Herbicide and Quinone Binding to Chromatophores and Reaction Centers from *Rhodobacter sphaeroides*

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Besides *s*-triazine and triazinone herbicides the chromone stigmatellin and tetrahalogen-substituted 1,4-benzoquinones are inhibitors of photosynthetic electron flow from reduced cytochrome *c* to ubiquinone-6 in isolated bacterial reaction centers. With isolated bacterial chromatophores binding experiments with radiolabeled herbicides can be performed in a similar way as with thylakoids from higher plants. Tetrahalogen-substituted 1,4-benzoquinones in a Michael type reaction can add onto nucleophilic groups in proteins. In bacterial reaction centers, a [^{14}C]tetrabromo-1,4-benzoquinone (bromanil) exclusively binds to the H-subunit.

Introduction

The recent X-ray crystallographic analysis of the reaction center of the photosynthetic bacterium *Rhodobacter viridis* [1] has tremendously increased our knowledge about the organization of this simple photosystem. It consists out of three protein subunits, known as the H- ("heavy"), M- ("medium") and L- ("light") subunit. The M- and L-subunit carry the primary and secondary quinone acceptor molecules, Q_A and Q_B, respectively, whereas the H-subunit stabilizes the reaction center core complex. Most recent comparisons of amino acid sequences have revealed a high sequence homology between the L-subunit and the D1-protein ("herbicide binding protein", "Q_B-protein") of photosystem II and between the M-subunit and the D2-protein of photosystem II [2]. This has led to the speculation that D1 and D2 constitute the photosystem II reaction center core complex and that this reaction center core complex is organized in a way similar to that of the bacterial reaction center [2]. Indeed, a photosystem II preparation has been isolated which consists out of D1, D2, and cytochrome b_{559} and performs the primary photochemistry of photosystem II [3]. Thus, the bacterial reaction center may be considered as a model system for photosystem II of higher plants and algae. We wish to report here on the inhibitory activity of common photosystem II herbicides and inhibitors in bacterial reaction centers and their binding characteristics. Furthermore, a newly synthesized [^{14}C]tetrabromo-1,4-benzoquinone (bromanil) was found to bind exclusively to the H-subunit of the bacterial reaction center.

Abbreviations: Bchl, bacteriochlorophyll; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; LDAO, lauryldimethylamineoxide; SDS, sodium dodecyl sulfate; UHDBT, 5-*n*-undecyl-6-hydroxo-4,7-dioxo-benzothiazole.

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Materials and Methods

Chromatophores and reaction centers from *Rhodobacter sphaeroides* (strain 2.4.1) were prepared according to Jolchine and Reiss-Husson [4] with the modification that the dissociation with 0.22% LDAO was performed twice. In a typical binding experiment with [^{14}C]metribuzin (spec. activity 25.7 mCi/mmol) chromatophores at a concentration corresponding to 100 µg Bchl were incubated in 2 ml of 20 mM tricine, pH 8.0, and 20 mM $MgCl_2$ for 10 min. Chromatophores were separated by centrifugation for 15 min in a Beckman SW60 Ti swinging bucket rotor at 230000 × *g*. Pellet and an aliquot of the supernatant were assayed for radioactivity in a 1219 Rackbeta liquid scintillation counter (LKB-Wallac) with automatic quench correction. Oxidation of reduced cytochrome c (from horse heart) by isolated bacterial reaction centers under anaerobic conditions in the light (610 nm) was followed spectrophotometrically at 550 nm according to Clayton *et al.* [5]. The reaction mixture contained in a volume of 1 ml 10 mM Tris buffer, pH 7.5; 0.05% LDAO; reduced cytochrome c, 10 µM; ubiquinone-6, 3 µM;

and reaction centers corresponding to 0.5 μM Bchl. [^{14}C]bromanil (spec. activity 45 mCi/mmol) was synthesized as described recently [6]. The assay of radioactivity distribution after gel electrophoresis was performed essentially as in [7].

Results and Discussion

Most of the commonly used photosystem herbicides of the DCMU-type and phenol type do not act or only moderately, as inhibitors of electron transport through the bacterial reaction center [8]. There exists, however, one exception: *s*-triazine herbicides are effective inhibitors in the bacterial system; terbutryn being the most active one [8]. In their system, Stein *et al.* [8] have used reaction centers from the carotenoidless mutant *Rhodobacter sphaeroides R-26,* whereas the wild type has been used in our studies. We have assayed steady state electron transport in the bacterial reaction center using reduced cytochrome *c* as the electron donor and ubiquinone-6 as the electron acceptor. The assay was run under anaerobic conditions. As Table I demonstrates, terbutryn is an excellent inhibitor in our testing system as well. Contrary to Stein *et al.* [8], metribuzin was also found to be active with a pI_{50}-value of 5.22. From the phenol type inhibitors tested, only 2-iodo-4-nitro-6-isobutyl-phenol exhibited inhibitory activity, whereas the others were much less active or not at all (Table I). Interestingly, the chromone type inhibitor stigmatellin [9] proved to be an efficient inhibitor. The same is true for the four tetrahalogen-substituted 1,4-benzoquinones [6], where inhibitory activity increased with increasing molecular weight of the halogen (Table I). Furthermore, UHDBT which is a powerful photosystem II inhibitor [10] was also active to some extent in the bacterial system. It should be noted that other halogen-substituted quinones as described in [6] could replace ubiquinone-6 as electron acceptor in a terbutryn-sensitive reaction.

Table I. pI_{50}-values for inhibition of photosynthetic electron transport by various herbicides and inhibitors in bacterial reaction centers.

