Photoaffinity labeling of the azidoatrazine receptor site in reaction centers of *Rhodopseudomonas sphaeroides*

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Photoaffinity labeling of photosynthetic reaction centers of *Rhodopseudomonas sphaeroides* by the herbicide inhibitor, azido[^{14}C]atrazine, occurs principally on the L-subunit. The specificity of labeling is greater at 77 than at 295 K. Kinetic studies of charge recombination in reaction centers indicate competition between azidoatrazine and ubiquinone-1 (Q-1) for binding to the reaction center. This competition occurs through the L-subunit binding site, as increasing concentrations of Q-1 decrease azido[^{14}C]atrazine labeling of this site. It is proposed that the inhibitor binding site, predominantly on the L-subunit, and the secondary quinone binding site on the M-subunit, are adjacent so that there is partial overlap by one molecule of the domain occupied by the other.

Bacterial reaction center *Azido[^{14}C]atrazine* *Herbicide inhibitor* *Photoaffinity labeling* Rhodopseudomonas sphaeroides *Ubiquinone*

1. INTRODUCTION

The reaction center of photosynthetic bacteria is the site in which light energy drives the transmembrane primary electron transfer of the photosynthetic electron transport chain. The reaction center of *Rhodopseudomonas sphaeroides* is composed of 3 polypeptides of 28, 32 and 36 kDa (L, M and H, respectively [1]) in a ratio of 1:1:1. The reaction center also contains 4 BChl, 2 BPheo [2], 1 Fe^{2+} [3] and 2 UQ-10 [4]. Analogous electron transport components are found in the reaction centers of Photosystem II of green plants.

The two quinones act as primary (Q_A) and secondary (Q_B) electron acceptors in both systems. Recent biophysical evidence [5–9] has suggested that inhibition of Q_A to Q_B electron transfer occurs by way of competition between secondary quinone and inhibitor for binding to the reaction center.

Subunits M and H of the reaction center have been implicated in Q_B binding [10] and one would predict the same localization for the binding of the inhibitor. Using azido[^{14}C]atrazine, one of a family of triazine herbicide inhibitors, we show here that the inhibitor binding site is located, instead, primarily on the L-subunit.

Abbreviations: azidoatrazine, 2-azido-4-ethylamino-6-isopropylamino-*s*-triazine; BChl, bacteriochlorophyll; BPheo, bacteriopheophytin; LDAO, lauryldimethylamine oxide; SDS–PAGE, polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate; terbutryn, 2-methylthio-4-ethylamino-6-*t*-butylamino-*s*-triazine; UQ-*n*, ubiquinone (*n* corresponds to the number of isoprenoid units)

2. MATERIALS AND METHODS

Reaction centers were isolated from *Rhodopseudomonas sphaeroides* strain R-26 as in [11] and were normally suspended in 10 mM Tris (pH 8.0), 0.025% LDAO. Under these conditions approx. 70% of the reaction centers were depleted of secondary acceptor, Q_B.

Spectroscopic measurements were performed in a flash-detection spectrophotometer similar to that in [12]. Rates of reduction of P-870$^+$, by charge recombination with the acceptor-side electron,

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were measured by detection of P-870$^+$ at 425 nm at various times following a saturating actinic flash. The fastest rate of charge recombination occurs from Q_A^- ($t_{1/2}$ = 80–100 ms) in centers where electron transfer to Q_B is blocked. Slower recombination reflects electron transfer to Q_B. An estimate of the extent of secondary electron transfer was obtained by measuring the amplitude of the slower phase. In the recombination and labeling experiments to be described, UQ-1 was added in ethanolic solution and functioned as secondary acceptor [13].

Photoaffinity labeling of reaction centers (10 μM) was performed in the presence of 1.5 μM azido[^{14}C]atrazine (49.4 mCi/mmol, Pathfinder Laboratories) through UV irradiation at either room temperature (295 K) or at 77 K. Fifty μl samples (1 mm optical path length) were placed 8 cm from two unfiltered 15 W germicidal lamps (General Electric). Following irradiation, the samples were suspended in 50 mM Na_2CO_3, 50 mM dithiothreitol and 2% SDS and electrophoresed [14] at room temperature on 12% polyacrylamide gels containing 0.1% SDS. The gels were first stained with Coomassie blue, then impregnated with En^{3}Hance (New England Nuclear) and dried for fluorography. Labeling by azido[^{14}C]atrazine was detected at −70°C with X-ray film (Kodak X-Omat, XAR-5).

