First Glance on the Three-Dimensional Structure of the Photosynthetic Reaction Center from a Herbicide-Resistant *Rhodopseudomonas viridis* Mutant

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A first model of the three-dimensional structure of the photosynthetic reaction center of the mutant T1 (SerL223 → Ala, ArgL217 → His) from *Rhodopseudomonas viridis,* resistant toward the triazine herbicide terbutryn (2-methylthio-4-ethylamino-6-*t*-butylamino-*s*-triazine), has been developed from X-ray data measured to a resolution of 2.5 Å. The secondary quinone, Q_B, which in T1 binds better than in the wild type, is present in the crystals. Both substituted residues are clearly visible in the difference fourier map. The replacement of these two residues in the Q_B site causes only minor changes in the overall structure of the protein.

Introduction

In the characterization of purposely modified proteins the combination of functional characterization by different spectroscopic techniques and structural characterization by X-ray crystallography increases our knowledge about macromolecular structure and function. With the crystallization and X-ray structure analyses of the photosynthetic reaction centers (RCs) of different purple bacteria a model of their three-dimensional structure became available [1–4]. On the other hand the RCs are spectroscopically and biochemically well characterized (for reviews see [5, 6]) and they are homologous to photosystem II (PS II) of higher plants (for review see [7]). Their electron acceptors Q_A and Q_B are chemically very similar and both quinones are magnetically coupled to a non-heme iron as shown by EPR spectroscopy [5, 8]. Herbicides of the triazine class block electron transfer in both systems by displacing the secondary acceptor, Q_B [9–11]. We used terbutryn in order to select several herbicide-resistant mutants of *Rhodopseudomonas (Rps.) viridis* [12, 13]. Its mode of binding to the RC of *Rps. viridis* has been established by X-ray crystallography [14]: a hydrogen bond between the ethylamino nitrogen of terbutryn and the side chain oxygen of serine L223, a second hydrogen bond between the peptide nitrogen of isoleucine L224 and N3 of the *s*-triazine ring system, and numerous van-der-Waals interactions that also contribute to inhibitor binding. The binding site of the herbicide overlaps partially with that of the secondary acceptor, which is a ubiquinone-9 in *Rps. viridis* [15]. In the refined electron density at 2.3 Å resolution the ubiquinone could be detected and the following hydrogen bonds seem likely [7, 16]: a hydrogen bond between the hydroxyl group of serine L223 and the quinone carbonyl oxygen-1 which is also hydrogen bonded to the backbone N–H of glycine L225, a hydrogen bond between the carbonyl oxygen-2 of the quinone to the imidazole nitrogen of histidine L190, which is a ligand to the non-heme iron (see Fig. 1a).

The amino acid substitutions that lead to herbicide resistance are located in the Q_B-binding site with one exception [13]. In one *Rps. viridis* mutant an additional amino acid in the Q_A site has been replaced. Here we describe structural changes in the RC from the mutant T1 (SerL223 → Ala and ArgL217 → His) from *Rps. viridis* as determined by X-ray crystallography prior to refinement. This mutant is the only one that shows a higher affinity for the secondary quinone than the wild type [13].

Materials and Methods

RCs of the herbicide-resistant mutant T1 have been isolated and crystallized by the sitting drop method using the slightly modified procedure of Michel [17]. The crystals were grown at pH 7.5

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from ammonium sulfate (1.75 M) at 18 °C within three weeks. The crystals used for X-ray diffraction had a size of about 1.0 × 0.3 × 0.3 mm. Diffraction data were collected from four crystals at the EMBL positron beam line X11 facility at DESY (Hamburg) on film to 2.5 Å resolution. The crystals were cooled to 0 °C during data collection. They are isomorphous with those of the wild type that crystallize in space group $P4_32_12$ [18]. Films were scanned with an Optronics P-1000 microdensitometer and processed using the FILME [19] program package, resulting in R_{merge} values between 0.098 and 0.115 for 61,787 unique reflections between 10.0 and 2.5 Å resolution ($R_{merge} = \Sigma(I - \langle I \rangle)/\Sigma I$). Refinement using the TenEyck-Tronrud (TNT) refinement program [20] and FRODO [21] is in progress.

Results and Discussion

The stability of the RCs of the mutant T1 is decreased compared to the wild type with respect to freezing or storage at 4 °C. The pH optimum is changed to somewhat higher pH values. The occupancy of the Q_B site has raised to 60% compared to the 35% found for the wild type under the same conditions [16]. Since the RC crystals of the mutant are isomorphous with those of the wild type the difference-Fourier technique has been used for analysis of the X-ray data.

As expected, the highest positive difference density peak in an F_{obs}(mutant) − F_{obs}(native) map is located at the ubiquinone, which binds like in the wild type structure. In the mutant T1 oxygen-1 of the quinone seems to be hydrogen bonded to the peptide nitrogen of glycine L225 and oxygen-2 to the imidazole nitrogen of histidine L190 (see Fig. 1). However, this is a preliminary model which may change during the refinement.

