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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Running Title: Ca^{2+}-binding enhances thermostability of *Tch. tepidum* LH1-RC

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***Thermochromatium (Tch.) 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 *et al*., (2007) *Biochim. Biophys. Acta* 1767, 1057-1063]. In this work, we demonstrate that thermal stability of the *Tch. tepidum* LH1-RC is much higher than that of its mesophilic counterparts and the enhanced thermal stability requires Ca^{2+} as a cofactor. Removal of the Ca^{2+} from *Tch. tepidum* LH1-RC resulted in a complex with the same degree of thermal stability to 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 Q_y transition between 915 nm for the native and 880 nm for the Ca^{2+}-depleted LH1-RC. Differential scanning calorimetry measurements reveal that degradation temperature of the native LH1-RC is 15 ˚C higher and the enthalpy change is about 28% larger than the Ca^{2+}-depleted LH1-RC. Substitution of the Ca^{2+} with other metal cations caused a decrease in thermal stability of extent depending on the properties of the cations. These results indicate that Ca^{2+} ions play a dual role in stabilizing the structure of the pigment-membrane protein complex and altering its spectroscopic properties, and hence provide insight into the adaptive strategy of this photosynthetic organism to survive in extreme environments using natural resources.**

Purple sulfur photosynthetic bacterium, *Thermochromatium (Tch.*) *tepidum*, was originally isolated from hot springs in Yellowstone National Park, and can grow anaerobically at optimum temperatures of 48 – 50 ºC with an upper limit of 58 ºC (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 ºC, and remained active at 60 ºC over 20 min, whereas the same enzyme from the closely related mesophilic bacterium *Allochromatium* (*Ach.*) *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 due to complex protein-protein and protein-detergent/lipid interactions (8). In the case of reaction center (RC)[1] 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 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* (*Rba.*) *sphaeroides* did not improve the thermal stability,

and the native RC of *Rba. sphaeroides* showed same degree of thermal stability to the *Tch. tepidum* RC under similar experimental condition (12). Therefore, it was concluded that the *Tch. tepidum* RC is not inherently more stable than the *Rba. 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 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, 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_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^{2+} binding to the LH1 is estimated to occur in a stoichiometric ratio of Ca^{2+}/LH1ab-subunit = 1:1. Excitation dynamics of the energy transfer have been compared in the Ca^{2+}-bound and Ca^{2+}-free forms (17). In this work, we present experimental evidence that the Ca^{2+} is required for the enhanced thermal stability of the *Tch. tepidum* LH1-RC complex. Effects of Ca^{2+} depletion and replacement of the Ca^{2+} with other metal cations on the stability were examined using absorption, circular dichroism (CD) and differential scanning calorimetry (DSC). Based on the results, the roles of Ca^{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 thermophilic 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 $CaCl_2$ from 10 mM to 25 mM were collected. The LH1-RC complexes from *Ach. vinosum* and *Rhodospirillum* (*Rsp.*) *rubrum* were used as references for a comparison with that of *Tch. tepidum*. Chromatophores from *Ach. 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 hr 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*-dodecylmaltoside (DDM). The LH1-RC fractions eluted with a linear gradient of NaCl from 75 mM to 160 mM were collected. Detergents in the LH1-RC samples from *Ach. 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^{2+}-depleted *Tch. tepidum* LH1-RC complex was prepared as described previously (16). The native LH1-RC complex, which has an LH1 Q_y absorption ~ 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^{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_y absorption ~ 880 nm and is therefore designated as B880. For metal cation-substitution experiments, 40 mM of Ca^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+} and Cd^{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_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 since this band has been shown to be unaffected by Ca^{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_y absorption intensity after 5 min incubation at a given temperature. Thermal stabilities of the metal-substituted LH1-RC complexes were assessed using the relative LH1 Q_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 mg/ml 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 in a range of 10˚C – 100˚C at a heating rate of 1 ˚C/min. The DSC measurement for the B880 was carried out in the absence of Ca^{2+}. For the measurement of B915, 20 mM of $CaCl_2$ 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 band width, and 2 sec response time.

