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Vibrational contributions of the quinones in *Rb. Sphaeroides* reaction centers by light-induced time-resolved FTIR spectroscopy

Daniel L. Thibodeau, Eliane Nabedryk, Werner G. Mäntele, Jacques Breton

In photosynthetic bacterial reaction centers of *Rh. sphaeroides,* the electron is transferred from the primary donor P (bacteriochlorophyll donor) to the primary quinone Q_A in 200 ps and to the secondary quinone Q_B in 100 μs. In the absence of an extrinsic electron donor to P, the charge recombination $P^+Q_A^-$-->PQ_A at 300 K, has a half-life of ~60 ms while P^+Q_B-->PQ_B takes place in a few seconds. Light-induced FTIR difference spectroscopy has been to characterize primary intermediates produced under steady-state conditions. However, in these conditions, the quinone bands have been difficult to assign owing to the large P^+/P contribution in light-induced FIIR spectra. In order to discriminate the individual contributions arising from the quinones and/or their binding sites in the protein during the charge separation, successive time-resolved FIIR difference spectra have been obtained. This method has the advantage of providing the differential spectra of both $P^+Q_A^-/PQ_A$ and $P^+Q_B^-$-->PQ_B states for a single actinic event, in the same conditions of temperature and chemical environment. In a series of time resolved FIIR spectra, where each spectrum is recorded in 100 ms, the time elapsed after illumination results in spectra containing only $P^+Q_B^-/PQ_B$ when the signal of $P^+Q_A^-/PQ_A$ has completely decayed. Thus, the difference of the normalized spectra of $P^+Q_A^-/PQ_A$ and $P^+Q_B^-/PQ_B$ states gives the contributions Q_A, Q_A^-, Q_B, Q_B^-, and/or their amino acid residue partners without the interference from P^+ and P.