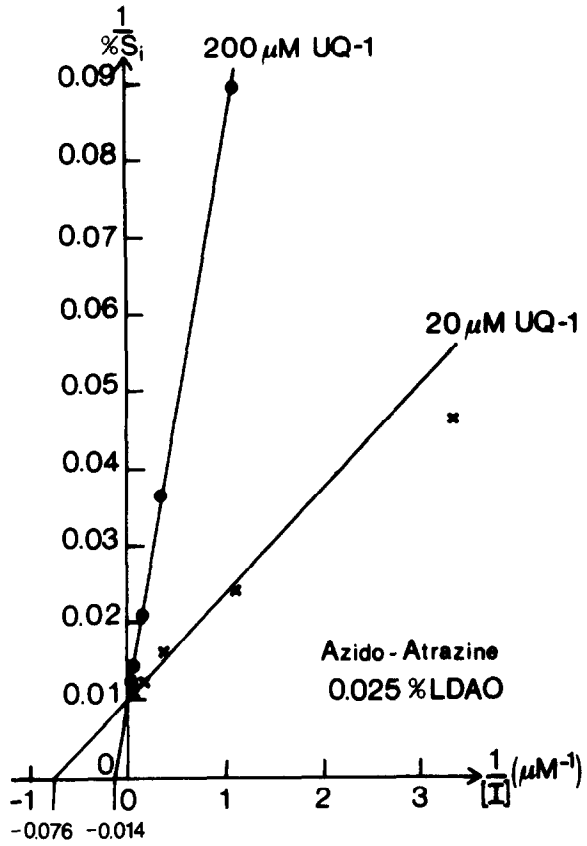

Fig.1. Double-reciprocal plot of the concentration of azidoatrazine *vs* the percent of reaction centers binding inhibitor at the indicated concentrations of UQ-1. Inhibitor binding was measured as the percent loss of the slow phase of charge recombination following a saturating laser flash. This percentage is given as $[A(i=0) - A(i)]/[A(i=0) - A(i=\infty)] \times 100$, where A is the amplitude of $\Delta I/I_{425nm}$ at 1 s after the flash for concentration, i, of inhibitor. Reaction centers (100 nM) were suspended in 0.025% LDAO, 10 mM Tris (pH 8.0) in the presence of 20 or 200 μM UQ-1.

3. RESULTS

3.1. *Azidoatrazine competes with UQ-1 for binding to reaction centers*

Reaction centers (10 μM) were equilibrated with either 20 or 200 μM UQ-1. The reduction of P-870$^+$ by charge recombination was measured at 425 nm, following a saturating actinic flash, in the presence of increasing concentrations of azidoatrazine. The $\Delta I/I_{425nm}$ at 1 s was taken as an indicator of the extent of secondary electron transfer. Normalization of these data to zero and to saturating concentrations of azidoatrazine permitted a measure of the percent inhibition as a function of inhibitor concentration. These data are presented as double-reciprocal plots in fig.1 and show that the binding affinity of the inhibitor is lowered by a factor of approx. 6 by the 10-fold increase in UQ-1 concentration. The I_{50} at 1 s and at 200 ms after the actinic flash were both 1.5 μM in the presence of 20 μM UQ-1 and 7.5 μM in the presence of 200 μM UQ-1.

This observation is similar to that observed with other inhibitors of Q_A to Q_B electron transfer, such as terbutryn, another triazine inhibitor, and *o*-phenanthroline. In all 3 cases [13] quinone competed with the inhibitor for binding to the reaction center.

3.2. *Identification of the subunit binding azidoatrazine*

Reaction centers, depleted of Q_B, were irradiated in the presence of 1.5 μM azidoatrazine at 77 and 295 K. In both cases (fig.2) L is the predominant subunit labeled. The specificity for labeling of this subunit is, however, considerably increased by the low temperature conditions, presumably because of the more restricted diffusion of the photoactivated inhibitor at 77 K. We note also that there is considerably less protein aggregation induced by irradiation under these conditions. No labeling occurs in the absence of irradiation (fig.3).

3.3. *UQ-1 displaces azidoatrazine from the L-subunit*

Reaction centers, irradiated under the low temperature conditions of fig.2, showed decreased labeling of the L-subunit in the presence of increasing concentrations of UQ-1 (fig.3). Densitometric traces (not shown) of the fluorograms indicate a 5-fold decrease in labeling of the L-subunit upon increasing the UQ-1 concentration from 0 to 200 μM. This decreased labeling is not due to an attenuation of the UV light intensity by UQ-1. Such attenuation is negligible at the quinone concentrations used. These experiments thus indicate that the competition observed between inhibitor and quinone (fig.1) occurs through the inhibitor binding site on the L-subunit.