RESULTS

Figure 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_y absorption band at 914.5 nm (15, 16). Overall spectral character, including LH1 Q_y (914.5 nm), Q_x (592 nm) and soret bands (379 nm), was firmly retained over 96 min incubation (Fig. 1A). The carotenoid bands at 486, 514 and 551 nm, as well as the protein bands at 280 nm remained almost unchanged by the thermal treatment. In contrast, the LH1 Q_y band of the Ca^{2+}-depleted B880 species rapidly decreased and completely disappeared after 48 min incubation (Fig. 1B). With increasing incubation time, the 879 nm-band decreased and a new band at 775.5 nm appeared with an isosbestic point at 802 nm. This indicates that the Ca^{2+}-depleted B880 complex is thermally more unstable than the B915 and the LH1 complex was directly decomposed into monomeric forms of the BChl *a*–bound α- and β-polypeptides upon the degradation. In addition, the carotenoid bands completely disappeared after 96 min incubation, and the denatured carotenoid molecules were presumed to contribute to the increase of the absorption intensities shorter than 410 nm.

The thermal stabilities of the B915 and B880 were further investigated over a wide range of temperature. Figure 2 shows the changes in relative LH1 Q_y band intensities of the two complexes as functions of time at various temperatures. The Q_y band of the B915 retained 98% intensities over 64 min up to 40 ˚C. The relative intensities deceased to 89% and 72% at 50 ˚C and 60 ˚C, respectively, during the same period, and completely disappeared in about 10 min at 70 ˚C. This suggests that decomposition of the B915 is markedly enhanced at the temperatures higher than 60 ˚C (Fig. 2A). In comparison, the Q_y band of the Ca^{2+}-depleted B880 apparently decreased with incubation time even at 30 ˚C and 40 ˚C, and rapidly diminished at temperatures 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 *Ach*. *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 Data). This indicates that 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 *Ach*. *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 Figure 3 for the four LH1-RC complexes. The *Tch. tepidum* B880, *Ach. 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 above measurements were made by monitoring the Q_y absorption bands of BChl *a* molecules which sensitively reflect the configuration of the chromophores in 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. Figures 4A and 4B show 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^{2+}-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 Figure 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 was 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^{2+} 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_y absorption. When the CD intensity of the B880 began to diminish rapidly at temperatures higher than 60 ˚C, the LH1 Q_y absorption band was completely eliminated. 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 *a*-bound α- and β-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. Figure 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 ˚C and 68 ˚C. Taking into account the temperature dependences of absorption and CD spectra, the DSC main peaks are considered to correspond to the temperatures at which the LH1 complexes are dissociated into

monomeric α- and β-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 ΔH of these transitions were calculated to be 6690 kJ/mol 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+}. Figure 6 shows the time changes of relative LH1 Q_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) ≈ Ca^{2+}(914.5) > Ba^{2+}(890) ≈ Sr^{2+}(890) > Mg^{2+}(887.5) > Cd^{2+}(887.5) > B880(880.5), where the LH1 Q_y peak positions in nanometer are indicated in the 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_y peak position between the metal ion-substituted complexes: the greater the red-shift, the more stable the LH1-RC complex.

DISCUSSION

The present 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 the Ca^{2+} to the B880. This reversible process is also accompanied by an interchangeable shift of the LH1 Q_y transition between 915 nm 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 temperature 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_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).

The present 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). The present 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 the 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 with 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 970 and *Roseospirillum 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 *a* molecules and specific interactions between BChl *a* and LH1 polypeptides, but thermal stability of these complexes has not been examined although the optimum growth temperature for the *Roseospirillum 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 *a*-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 evidences 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* $\alpha\beta$(BChl*a*)$_2$ subunit into LH1 complexes (33-35). Carotenoids were not incorporated in these reconstitution experiments. The large enthalpy changes in the present work may be attributed to (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 to 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 vs 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 to 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 to engineering new pigment-membrane protein complexes with controlled thermodynamic and spectroscopic functions.

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FOOTNOTES

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[1]Abbreviations: BChl, bacteriochlorophyll; LH, light-harvesting; RC, reaction center; CD, circular dichroism; DDM, *n*-dodecylmaltoside; DPC, *n*-decylphosphocholine; DSC, differential scanning calorimetry; LH, light-harvesting; OG, *n*-octyl-β-D-glucopyranoside; RC, reaction center

FIGURE LEGENDS

FIGURE 1. **Changes in the absorption spectra on thermal degradation at 50 °C for the native B915 (A) and Ca^{2+}-depleted B880 (B) from *Tch. tepidum* as functions of time.** The samples were dissolved

in 20 mM Tris-HCl (pH7.5) and 0.8% (w/v) DPC. Concentrations of the starting samples were adjusted to A_{514} = 0.356.

