DNA Binding Characteristics of CrtJ

A REDOX-RESPONDING REPRESSOR OF BACTERIOCHLOROPHYLL, CAROTENOID, AND LIGHT HARVESTING-II GENE EXPRESSION IN RHODOBACTER CAPSULATUS

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Stephen N. Ponnampalam§ and Carl E. Bauer††

From the §Biochemistry Program, Department of Chemistry, and the †Department of Biology, Indiana University, Bloomington, Indiana 47405

Previous genetic analysis indicated that the photosynthesis gene cluster from Rhodobacter capsulatus coded for the transcription factor, CrtJ, that is responsible for aerobic repression of bacteriochlorophyll, carotenoid, and light harvesting-II gene expression. In this study, we have heterologously overexpressed and purified CrtJ to homogeneity and shown by gel mobility shift assays that CrtJ is biologically active. DNase I footprint analysis confirms molecular genetic studies by showing that CrtJ binds to conserved palindromic sequences that overlap the −10 and −35 promoter regions of the bchC operon. Graphs of the percentage of DNA bound versus protein concentration show sigmoidal curves, which is highly indicative of cooperative binding of CrtJ to the two palindromic sites. A binding constant for interaction of CrtJ with the palindrome that spans the −10 region was calculated to be 4.8 × 10⁻¹⁰ M, whereas affinity for the palindrome that spans the −35 region was found to be 2.9 × 10⁻¹⁰ M. Binding of CrtJ to the bchC promoter region was also found to be redox-sensitive, with CrtJ exhibiting a 4.5-fold higher binding affinity under oxidizing versus reducing conditions.

Rhodobacter capsulatus is a purple nonsulfur photosynthetic bacterium that regulates synthesis of its photosynthetic apparatus in response to alterations in oxygen tension and light intensity. Oxygen tension at atmospheric levels (21%) results in virtual suppression of photopigment production, whereas reduced oxygen tension (<1%) promotes cellular differentiation that results in the formation of an intracellular membrane that houses the light-driven energy-generating photosystem (1). Oxygen regulation of photosystem synthesis is known to be controlled, in part, by regulating transcription of photosynthesis genes (reviewed in Refs. 2 and 3).

Regulation of photosynthesis gene expression by oxygen tension is known to be controlled by many different transcription factors (reviewed in Refs. 2 and 3). One regulatory circuit, RegB-RegA, is a phosphorylation cascade responsible for an aerobic activation of light harvesting and reaction center structural genes that are coded by the psa, puh, and puc operons. RegB is a histidine kinase that initiates a phosphorylation cascade when the cells are growing in an anaerobic environment (4–6). In a previous study, we observed that disruption of the open reading frame termed crtJ (ORF469) resulted in the aerobic production of photopigments (7). Genetic and transcriptional studies have indicated that crtJ appears to code for an aerobic repressor of bacteriochlorophyll (bch), carotenoid (crt), and light harvesting-II (puc) gene expression (8–12). We also observed that cell-free extracts derived from wild type R. capsulatus cells are able to promote the formation of a stable gel mobility shift with a bchC promoter fragment, whereas extracts from a crtJ-disrupted strain were unable to do so (11).

Gel shift (11) and mutational analysis (12), have indicated that CrtJ is most likely interacting with a conserved palindrome (gTGT-N₁₂-ACAr) that is present in the CrtJ-regulated bch, crt, and puc promoters. However, direct proof that CrtJ recognizes the conserved palindrome sequence has not been demonstrated.

In this study, we have heterologously overexpressed and purified CrtJ to homogeneity. Gel mobility and DNase I protection analysis were utilized to demonstrate that CrtJ indeed binds to a conserved palindromic sequence that is present in two copies of the bchC promoter. We also demonstrate that binding of CrtJ to the bchC promoter is affected by the redox state of the binding buffer.

MATERIALS AND METHODS

Strains, Media, and Growth Conditions—Strain NM522 was used for routine cloning procedures. For overexpression of CrtJ, strain BL21(DE3) was utilized (13). Plasmids pRPS404 (14), pUC19 (15), pUT7–7 (16), and pET28a(+) (16–18) have been described previously. Luria broth was used for agar solidified plates and for liquid cultures (19). Ampicillin and kanamycin were used at 100 and 30 μg/ml, respectively.

