Fluorescence Induction Characteristics of Wild-Type and Herbicide-Resistant Strain of the Photosynthetic Bacterium *Rhodobacter capsulatus*

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Fluorescence induction characteristics have been studied in wild-type and atrazine-resistant mutant of *Rhodobacter capsulatus*. Fluorescence induction was found to be a useful technique to monitor the altered electron transfer in the atrazine-resistant mutants as well as in the different membrane fractions of wild-type *R. capsulatus*. In both cases, the proportion of the fast rise of variable fluorescence was increased indicating the enhancement of Q_A^-. In the mutant strain, the I_{50} value of triazine herbicide terbutryn was increased by 100-fold whereas the natural resistance of *R. capsulatus* against diuron was abolished by the mutation.

Introduction

Many herbicides, including *s*-triazines, inhibit the light reactions of photosynthesis by blocking electron transfer to quinones in the reaction centers of photosynthetic bacteria and higher plants [1–3]. Understanding the mechanism of herbicide action in bacterial reaction centers may help to explain the mode of action of these herbicides in plants. A promising approach for studying the mechanism of herbicide action involves the isolation and characterization of herbicide-resistant mutants of different photosynthetic bacterial species. The herbicide-resistant mutants isolated by various groups [4–9] showed decreased sensitivity to inhibition of electron transfer to Q_B by triazine herbicides and altered electron transfer properties. A simple and rapid method for determining herbicide inhibition of these electron transfer events is to measure the fluorescence induction kinetics of isolated photosynthetic membranes in higher plants or bacterial chromatophores [4, 10]. In this report, we have analyzed the fluorescence induction characteristics of wild-type and spontaneously occurring triazine-resistant mutant of *Rhodobacter (R.) capsulatus*.

Materials and Methods

The wild type *R. capsulatus,* strain B-10 was grown photosynthetically under anaerobic conditions on a succinate based medium as described previously [11]. The isolation of atrazine-resistant mutant strain designated AR 201 was achieved by transfer of wild type cells to a culture medium supplemented with 200 μM atrazine, followed by selection for spontaneous mutants. Chromatophores of the wild type and resistant strain were isolated with a French press and purified by differential centrifugation. Further resolution of subpopulations of chromatophores differing in sedimentation coefficient was performed according to the method described in [12, 13].

Fluorescence induction transients were measured according to the method of Paterson and Arntzen [10]. The assay medium contained 10 mM phosphate (pH 7.6), 0.1 mM phenazine methosulfate, 2 μM antimycin A, 0.2 mM diaminodurene and 1 mM Na-ascorbate. Bacteria or chromatophores were added to a final concentration of 10–15 μg bacteriochlorophyll per ml [4]. Before the measurements, both bacteria and chromatophores were incubated in the dark for 3 min at room temperature.

Results and Discussion

Fluorescence spectroscopy is a highly sensitive method for studying certain biophysical aspects of the electron transfer in the reaction center of both bacteria and higher plants [14–16]. Fig. 1 shows that both the whole cells of the wild type *R. capsulatus* and its isolated chromatophores exhibit typical fluorescence induction curves. The small initial rise from the non-variable level (F_o) to the intermediate plateau level (F_{pl}) is followed by a smaller but more pronounced fluorescence increase to F_{max} level. It was recently demonstrated in chloroplasts that the initial rise from F_o to F_{pl} was due to the

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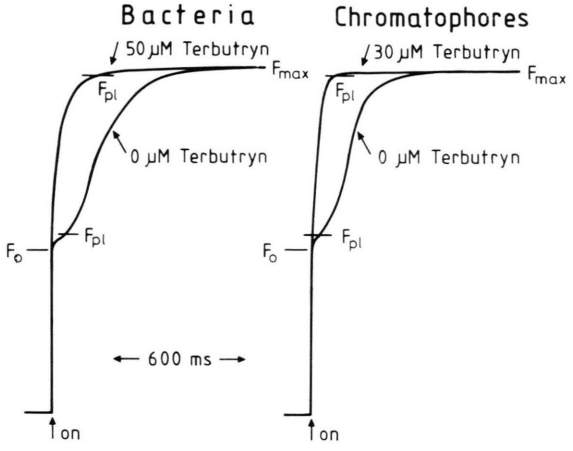

Fig. 1. Fluorescence induction kinetics of whole cells and isolated chromatophores of wild type *R. capsulatus* in the presence and absence of terbutryn.

