X-ray crystal structure of the YM210W mutant reaction centre from *Rhodobacter sphaeroides*

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Abstract The X-ray crystal structure of a reaction centre from *Rhodobacter sphaeroides* with a mutation of tyrosine M210 to tryptophan (YM210W) has been determined to a resolution of 2.5 Å. Structural conservation is very good throughout the body of the protein, with the tryptophan side chain adopting a position in the mutant complex closely resembling that of the tyrosine in the wild-type complex. The spectroscopic properties of the YM210W reaction centre are discussed with reference to the structural data, with particular focus on evidence that the introduction of the bulkier tryptophan in place of the native tyrosine may cause a small tilt of the macrocycle of the B_L monomeric bacteriochlorophyll.

Key words: Reaction center; Photosynthesis; Crystallography; Mutant; M210

1. Introduction

The reaction centre (RC) from the purple bacterium *Rhodobacter sphaeroides* is the best characterised example of a group of membrane proteins that are responsible for the transduction of light energy into a biologically useful form in bacteria, algae and higher plants. X-ray crystallography has revealed that the arrangement of cofactors and amino acids of the L and M subunits in the RC shows an approximate two-fold symmetry (Fig. 1), with the symmetry axis running through the centre of the protein in a direction perpendicular to the plane of the bacterial membrane [1–4]. The bacteriochlorin and ubiquinone cofactors of the RC are arranged around this symmetry axis in two branches that span the membrane (Fig. 1), but only one of these branches is used for transmembrane electron transfer. The source of this asymmetry is the protein, which tunes the optical and electrochemical properties of the cofactors, acts as a scaffold holding the cofactors at precise distances and orientations, and provides the medium through which the electron moves during transmembrane electron transfer.

One of the most intensively studied residues in the RC complex is Tyr M210 (Fig. 1). This residue is in a key location, within 4 Å of all of the bacteriochlorin cofactors that participate in the primary electron transfer reaction. These cofactors are the pair of excitonically coupled bacteriochlorophyll (BChl) molecules that are the primary donor of electrons (P), a molecule of bacteriopheophytin (BPhe) termed H_L, located approximately half-way cross the membrane dielectric, and a monomeric BChl (B_L) that is located between P and H_L (Fig. 1). Primary electron transfer involves the reduction of H_L by the first excited singlet state of P (P*), in approximately 3–4 ps at room temperature (see [5,6] for reviews of the functional and spectroscopic properties of the RC). It is now becoming accepted that primary electron transfer is a two-step reaction involving the reduction and (more rapid) reoxidation of the monomeric BChl, B_L. Replacement of Tyr M210 with residues such as Phe, Leu or Trp brings about a significant slowing of primary electron transfer [7–14], the lifetime of P* changing from 3–4 ps to several tens of picosecpmds in the case of the YM210W RC [12,13,15,16]. As a result, the Tyr M210 residue and the symmetry-related Phe L181 have received a great deal of experimental attention [9–11,16–27].

In a previous manuscript [28], we reported an X-ray crystal structure for the YM210W RC at 3.4 Å resolution which showed, within the limits imposed by the resolution, that the overall structure of the YM210W RC is well conserved with no major changes in the position of the RC cofactors. In the present study we have determined the structure of the YM210W mutant RC to a resolution of 2.5 Å. We discuss the details of the structure with reference to the extensive spectroscopic data available for the YM210W mutant.

2. Materials and methods

2.1. Experimental material

An antenna-deficient strain of *Rb. sphaeroides* containing a RC with the mutation Tyr M210 to Trp (YM210W) was constructed as described previously [13]. Cell growth, membrane preparation, RC purification and crystallisation were carried out as reported recently [29]. Trigonal crystals appeared within 1–4 weeks and grew as prisms of variable size, ranging from 0.3 mm to 1 mm in the longest dimension. The crystals belonged to the space group $P3_121$ and had unit cell dimensions of $a = b = 142.4$ Å, $c = 188.0$ Å, $\alpha = \beta = 90°$, $\gamma = 120°$.