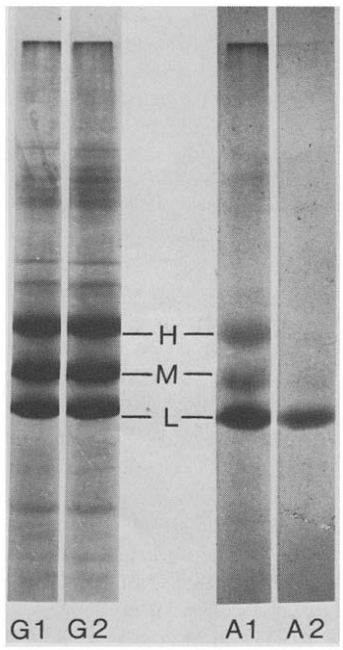

Fig.2. Protein profile (G1 and G2, stained with Coomassie blue) and fluorogram (A1 and A2) of reaction centers (10 μM) following photoaffinity labeling with azido[^{14}C]atrazine (1.5 μM) and SDS–PAGE. The UV irradiation lasted for 90 s at 295 K (G1 and A1) and for 10 min at 77 K (G2 and A2).

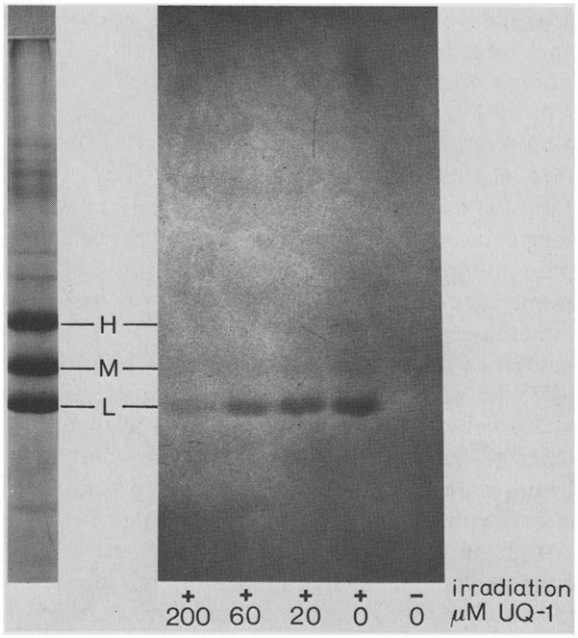

Fig.3. Protein profile (left, stained with Coomassie blue) and fluorogram of reaction centers (10 μM) on SDS–PAGE following photoaffinity labeling for 10 min (77 K) in the presence of 1.5 μM azido[^{14}C]atrazine and at the indicated concentrations of UQ-1. The last column on the right shows non-irradiated reaction centers incubated in the presence of azido[^{14}C]atrazine in the absence of UQ-1.

Densitometric traces of the fluorograms indicate that even upon irradiation at 77 K there is some labeling of the M-subunit, approx. 5-times less than that of L. This labeling was also diminished by increasing concentrations of UQ-1.

Addition of 5 mM *o*-phenanthroline (not shown) completely abolished azidoatrazine labeling (conditions of fig.3, without UQ-1) of the L- and M-subunits. This result is in perfect agreement with our previous observation [13] of total

dissociation of terbutryn from the reaction center by 5 mM *o*-phenanthroline.

4. DISCUSSION

In higher plants and algae, azidoatrazine has been shown to bind to a 32 kDa polypeptide [15], probably involved in the binding of Q_B [16]. The amino acid sequence for subunit M has been recently reported [17] and shown to have considerable homology to this 32 kDa polypeptide [18] with respect to the primary structure and the hydropathy profile. Authors in [17] further reported that there is considerable homology between subunits L and M. It is therefore not a complete surprise that L should bind azidoatrazine.

The simplest model to explain these results would be one in which quinone and inhibitor bind to the same site, i.e., to the L-subunit. Authors in [19] have shown, however, that Fab fragments of anti-M antibodies block reassociation of UQ-10 with the Q_A site in Q_B-depleted reaction centers. While anti-H antibodies were practically without effect, removal of the H-subunit greatly decreased the quinone binding affinity [10,19]. Anti-L antibodies were without effect on the quinone reassociation. The results in [19] implicate M and H in Q_B binding, while our results implicate L and, to a slight degree, M in inhibitor binding. Together, they suggest separate binding sites for inhibitor and quinone. One could reconcile these results by an allosteric model in which quinone and inhibitor bind to different and distant regions of the reaction center and where each lowers the binding affinity at the other site.

Evidence of our own involving terbutryn binding to bacterial reaction centers [13] and of authors in [21] on atrazine binding to the 32 kDa polypeptide could also be explained by partial overlap between adjacent inhibitor and quinone binding sites. From the data presented here we would, in a simpler model, locate these sites at a contact point between the L- and M-subunits. The existence of adjacent sites would be consistent with:

(i) The labeling of L and M by azido [^{14}C]atrazine;

(ii) The apparent partial overlap of the domains occupied by the inhibitor and the quinone head group [13,20] and

(iii) The total displacement of both by *o*-phenanthroline ([13] and here).

Finally, the role of H could be one of facilitated binding of UQ-10 to the reaction center through interaction with the extended isoprenoid tail, which we know to increase the binding affinity of ubiquinone by a factor of 5–10 [13].

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