4. Proton transfer in bRC

Seven residues, namely His-H126, His-H128, Asp-M17, Asp-L210, Glu-L212, Asp-L213 and Ser-L223, were suggested to be involved in these PT events in WT-bRC (Figure 4-2-1, reviewed in refs. (Okamura et al., 2000; Paddock et al., 2003a)). These seven residues are located along water channels (reviewed in Ref. (Abresch et al., 1998)) found in the crystal structure of bRC at 2.2 Å resolution (Stowell et al., 1997).

4.1. PT inhibition in D(L213)N mutant and revertant

4.1.1. Overview

A) AA mutant. In the double mutant E(L212)A and D(L213)A (**AA mutant**) of RC from *Rb. capsulatus*, the first ET from Q_A^- to Q_B occurs, but the photo-cycle is interrupted at this point (Hanson et al., 1992a), presumably because Q_B cannot be protonated due to the lack of Glu-L212 and Asp-L213. The crystal structure of the AA mutant from *Rb. sphaeroides* revealed an expanded Q_B cavity with respect to the native bRC (Pokkuluri et al., 2002). Although the crystal structure of the AA mutant was obtained in the dark, Q_B is located at the proximal binding position as is the light-exposed structure of the native bRC (Stowell et al., 1997). Furthermore, the chain segment of residues L207 – L213 is displaced by 0.5 Å and of residues L223 – L227 by 0.3 – 0.7 Å with respect to the native RC (Pokkuluri et al., 2002).

B) Single mutation of Asp-L213 to Asn (D(L213)N). The single point mutation of Asp-L213 to Asn (D(L213)N) decreases $k_{AB}^{(1)}$ by a factor of 10 (**inhibited mutant**), implying that in kinetic phase 1 the PT to Glu-L212 (Eq. 3-1a) is less efficient than in WT-bRC (Paddock et al., 1994; Paddock et al., 1998). In the same mutant a dramatic 6000-fold decrease of $k_{AB}^{(2)}$ was observed, rendering this rate independent of the ET driving-force. This indicates that in the D(L213)N mutant bRC the PT for kinetic phase 2 (Eq. 3a) becomes rate-limiting in contrast to WT-bRC (Paddock et al., 1994; Paddock et al., 1998).

C) PT revertant mutant for D(L213)N. The decreased PT rate for the single mutant can be recovered by an additional mutation of Asn-M44 to Asp, since the side chain of Asp at M44 can substitute the removed carboxylate at L213 in the mutant bRC (Hanson et al., 1992b; Rongey et al., 1993; Paddock et al., 2003b). On the other hand, the PT in the D(L213)N mutant bRC was also restored by mutations of Arg-M233 to Cys or Arg-H177 to His; namely the double mutants D(L213)N/R(M233)C or D(L213)N/R(H177)H, respectively (**revertant mutant**) (Paddock et al., 1998; Paddock et al., 2003a) (Figure 4-2-1). However, in these two mutants, no carboxylate is re-introduced and, based on the wild type bRC structure, the corresponding two mutated sites M233 and H177 have a distance of more than 10 Å from residue L213 i.e. 13 Å and 17 Å, respectively (Stowell et al., 1997). Based on the observed structural changes close to Glu-H173 from wild type to revertant mutant bRC, the proposed mechanism to recover PT in the revertants involves Glu-H173 in the PT pathway (Paddock et al., 2003a; Xu et al., 2004).

4.1.2. Proton uptake of Glu-H173 in revertant mutants

As in the wild type bRC, we observed a large proton uptake at Glu-L212 upon formation of the Q_B^- state in both revertant and inhibited mutant bRC (Ishikita and Knapp, 2005b). The revertant mutants showed a small but significant increase of protonation also at Glu-H173, while the WT-bRC does not show a protonation at this residue (Ishikita and Knapp, 2005b).

