Thiazolylidene-Ketonitriles are Efficient Inhibitors of Electron Transport in Reaction Centers from Photosynthetic Bacteria

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Thiazolylidene-ketonitriles are efficient inhibitors of photosynthetic electron flow in reaction centers from either *Rhodobacter sphaeroides* or *Rhodobacter capsulatus*. Some compounds of this class exhibit a higher inhibitory potency in the bacterial system as compared to photosystem II. Up to now, photosystem II inhibitors were generally less active in photosynthetic bacteria. An azido-thiazolylidene-ketonitrile upon illumination almost exclusively tags the L-subunit in the bacterial reaction center.

Introduction

Due to a X-ray crystallography, the architecture of the reaction center of photosynthetic bacteria is well understood [1]. The bacterial reaction center consists out of 3 subunits, the L-, M-, and H-subunit. The L- and M-subunits carry the photosynthetic pigments, necessary for the light-induced primary charge separation. The primary quinone acceptor (Q_A) is oriented towards the M-subunit, whereas the secondary quinone acceptor (Q_B) is oriented towards the L-subunit. Q_B is bound loosely and lost upon crystallization [1]. Furthermore, certain inhibitors can bind strongly to the Q_B-site. In this way they prevent binding of the native ubiquinone to the Q_B-site, which leads to an interruption of photosynthetic electron flow. For the inhibitors terbutryn (an *s*-triazine) and *o*-phenanthroline the orientation within the Q_B-binding niche is well known, because X-ray crystallographic data are available [2].

It became soon clear that the photosystem II reaction center core complex of higher plants and algae is homologous to the bacterial reaction center [3, 4]. In the photosystem II core complex the Q_B-site is located at the D-1 protein which corresponds to the L-subunit and is likely prone to attack by inhibitors. Many of these inhibitors are used as powerful herbicides. Since the architecture of the photosystem II reaction center core complex is not known due to a lack of crystallographic data, the bacterial reaction center can serve as a model for new efficient photosystem II herbicides. However, this model has two important shortcomings.

First, most of the powerful photosystem II herbicides, like ureas, anilides, carbamates, uracils, triazinones and phenols are completely inactive in the bacterial system. Second, those herbicides which are active exhibit a much lower inhibitory potency as compared to photosystem II [5]. For example, terbutryn which has been recognized so far as the most potent inhibitor of the bacterial system, exhibits a pI_{50}-value of 6.25 [6]. In plant thylakoids the pI_{50}-value of terbutryn is 7.52 [5], which is more than one order of magnitude higher as compared to bacterial reaction centers.

In thiazolylidene-ketonitriles (Fig. 1) recently effective inhibitors of photosystem II have been found [7]. As judged from a photoaffinity labeling experiment with a radioactive azido-analogue, thiazolylidene-ketonitriles preferentially bind to the D-1 protein of photosystem II [7]. As we wish to report here, thiazolylidene-ketonitriles are also

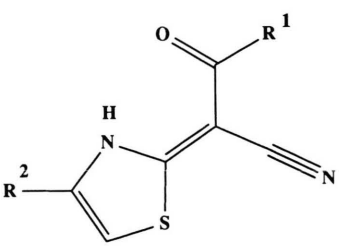

Fig. 1. Structural formula of thiazolylidene-ketonitrile.

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efficient inhibitors of photosynthetic electron transport in reaction centers from *Rhodobacter sphaeroides* or *Rhodobacter capsulatus*. In this chemical class, some compounds – contrary to the normal rule – have a higher inhibitory potency in the bacterial reaction center than in photosystem II. Furthermore, the azido-analogue mentioned above in reaction centers preferentially tags the L-subunit.

Materials and Methods

The synthesis of the thiazolylidene-ketonitriles (Fig. 1 and Table I, **1–8**) is described in [7]. The thiazolylidene-ketonitriles and 2-[(4-azidophenyl)-2,3-dihydro-4-[^{14}C]thiazol-2-yliden]3-oxobutyronitrile [7] (Fig. 1; $R^1 = CH_2C_6H_5$, $R^2 = 4\text{-}N_3\text{-}C_6H_4$) at a spec. activity of 1835 MBq/mmol were generous gifts by Schering AG, Berlin.

Reaction centers from *R. sphaeroides* and *R. capsulatus* were prepared according to [6] or [8], respectively. Inhibitory activity of compounds in photosynthetic electron transport in isolated reaction centers was assayed in a system using reduced cytochrome *c* as the electron donor and ubiquinone-6 as the acceptor [6]. Photoaffinity labeling experiments were performed according to protocols in [7, 9].

Results and Discussion

The pI_{50}-values of electron transport inhibition in reactions centers from *R. sphaeroides* and *R. capsulatus* for various thiazolylidene-ketonitriles together with the previously published pI_{50}-values for photosystem II [7] are given in Table I. From the 8 compounds tested, 5 compounds (No. **2, 5–8;** Table I) were better inhibitors in photosystem II as compared to the bacterial systems. This is usual the case for all photosystem II herbicides [6]. However, 3 compounds (No. **1, 3–4;** Table I) exhibited higher pI_{50}-values in both bacterial systems as compared to photosystem II. This behaviour has not yet been found for any other chemical class investigated in the bacterial system so far. Compound **4,** which is the most active thiazolylidene-ketonitrile, is almost two orders of magnitude more active in reaction centers from *R. sphaeroides* as compared to photosystem II (6.33 *versus* 4.52; Table I). It is also better than terbutryn. Chlorine-substitution in either of the aromatic moieties generally increases inhibitory potency; in this respect, two chlorine atoms are more effective than one (compare compounds **2–4** and **7** and **8;** Table I). This "chlorine effect" is common to photosystem II herbicides (for example, pI_{50}-values: 3-phenyl-; 4.4; 3-(4′-chlorophenyl)-; 5.4; 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; 6.7; see [10]).