Construction of a CrtJ Overexpression Vector—CrtJ was expressed in Escherichia coli using a T7 RNA polymerase-based overexpression system that appended the amino terminus of CrtJ with a His₉ tag (Novagen). For this construction, the CrtJ coding region (14,821–16,209 base pairs of the photosynthesis gene cluster; Ref. 20) was amplified using a polymerase chain reaction (PCR). The upstream oligonucleotide primers, 5′CCCATATGCGACGGGAGGCCTTGCA and 5′CCCTCTAGAACGGGAGTCCTTGCA and 5′CCCTCTAGAACGGGAGTCCTTGCA, respectively, were designed to contain an Ndel restriction site at the start codon and an XcoI site at the stop codon (underlined bases). The PCR-amplified fragment was subsequently cloned into SmaI-digested pUC19 and then subcloned into the Ndel-HindIII restriction sites of pET28a(+) (Novagen), resulting in the recombinant plasmid, pET28:CrtJ.

Overexpression and Purification of CrtJ—The expression plasmid pET28:CrtJ was transformed into the pT7 RNA polymerase expression strain BL21(DE3), and CrtJ was then expressed to high levels by isopropyl-β-D-thiogalactopyranoside (IPTG) induction as described previously (16, 17). The cell pellet from a 100-ml culture was then resuspended in 4 ml of ice-cold binding buffer composed of 20 mM Tris-HCl, pH 7.9, 5 mM imidazole, 0.5 mM NaN₃, 0.1% Nonidet P-40, and the cells were then disrupted by sonication. The lysate was then clarified by
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centrifugation at 39,000 \times g for 20 min with the supernatant further clarified by filtration through a 0.45-\mu m membrane (Gelman Sciences) to prevent clogging of the resin during column chromatography. Purification of His6-appended CrtJ was performed using nickel column affinity chromatography as described by the resin manufacturer (Novagen). Chlorins containing the highest CrtJ concentrations as visualized by SDS-polyacrylamide gel electrophoresis were pooled, yielding a typical concentration of 0.30 mg/ml and dialyzed overnight in 1 liter of buffer composed of 50 mM Tris-HCl, pH 7.9, 50 mM potassium acetate, 1 mM EDTA, and 20% glycerol. The purified protein was then partitioned into 30-\mu l aliquots, subjected to rapid freezing using a dry ice/ethanol mixture, and stored at −70°C.

**Gel Mobility Shift Assay—PCR amplification and purification of a**

[22P-labeled DNA probe containing the \textit{bchC} promoter region was prepared as described by Ponnampalam et al. (11). For determination if purified CrtJ was active, a gel mobility shift assay was performed that compared DNA binding properties of purified CrtJ with that of a crude lysate that was prepared as described by Ponnampalam et al. (11). For this analysis, 3 \mu l of 22P-end-labeled \textit{bchC} DNA probe (10 fmol/3 \mu l) was added to 2.5 \mu l of poly(dI:dC) nonspecific competitor DNA (0.20 \mu g/\mu l). The DNA solution was then added to a 15-\mu l reaction containing either purified CrtJ protein (0.50–1.0 \mu g) or crude lysate (2 \mu g) in a reaction buffer composed of 50 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.1, 50 mM potassium acetate, 20% glycerol (v/v). Each reaction was then incubated for 20 min at 30 °C, loaded on a native 6% Tris-glycine-EDTA-buffered polyacrylamide gel, and electrophoresed at 18 mA for 2.5 h at 4 °C.