FIGURE 2. **Changes in the relative LH1 Q_y absorption intensities of *Tch. tepidum* B915 (A) and B880 (B) at different temperatures as functions of time.**

FIGURE 3. **Comparison of the temperature dependences of the LH1-RC complexes from *Tch. tepidum*, *Ach. vinosum* and *Rsp. rubrum*.** The relative LH1 Q_y absorption intensities were measured after incubation for 8 min (A), 16 min (B) and 64 min (C) at different temperatures.

FIGURE 4. **Circular dichroism spectra of the *Tch. tepidum* B915 (A) and B880 (B) in the 205 – 250 nm region at different temperatures.** (C) Temperature dependence of the CD intensities at 222 nm (solid lines and closed symbols) and the LH1 Q_y absorption intensities (dashed lines and open symbols) for B915 (circle) and B880 (square).

FIGURE 5. **DSC scans of the *Tch. tepidum* B915 (solid curve) and B880 (dashed curve).** The concentrations were 3.1 mg/ml and 2.6 mg/ml for B915 and B880, respectively. The buffer contained 20 mM Tris-HCl (pH7.5) and 0.8% (w/v) DPC. Scan rates were 1 ˚C per min.

FIGURE 6. **Changes in the relative LH1 Q_y intensities at 50 °C for the metal ion-substituted *Tch. tepidum* LH1-RC complexes as functions of time.** For comparison, the data for native B915 and Ca^{2+}-depleted B880 are also plotted.

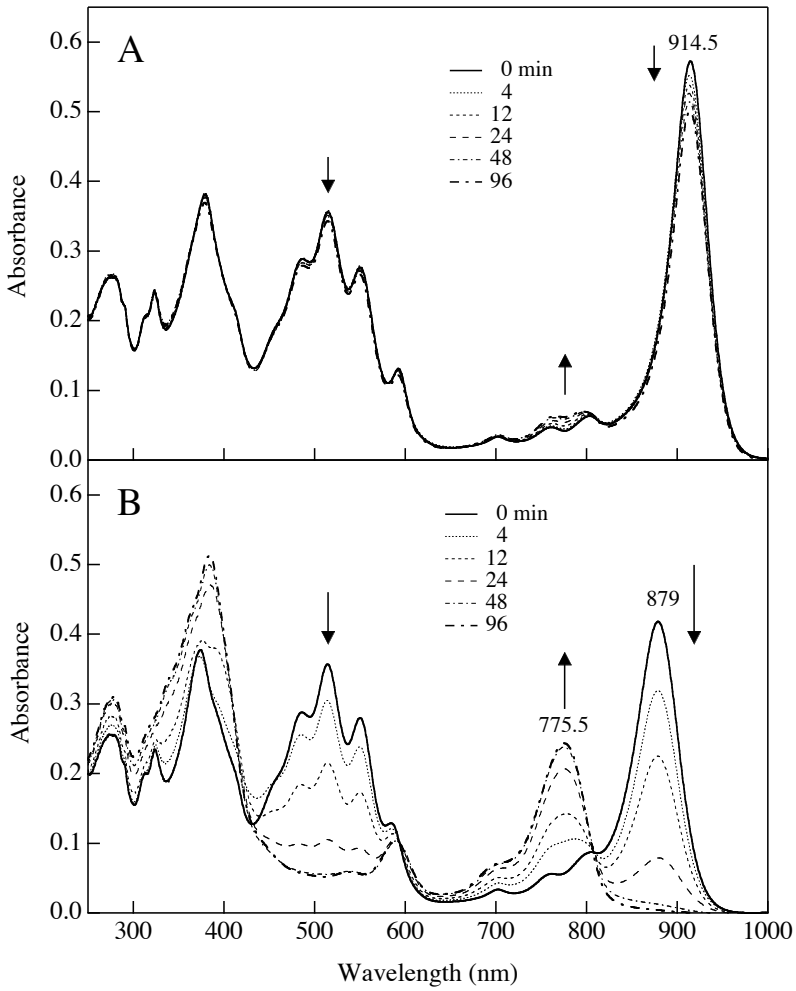

Fig. 1 Kimura et al.

