Photostimulation of a Sensory Rhodopsin II/HtrII/Tsr Fusion Chimera Activates CheA-Autophosphorylation and CheY-Phosphotransfer in Vitro†

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ABSTRACT: A chimeric fusion protein consisting of *Natronomonas pharaonis* sensory rhodopsin II (SRII), fused by a flexible linker to the two transmembrane helices of its cognate transducer protein, HtrII, followed by the HtrII membrane-proximal cytoplasmic fragment joined to the cytoplasmic domains of the *Escherichia coli* chemotaxis receptor Tsr, was expressed in *E. coli*. Purified fusion chimera protein reconstituted in liposomes binds to *E. coli* CheA kinase in the presence of the coupling protein CheW, and activates CheA autophosphorylation activity. CheA kinase activity is stimulated by photoexcitation of the SRII domain of the fusion protein, as shown by the wavelength-dependence of photostimulated phosphotransfer to the *E. coli* flagellar motor response regulator CheY in the purified in vitro liposomal system. Further confirming the fidelity of the in vitro system, increased and decreased levels of CheA activation in vitro result from overmethylated and undermethylated fusion protein purified from methylesterase and methyltransferase-deficient *E. coli*, respectively. Photoexcitation of the undermethylated fusion protein resulted in a 3-fold increase in phosphotransfer over that of the dark state. The results directly demonstrate the coupling of SRII photoactivated states to histidine kinase activity, previously predicted on the basis of sequence homologies of the haloarchaeal phototaxis system components to those of *E. coli* chemotaxis. The fusion chimera provides the first tool for in vitro measurement of photosignaling activity of SRII−HtrII molecular complexes.

Sensory rhodopsin II (SRII),[1] a repellent phototaxis receptor in the haloarchaeon *Natronomonas pharaonis*, is one of the best characterized members of the large seven-helix retinylidene protein family found in bacteria, archaea, and eukaryotic microorganisms (type 1 rhodopsins, *1*). Atomic resolution structures are available for the receptor (*2*, *3*) and for the receptor bound to a fragment of its cognate transducer HtrII (*4*), and extensive characterization of its spectroscopic properties and photochemical reactions has been accomplished in several laboratories (for reviews, see refs *5*−*7*).

The SRII−HtrII complex is believed to mediate phototaxis responses in *N. pharaonis* cells by light-stimulated activation of autophosphorylation activity of a histidine kinase (CheA)/response regulator (CheY) "two-component" phosphotransfer system (*8*, *9*). In enteric chemotaxis CheA activity is further influenced by other *che* gene products interacting with the methyl-accepting receptor/transducers, in particular, coupling protein CheW, carboxylmethyltransferase CheR, and methylesterase CheB, (*8*, *9*). The putatively similar roles of *che* genes in haloarchaeal phototaxis are based on the presence of sequence-predicted CheA-binding domains on archaeal sensory rhodopsin transducers (*10*−*12*), the similar effects of SRII stimulation on HtrII carboxylmethylation as in enteric chemotaxis receptors (*13*), and the overall homology of sensory rhodopsin transducers to chemotaxis receptors in bacteria, such as Tsr, the *Escherichia coli* receptor for chemotaxis to serine and avoidance of several repellents. Furthermore, *cheA*, *cheW*, *cheR*, *cheB*, *cheY* homologues to the corresponding *E. coli.* chemotaxis genes are present in the *Halobacterium salinarum* genome (*14*) and have been shown by deletion studies to function in phototaxis signaling (*15*). Supporting this view of archaeal sensory rhodopsin function, chimeric fusion proteins containing the SRII protein fused to the N-terminal portion of HtrII fused in turn to cytoplasmic signaling and adaptation domains of *E. coli* chemotaxis receptors were shown to mediate phototaxis responses in *E. coli* cells (*16*).

The only available assays for sensory rhodopsin signaling are in vivo assays of phototaxis responses by motile cells (*17*, *18*). An in vitro measurement system with purified components would be valuable for elucidation of the SRII activation mechanism, relay of the signal to HtrII, and propagation of the signal to the CheA-binding domain. In vitro systems using purified enteric chemotaxis receptors with purified pathway components, namely, the coupling protein

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[1] SRII, sensory rhodopsin II from *Natronomonas pharaonis*; HtrII, transducer protein associated with SRII; CheA, chemotaxis protein A, a histidine kinase; CheW, chemotaxis protein W, a coupling protein; CheY, chemotaxis protein Y, a response regulator; CheR, S-adenosylmethionine-dependent carboxylmethyltransferase; CheB, carboxylmethylesterase; *E. coli*, *Escherichia coli*; *H. salinarum*, *Halobacterium salinarum*; Tsr, *E. coli* receptor for chemotaxis to serine; UM, undermethylated protein; WT, wild type; ME, overmethylated protein; TM1 and TM2, first (N-terminal) and second HtrII transmembrane helices, respectively.