To ascertain if DNA binding of purified CrtJ is redox-sensitive, purified CrtJ was diluted into a 30-\mu l reaction buffer containing 9 fmol of 22P-end-labeled \textit{bchC} DNA probe and 3 \mu l of a 10 mg/ml heparin solution. To facilitate the transport of electrons from the oxidizing/reducing agent to the protein center, 1.5 \mu l of ethanol and 1.0 \mu l of water-soluble redox mediators were also added to the mix (21). The ethanol-soluble mediators used were 1,4-benzoquinone (2.5 \mu g/ml), N,N,N,N′-tetramethylphenylenediamine (3.0 \mu g/ml), diamonodurene (12 \mu g/ml), 1,2-naphthoquinone (3.0 \mu g/ml), 5-hydroxy-1,4-naphthoquinone (3.0 \mu g/ml), duroquinone (4.0 \mu g/ml), 2,5-dihydroxy-1,4-benzoquinone (3.5 \mu g/ml), anthraquinone (saturated), and 2-hydroxy-1,4-naphthoquinone (3.0 \mu g/ml). The water-soluble mediators used were phenazine methosulfate (7.0 \mu g/ml), anthraquinone-2,6-disulfonate (7.5 \mu g/ml), anthraquinone-2-sulfonate (7.5 \mu g/ml), benzyl viologen (1.0 \mu g/ml), and methyl viologen (1.0 \mu g/ml). The protein samples were incubated for 30 min on ice in the presence of 10 mM sodium dithionite, argon gas, oxygen gas, or 20 mM potassium ferriyanide. Oxygen gas was bubbled through the sample continuously for 5 min. Argon gas was bubbled through the protein sample alternately with degassing by vacuum several times prior to addition of argon. After the 30-min redox incubation period, 3 \mu l of 22P-end-labeled probe (10 fmol) was added to the samples, which were then incubated for an additional 20 min at 30 °C. 3 \mu l of heparin (10 \mu g/ml) was then added as a competitor, and the samples were then electrophoresed at 4 °C as described above.

**DNAase I Footprint—**A 183-base pair DNA segment containing the \textit{bchC} promoter region was obtained by PCR amplification using oligonucleotide primers 5′-GTTCGGACCCGGCTTTGACC and 5′-TTCCACAAAGGTGCCTGAACCG with amplification conditions as described for the gel mobility shift assays. For selective labeling of DNA strands, one of the primers in the PCR reaction was \textit{EcoR I} labeled prior to amplification. The amplified segment was purified as described for the gel mobility shift assay. Binding of purified CrtJ to the DNA fragment prior to DNAase I digestion involved conditions similar to those described for the gel shift assays with the reaction mix composition containing 4 \mu l of end-labeled DNA (12 fmol), 82 \mu l of gel mobility shift buffer, and 9 \mu l of CrtJ at varying concentrations. After incubating the binding reaction at 30 °C for 20 min, DNAase I (0.50 \mu g/ml) was added to the mixture, and the digestion was allowed to proceed for 2 min at room temperature. The reaction was then stopped by the addition of 100 \mu l of DNAase I stop solution and electrophoresed on a 5% urea-denaturing Long Ranger polyacrylamide gel as described previously (22). A modified Maxam and Gilbert G + A chemical sequencing reaction was used for determining the location of DNAase I protection.

**EC50 (Effective Concentration for 50% Response) Value Determination**—The EC50 values for CrtJ binding to the \textit{bchC} promoter region were calculated using a modified method of Brenowitz et al. (24). Briefly, CrtJ DNA binding isotherms to the \textit{bchC} promoter region were generated by DNAase I footprint titration assays using 2-fold protein dilutions ranging from 4 ng to 4 \mu g. The gel was then analyzed for CrtJ binding isotherms using a PhosphorImager (Molecular Dynamics) to quantitate the level of CrtJ protection of a single band in the upstream and downstream palindromes. Values were corrected for loading by normalizing with a band from an unproTECTED region.