The trends in biological activity for thiazolylidene-ketonitriles are the same in *R. sphaeroides* and *R. capsulatus*. However, there are differences in the pI_{50}-values. The highest difference is found for compound **4,** which is about four times more active in *R. sphaeroides* as compared to *R. capsulatus*. We attribute these differences to the different amino acid sequences of the L-subunits of both organisms. An alignment of the herbicide binding re-

Table I. pI_{50}-Values for inhibition of photosynthetic electron transport in reaction centers from *Rhodobacter sphaeroides* (sph), *Rhodobacter capsulatus* (cap), and photosystem II (PS II) by thiazolylidene-ketonitriles. Their general formula is given in Fig. 1. Data for photosystem II were taken from [7].

No.	R^2	R^1	sph	cap	PS II
1	phenyl	phenyl	5.23	5.10	5.00
2	phenyl	benzyl	4.46	4.77	5.22
3	phenyl	4-Cl-benzyl	5.45	5.29	4.70
4	phenyl	2,4-diCl-benzyl	6.33	5.75	4.52
5	*t*-butyl	benzyl	4.39	4.64	6.15
6	2-naphthyl	benzyl	5.54	5.57	6.70
7	4-Cl-phenyl	benzyl	5.70	n.d.	6.10
8	3,4-diCl-phenyl	benzyl	5.90	6.02	6.70

n.d., not determined.

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              190       200       210       220       230       240
vir.  FVNAMALGLHGGLILSVANPGDGDKVKTAEHENQYFRDVVGYSIGALSIHRLGLFLASNI
cap.  FTTAWALAMHGALVLSAANPVKGKTMRTPDHEDTYFRDLMGYSVGTLGIHRLGLLLALNA
sph.  FTNALALALHGALVLSAANPEKGKEMRTPDHEDTFFRDLVGYSIGTLGIHRLGLLLSLSA
      --------------------|                           |----------
                IV                                          V
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Fig. 2. Amino acid alignments of the herbicide binding regions of the L-subunits of *Rhodopseudomonas viridis* (vir), *Rhodobacter capsulatus* (cap), and *Rhodobacter sphaeroides* (sph) according to [11]. Amino acids which are conserved through all three subunits are set in bold. Amino acids in the L-subunit of *R. viridis* which are involved in the binding of either terbutryn or *o*-phenanthroline are set in italics.

gions of the L-subunits of *Rhodopseudomonas viridis, R. capsulatus* and *R. sphaeroides* (according to [11]) are given in Fig. 2. According to Michel *et al.* [2] amino acids E_{212}, N_{213}, F_{216}, V_{220}, S_{223}, I_{224} and I_{229} are involved in terbutryn binding and amino acids H_{190}, L_{193} and I_{229} are involved in *o*-phenanthroline binding. We note that I_{224} is conserved in *R. sphaeroides* but exchanged with V in *R. capsulatus.* In addition, V_{220} is substituted by M in *R. capsulatus.* All other amino acids participating in herbicide binding are conserved, with the exception of N_{213}, which is replaced by D in both organisms. Changes in amino acid composition at positions 220 and 224 may be responsible for the different sensitivity towards thiazolylidene-ketonitriles in both organisms. It cannot be excluded, however, that also amino acid substitutions at other positions (see Fig. 2) may be the cause. It should be noted that thiazolylidene-ketonitriles have been classified as belonging to the "histidine-family" [3, 7] and in this respect resemble *o*-phenanthroline. π-charge distributions as calculated by MNDO in thiazolylidene-ketonitriles make it likely that they bind as the tautomeric hydroxy- rather than the keto-form (Fig. 1) [7].

Further proof for a preferential orientation of the thiazolylidene-ketonitrile molecule towards the bacterial L-subunit comes from a photoaffinity labeling experiment. If isolated reaction centers from *R. sphaeroides* are UV-illuminated in the presence of [^{14}C]azido-thiazolylidene-ketonitril (Fig. 1, R^1 = benzyl; R^2 = 4-azido-phenyl), the highest amount of radioactivity is found to be incorporated into the L-subunit (Fig. 3). The H-subunit is labeled to a much lesser extent and almost no radioactivity is found in the M-subunit

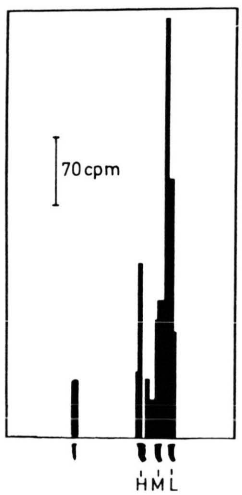

Fig. 3. Photograph of a SDS-PAGE gel (10–15%) of reaction centers from *Rhodobacter sphaeroides* labeled under UV-light (15 min) in the presence of [^{14}C]azido-thiazolylidene-ketonitrile (10 nmol/mg bacteriochlorophyll) (bottom) and radioactivity distribution therein (top).

(Fig. 3). As previously reported, [^{14}C]azido-atrazine also binds almost exclusively to the L-subunit [12, 13]. This result stresses the importance of the L-subunit for herbicide-binding and its homology to the D-1 protein of the photosystem II reaction center core complex.

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