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CheW, CheA, and CheY, have been developed (*19*). Accordingly, we purified an SRII−HtrII−Tsr fusion chimera, reconstituted it in liposomes, and tested its photoactivity with purified *E. coli* post-transducer components. We found that light and carboxylmethylation of Tsr in the fusion chimera regulate kinase activity in vitro. The basal extent of phosphorylation of the undermethylated fusion chimera is enhanced 3-fold by photostimulation of its SRII domain. The results confirm that the SRII−HtrII complex photomodulates histidine kinase activity and provides an approach that may be used as an in vitro assay for SRII−HtrII signaling function.

MATERIALS AND METHODS

Strains and Plasmids. E. coli strain RP3098 (Δ (flhA-flhD), a K-12 derivative and c*he* genes deletion mutant) used for expression of *che* gene products (*20*) was provided by Dr. J. S. Parkinson (University of Utah, Salt Lake City). *E. coli* strains, RP4968 (*cheR* deletion) and RP4972 (*cheB* deletion) were used to express the undermethylated and overmethylated fusion proteins, respectively. We assume that the *cheR* deletion strain-expressed fusion chimera is undermethylated and *cheB* deletion strain-expressed fusion chimera is overmethylated with respect to wild type, based on the known enzymatic activities of the CheR and CheB proteins. An outer membrane protease-deficient *E. coli* strain, UT5600, was used for expression of fusion protein with wild-type steady-state methylation, as described (*16*). Plasmids for expression of CheA (pKJ9), CheY (pAR::CheY), and CheW (pJL63) were provided by Dr. J. S. Parkinson.

Overexpression and Purification of Che Proteins. CheA and CheY were purified by dye-ligand chromatography and gel filtration following the procedure of Hess et al. (*21*) with minor modifications. CheW was purified by the method of Stock et al. (*22*). In brief, the total soluble protein fraction in TEDG-10 was precipitated by ammonium sulfate (30−50%), dialyzed in 50 mM MES buffer, pH 6.0 and subjected to ion exchange chromatography on a DE-52 column. Protein concentration was established either by the BIORAD protein estimation kit using bovine serum albumin (or gamma globulin) and/or by extinction coefficients of 8.25 mM^{-1} cm^{-1} for CheY, 16.3 mM^{-1} cm^{-1} for CheA, and 5.12 mM^{-1} cm^{-1} for CheW at 280 nm (*23*).

Purification and Reconstitution of Tsr-Fusion Protein. NpSRII−HtrII−Tsr fusion chimeras with a 9-histidine tag at the carboxyl terminus were expressed in *E. coli* strain UT5600 as described (*16*). Chimera construction details are in ref *16*. Recombinant PCR was used to introduce factor Xa cleavage site (IEGR) adjacent to the 9-histidine tag. The 1.5% octyl glucoside-solubilized membrane fraction was used for single step Ni^{2+}-NTA chromatography for purification. Protein concentration was measured by the BIORAD protein estimation kit. Photochemical activity and expression level were confirmed by absorption spectroscopy and flash photolysis of membranes as described (*16*) and similarly in purified samples. The 9-histidine tag was removed by application of the factor Xa cleavage, capture kit (Novagen, Madison, WI). A balanced steady-state methylation (WT) was obtained with protein expressed in strain UT5600. An undermethylated protein (UM) was expressed and purified from the CheR-deficient strain, and an overmethylated form (ME) was obtained by using the CheB-deficient strain.

Purified fusion proteins were reconstituted into *E. coli* polar lipids (Avanti Polar Lipids, Alabaster, AL). A 300 μL stock lipid suspension (10 mg/mL) in TKMD buffer (50 mM Tris-HCl, 50 mM KCl, 10 mM $MgCl_2$, 0.5 mM DTT, pH 7.5) with 1% octyl glucoside was prepared and mixed with 200 μL of the purified protein (in TKMD buffer with 1% octyl glucoside). The suspension was subjected to low pulse sonication and to dialysis for 96 h to remove detergent. Control liposomes were prepared in the same manner without fusion protein addition. Liposome and proteoliposome suspensions were prepared fresh prior to each experiment.