**Nitrocellulose Filter Binding Assays**—Nitrocellulose filter binding assays according to the method of Withereil and Uhlenbeck (25), were utilized to determine the fraction of isolated CrtJ that was active in DNA binding, the level of which was then used to correct the EC50 values for CrtJ binding to the \textit{bchC} promoter region. For this determination, 1.2 pmol (62.5 ng) of purified CrtJ was incubated with varying amounts (from 0.04 to 3.65 pmol) of 32P-labeled \textit{bchC} DNA probe that was identical to the probe used for the DNAase I footprint titration assay. The sample volume used for each reaction was 30 \mu l consisting of 10 \mu l of DNA sample (of varying dilutions), 5 \mu l of heparin (1000 \mu g/ml), 9 \mu l of CrtJ (1.2 pmol), and 6 \mu l of binding buffer. The reactions were incubated at 30 °C for 10 min and then filtered through 0.45-\mu m pore size pure nitrocellulose filters (number 91382, Schleicher and Schuell), which had been previously soaked in binding buffer (50 mM Tris-HCl, pH 8.0, 50 mM potassium acetate, and 20% glycerol) for 2 h at room temperature. The filtration time was relatively rapid, with most of the filtrate passing through the nitrocellulose filter within 60 s. The filters were then air-dried for 30 min and counted in a Packard scintillation counter (model Tri-Carb 2100TR) using Bio-Safe II Biodegradable Counting Mixture (Research Products International Corporation). Retention efficiencies for the 32P-labeled DNA calculated as the number of counts retained on the nitrocellulose filter compared with the number of counts retained on the filter at saturating levels of the protein ranged from 60 to 95%. A control assay was also done in which no protein was added to the varying amounts of DNA used to ascertain the background level of DNA that bound to the nitrocellulose filter.

**Hill Coefficient Determination**—The Hill coefficient was determined by using a nonlinear regression analysis to fit the Hill equation given by $y = a + (x \cdot E)^{-b}$, where $a$ is the maximum value of $y$ as $x$ approaches infinity, $y$ corresponds to the fractional occupancy of the binding site on the DNA molecule, $x$ is the protein concentration, $E$ is the EC50 value, which corresponds to half-maximal saturation of each binding site, and $b$ is the Hill coefficient. The Hill coefficients for the binding of CrtJ to both the upstream and downstream palindromes were obtained from a DNAase I footprint titration assay from which site binding isotherms were generated. The Hill coefficients were then determined from a graph of fractional saturation versus protein concentration.

**RESULTS**

**Purification of Biochemically Active CrtJ**—A His-tagged version of CrtJ was overexpressed in E. coli using a T7-based overexpression system. As demonstrated by the SDS-polyacrylamide gel electrophoresis polypeptide profile in Fig. 1, IPTG-based induction of T7 RNA polymerase resulted in high level overexpression of CrtJ, which was predominantly found in the soluble fraction (lane 3). CrtJ was subsequently isolated to a high level of purity (>95%) as judged by Coomassie Blue staining (lane 4) by affinity binding of the His6-tagged CrtJ to a Ni2+ resin.

Gel retardation (mobility shift) assays were subsequently performed to ascertain if isolated CrtJ was active. As a control, we performed a gel retardation assay with crude cell lysates obtained from wild type and CrtJ-disrupted \textit{R. capsulatus} cells. In confirmation of previous results (11), we observed that a
DNA fragment that contains the \textit{bchC} promoter region was shifted to a reduced electrophoretic mobility when incubated with the wild type extract (Fig. 2, lane 2). This mobility shift is absent in extracts derived from the \textit{crtJ}-disrupted strain DB469 (lane 3), suggesting that the mobility shift observed with the wild type extract resulted from an interaction of CrtJ with the \textit{bchC} promoter region. This supposition is supported by mobility shifts obtained with increasing concentrations of purified CrtJ (lanes 4–6) that has an electrophoretic mobility identical to that observed with wild type crude extracts. These results indicate that heterologously expressed CrtJ was isolated in a properly folded form and that it has specificity for a sequence within the \textit{bchC} promoter region.

**CrtJ Binds to a Duplicated Palindrome in the \textit{bchC} Promoter Region—**DNase I protection (footprint) analysis of CrtJ binding to the \textit{bchC} promoter region was performed on both DNA strands by selectively 5’-end labeling either the top or bottom strand. As seen from the DNase I digestion patterns in Fig. 3, CrtJ protects a region of the top strand ranging from -3 to -52 and on the bottom strand from -1 to -49. Inspection of the protected DNA sequence (Fig. 3) indicates that this region contains two copies of the palindromic sequence TGT-N$_2$-TCAA-N$_2$-ACA, one of which flanks the -10 and the other the -35 region of the \textit{bchC} promoter (11, 12, 24). Several sites hypersensitive to DNase I digestion are also observed on the top and bottom strands (Figs. 3 and 4). These hypersensitive strands extend 36 base pairs upstream and 58 base pairs downstream from the region of CrtJ protection.