Mutation of Glu-H173 to Gln resulted in the decrease in the rates for the first and second ET from Q_A^- to Q_B, presumably by affecting the kinetics of PT to Q_B in which Glu-H173 may participate (Takahashi and Wraight, 1996). On the other hand, in steady-state FTIR measurements (Nabedryk et al., 1998) and our previous computation (Rabenstein et al., 2000; Ishikita and Knapp, 2004), Glu-H173 in WT-bRC remains deprotonated regardless of the redox state of Q_B. The latter fact implies a small pK_a value for Glu-H173 in wild type bRC, without excluding a possibility of its transient protonation during the PT to Glu-L212 (i.e. proton uptake of Glu-L212 in kinetic phase 1). Hence, the significant change in protonation state of Glu-H173 upon revertant mutation (Ishikita and Knapp, 2005b) may suggest a participation of Glu-H173 in the PT in kinetic phase 1 (PT that is coupled to the first ET from Q_A^- to Q_B) (Paddock et al., 2003a; Gunner and Zhu, 2004; Xu et al., 2004).

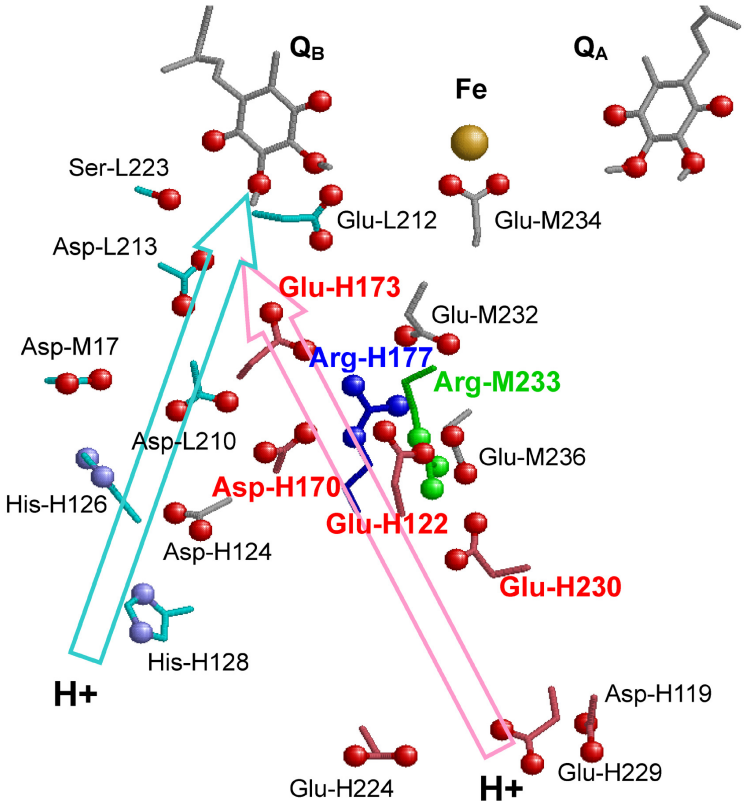

Figure 4-2-1. Structural overview of titratable residues at the Q_B binding site based on the crystal structure of the wild-type bRC (PDB 1AIG) (Stowell et al., 1997). The transparent blue arrow indicates PT pathways proposed in previous studies as reviewed in (Nabedryk et al., 2001). The transparent purple arrow denotes an alternative PT pathway suggested in the present work. Residues, which belong to these PT pathways, are colored with cyan and pink, respectively. Residues, which showed significant changes in the calculated pK_a upon mutation, were labeled in bold red color letters. Bold color letters were also used for the mutated Arg.

4.1.3. pK_a shift of Glu-H173 upon revertant mutation

The observed proton uptake of Glu-H173 in revertant mutant bRC implies its pK_a increase upon formation of the Q_B^- state. Indeed, with respect to wild type bRC, we observed a considerable increase in the pK_a value of 3.5 units for Glu-H173 in both revertant mutants (Ishikita and Knapp, 2005b).

It was previously suggested that a rearrangement of the side chains of charged

residues e.g. Arg-H177 upon revertant mutations increases the pK_a value for Glu-H173, giving rise to Glu-H173 being capable of functioning as both proton donor and acceptor in the PT pathways (Gunner and Zhu, 2004; Xu et al., 2004). Indeed, our computations showed that the pK_a value for Glu-H173 is above 6 in both revertant mutants. Contrary to the revertant mutants, the inhibited and the AA mutants showed lowered pK_a values for Glu-H173 than that in the wild type bRC, being lowered by 1.1 in the former and 3.2 units in the latter (Ishikita and Knapp, 2005b).