Autophosphorylation of Histidine Kinase, CheA. Autophosphorylation reactions of CheA were carried out in 30 μL of TKMD buffer. Reactants were mixed and incubated 5 min at 25 °C prior to addition of 0.1 mM [γ-^{32}P]ATP (specific activity ∼8000 cpm/pmol and subsequently quenched by 30 μL 2× LSB (Laemmli sample buffer, 62.5 mM Tris-Cl, 6.8, 4% sodium dodecyl sulfate, 20% glycerol, 10% 2-mercaptoethanol, and 0.1% bromophenol blue) containing 50 mM EDTA.

Phosphotransfer to Response Regulator, CheY. Phosphotransfer to CheY was performed in 30 μL of TKMD buffer in the presence of 10-fold excess of CheY over CheA. Reactants were mixed and incubated for 5 min at 25 °C, and phosphorylation initiated by addition of 0.2 mM [γ-^{32}P]-ATP (specific activity ∼40 000 cpm/pmol) and subsequently quenched by 30 μL of 2× LSB (Laemmli sample buffer containing 50 mM EDTA).

Photoactivation of Phosphotransfer Reactions. Photostimuli were delivered by a tungsten−halogen source (100 W) focused on the incubation chamber after passing the beam through band-pass interference filters (Corion Corp. Holliston, MA), two heat-absorbing filters, and 2.4 cm of 5% copper sulfate solution to block heating effects. Interference filters transmitting 500 ± 5 nm, 600 ± 5 nm, or 450 ± 20 nm were used for spectral selection and neutral density filters were used to reduce illumination intensity. Illumination fluence rates were measured at the chamber surface with a Kettering Radiant Power meter (Scientific Instruments Inc, FL).

Phosphorylated protein samples separated by SDS−PAGE were directly quantified using an instant phosphorimager (Packard Instant Imager, Meriden, CT).

RESULTS

Expression, Purification, and Reconstitution of the SRII−HtrII-Tsr Fusion Proteins. The purified octyl glucoside-solubilized fusion protein (Figure 1A) was obtained with typical yields of ∼1 mg from a 1-L *E. coli* culture and its absorption spectrum was stable for weeks when stored at 4 °C in TKMD buffer, pH 7.5 containing 1% octyl glucoside without noticeable degradation of the protein (Figure 1B). Immunoblot analysis with anti-histag antibody also showed a single band indicating no degradation (data not shown). The isolated CheA, CheW, and CheY proteins were also electrophoretically pure (Figure 1B). The observed molecular masses for each component, namely, fusion protein (70 kDa), CheA (75 kDa), CheW (18 kDa), and CheY (12 kDa), were consistent with their expected molecular weights.

The lipid-reconstituted fusion proteins formed a ternary complex with CheW and CheA, as shown by the decrease