**Determination of Percentage of Active CrtJ**—The fraction of CrtJ that is active in DNA binding was determined according to the filter binding method of Withereell and Uhlenbeck (25), which involves quantitating the amount of maximal DNA probe bound with a known amount of protein. If the oligomerization state of the protein bound to the DNA probe is known, then it is possible to calculate the percentage of fraction of protein that is active. For our analysis, the CrtJ protein concentration was held constant at 1.2 pmol (at approximately half saturation) with the $^{32}$P-labeled \textit{bchC} DNA probe varied in the vicinity of the protein (CrtJ) concentration. As shown in the DNA titration curve in Fig. 5, the amount of DNA bound by the filter rises to a saturation point of 0.11 pmol of DNA (note that this value has been corrected for the efficiency of probe retention by the filter as described under “Materials and Methods”). Assuming that the DNA probe maximally binds to the filter when both palindromes are fully occupied and that two CrtJ bind per palindrome for a total of four CrtJ/probe, then at saturating levels of input DNA bound (0.11 pmol) there must be 0.44 pmol of protein bound by the filter. Thus, the fraction of
protein that is active corresponds to 0.44 pmol of protein retained by the filter/1.2 pmol of input protein in the assay, which corresponds to an active protein fraction of 36.7%.

**EC$\text{_{50}}$ Values and Hill Coefficient Determination of CrtJ Binding to the Upstream and Downstream Palindromes of the bchC Promoter Region**—Binding isotherms for interactions of CrtJ to each of the individual palindromes were determined using a DNase I footprint titration assay according to the method of Brenowitz et al. (24). This method involves quantitating, by PhosphorImager analysis, CrtJ-mediated protection of a DNase I digestion site in each of the palindromes using small increments of CrtJ and correcting for background. The optical density ratios are then converted to fractional protection values. However, because even at saturating levels of protein, the DNA is not completely protected from DNase I digestion, the fractional protection values have to be converted to fractional saturation values. The fractional saturation values are then plotted against the logarithm of the protein concentration (Fig. 6). As shown in Fig. 6, CrtJ protection of the upstream (−21 to −52) and downstream (−1 to −20) palindromes exhibits sigmoidal curves of protection with an average Hill coefficient of 2.5 for each binding site. Since the Hill coefficient is greater than 2, it indicates that there are cooperative interactions among three or more protein subunits that are binding to the two palindromic binding sites. This further supports the hypothesis that CrtJ binds in a multimeric form, possibly as a dimer to each palindromic site and that tetramerization of these dimers induces a cooperative interaction (see “Discussion”). From the fractional saturation values, we can also determine an EC$\text{_{50}}$ value for binding of CrtJ to the upstream and downstream palindromes. The values obtained, after correcting for 37% active protein, are values of 2.9 × 10$^{-9}$ M for binding to the upstream palindrome and 4.8 × 10$^{-9}$ M for binding to the downstream palindrome.

**CrtJ Binding to the bchC Promoter Region Is Redox-sensitive**—Since previous genetic analysis indicated that CrtJ most likely functions as an aerobic repressor (11), we next addressed whether binding of CrtJ to the bchC promoter was affected by alterations in the redox state of the binding buffer. For this analysis, we first preincubated CrtJ for 30 min in the presence of a binding buffer that contained soluble redox mediators (to facilitate the transport of electrons from the oxidizing/reducing agent to the protein center) (22) as well as various oxidizing/reducing agents. After preincubation, a $^{32}$P-end-labeled bchC DNA probe was added to the samples, incubated for an additional 20 min, and then subjected to gel electrophoresis. As shown in the gel shift assays in Fig. 7, preincubation of CrtJ in the presence of a binding buffer that was saturated with oxygen (by bubbling molecular oxygen into the binding buffer) or 20 mM potassium ferricyanide, which have redox values of approximately +800 and +450 mV, respectively, promoted excellent binding conditions. In contrast, preincubation of CrtJ under conditions where oxygen was replaced with the redox neutral gas argon or with 10 mM of the strong reducing reagent sodium dithionite (approximately −600 mV) resulted in a significant reduction of binding activity.