4.1.4. Main effect of pK_a shifts upon revertant mutation: charge effect or induced conformational change?

One might anticipate that the main effect of pK_a shifts observed with mutations in bRC is due to changes in the net charge of a residue. Accordingly, the elimination of a positive charge at Arg-M233 or Arg-H177 should result in a pK_a shift for Glu-H173 and Asp-M17.

To mimic the action of the D(L213)N/ R(M233)C double mutant, we used the crystal structure for the WT-bRC (Stowell et al., 1997), constrained Asp-L213/ Arg-M233 to be protonated/ deprotonated, and calculated the pK_a for Glu-H173. Surprisingly, this constraint resulted in a decrease of the pK_a for Glu-H173 by 1.8, as opposed to that calculated from the crystal structure of the D(L213)N/ R(M233)C mutant. At the same time, this constraint shifted the pK_a for Glu-H230 from 0.9 (in the original WT-bRC) to 11.9, resulting in a fully protonated Glu-H230. Therefore, the increase of pK_a calculated for Glu-H173 in revertant mutants is not merely due to a decrease of the net charge in the neighborhood of this residue, but requires also a reorientation of the corresponding amino acid side chains, as previously suggested by Paddock *et al*. (Paddock et al., 2003a).

Hence, in addition to a change of net charge around Glu-H173 upon revertant mutations, we conclude that the participation of Glu-H173 in the PT requires also a suitable rearrangement of the participating amino acid side chains. Indeed, the crystal structures of revertant mutant bRC show a drastic rearrangement of side chains and change in salt-bridge pattern around Glu-H173 relative to either wild type or inhibited mutant bRC (Xu et al., 2004).

Conclusion:

The reorientation of side chains, but not the net charge, is the main mutational effect for the recovery of PT in the revertant mutants. In this case, the change of the net charge upon mutation is rather compensated by the reorientation of the side chains (Ishikita and Knapp, 2005b)**.**

The importance of the reorientation of residues on pK_a can be understood by **the electrostatic domino effect** (Sebban et al., 1995b), in which successive reorientation of residues propagate over a large distance (see details in the next section **4.2** (Ishikita and Knapp, 2005c))**.**

4.2. PT inhibition by metal binding

4.2.1. Overview

It has been established that the addition of Cd^{2+} (or Zn^{2+}) ions to bRC diminishes $k_{AB}^{(1)}$ and $k_{AB}^{(2)}$ 10-fold and 20-fold, respectively (Utschig et al., 1998; Paddock et al., 1999; Ädelroth et al., 2000; Paddock et al., 2000; Gerencsér and Maróti, 2001). Furthermore, in these metal-bound bRC $k_{AB}^{(2)}$ of kinetic phase 2 becomes independent of the ET driving-force, switching the rate-limiting step from ET (Eq. 3-2b) to PT (Eq.

3-2a) events [i.e. $k_{AB}^{(2)}$ essentially equals the PT rate, implying a reduction of the PT rate by more than 10^2 (Paddock et al., 1999; Paddock et al., 2000)]. The refinement of the crystal structures for Cd^{2+}/Zn^{2+} bound bRC confirm that these metal ions bind at Asp-H124, His-H126 and His-H128 (Axelrod et al., 2000) (Figure 4-2-2), although a recent XAFS study implied a second potential binding site for Zn^{2+} (Giachini et al., 2005). The double mutation of His-H126 and His-H128 to Ala resulted in a decrease of $k_{AB}^{(1)}$ and $k_{AB}^{(2)}$ by 10-fold and 4-fold, giving rise to PT as the rate-limiting process of kinetic phase 2 (Ädelroth et al., 2001).

The exact mechanism of PT inhibition by metal binding is a matter of debate. Mainly, three mechanisms have been proposed: Cd^{2+} binding induces

(i) inhibition of conformational gating for ET from Q_A to Q_B, i.e. Eq. 3-1b (Utschig et al., 1998) (**gating inhibition mechanism**), or

(ii) blocking the ability of the residues at the proton entry point (His-H126/128) to vary their protonation states as proton donor/ acceptor groups (Ädelroth et al., 2001; Paddock et al., 2003b) (**His-entry inhibition mechanism**), or

(iii) pK_a shifts of residues in the PT pathways (Gerencsér and Maróti, 2001) (**pK_a inhibition mechanism**).