2.2. X-ray crystallography

High resolution X-ray diffraction data (2.5–11.0 Å) were collected

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Abbreviations: BChl, bacteriochlorophyll; BPhe, bacteriopheophytin; P, primary donor; *Rb*, *Rhodobacter*; RC, reaction centre; Ubi, ubiquinone; WT, wild type

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at room temperature on a 30 cm MAR image plate on station 9.6 of the Daresbury synchrotron facility. Low resolution data (3.4–40.0 Å) were collected on a Nonius-Enraf DIP2020 system at the University of Glasgow. Crystals were translated at least twice to minimise problems associated with radiation damage. Radiation damage led to a gradual loss of the high resolution terms over time, and so the effective isotropic resolution of the data set was limited to the resolution at which 50% of reflections in the outer shell had $I > 2\sigma(I)$. The data were processed, scaled and merged using DENZO and SCALEPACK [30]. The co-ordinates of the RC from *Rb. sphaeroides* strain RCO2 [29] with the M210 residue and solvent molecules omitted were used as the starting model, and a rigid body refinement was performed in XPLOR 3.1 [31]. Maps calculated at this point immediately suggested that the mutation had caused a small movement of the B_L BChl. This BChl was omitted and restrained maximum likelihood refinement carried out using REFMAC [32]. The mutated residue and the B_L BChl were rebuilt in O [33] and refinement was continued in REFMAC. Water molecules were fitted using ARPP [34]. All of the collected data, from 40 to 2.5 Å resolution, were used in the refinement and an XPLOR bulk solvent correction was applied. Data collection and refinement statistics are shown in Table 1. Figures were prepared using the programs Molscript [35], Raster3D [36] and O [33] or XtalView [37].

3. Results and discussion

3.1. Structure of the YM210W RC protein

The X-ray crystal structure of the YM210W mutant RC was determined at a resolution of 2.5 Å, as described in Section 2, and was compared with a structure of the wild-type (WT) RC, determined in previous studies at a resolution of 2.6 Å [29]. The two structures had estimated co-ordinate errors of ~0.24 Å and ~0.26 Å, respectively. The comparison showed that there was good structural conservation throughout the bulk of the protein–cofactor system, with significant changes in structure attributable to the mutation confined to the immediate vicinity of the M210 residue (see below). The structural models of the WT and YM210W RC were overlaid using the program LSQKAB [38]. There was no significant deviation of the polypeptide backbone of the L, M or H subunit; the overall root mean square displacement between the C carbon atoms of the amino acids of the L, M and H subunits was 0.18 Å. The one significant exception to this was a very small movement of the backbone atoms of the M210 residue (Fig. 2).

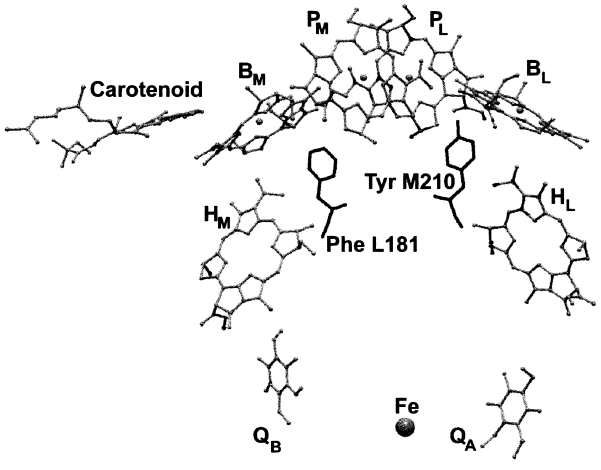

Fig. 1. Arrangement of the cofactors of the *Rb. sphaeroides* RC, and the positions of residues Tyr M210 and Phe L181. The side chains of the bacteriochlorin and ubiquinone cofactors have been omitted for clarity.