A. "DCMU-type"		dinoterb	none
terbutryn	6.25		
metribuzin	5.22	C. Chromone type	
DCMU	4.30	stigmatellin	6.09
phenisopham	none		
		D. Quinone type	
B. Phenol type		(1,4-benzoquinone)	
2-iodo-4-nitro-6-		tetrafluoro-	5.00
isobutylphenol	4.80	tetrachloro-	5.25
picric acid	<4	tetrabromo-	5.60
ioxynil	<4	tetraiodo-	6.00
i-dinoseb	none	UHDBT	4.60

So far no binding experiments with radioactively labeled herbicides or inhibitors and bacterial chromatophores have been performed. This technique, as introduced by Tischer and Strotmann [11], is widely used with isolated thylakoids. For separation of chromatophores from the supernatant in the binding assay much higher centrifugal forces than for thylakoids are required. Fig. 1 shows the binding of [^{14}C]metribuzin to isolated bacterial chromatophores and the inset in Fig. 1 the corresponding Eadie-Scatchard plot for the binding data. In both presentations clearly a high and a low affinity binding can be distinguished. A binding constant $K_b = 90$ nM ($pK_b = 7.02$) and a number of binding sites $x_t = 0.12$ nmol/mg Bchl were calculated for the high affinity binding. There is a discrepancy of more than one order of magnitude between the pI_{50}-value (Table I) and the pK_b-value of metribuzin. In thylakoids, these values are identical [11]. The reason for this discrepancy is not known yet. For terbutryn, whose binding characteristics are similar to that of metribuzin, pI_{50}- and pK_b-value are in the same range [12]. Other DCMU-type inhibitors like phenisopham and DCMU itself show no high affinity binding. The same is true for phenol type herbicides

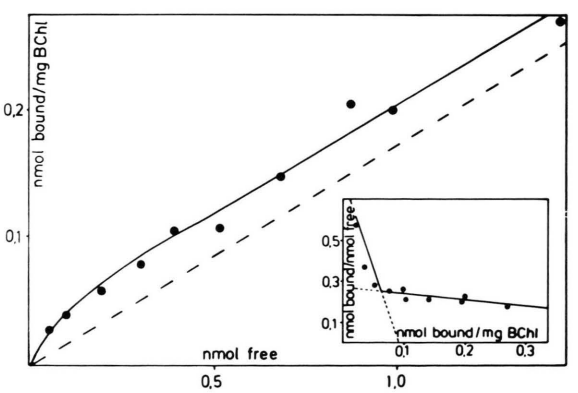

Fig. 1. Binding of [^{14}C]metribuzin to isolated bacterial chromatophores. The dashed line indicates the amount of low affinity (unspecific binding). Inset: Eadie-Scatchard plot of binding data. Free = unbound labeled metribuzin; BChl = bacterial chlorophyll.

with the exception of 2-iodo-4-nitro-6-isobutylphenol.

The herbicide-derived photoaffinity label azidoatrazine in isolated bacterial reaction centers exclusively binds to the L-subunit [13, 14], whereas an azido-anthraquinone tags the M-subunit [15]. These results localize the Q_A binding site on the M-subunit and the Q_B binding site on the L-subunit, in agreement with the X-ray crystallographic data [1]. We have recently demonstrated that halogen-substituted 1,4-benzoquinones in a Michael type reaction can add onto nucleophilic groups in soluble molecules and membrane bound proteins under formation of a covalent linkage [6]. As already stressed, tetrahalogen-substituted 1,4-benzoquinones are also inhibitors of electron transport in the bacterial reaction (Table I). In isolated bacterial reaction centers a [^{14}C]bromanil [6] exclusively binds to the H-subunit (Fig. 2). So far, no functional role in quinone binding has been attributed to the H-subunit. However, recent results have demonstrated that the H-subunit plays a major role in defining the Q_B binding site [16]. Upon removal of the H-subunit from the reaction center complex the rate of electron transfer from Q_A to Q_B is greatly diminished and sensivity of this electron transfer towards inhibitors like terbutryn is greatly decreased [16]. The labeling of the H-subunit by bromanil further stresses the importance of the H-subunit for the stability of the Q_B-site and the intactness of the reaction center core complex.

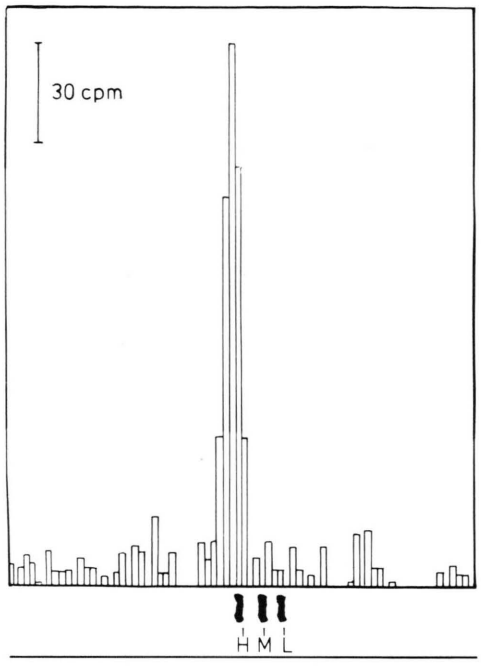

Fig. 2. Photograph of a SDS polyacrylamide electrophoresis gel (10–15%) (bottom) and radioactivity distribution therein of bacterial reaction centers labeled with [^{14}C]bromanil (10 nmol/mg bacterial chlorophyll).

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