Based on the decrease in $k_{AB}^{(1)}$ upon Zn^{2+} binding Utschig et al. (Utschig et al., 1998) proposed a gating inhibition mechanism, mainly because in WT-bRC the rate-limiting step in kinetic phase 1 was suggested to be the conformational gating step (Eq. 3-1b) (Graige et al., 1998). However, more recent studies of the E(L212)N mutant by Ädelroth et al. (Ädelroth et al., 2000) indicated that the inhibiting step in kinetic phase 1 of Cd-bRC is PT to Glu-L212 (Eq. 3-1a) rather than the conformational gating step (Eq. 3-1b).

Instead of gating inhibition the His-entry inhibition was proposed as key inhibition mechanism (Ädelroth et al., 2001; Paddock et al., 2003b). This mechanism is consistent with the location of the Cd^{2+}/Zn^{2+} binding site at His-H126/128 in the crystal structure of metal bound bRC (Axelrod et al., 2000) and a significant decrease of $k_{AB}^{(1)}$ and $k_{AB}^{(2)}$ by 10-fold and 4-fold in the double mutant H(H126)A/H(H128)A (Ädelroth et al., 2001). Nevertheless, the decrease of $k_{AB}^{(2)}$ is smaller in this double mutant bRC than the 20-fold decrease observed upon Cd^{2+} binding in the WT-bRC (Paddock et al., 1999; Paddock et al., 2000). Therefore, instead of the His-entry inhibition mechanism, Gerencsér and Maróti proposed that the PT inhibition by the metal binding is due to induced pK_a shifts of residues along the PT pathways (Gerencsér and Maróti, 2001). However, this pK_a inhibition mechanism may be in contradiction to the essentially unaffected rate of $P^+Q_B^-$ charge recombination in the metal bound bRC, which is assumed to be sensitive to pK_a changes of titratable residues in the PT pathways (discussed in Ref. (Ädelroth et al., 2001)).

4.2.2. PT pathways: pK_a decrease upon Cd^{2+} binding

It is commonly accepted that Cd^{2+} binding has little impact on charge recombination rate of $P^+Q_B^-$ in spite of the positive Cd^{2+} charge near Q_B (Utschig et al., 1998; Paddock et al., 1999; Ädelroth et al., 2000; Gerencsér and Maróti, 2001). To elucidate the electrostatic influence of Cd^{2+} binding on residues involved in PT events, we calculated the pK_a for those residues along the PT pathways for the $P^+Q_B^-$ charge state. With Cd^{2+} binding at bRC, several acidic residues in the PT pathway showed significant decrease in pK_a by up to 3.9 units: for instance, 1.1, 1.3, 1.5, 1.8, 2.5 and 3.9 units for Glu-H230, Asp-L213, Asp-M17, Glu-M236, Asp-H170 and Asp-H119, respectively (Ishikita and

Knapp, 2005c) (see also Figure 4-2-2).