Three separate reactions were performed to determine if the observed redox response was reversible. In the first reaction, CrtJ was incubated separately with either 10 mM sodium dithionite or 20 mM potassium ferricyanide for 1 h. In a second reaction, CrtJ was first incubated for half an hour with 10 mM sodium dithionite, an excess of potassium ferricyanide (20 mM) was added, and then the reaction was incubated for a further 30 min. As shown in Fig. 8, the inhibitory effect of reducing conditions is indeed reversible, since the addition of the excess potassium ferricyanide to the CrtJ sample that had been inhibited with dithionite promoted the formation of a stable DNA-protein complex in the gel mobility shift assay (lanes 8 and 9). Thus, CrtJ appears to be capable of sensing environmental redox values and adjusting binding accordingly.

We next performed gel mobility shift assays with small increments (0.05–0.10 µg) of CrtJ to generate binding isotherms for the determination of EC$\text{_{50}}$ values (effective concentration of CrtJ for 50% response) of CrtJ binding to the bchC promoter.

![Fig. 5. Active fraction of CrtJ as estimated by a DNA excess binding curve.](image)

![Fig. 6. Site binding isotherms generated from DNase I protection patterns.](image)
from the 0, 0.50, and 1.0 m ferricyanide.
4–6 with argon, Lanes 1–3 phoresis. Lanes 1–3 are of CrtJ treated with sodium dithionite, lanes 4–6 with argon, lanes 7–9 with oxygen, and lanes 10–12 with potassium ferricyanide.

Purified CrtJ (0, 0.25, 0.50 M under oxidizing conditions (oxygen) and 2.7 M for reducing conditions (sodium dithionite). Intermediate EC 50 values were obtained for less severe oxidizing/reducing conditions. Cooperative binding of CrtJ to the two palindromic sites of the \textit{bchC} promoter region shows an area of protection extending approximately 52 base pairs from the promoter region shows an identical shift with that of the crude lysate of wild type \textit{R. capsulatus} cells and by the results of our DNase I footprint titration assays. The protection pattern of CrtJ binding to both the top and bottom strands of the \textit{bchC} promoter region shows an area of protection extending approximately 52 base pairs from –1 to –51. This region contains two conserved palindromic sequences TGT-N12-ACA that are centered around the \sigma^{70}-like promoter sequences present at the –35 and –10 regions of the \textit{bchC} operon (11, 12, 26). Recognition of this palindrome by CrtJ is also supported by prior mutational analysis, which indicated that they were involved in the binding of an aerobic repressor (12).

The EC_{50} values of CrtJ binding to the \textit{bchC} promoter region under oxidizing conditions as calculated by gel mobility shift analysis

| Redox reagent            | EC_{50} | Corrected EC_{50} value |
|--------------------------|---------|-------------------------|
| Oxygen                   | 0.065   | 6.0 \times 10^{-9}      |
| Potassium ferricyanide   | 0.092   | 8.4 \times 10^{-9}      |
| Argon                    | 0.21    | 1.9 \times 10^{-8}      |
| Sodium dithionite        | 0.29    | 2.7 \times 10^{-8}      |

is active as evidenced from gel mobility shift assays where purified CrtJ shows an identical shift with that of the crude lysate of wild type \textit{R. capsulatus} cells and by the results of our DNase I footprint titration assays. The protection pattern of CrtJ binding to both the top and bottom strands of the \textit{bchC} promoter region shows an area of protection extending approximately 52 base pairs from –1 to –51. This region contains two conserved palindromic sequences TGT-N12-ACA that are centered around the \sigma^{70}-like promoter sequences present at the –35 and –10 regions of the \textit{bchC} operon (11, 12, 26). Recognition of this palindrome by CrtJ is also supported by prior mutational analysis, which indicated that they were involved in the binding of an aerobic repressor (12). Evidence at hand indicates that cooperative interactions occur between CrtJ bound at the two palindromes. Cooperativity is indicated by the formation of sigmoidal curves and by the Hill coefficient obtained for CrtJ binding to the two palindromic sites, which indicates that this interaction involves more than 2.5 CrtJ polypeptides. We also have unpublished data that indicates that CrtJ binds very poorly to DNA segments that contain only one of the two identified \textit{bchC} palindromes.2 Given well characterized interactions observed among other prokaryotic transcription factors such as the \textit{lac}I repressor, the nitrogen regulator NtrC, and the \textit{lacI} repressor in \textit{E. coli} (27–29), the most likely scenario is that CrtJ binds as a dimer to each of the palindromes and that the dimers cooperatively interact to form stable tetramers.