Table 1
Crystallographic statistics for data collection and refinement

	YM210W RC
Collection statistics	
No. of unique reflections	72390
Completeness[a]	94.4% (94.8%)
Multiplicity	3.4 (2.6)
R_{merge}[a,b]	12.1% (54.0%)
Refinement statistics	
Resolution range	40.0–2.5 Å
R factor[c]	19.6%
Free-R factor[d]	22.5%
Average B factor for buried atoms[e]	51 Å^2
Geometry	
RMSD from ideality	
bonds	0.013 Å
angles	2.5°
Ramachandran plot, residues in:[f]	
Most favoured areas	91.8%
Additional allowed areas	7.9%
Generously allowed areas	0.3%
Disallowed areas	0.0%
Co-ordinate error[g]	0.24 Å
Model	
No. of protein residues	837
No. of cofactors	4BChl, 2 BPhe, 2 Ubi, 1 Spo, 1 Fe
No. of waters	97
No. of detergents	1
No. of phosphates	0
No. of lipids	1

[a]Figures within parentheses refer to the statistics for the outer resolution shell (2.59–2.5 Å).
[b]$R_{merge} = \Sigma_h \Sigma_i |\langle I(h)\rangle - I(h)_i| / \Sigma_h \Sigma_i I(h)_i$ where $I(h)$ is the intensity of reflection h, Σ_h is the sum over all reflections, Σ_i is the sum over all i measurements of reflection h.
[c]R factor is defined by $\Sigma \| F_O | - | F_C \| / \Sigma | F_O |$.
[d]Free-R was calculated with 5% reflections, selected randomly [45].
[e]Calculated by the program WHAT CHECK [46].
[f]Ramachandran plot was produced by Procheck version 3.0 [47].
[g]Co-ordinate error was estimated by Cruickshank's DPI [48].

In the overlaid structures the mutant Trp M210 residue was co-planar with the WT Tyr M210 residue (Fig. 2A,B). In order to accommodate the Trp residue in the volume normally available to the WT Tyr residue there was a small amount of movement (maximally 0.4 Å) of the backbone atoms of the M210 residue in the YM210W RC (Fig. 2A,B), but this movement did not extend to the flanking amino acids Leu M209 or Gly M211. Despite the fact that Trp has a larger volume than Tyr, there was no significant movement of surrounding amino acids. This was largely due to the fact that the bulk of the 'binding pocket' of the M210 residue comprises atoms of the P BChls, the B_L BChl and the H_L BPhe.

3.2. Structure of the YM210W RC cofactors

As with the protein, there was good conservation of the positions and conformations of the RC cofactors when the YM210W structure was compared with the WT structure. However, upon close scrutiny a small rearrangement appeared to have occurred where the bulkier Trp residue comes within close proximity to the B_L BChl (Fig. 2C). In the WT RC, Tyr M210 points in the direction of rings II and III of the B_L BChl, the closest approaches between the two being between the Tyr hydroxyl oxygen and B_L carbon Cβ that connects rings II and III (3.4 Å) and carbon C15 of ring III (3.6 Å) (the commonly used Fischer numbering system is used for the