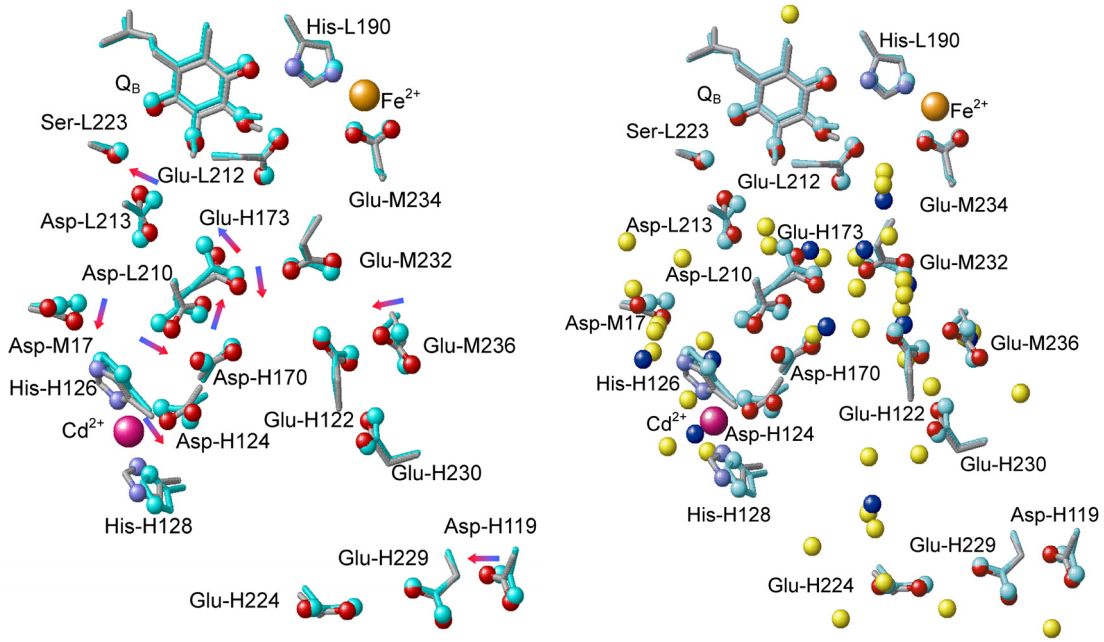

Figure 4-2-2. Rearrangement of side- chains upon Cd^{2+} binding along the PT pathways of bRC. Residues from the Cd-bRC structure (PDB 1DV3) (Axelrod et al., 2000) or the WT-bRC structure (PDB 1AIG) (Stowell et al., 1997) are colored in CPK atom colors (red for oxygen and blue for nitrogen) or cyan, respectively. **Left)** Direction of the atomic coordinate change in the side-chains are indicated with arrow. **Right)** Position of crystal water molecules along the PT pathways in the Cd-bRC structure (yellow) and WT-bRC structures (blue).

Gerencsér and Maróti (Gerencsér and Maróti, 2001) observed that $k_{AB}^{(1)}$, the rate of kinetic phase 1 ($k_{AB}^{(1)}$), is constant at low pH but starts to decrease above a critical pH value. This effect occurs at pH 9.2 in WT-bRC and is thus connected to a moiety of titratable residues with corresponding apparent pK_a, which was down-shifted by 1.8 pH units to 7.4 in Cd-bRC. On the other hand, we calculated a pK_a of 8.9 and 7.6 for Asp-L213 in WT-bRC and in Cd-bRC, respectively (Ishikita and Knapp, 2005c). Taking into account the experimental evidence for the importance of Asp-L213 in PT (reviewed in ref. (Okamura et al., 2000)), we tentatively conclude that one of the main titratable groups observed in the study of Gerencsér and Maróti (Gerencsér and Maróti, 2001) is Asp-L213.

4.2.3. Identification of the apparent pK_a of $k_{AB}^{(1)}$

Identification of the origin of kinetic phase 1 i.e. $k_{AB}^{(1)}$-related pK_a is yet an open question. The measured pH limit, above which $k_{AB}^{(1)}$ starts to decrease, varies slightly with the experimental conditions used by Paddock et al. (Paddock et al., 1989), by Gerencsér and Maróti (Gerencsér and Maróti, 2001) or by Takahashi and Wraight (Takahashi and Wraight, 1992), being 8.5, 9.2 and 9.5, respectively. From the midpoint of the pH-dependent region, Paddock et al. deduced an apparent pK_a value of 9.5 related to $k_{AB}^{(1)}$ (Paddock et al., 1989). On the other hand, Takahashi and Wraight obtained two pK_a values, 9.5 and 11, which they deduced from both edges of the pH-dependent region and assigned to Q_B and Q_B^-, respectively, (Takahashi and Wraight, 1992). We use the calculated pK_a as the protonation midpoint of a titratable group (Ishikita and Knapp, 2005c), which might be more related to the determination of pK_a by Paddock et al. (Paddock et al., 1989).

Mainly by mutational studies for either E(L212)Q or D(L213)N bRC, this apparent pK_a was believed to refer to Glu-L212 as a single residue (Paddock et al., 1989; Takahashi and Wraight, 1992). The calculated pK_a values for Glu-L212 are 6.1 for Q_B and 9.4 for Q_B^- (Ishikita and Knapp, 2005c), indicating a proton uptake of this residue at pH 7 as observed in IR (Hinerwadel et al., 1995) and FTIR (Nabedryk et al., 1995) studies. Nevertheless, the assignment of Glu-L212 as a single residue to the apparent pK_a seems too oversimplified, because Q_B is located in region of strongly interacting titratable residues.