The changes due to the amino acid substitutions are clearly visible in the difference-Fourier map at 2.5 Å resolution. There is a well defined negative difference density at the guanidinium group of arginine L217 and a positive density which is attributed to the histidine ring system. Another well defined negative difference density at the serine OH group extends toward the side chain of asparagine L213. A positive difference density close by suggests a rotation of asparagine L213 toward histidine L217. Fig. 1 shows part of the structure of the Q_B site of the wild type (a) compared to that of T1 (b) after 12 cycles of refinement. The coordinates of the wild type are from the current model, refined at 2.3 Å resolution [16]. In Fig. 2 the structure of a larger part of the Q_B site of the mutant T1 is superimposed on the wild type. It shows clearly that there are only minor shifts in the orientation of residues others than L213. Histidine L217 in the mutant has an orientation similar to arginine in the wild type. Differences between both structures could also be due to the lower occupancy of the Q_B site in the wild type RCs.

Because the side chain oxygen is missing in alanine L223 in T1, this residue is no longer able to act as hydrogen bond donor to the side chain oxygen of asparagine L213 and the rotation of this asparagine towards histidine L217 into a more favorable orientation becomes possible. This reorganization seems to require the replacement of arginine L217 by histidine and seems to be stabilized by a hydrogen bond between the imidazole nitrogen of histidine L217 and the asparagine side chain (Fig. 1b). Therefore, the double mutation in T1 might be explained from structural requirements rather than from statistical reasons. Asparagine L213 is an aspartic acid in *Rhodobacter (Rb.) sphaeroides* and *Rb. capsulatus* (for sequence alignment see Fig. 10 in [13]). A salt bridge was suggested between arginine L217 and either L210 or L213 in *Rb. sphaeroides* [22] and aspartic acid L213 in the RC from *Rb. sphaeroides* has an orientation similar to asparagine L213 in the T1 mutant of *Rps. viridis*. This could explain why there is no herbicide-resistant mutant from *Rb. sphaeroides* with a change of arginine L217. However, it could also be due to the low number of mutants that have been characterized so far.

The increased binding affinity of Q_B in the T1 mutant is difficult to understand. Serine L223 is involved in quinone and terbutryn binding [7, 15], therefore, its removal has been expected to lead to a decreased binding of both. This was found for the *Rb. sphaeroides* mutant S223P mutant (SerL223 → Pro) [23]. A role of serine L223 in the protonation of Q_B has been proposed by Paddock *et al.* [24]. The removal of serine L223 leaves the ethylamino nitrogen of terbutryn unpaired which decreases the binding of terbutryn and other triazine herbicides to chromatophores of T1 by several orders of magnitude [25]. In *Rb. capsulatus* the