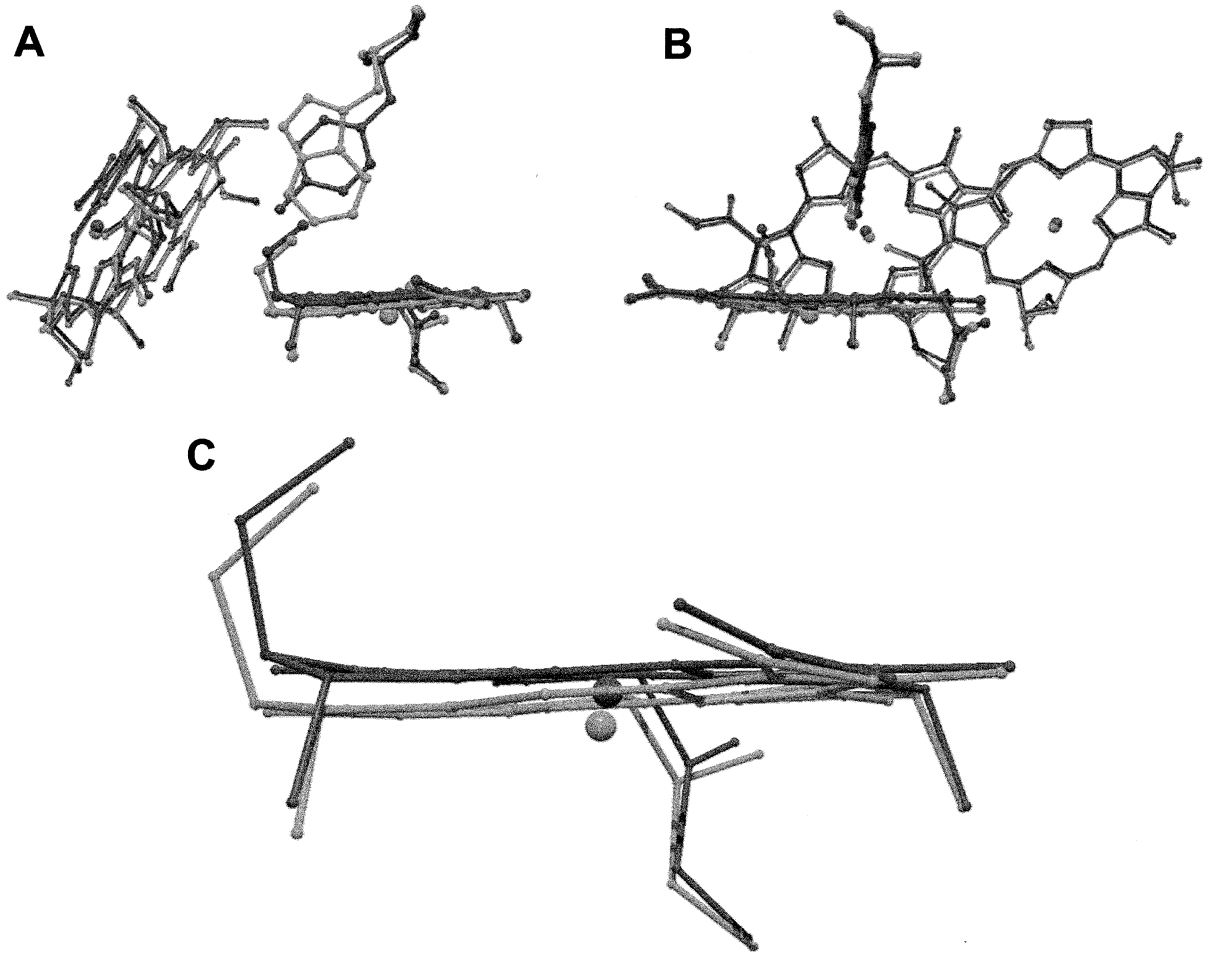

Fig. 2. Superimposition of structural models of the WT RC (dark grey) and YM210W RC (light grey), showing BChls P_L, P_M and B_L, and residue M210. A: View approximately perpendicular to the plane of the ring of the M210 residue. B: View parallel to the plane of the ring of the M210 residue. C: Enlarged view of the B_L BChl, highlighting the tilt of the macrocycle that accompanies mutation of Tyr M210 to Trp. The direction of this view is as for A.

bacteriochlorin molecules; see [39]). There is thus very little space between residue Tyr M210 and the B_L BChl. In the YM210W RC there was a similar spacing, the closest approaches being between carbon CH of the Trp ring and B_L carbon Cβ (3.5 Å) and carbon C15 of ring III (3.3 Å). In order to achieve this, the B_L BChl appeared to have undergone a small tilt, mainly affecting the positions of rings II and III, rather than a translation of the whole BChl ring. Evidence for a tilt was observed in the original electron density maps, and this shows up very clearly in difference electron density maps comparing the data for the YM210W RC with that for the WT RCO2 RC (Fig. 3).

