Resistance of reaction centers from *Rhodobacter sphaeroides* against UV-B radiation. Effects on protein structure and electron transport

Júlia Tandori¹, Zoltán Mátyás, Péter Maróti¹ & Imre Vass²,*
¹Department of Biophysics, József Attila University, Szeged, Hungary; ²Institute of Plant Biology, Biological Research Center, P.O. Box 521, H-6701, Szeged, Hungary; *Author for correspondence

Received 9 January 1996; accepted in revised form 22 October 1996

Key words: UV-B radiation, bacterial reaction center, protein structure

Abstract

Inhibition of electron transport and damage to the protein subunits by ultraviolet-B (UV-B, 280–320 nm) radiation have been studied in isolated reaction centers of the non-sulfur purple bacterium *Rhodobacter sphaeroides* R26. UV-B irradiation results in the inhibition of charge separation as detected by the loss of the initial amplitude of absorbance change at 430 nm reflecting the formation of the P⁺(QAQB⁻) state. In addition to this effect, the charge recombination accelerates and the damping of the semiquinone oscillation increases in the UV-B irradiated reaction centers. A further effect of UV-B is a 2 fold increase in the half-inhibitory concentration of o-phenanthroline. Some damage to the protein subunits of the RC is also observed as a consequence of UV-B irradiation. This effect is manifested as loss of the L, M and H subunits on Coomassie stained gels, but not accompanied with specific degradation products. The damaging effects of UV-B radiation enhanced in reaction centers where the quinone was semiredused (QB⁻) during UV-B irradiation, but decreased in reaction centers which lacked quinone at the QB binding site. In comparison with Photosystem II of green plant photosynthesis, the bacterial reaction center shows about 40 times lower sensitivity to UV-B radiation concerning the activity loss and 10 times lower sensitivity concerning the extent of reaction center protein damage. It is concluded that the main effect of UV-B radiation in the purple bacterial reaction center occurs at the QAQB quinone acceptor complex by decreasing the binding affinity of QB and shifting the electron equilibration from QAQB⁻ to QA⁻QB. The inhibitory effect is likely to be caused by modification of the protein environment around the QB binding pocket and mediated by the semiquinone form of QB. The UV-resistance of the bacterial reaction center compared to Photosystem II indicates that either the QAQB acceptor complex, which is present in both types of reaction centers with similar structure and function, is much less susceptible to UV damage in purple bacteria, or, more likely, that Photosystem II contains UV-B targets which are more sensitive than its quinone complex.

Abbreviations: Bchl – bacteriochlorophyll; P – Bchl dimer; QA – primary quinone electron acceptor; QB – secondary quinone electron acceptor; RC – reaction center; UV-B – ultraviolet-B

Introduction

The recent enhancement of solar ultraviolet-B (280–320 nm) radiation on the surface of Earth is of concern due its potential damage to living organisms, including photosynthetic systems (Smith et al. 1995). An important action site of UV-B radiation in oxygenic photosynthetic organisms is Photosystem II (PS II), whose electron transport is inhibited and the D1 and D2 reaction center subunits are damaged (for reviews see Bornman 1939; Vass 1996). Within PS II, the QA and Qb quinone electron acceptors (Greenberg et al. 1989), the Tyr-Z and Tyr-D redox active tyrosines (Yerkes et al. 1989) as well as the catalytic manganese cluster of the water-oxidizing complex (Renger et al. 1989) are believed to be essential targets. Although various
lines of evidence support the primary sensitivity of the water-oxidizing complex, no consensus has yet been reached concerning the importance of its damage relative to the destruction of the quinone acceptors and tyrosine donors in the overall UV-B effect in PS II (see Bornman 1989; Vass 1996).

In contrast to the well documented damage of PS II by UV-B radiation, there is a hiatus in our knowledge regarding the effects of UV-B on the structure and function of the photosynthetic apparatus in photosynthetic bacteria, which developed early in the evolution when the atmosphere contained very low amounts of UV screening ozone (Kasting 1993). The response of purple bacteria to UV-B radiation is of special significance considering the important similarities and differences in the protein structure and electron transport of its reaction center (RC) relative to that of PS II. The RCs of both purple bacteria and PS II consist of a heterodimer of two homologous protein subunits, the L and M in purple bacteria, and the D1 and D2 in PS II (see Michel and Deisenhofer 1988). The redox cofactors which mediate electron transfer from the RC dimer to the acceptor side are highly homologous. The primary electron acceptor is (bacterio)pheophytin, which transfers the electron to the so called two-electron gate of two serially connected quinones, QA and QB (reviewed by Andersson and Styring 1991; Shinkarev and Wraight 1993). However, the homology is not conserved in the electron transport at the donor side. In PS II, the final electron source is water, where from the transfer of electrons to the reaction center chlorophyll, P680, is mediated by a redox-active tyrosine residue, Tyr-Z, of the D1 subunit (see Andersson and Styring 1991). In contrast, the bacterial RC can not oxidize water. The electron donor of Rhodobacter (Rb) sphaeroides is a water soluble cytochrome c2 which reduces directly the oxidized bacteriochlorophyll dimer, P860+, without involving redox-active residues of the protein matrix (see Rosen et al. 1980; Mathis 1994).

Based on the similar structure and function of PS II and purple bacteria at the acceptor side and their largely different electron transport at the donor side, the RC of purple bacteria may provide a useful experimental system to study the detailed mechanism of the UV-B effect in photosynthetic systems. Importantly, a better insight is expected into the UV-sensitivity of the quinone electron acceptors concerning their putative role in the impairment of electron transport and sensitizing the degradation of the RC protein subunits (Greenberg et al. 1989; Trebst and Depka 1990; Melis et al. 1992).

In the present work we characterized for the first time the effect of UV-B radiation on the electron transport activity and protein structure of isolated RC complexes from Rb. sphaeroides. Our results show that the bacterial RC is much less sensitive to UV-B radiation than PS II, indicating that the presence of quinone acceptors does not necessarily render photosynthetic RCs highly susceptible to damage by UV-B radiation.

**Materials and methods**

**Chemicals**

Ubiquinone-10 (UQ10, 2,3-dimethoxy-5-methyl-6-decaisoprenyl-1,4-benzoquinone, Sigma) was solubilized by sonication in 30% Triton X-100 (Serva). The buffer TRIS (2-amino-2-(hydroxymethyl)-1,3-propanediol) was obtained from Sigma. Solution of ferrocene (Sigma) was prepared in ethanol prior to use.

**Strains and growth conditions**

The carotenoidless mutant of Rb. sphaeroides strain (R26) was grown phototrotophically under anaerobic conditions in a medium supplemented with potassium succinate as described earlier (Ormerod et al. 1961). Growth temperature was 30°C, and the light intensity of 90 μE m\(^{-2}\) s\(^{-1}\) was provided by Tungsten lamps (Nagy et al. 1991).

**Preparation of RCs**

Cells were broken by French press (150 MPa) and after removing unbroken cells and cell debris by centrifugation (20 min, 40,000 × g) the chromatophores were sedimented by ultracentrifugation (240,000 × g). RCs were prepared by LDAO (lauryldimethilamine N-oxide, Fluka) solubilization and standard protein purification methods (ammonium sulfate precipitation, DEAE Sephacell (Sigma) column chromatography and ultrafiltration) as described previously (Maróti and Wraight 1988). QB was reconstituted by adding 10-fold excess of UQ10 to the RC solutions. RCs with one quinone were prepared with minor modifications of the method of Okamura et al. (1975).