Determination of the oxidation reduction potential of bacterial reaction centers

Application Note

Author

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Introduction

Light is collected and converted into chemical energy by both plants and bacteria in the process known as photosynthesis. The primary process in photosynthesis is the capture of light energy followed by the formation of a charge separated state across the membrane. This process occurs in a pigment-protein complex imbedded in the photosynthetic membrane called the reaction center. The Rhodobacter sphaeroides bacterial reaction center is composed of three protein subunits, L, M and H, four bacteriochlorophyll (Bchls) a molecules, two bacteriopheophytin a molecules, two quinones, a carotenoid, and a non-heme iron. Two of the bacteriochlorophyll molecules form a dimer (P) which serves as the electron donor. After the absorption of light, an electron is transferred from the excited donor to a series of acceptors. Associated with the creation of P+ is the bleaching of the protein's optical absorption band at 865 nm.

One of the outstanding questions of the reaction center structure-function relationship is how each of the four BacterioChlorophylls is involved in the primary process of electron transfer. To address this question we have designed modifications of the reaction center to introduce hydrogen bonds to the 9-keto groups of the bacteriochlorophyll of P.[1] Mutations were made at two residues: L13l Leu to His and M160 Leu to His. The addition of a hydrogen bond to the keto group should perturb the electronic structure of the specific Bchl. In this report we describe measurements of the P/P+ midpoint potential of the mutant and wild type reaction centers.

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Experimental

Equipment

- Cary 5 spectrophotometer
- Radiometer model P101 Pt electrode with model K401 calomel reference electrode
- Orion Research model 701A/digital ion analyzer

Reagents

- Potassium hexacyanoferrate (III) (Fluka Biochemika)
- L(+) ascorbic acid, sodium salt (Fluka Biochemika)
- Tris = hydroxymethylamino (methane) buffer (Schwarz-Mann Biotechnology)
- EDTA (Baker)
- Triton-X-100 (Fluka Biochemika)
- Quinhydrone (1:1 hydroquinone:benzoquinone) (Sigma)

Methodology

The P/P+ midpoint potential has been determined by monitoring the absorption at 865 nm during chemical titration studies using the Cary 5. The P/Pt midpoint potential of both wild type and mutant reaction centers was determined by adding aliquots of chemical titrants to isolated reaction centers and then recording both the ambient potential and the absorbance spectrum from 760 to 1000 nm. Potassium hexacyanoferrate (III) and L(+) ascorbic acid, sodium salt were used as the oxidant and reductant, respectively. Purified reaction centers were titrated in 100 mM Tris , pH 8.0, 1 mM EDTA and 0.05% Triton X-lOO. Titrants were made at varying concentrations from 100 mM to 1 M fresh daily in 10 mM Tris, pH 8.0, 1 mM EDTA and 0.05% Triton X-lOO.

For most titrations, 10 mL samples with an absorbance at 800 nm of 0.2–0.6 were continuously stirred and pumped through a flow cuvette situated in the sample position of a double beam Cary 5 UV-Vis-NIR spectrophotometer. Reaction center samples were kept at room temperature and in the dark throughout the titration. The Pt electrode Radiometer and calomel reference electrode, attached to a ionanalyzer, were immersed in the sample and monitored the ambient potential. Titrants were added directly to the sample.

All measured potentials were referenced to a calomel electrode. In order to reference these values to the standard hydrogen electrode (SHE), 244 mV was added to each measured value.[2] The potentiometer and electrodes were calibrated by two different methods. First, solutions were prepared containing different known ratios of potassium hexacyanoferrate(III) and potassium hexacyanoferrate(II) trihydrate. The potentials of these solutions, ranging from 400–550 mV (SHE), were measured and plotted against calculated values for the potentials according to the Nernst equation (n=l),

$$Eh = EO + (RT/nF)\ln [ox]/[red] \quad (1).$$

The data were fitted to a line using a least squares fit analysis with a slope of 1.00 and a y- intercept of -21 mV. The SHE referenced potentials were then corrected by subtracting 21 mV, yielding the final corrected value.

A second calibration was performed by measuring potentials of saturated quinhydrone solutions prepared in several buffers at different pHs. Again, the measured potentials, ranging from 200 to 400 mV, were plotted against calculated values, according to the following equation,[3]

$$Eh = 700 - 59.2 * pH(mV) \text{ at } 25\ ^\circ C \quad (2),$$

and the data were fitted to a line by least squares fit analysis, yielding a slope of 1.00 and a y- intercept of -5.6 mV. This second calibration was not used to correct the measured values because it does not cover the range in which the titrations were performed, but it does provide another estimation of the error in the measured potentials.