Upon mutation of Glu-L212 to Ala, bRC from *Rb. capsulatus* loses the acidic residue Glu-L212. Nevertheless, the same pH-dependence was observed (Hanson et al., 1992a; Maróti et al., 1994), implying that Glu-L212 cannot be the only residue determining the apparent pK_a for $k_{AB}^{(1)}$ (Sebban et al., 1995a; Sebban et al., 1995b; Gerencsér and Maróti, 2001). Indeed, if the two pK_a values of 9.5 and 11 for Q_B and Q_B^- were assigned just to Glu-L212 (Takahashi and Wraight, 1992), a straightforward interpretation leads to the conclusion that this residue is not involved in proton uptake upon formation of the Q_B^- state at pH 7, which is in conflict with the stoichiometry of proton uptake observed in IR (Hinerwadel et al., 1995), FTIR (Nabedryk et al., 1995) and electrostatic (Rabenstein et al., 2000; Ishikita et al., 2003; Ishikita and Knapp, 2004) studies and the calculated pK_a.

Hence, a cluster of strongly interacting titratable residues near Q_B hinders a clear assignment of this apparent pK_a to a specific residue (discussed in refs. (Hinerwadel et al., 1995; Sebban et al., 1995a; Sebban et al., 1995b; Gerencsér and Maróti, 2001)). Consistently, recent FTIR studies for E(L212)D/D(L213)E double mutant bRC also demonstrated a strong interaction between carboxylic acids specifically in the Q_B region, which cannot be interpreted with a simple, conservative concept of amino acid replacement effect between Glu and Asp (Nabedryk et al., 2004). More recently, ENDOR studies suggested a significant role of Asp-L213 in the rate-limiting step for kinetic phase 1 ($k_{AB}^{(1)}$) by rotating the hydroxyl group of Ser-L223 to Q_B^- (Paddock et al., 2005), as previously predicted in electrostatic computations (Alexov and Gunner, 1999; Ishikita and Knapp, 2004). Unlike Glu-L212, Asp-L213 is generally considered not to be involved in proton uptake upon formation of the Q_B^- state, but this does not imply that Asp-L213 cannot participate in PT (reviewed in ref. (1)).

Thus, we conclude that Asp-L213 is one of the main contributors to the apparent pK_a shift for $k_{AB}^{(1)}$ upon Cd^{2+} binding, without excluding a substantial contribution of nearby interacting residues such as Glu-L212 or Asp-M17 (Ishikita and Knapp, 2005c). This agrees with the deduced conclusion in former studies that not only Glu-L212 but also a cluster of strongly interacting titratable residues contribute to the pH-dependence of $k_{AB}^{(1)}$ (Sebban et al., 1995a; Sebban et al., 1995b; Gerencsér and Maróti, 2001).

4.2.4. Asp-L213, a residue in PT pathways

Formerly the decrease of $k_{AB}^{(1)}$ upon Zn^{2+} binding was interpreted as being due to the inhibition of the conformational gating step (Eq. 3-1b) (Utschig et al., 1998). However, more recent kinetic studies suggest that the decrease of $k_{AB}^{(1)}$ upon metal binding (by 10-fold with respect to wild type bRC) is due to the inhibition of PT to Glu-L212 rather than the conformational gating step (Ädelroth et al., 2000). An equal amount of reduction in $k_{AB}^{(1)}$ for the D(L213)N mutant was previously attributed to the inhibition of PT to Glu-L212 via Asp-L213 (i.e. proton uptake by Glu-L212 belonging to kinetic phase 1) (Paddock et al., 1994) (see also Figure 1a for the location of Asp-L213 and Glu-L212). From these experimental observations, we believe that Cd^{2+} binding inhibits the function of Asp-L213 as PT mediator in kinetic phase 1 (Eq. 3-1a), which slows

down the PT to Glu-L212. Since Asp-L213 is directly involved in the PT pathway to Q_B^- of kinetic phase 2 (Eq. 3-2a), the inhibition of Asp-L213 function in PT should also reduce $k_{AB}^{(2)}$ dramatically, thus changing the energetics of kinetic phase 2 from a ET driving-force dependent to driving-force independent reaction.