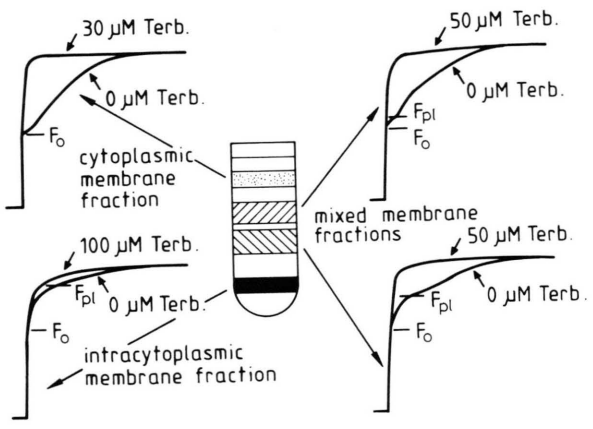

Fig. 2. Fluorescence induction transients of various membrane fractions separated in a 20–50% sucrose gradient according to [12, 13]. Terb, terbutryn.

prompt photoreduction of the primary quinone acceptor Q_A whereas the lag and subsequent rise of fluorescence from F_{pl} to F_{max} is indicative of the quinone pool photoreduction [17]. Addition of herbicide terbutryn to the chromatophores of *R. capsulatus* stimulates the rapid phase of the fluorescence transient, indicating a more rapid accumulation of Q_A^-. This is consistent with previous results obtained with *o*-phenanthroline and triazine herbicides on *R. sphaeroides* [4, 15].

It is well established that cytoplasmic and intracytoplasmic membranes form a continuous membrane system in purple bacteria. The two types of the membrane of wild type *R. capsulatus* were found different in a number of physical and chemical properties (*i.e.* bacteriochlorophyll content, number and size of photosynthetic units, phosphorylating activity, *etc.*) [12, 13]. As it is shown in Fig. 2, the membrane heterogeneity is strongly reflected in the fluorescence induction kinetics of the cytoplasmic and intracytoplasmic membranes separated on sucrose gradient. The cytoplasmic membrane fraction exhibits "normal" fluorescence induction and high herbicide sensitivity. The fluorescence transient of the intracytoplasmic membrane, however, has an elevated F_{pl} level and it is insensitive even against the high concentration of herbicide indicating that the Q_A-Q_B electron transfer may be altered in this type of membranes.

Herbicide-resistant mutants of photosynthetic bacteria isolated previously showed alterations in electron transfer characteristics within the quinone complex as measured by cytochrome photooxidation, P^+ back reaction [4, 5, 7]. The altered electron transport in herbicide-resistant chloroplasts of higher plants is well characterized by the elevated F_{pl} level of the fluorescence induction curves [18, 19]. As it is shown in Fig. 3, the fluorescence induction curve of our AR 201 triazine-resistant mutant

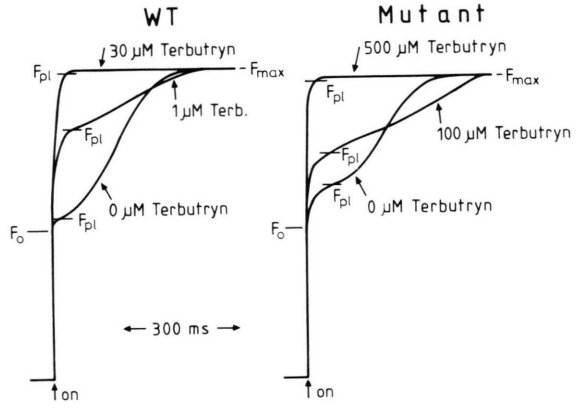

Fig. 3. Fluorescence induction curves of wild type and resistant *R. capsulatus* measured at various herbicide concentrations.

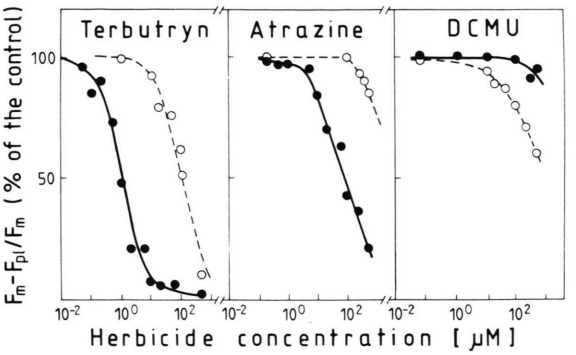

Fig. 4. The effect of the herbicides on the rapidly rising portion of the variable fluorescence changes.

of *R. capsulatus* also has an increased F_{pl} level as compared to that of the wild type. This might indicate the enhancement of Q_A^- in AR 201. Addition of terbutryn (which completely blocks electron transfer between Q_A and Q_B) increases F_{pl} nearly to the level of F_{max}. For the inhibition of electron transport, the resistant strain requires much higher concentration of herbicide than the wild type. The percentage of fluorescence rise occurring as a fast phase was calculated at several herbicide concentrations. The results obtained for atrazine, terbutryn and diuron are shown in Fig. 4. The I_{50} value (the herbicide concentration at which 50% inhibition can be observed) was 1 μM terbutryn in the wild type and 100 μM in the resistant strain AR 201. Similar increase of I_{50} value was observed when atrazine was used for titration. In agreement with earlier observations, diuron has no effect on fluorescence induction of wild type *R. capsulatus* [1, 2, 8]. The AR 201 mutants, however, showed an increased sensitivity against diuron. Similar increase of diuron sensitivity was recently described with a terbutryn-resistant mutants T 4 of *Rps. viridis* in which Tyr L 222 was replaced by Phe. Since the increased sensitivity toward diuron makes our bacterial mutant specifically interesting for studying herbicide binding, the sequencing of the gene coding the L-subunit is in progress in our laboratory.

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