All titrations were begun by the addition of 10 mL 100 mM potassium hexacyanoferrate(III) and 5 mL 100 mM sodium ascorbate. Small aliquots of potassium hexacyanoferrate(III) were then added (the potential was increased by 3 to 6 mV per addition), the potentials

recorded and spectra acquired until the ambient potential of the sample reached approximately 540 mV. The optical spectrum from 760 to 1000 nm was measured at each potential on a Cary 5 spectrophotometer (Figure 1). The spectra were normalized at 800 nm to correct for degradation of the reaction centers during the titration. The fraction of the reaction centers that were reduced was determined by comparing the normalized absorbance at 865 nm to the maximum absorbance at 865 nm (100% reduced) and the minimum absorbance at 865 nm (0% reduced). The minimum absorbance of the dimer band was determined by measuring the spectrum in the presence of a saturating light source. The data were fitted to the Nernst equation (n=l).

Results

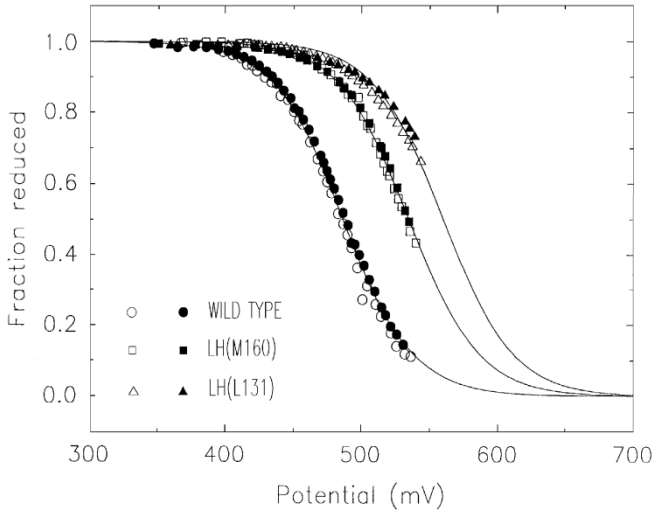

Figure 1. The near infrared spectrum, 760 nm to 1000 nm, for isolated wild type reaction centers from Rhodobacter sphaeroides at different potentials. Note that the 865 nm band associated with the electron donor P bleaches as the state P+ is formed but that the other bands are largely unchanged.

The redox potentials of cofactors in proteins, such as the hemes in cytochromes, vary by several hundred mV, due to differences in ligations, the polarity of nearby residues and other interactions.[4,5] The introduction of hydrogen bonds to the dimer Bchls in reaction centers would be expected to alter the P/P+ redox midpoint potential. Redox titrations of reaction centers with mutations near the dimer are compared to wild type reaction centers in Figure 2.

The value of the midpoint potential of wild type reaction centers was found to be approximately 480 mV, in agreement with recent results obtained using an electrochemical cell.[6] The Leu to His at L13l mutant and the Leu to His at M160 mutant showed increases of approximately 80 and 55 mV, respectively, in their midpoint potentials relative to the wild type (Table 1).

Table 1. Redox data for wild type and mutants

Mutation	P/P+ mid-point potential
none- wild type	480 ± 10 meV
Leu to Kis at L 131	560 ± 10 meV
Leu to His at M 160	535 ± 10 meV

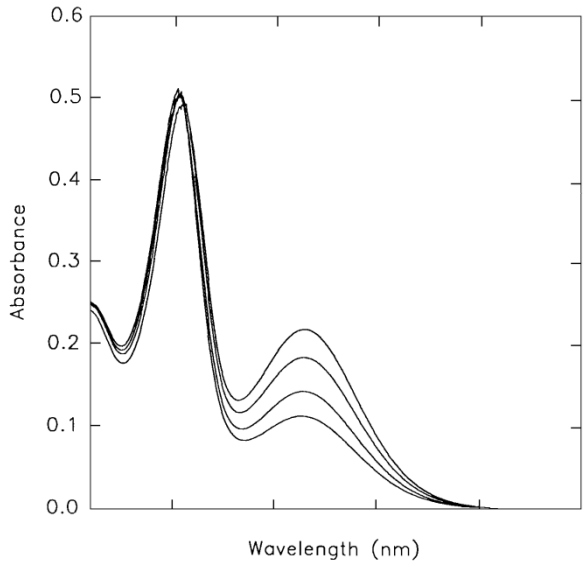

Figure 2. Redox titrations of reaction centers. The extent of reduction of the dimer was determined by measuring the absorbance of the dimer peak (865 nm in the wild type), while monitoring the ambient redox potential. The open symbols represent data from an oxidative titration and the closed symbols represent data from a subsequent reductive titration on the same sample. The solid lines depict the fits of the Nernst equation (n=l) for the combined data using a single parameter, Em, the midpoint potential. Figure is reproduced from Reference 1.

References

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