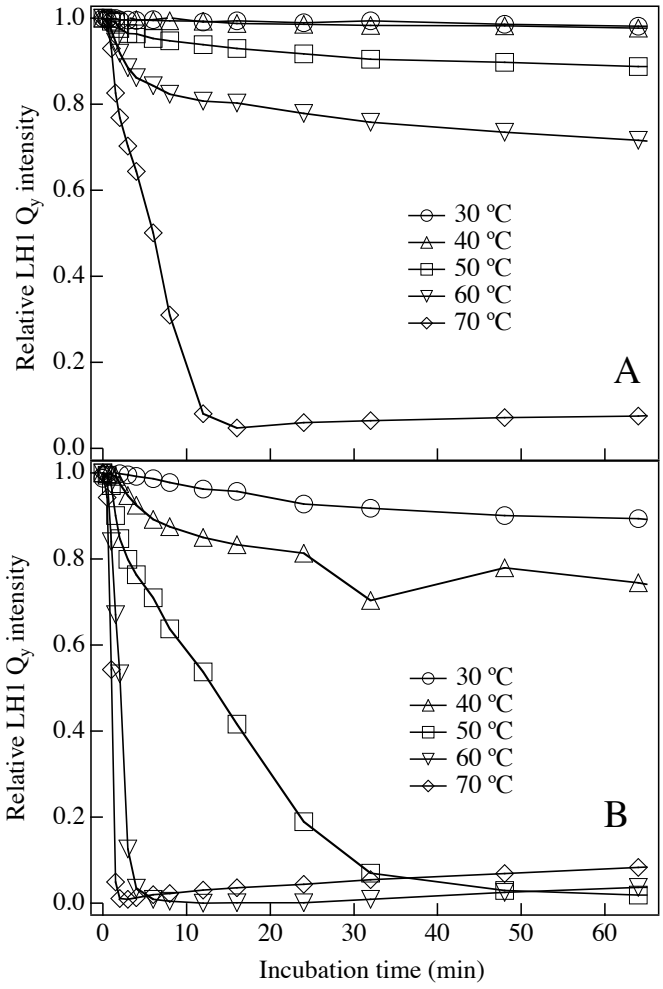

Fig. 2 Kimura et al.