Table 4-2-1. pK_a of acidic residues in the $Q_AQ_B^-$ state.

pK_a	WT-bRC [a]	Cd-bRC [b]		pK_a shift on Cd^{2+} binding, influence from:		
		with Cd^{2+}	without Cd^{2+}	Cd^{2+} charge [c]	conformational change [d]	total [e]
residues of subunit H (inside)						
Glu-H122	1.5	2.7	3.1	–0.4	**1.6**	**1.2**
Asp-H170	–4.7	–7.2	–6.5	–0.7	**–1.8**	**–2.5**
Glu-H173	2.7	3.4	3.6	–0.2	0.9	0.7
Glu-H230	0.9	–0.2	0.0	–0.2	–0.9	**–1.1**
residues of subunit H (surface)						
Asp-H119	4.6	0.7	0.7	0.0	**–3.9**	**–3.9**
Glu-H224	3.8	4.4	4.5	–0.1	0.7	0.6
Glu-H229	4.3	3.8	3.8	0.0	–0.5	–0.5
residues of subunit L						
Asp-L210	3.0	2.8	4.9	**–2.1**	**1.9**	–0.2
Glu-L212	9.4	11.7	11.8	–0.1	**2.4**	**2.3**
Asp-L213	8.9	7.6	8.0	–0.4	–0.9	**–1.3**
residues of subunit M						
Asp-M17	5.4	3.9	4.5	–0.6	–0.9	**–1.5**
Glu-M232	–0.7	2.7	2.6	0.1	**3.3**	**3.4**
Glu-M236	8.4	6.6	6.9	–0.3	**–1.5**	**–1.8**

Significant Cd^{2+} binding effects on pK_a (shifts larger than |1.0|) are marked in bold font.
[a] Light exposed structure for wild type bRC (PDB 1AIG) (Stowell et al., 1997).
[b] Light exposed structure for Cd^{2+} bound wild type bRC (PDB 1DV3) (Axelrod et al., 2000).
[c] Influence of Cd^{2+} charge on pK_a shift: $\Delta pK_a(Cd^{2+}\text{ charge}) = pK_a(\text{with } Cd^{2+}) - pK_a(\text{without } Cd^{2+})$.
[d] Influence of conformational change between WT and Cd^{2+} bound bRC on pK_a shift:
$\Delta pK_a(\text{conformation}) = pK_a(\text{without } Cd^{2+}) - pK_a(\text{WT})$.
[e] $pK_a(\text{with } Cd^{2+}) - pK_a(\text{WT})$, i.e. $pK_a(Cd^{2+}\text{ charge})^c + pK_a(\text{conformation})^d$.

The drastic 10-fold decrease in $k_{AB}^{(1)}$ and 6000-fold decrease in $k_{AB}^{(2)}$ upon mutation from-Asp-L213 to Asn indicates the importance of the role of Asp-L213 in efficient PT events of WT-bRC (Paddock et al., 1994; Paddock et al., 1998). In turn, this implies already that, even in WT-bRC, only a small perturbation of this residue might result in serious retardation of the PT for both kinetic phases. Thus, we interpret the decrease in pK_a for Asp-L213 upon Cd^{2+} binding in our computation (Ishikita and Knapp, 2005c) as being one of the important factors of the mechanism of PT inhibition.

4.2.5. Electrostatic domino: a mechanism of long-range electrostatic influence

On the other hand, the distance between Cd^{2+} and Asp-L213 is ~12 Å (see also Figure 4-2-2), which may be too large for a significant direct electrostatic interaction (Cd^{2+} ionic effect). Remarkably, similar long-range electrostatic interactions were formerly proposed by Sebban et al. (Sebban et al., 1995b) with their study of mutant bRC from *Rb. capsulatus*, which possesses Arg-M231 and Asn-M43 at distances of 9 and 15 Å

from Q_B, respectively. Each single mutation of Arg-M231 to Leu or Asn-M43 to Asp shifted the pK_a of titratable residues with respect to WT-bRC, and resulted in a similar level of electrostatic influence on Q_B. They interpreted these long-range electrostatic influences as the consequence of a rearrangement of a combined salt-bridge/H-bond network propagated between the mutation site and Q_B, as an "electrostatic domino effect" (Sebban et al., 1995b).