Although this putative tilt was small, involving a change in angle of only approximately 3°, it was quite distinct in the electron density maps (Fig. 3). However, the maximal movement measured from the position of an atom in the B_L BChl in the mutant structure as compared with that in the WT structure was of the order of just 0.5 Å, and care has to be taken when describing such small differences. When comparing two crystal structures it is important to acknowledge that both are associated with an inherent co-ordinate error. In the present case the YM210W structure has an estimated co-ordinate error of ~0.24 Å, and the WT structure a similar value of ~0.26 Å. Therefore, taking the two estimated co-ordinate error values together, a separation of 0.5 Å is of borderline significance. Our conclusion, therefore, is that there is some evidence that the B_L BChl undergoes a tilt of a few degrees in order to accommodate the bulkier Trp residue at the M210 position, but we cannot state unequivocally that this has occurred because the movement involved is relatively small.

3.3. Comparisons with spectroscopic data on cofactor conformation in the YM210W RC

In a study of the 800 nm region of the RC spectrum by ADMR spectroscopy, Hoff and co-workers concluded that differences between the spectroscopic properties of the YM210W and WT RC could be accounted for by an increase in the interaction of B_L and H_L, arising either from a change in the position of the B_L BChl and/or the H_L BPhe, or a rotation of the Q_y transition moment of B_L towards the axis of two-fold symmetry [15]. A rotation of this sort would also be expected to decrease the interaction between B_L and P, through a decrease in orbital overlap, and this would also help to explain effects of the mutation on LD-ADMR spectra [15]. These findings would be consistent with a small tilt of the macrocycle of the B_L BChl of the sort illustrated in Fig. 2C, as this would cause the Q_y transition moment of the B_L BChl to form a smaller angle with the C_2 symmetry axis.

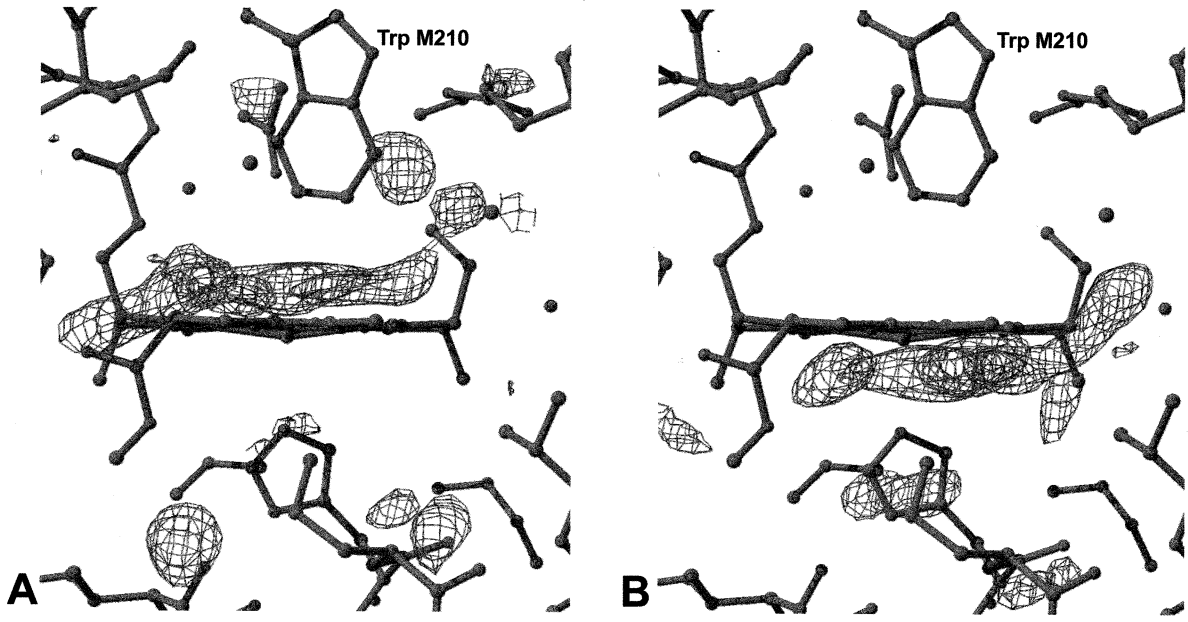

Fig. 3. Difference electron density maps of the B_L BChl, and the structural model of the YM210W RC. The maps were calculated after rigid body refinement. A: The B_L BChl shown with negative difference density. B: The same view of the B_L BChl shown with positive difference density.