The EC_{50} values of CrtJ binding to the \textit{bchC} promoter region under oxidizing conditions is 6.0 \times 10^{-9} m, as based on gel mobility shift results, which is the same order of magnitude as that of the \textit{lacI} repressor binding to the right operator site, O_{R1} (under "physiological conditions", cl repressor has a K_{D} of 3 \times 10^{-9} m for O_{R1} (13, 30, 31)). This value is, however, several

\textsuperscript{2}S. Ponnampalam and C. Bauer, unpublished results.
orders of magnitude lower than that observed for other repressors, such as the lac repressor, which has a binding affinity of $10^{-12}$ M (32). The modest affinity of CrtJ binding to the bchC promoter may reflect the fact that CrtJ-regulated promoters are only moderately repressed (1.5–2-fold) by CrtJ (11, 12), which is contrasted by the 1,000-fold (32) repression exhibited by the lac repressor.

Since previous genetic analysis indicated that CrtJ functions as an aerobic repressor (8–12), it was perhaps not surprising to find that CrtJ binds to the bchC DNA fragment better under oxidizing versus reducing conditions. The calculated EC$_{50}$ value of CrtJ binding to the bchC promoter region under oxidizing conditions is 4.5-fold higher than that observed under reducing conditions, suggesting that CrtJ may have redox sensing capabilities. The effect of altering the redox state on CrtJ binding is reversible, suggesting that reducing conditions are not nonspecifically affecting DNA binding activity. Furthermore, CrtJ binding is best under highly oxidizing conditions (such as oxygen-saturated binding buffer), which is a condition that inhibits binding of other redox-responding DNA binding proteins such as FNR, DtxR, SoxR, and Fur. These latter redox-responding proteins have oxygen-labile iron or iron-sulfur clusters that are required for optimal DNA binding capabilities (32–40). Although the redox-sensing component within CrtJ is yet to be elucidated, we feel that it is unlikely to have a redox-responding iron center. Inspection of the CrtJ sequence indicates no obvious Cys-rich iron binding motifs such as that found in iron binding transcription factors. Atomic absorption spectroscopy and EPR of isolated CrtJ also shows no significant levels of iron in the isolated protein preparations. A recent study by Gemelosy and Kaplan (41) indicates that in Rhodobacter sphaeroides a second protein (AppA) may also be involved in controlling redox-sensitive binding by CrtJ. Although no experimental evidence has been obtained for a function for AppA in controlling CrtJ activity, it is possible that AppA may be sensing redox and somehow transmitting this information to CrtJ. A homolog for AppA in R. capsulatus has not yet been described, so at this time we are unable to biochemically ascertain whether AppA affects the in vitro redox response of CrtJ DNA binding.

Finally, there are several studies that indicate that CrtJ may be a global repressor of pigment biosynthesis genes and that its presence is conserved in diverse species of anoxygenic photosynthetic bacteria. For example, sequence and genetic analysis of the R. capsulatus photosynthesis gene cluster indicates the presence of similar palindromic motifs within putative promoter sequences for bacteriochlorophyll, carotenoid, and light harvesting-II structural genes (2, 12, 20, 26, 42, 43). Sequence analysis of the puc promoter from such diverse species as R. sphaeroides (44), R. capsulatus (45), and Rhodopseudomonas palustris (46) also shows the presence of the palindrome that is characterized in this study, indicating that CrtJ may be conserved among a diverse group of anoxygenic photosynthetic bacteria. Thus, any insights into the mechanism of redox responsiveness of CrtJ binding to photosystem promoters could also provide insight into how these additional organisms control photosystem development.