the dissociation temperature of the B915 was 15°C higher than that of B880, and the enthalpy change for the B915 dissociation was about 28% larger than that for B880. The enthalpy changes measured by DSC for both B915 and B880 are much larger than that determined by the spectroscopic method for an association of Rsp. rubrum αβ(BChl$\alpha$), subunit into LH1 complexes (33–35). Carotenoids were not incorporated in these reconstitution experiments. The large enthalpy changes in this study may be attributed to the following: (a) the strong interaction between LH1 and RC; (b) stabilization of the LH1 complex by carotenoids, and (c) stabilization of the whole LH1-RC complex by the phosphocholine detergent. Actually, enthalpy changes estimated from the spectroscopic analysis for the B915 and B880 were comparable with those from the DSC measurements (data not shown).

Metal substitution experiments indicated that the thermal stability of Tch. tepidum LH1-RC strongly depends on properties of the metal cations used (Fig. 6). Generally, ligands involved in the Ca$^{2+}$-binding site are mainly oxygen atoms in carboxylate groups of acidic residues, amide bonds, and water molecules. In addition to the negative charge of the oxygen atoms, recent studies have revealed that the mode of carboxylate binding (monodentate versus bidentate) also plays an important role in recognition of a native metal cofactor (36). Based on a statistical analysis of the known structures of metalloproteins, factors governing the carboxylate-binding modes have been proposed, and the difference in the binding mode in Mg$^{2+}$- and Ca$^{2+}$-containing proteins can be rationalized. The Ca$^{2+}$-binding site was shown to prefer a bidentate form compared with the Mg$^{2+}$-binding site, and a carboxylate monodentate-bidentate switch could be used to fine-tune the metal-binding site affinity and/or selectivity, thus modifying the function/character of the metalloproteins (36). Our results in this work show that metal cations can regulate thermal stability of a large pigment-membrane protein complex, and the subtle changes in the metal-binding site can be sensitively monitored by the LH1 Q$_y$ transition. The highly selective property of metal ion binding to the Tch. tepidum LH1-RC may be used as a useful model for investigation of the mechanism of molecular recognition, and provide a potential design tool that could be employed for engineering new pigment-membrane protein complexes with controlled thermodynamic and spectroscopic functions.

Acknowledgments—We thank Jian-Ping Zhang, Peng Wang, and Fei Ma for useful discussions and M. Nakamura and K. Horiguchi for technical assistance.
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