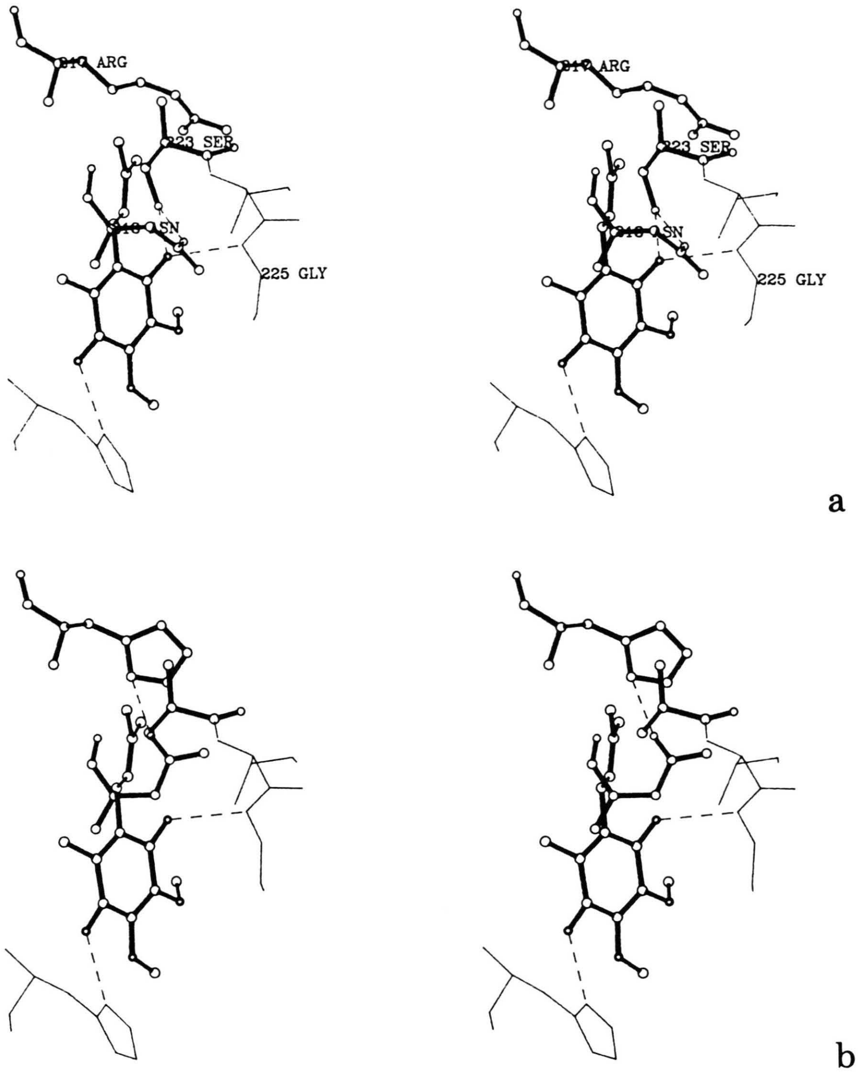

Fig. 1. Stereo plots of part of the Q_B site of a) *Rps. viridis* wild type; b) mutant T 1 (SerL 223 → Ala, ArgL 217 → His). Possible hydrogen bonds are drawn as dashed lines. The quinone oxygen which is hydrogen bonded to serine L 223 in the wild type will be referred to as oxygen-1 and that, which is hydrogen bonded to histidine L 190 as oxygen-2. Ubiquinone, arginine L 217, serine L 223 and asparagine L 213 are shown as atomic models. Histidine L 190, isoleucine L 224 and glycine L 225 are drawn in a skeletal mode. Fig. 1 and 2 were produced by a computer program written by Lesk and Hardman [28].

replacement of serine L 223 by alanine without a second compensating mutation results in the loss of photosynthetic growth [26]. To date, no mutant of a purple bacterium with the single change of arginine to histidine has been reported. It would be interesting to test by site-directed mutagenesis, whether the change of arginine L 217 to histidine in *Rps. viridis* would affect herbicide binding. Long before structural data or protein sequences from the RCs of purple bacteria and PS II were available, a positive charged amino acid like arginine was already suggested to be important for herbicide binding to the 32 kDa protein of PS II [27]. It is now tempting to assign arginine L 217 in *Rps. viridis* to this residue. Arginine L 217 is conserved in *Rb. capsulatus, Rb. sphaeroides, Rps. viri-*

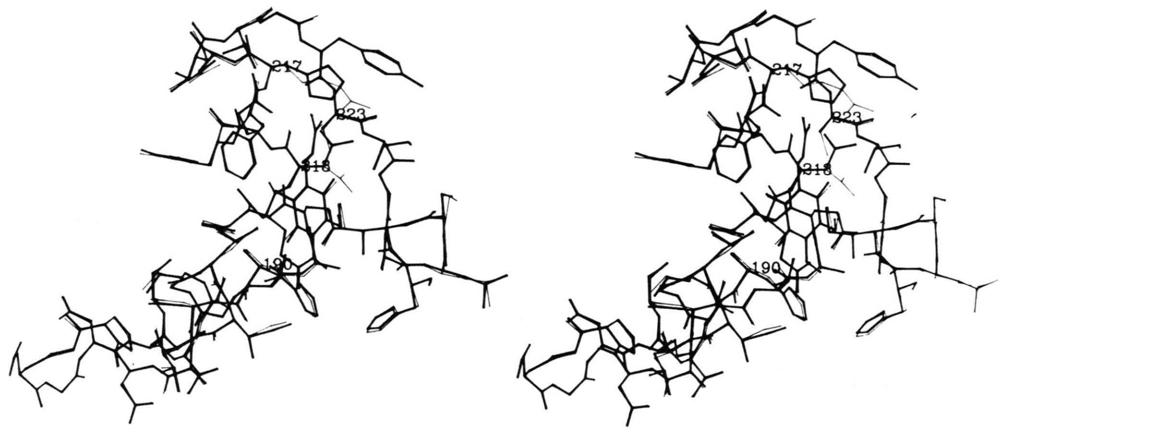

Fig. 2. Stereo plot of a larger part of the Q_B site from *Rps. viridis* wild type (narrow lines) superimposed on that of the mutant T1 (bold lines). The residues L190 to L230 and the quinone are shown. L190, L213, L217 and L223 are labeled at their α carbon atoms.

dis and *Chloroflexus aurantiacus,* but in *Rhodospirillum (R.) rubrum* a glutamine is at position L217 (see Fig. 10 in [13]). There are no data about herbicide binding to RCs or chromatophores of *R. rubrum.* We would expect atrazine to be less effective an inhibitor in *R. rubrum* than in *Rps. viridis* or *Rb. sphaeroides.*

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