3.4. Specificity of the changes in structure observed in the YM210W RC

Replacement of Tyr M210 with a Phe, Leu, Ile or Trp residue produces small red shifts (3–5 nm) of the Q_x and Q_y absorbance bands of the B_L BChl [12,15,21]. The X-ray structure of the YM210W RC shows in some detail the effect of this particular mutation on the B_L BChl, but does not provide an obvious explanation for the change in the energy of the B_L Q_y transition. In fact, in looking for the structural basis for these small spectral shifts some care should be taken, as it is possible that the change in the detailed conformation of the B_L BChl indicated by the electron density maps obtained in this study is particular to the Tyr to Trp mutation, arising from the fact that Trp is a slightly bulkier residue than Tyr. The reverse is true for other mutations such as Phe, Leu or Ile, where these small spectral shifts are also seen. An X-ray crystal structure of the YM210F mutant RC has been reported [40], and this did not show a change in the position of the B_L BChl. However, the resolution of that structure (3.0 Å, based on data that was 64.3% complete) was lower than that described in the present study, and in this context it should be noted that in an earlier structure for the YM210W RC, at 3.4 Å resolution, there was no indication of a shift or tilt of the B_L BChl. Clearly, further high resolution crystallographic data are required on one or more additional M210 mutant complexes before any attempt can be made to draw general conclusions in this area.

3.5. Functional consequences of the Tyr to Trp mutation

The YM210W mutation has a stronger effect on the rate of primary electron transfer than any other single mutation reported (excluding mutations that affect the cofactor composition of the RC). At room temperature, the time constant for primary charge separation in membrane-bound RCs is changed from 4.6 ps in the WT RC to 72 ps in the membrane-bound YM210W RC [13]. Similar observations have been made for purified YM210W RCs [12,15]. At cryogenic temperatures, in contrast to the acceleration of primary electron transfer seen in the WT RC, there is an even more dramatic slowing of this reaction in the YM210W RC [12,16,24]. This slowing also reduces the yield of primary electron transfer [13,16]. Mutation of Tyr M210 to Phe, Leu or Ile also adversely affects the rate and yield of primary electron transfer, and changes the temperature dependence of the reaction, although the effects of these mutations are less severe [7–14].

The YM210W mutation increases the mid-point potential of the P/P^+ redox couple (E_m P/P^+) by 50 mV [12,13], but has only limited effects on other properties of the P dimer. A number of spectroscopic studies have shown that replacement of Tyr M210 by Phe, Leu or Ile does not have any major effects on the optical properties of the P BChls [15,21]. FT-Raman spectroscopy has shown that these mutations do not have any significant effect on the frequencies of vibrational modes associated with the ground electronic state of P, and in particular do not affect the number or strength of H-bond interactions between the protein and conjugated carbonyl groups of the P BChls [18,21]. Mutations to Phe, Leu, His or Trp do not affect the frequency spectrum of vibrational modes coupled to the P* state, as detected by time-resolved spectroscopy [16,41]. ENDOR studies have shown that Phe and Leu mutations at M210 have little effect on the electronic structure of the P^+ cation, including the distribution of positive charge over the two halves of the P^+ dimer [14], conclusions which are supported by FT-Raman spectroscopy [21]. Finally, ADMR and EPR measurements have shown that the YM210W mutation does not affect the characteristics of the P triplet state [15].

The overall conclusion to be taken from these studies is that replacement of Tyr M210 by residues such as Phe, Leu, Ile or Trp has little effect on the properties of P, other than some minor spectral shifts and an increase in E_m P/P^+. The details of the structure of the YM210W RC are in general accord

with this conclusion, with no discernible changes in the conformation of the P BChls or their interactions with the surrounding protein (except for a possible small change in the interaction with the B_L BChl, outlined above).