The comparison of the side-chain orientation in the crystal structures between Cd-bRC and WT-bRC clearly indicates the "electrostatic domino effect" among a number of residues along the water channels to Q_B (Figure 4-2-2, right). The side-chain reorientation of Asp-L213 upon Cd^{2+} binding is likely to propagate via Asp-H124, His-H126, His-H128, Asp-H170, Glu-H173, Asp-L210 and Asp-M17 (Figure 4-2-2, left). The majority of these residues were proposed to participate in the main PT pathway (Okamura et al., 2000) to Glu-L212 or Q_B.

4.2.6. Main electrostatic effects of Cd^{2+} binding

Quite generally, pK_a shifts of titratable groups in proteins, computed relative to the corresponding model compounds in solution, originate from electrostatic interactions with the protein environment. Cofactors like Cd^{2+} are part of this environment in the case of Cd-bRC. The total electrostatic interaction of Cd^{2+} with a titratable residue can be split into (a) the bare influence from the positive charge on Cd^{2+}, which includes also changes in protonation pattern (**Cd^{2+} ionic effect**) and (b) the remaining part, which involves conformational changes induced by Cd^{2+} binding (**the conformation effect**). Accordingly, we defined the Cd^{2+} ionic effect on the pK_a of a titratable group as

$\Delta pK_a(Cd^{2+}$ charge)
= pK_a(with Cd^{2+} in the Cd-bRC structure) – pK_a(without Cd^{2+} in the Cd-bRC structure)

and the conformation effect as

$\Delta pK_a(Cd^{2+}$ conformation)
= pK_a(without Cd^{2+} in the Cd-bRC structure) – pK_a(WT-bRC structure)

(Table 4-2-1). Notably, the two light-exposed crystal structures of bRC (WT-bRC and Cd-bRC) were obtained in the same laboratory (Stowell et al., 1997; Axelrod et al., 2000). Comparison of the atomic coordinates of the two bRC structures yields a root-mean-square difference of 0.65 Å, which is considerably larger than uncertainties in the atomic coordinates (see ref. (Axelrod et al., 2000)).

We conclude that the pK_a shifts can predominantly be attributed to conformational changes induced by Cd^{2+} binding and not by Cd^{2+} ionic effect (Table 4-2-1) (Ishikita and Knapp, 2005c). This holds true for all residues that showed significant pK_a shifts upon Cd^{2+} binding. Indeed, those residues experiencing pK_a shifts upon Cd^{2+} binding also display considerable rearrangements of their own side-chain and nearby surrounding (Ishikita and Knapp, 2005c) (Figure 4-2-2).

The direct-charge effect of Cd^{2+} binding was small and, in most cases, even negligible with respect to the effect caused by conformational changes. It is of interest to understand how the direct-charge influence of the cationic Cd^{2+} is weakened (Table 4-2-1). Asp-L210, located proximal to the Cd^{2+}-protein complex, is the only residue whose pK_a is significantly affected (down-shift of 2 units) by the Cd^{2+} ionic effect (Table 4-2-1). However, the pK_a of this residue is simultaneously up-shifted by the same amount on account of the "conformational change effect", resulting in a vanishing pK_a

shift. Thus, to resist electrostatic perturbations applied to the protein environment upon Cd^{2+} binding, some residues are apt to neutralize the Cd^{2+} ionic effect on their pK_a by a conformational change (i.e. conformational change effect). This remarkable compensation of the direct-charge effect of Cd^{2+} (Ishikita and Knapp, 2005c) is consistent with the small effect on the rate of charge recombination for $P^+Q_B^-$ that is commonly observed with Cd^{2+} binding (Utschig et al., 1998; Paddock et al., 1999; Ädelroth et al., 2000; Gerencsér and Maróti, 2001).

Conclusion:

Cd^{2+} binding induces rearrangements of side-chains of titratable groups along the PT pathways, leading to significant pK_a shifts. Especially, inhibitions of residues in the immediate vicinity of Q_B, namely of **Asp-L213, are assumed to affect the PT kinetics most dramatically. The electrostatic influence of Cd^{2+} over a large distance (~12 Å) can be understood by the electrostatic domino effect (Sebban et al., 1995b), in which successive reorientation of residues (Asp-H124, His-H126, His-H128, Asp-H170, Glu-H173, Asp-M17 and Asp-L210) propagate over a large distance. The direct electrostatic influence of the Cd^{2+} ionic charge on these pK_a shifts is small and, in most cases, even negligible.** The results of the present study are consistent with the pK_a inhibition mechanism (Gerencsér and Maróti, 2001).