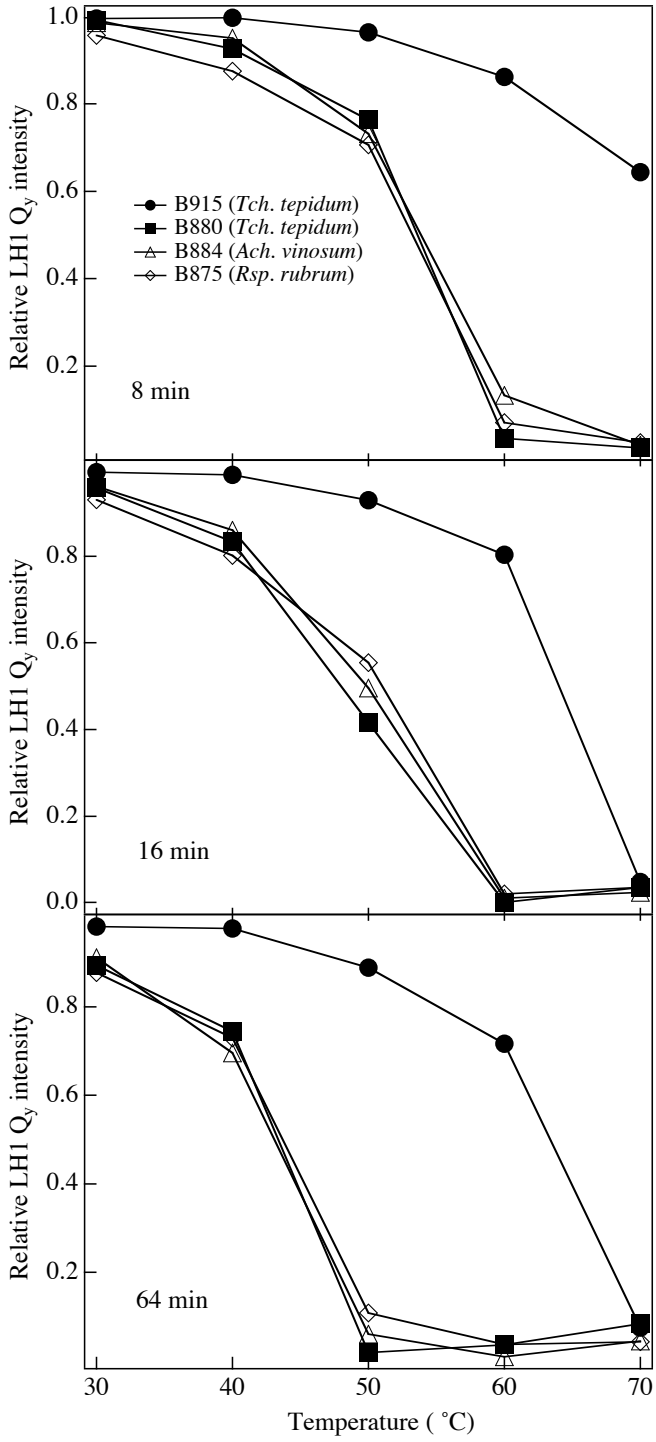

Fig. 3 Kimura et al.

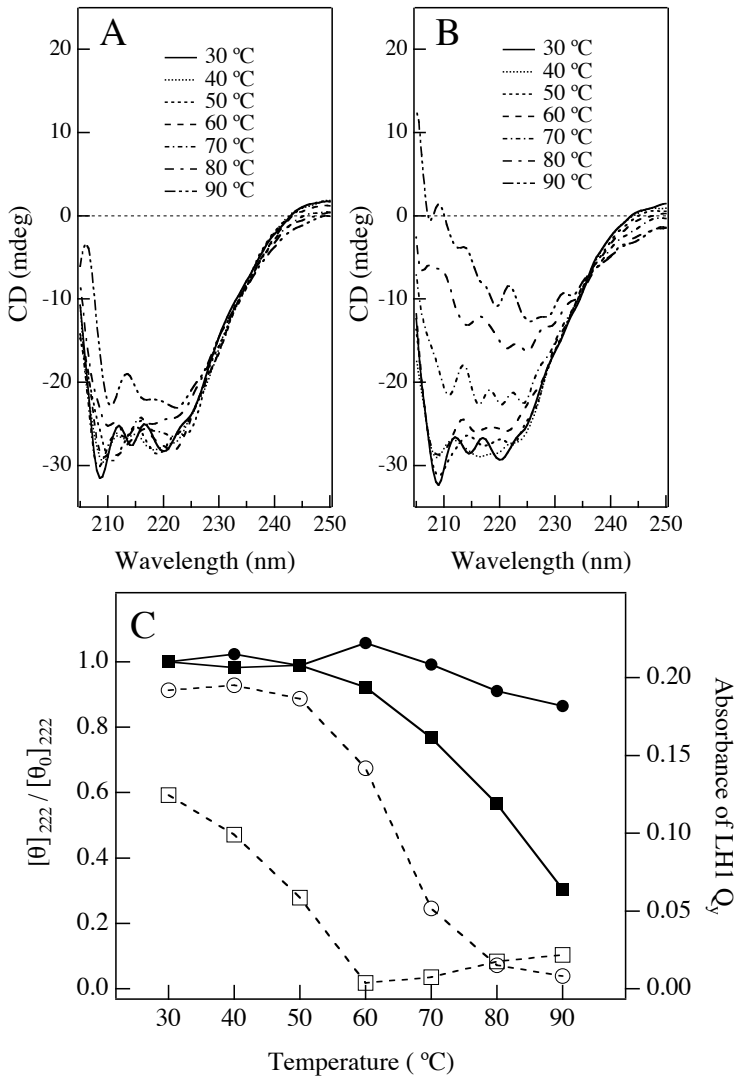

Fig. 4 Kimura et al.