As indicated above, the YM210W mutation brings about a 50 mV increase in E_m P/P^+ [12,13]. However, the X-ray crystal structure of the YM210W RC did not reveal any significant changes in the structure of the P BChls or their environment, other than the Tyr to Trp mutation and the possible changes relating to the interaction with the B_L BChl documented above. Given the conformation of the Trp residue in the YM210W RC, with the apolar part of the side chain being located closest to the P BChls, the main effect of the mutation is therefore to remove the polar hydroxyl group of the native Tyr residue and replace it with an apolar group. As with Tyr to Phe and Tyr to Leu/Ile mutations at the M210 position, that also bring about an increase in E_m P/P^+, the main effect of the Tyr to Trp mutation is therefore to bring about a decrease in the polarity of the protein medium in the immediate vicinity of both BChls of the P dimer.

The 50 mV increase in E_m P/P^+ observed in the YM210W RC goes some way to explaining the slow rate of primary charge separation in this complex. Such a change will increase the free energy of the $P^+B_L^-$ and $P^+H_L^-$ states, so decreasing the driving force for primary charge separation from P*, and there is experimental evidence that supports such a decrease [12,42]. However, changes of other residues in the vicinity of the P BChls that bring about similar (or larger) changes in E_m P/P^+ have much weaker effects on the rate of this reaction. This suggests that replacement of Tyr M210 by Trp or other apolar amino acids has more widespread effects that simply modulating E_m P/P^+. On the basis of computational studies using the X-ray crystal structure of the WT RC, it has been suggested that the OH group of Tyr M210 has a stabilising effect on the B_L anion, which forms part of the $P^+B_L^-$ state that is the first intermediate in primary electron transfer [43,44]. Thus the unusually strong effect of the replacement of Tyr M210 by Trp or other apolar amino acids is explained by an increase in the free energy of the $P^+B_L^-$ state brought about by a destabilisation of both P^+ and B_L^-. On the basis of the current findings, it is possible that the change in geometry of the interaction between the P BChls and B_L has an additional effect on the rate of primary electron transfer. However, it should be noted that this change in geometry is small, and as discussed above it may be an effect that is specific to a Tyr to Trp mutation at M210.

It is also apparent that the YM210W mutation brings about a modest slowing of the rate of secondary electron transfer from H_L^- to Q_A, which could indicate an effect of the mutation on the redox potential of the H_L/H_L^- couple [13]. Such a change is not due to a change in the position of the H_L BPhe relative to the Q_A ubiquinone, or a new interaction of the H_L BPhe with the side-chain nitrogen of Trp M210, as the geometry of the residue does not allow this.

3.6. Conclusions and outlook

The improved structural model of the YM210W RC shows that structural conservation is very good throughout the body of the protein, with changes in structure confined to the immediate vicinity of the M210 residue. The main effect of the mutation appears to be to cause a small tilt of the macrocycle of the B_L BChl. This tilt, together with some flexing of the backbone atoms of the M210 residue, results from the need to accommodation the bulkier Trp in place of the native Tyr residue. The observed tilt of the B_L BChl could not be determined with absolute certainty from the crystal structure alone, as it was sufficiently small as to lie within the estimated co-ordinate error. However there were good indications from the electron density maps that the tilt had occurred, and a tilt of this magnitude would explain some of the spectroscopic properties of the YM210W RC, in particular results from ADMR spectroscopy [15]. The small change in geometry of the interaction between B_L and the P BChls may be a contributory factor in determining the slow rate of primary electron transfer in this mutant, but it seems likely that this structural change is specific to the YM210W RC, and so probably does not contribute to the generic effects of replacing Tyr M210 with a non-polar residue.

To end on a cautionary note, although the 2.5 Å resolution X-ray structure of the YM210W RC provides a great deal of valuable information on the effects of this mutation, it does not provide obvious explanations for subtle effects such as the small spectral shifts seen in absorbance spectra of the RC cofactors. These effects may arise from changes in structure which are too small to be detectable from X-ray crystal structures where the expected co-ordinate error is of the order of 0.2–0.3 Å. It should also be cautioned that processes such as energy transfer and primary electron transfer are expected to be very sensitive to changes in inter-cofactor distance of the order of a few tenths of an Ångstrom, and so changes in structure that bring about distinct changes in the rates of these processes may be too small to be reliably detected by X-ray crystallography as currently applied to the bacterial RC.

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