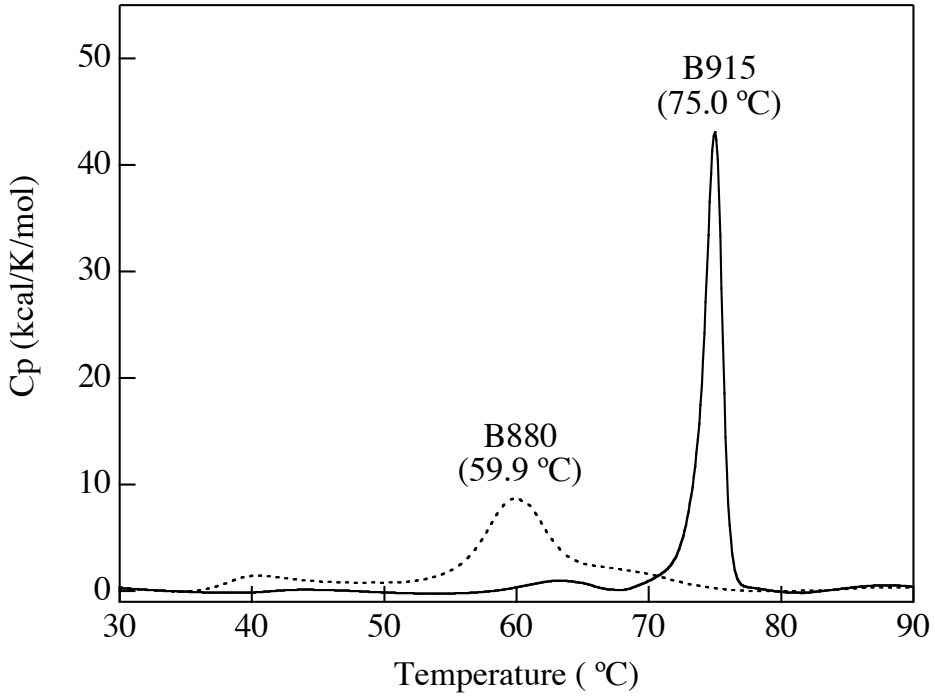

Fig. 5 Kimura et al.

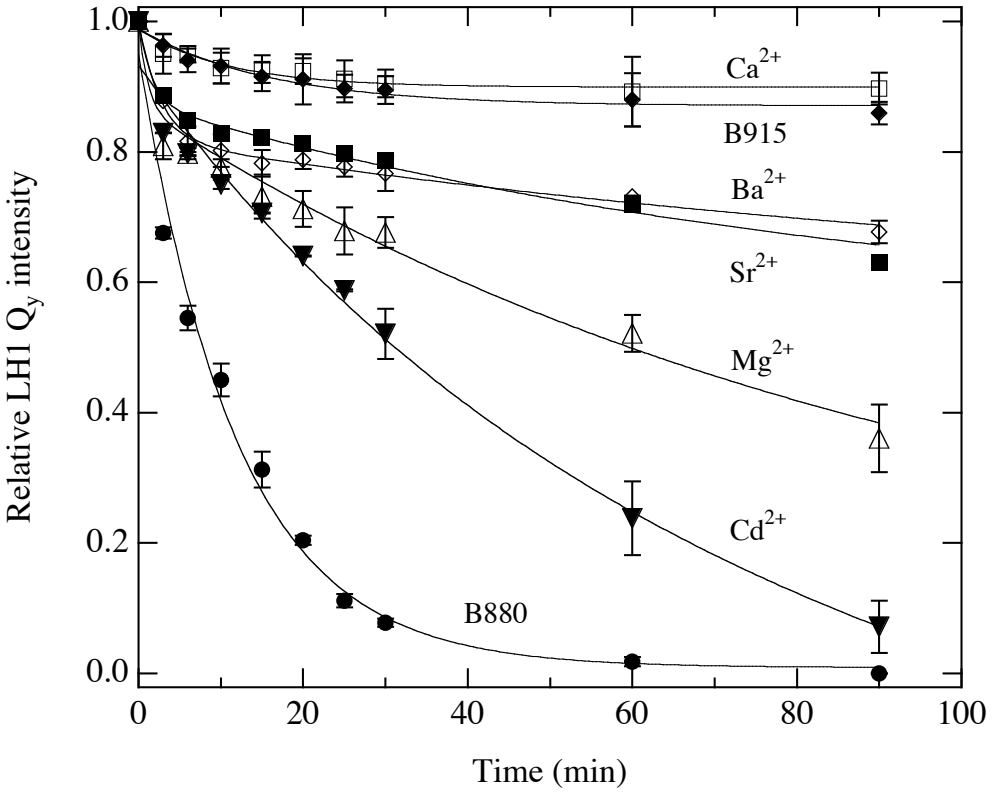

Fig. 6 Kimura et al.