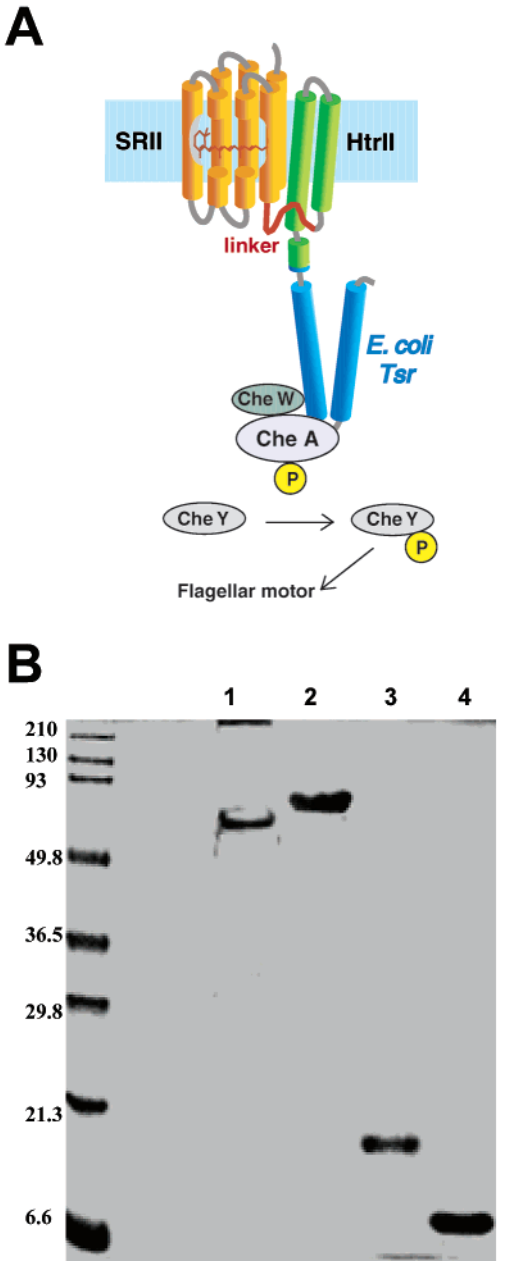

FIGURE 1: (A) Components of the photosignaling system. Sensory rhodopsin II (SRII) (orange) from *N. pharaonis*, fused by a flexible linker to the N-terminal portion of its cognate transducer protein, HtrII (green), fused in its cytoplasmic region to the homologous region of the *E. coli* chemotaxis receptor Tsr (blue). The module is shown complexed with the coupling protein CheW and autophosphorylating histidine kinase, CheA, which transfers a phosphate group to the flagellar motor switch regulator CheY. For simplicity, all proteins are depicted as monomers, but the fused photosignaling complex is known to be dimeric. (B) SDS−PAGE profile of the purified components. Coomassie-stained electrophoretic profile of the fusion protein (lane 1), CheA (lane 2), CheW (lane 3), and CheY (lane 4) molecules on a 15% SDS−polyacrylamide gel (~6 μg of each protein). Molecular weight markers (in kDa) are in the leftmost lane.

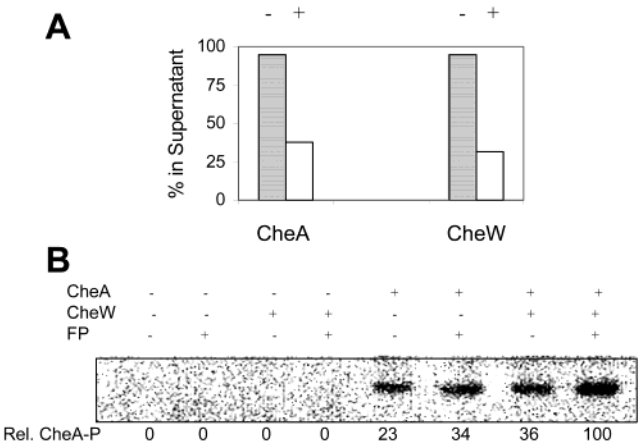

FIGURE 2: Formation of autophosphorylating ternary complex from Tsr fusion protein, CheW and CheA. (A) 2 μM CheA and 5 μM CheW were incubated with liposomes with and without 5 μM fusion protein for 30 min at 25 °C. The contents were centrifuged at 12640*g* and the supernatant was assayed for CheA and CheW proteins on SDS−PAGE and quantified by densitometry. 55 and 60% of CheA and CheW, respectively, bound to the fusion-protein-containing liposomes. (B) Proteins were mixed on ice in the various combinations. The final concentrations of each component were 2 μM Tsr fusion protein, 0.4 μM CheA, and 1 μM CheW. Liposomes were prepared with or without Tsr-fusion protein. The autophosphorylation reaction was initiated by addition of [γ-^{32}P]ATP, and the reaction was quenched after 2 min and analyzed for CheA-P by SDS−PAGE autoradiography. The extent of CheA phosphorylation (CheA-P) in the absence and presence of various component is shown relative to the maximal case.

in soluble CheA and CheW amounts (Figure 2A) when incubated with liposomes containing the fusion protein. From the decreased amounts, we calculate a molar ratio of fusion protein/CheW/CheA of 5:3:1, similar to the ratio of 6:4:1 for Tsr/CheW/CheA recently reported (*24*). Recent evidence suggests that chemotaxis receptors (e.g., Tsr and Tar) assemble in *E. coli* membranes as trimers of dimeric units (*25*, *26*), and an analysis of kinase activity at various subunit stoichiometries in Tsr and Tar signaling complexes indicates that ~6:4:1 is optimal for kinase activity (*24*).

The activity of the ternary complex was established by the stimulation of CheA autophosphorylation. In the autophosphorylation reaction, the γ-phosphoryl group of ATP is transferred to His-48 in CheA (*27*). Chemotaxis receptors such as Tar stimulate CheA autophosphorylation in a CheW-dependent manner (*19*, *28*, *29*). Liposomes containing the SRII−HtrII−Tsr fusion protein increased CheA autophosphorylation only in the presence of CheW (Figure 2B), indicating that the fusion protein forms an active ternary complex with CheA and CheW. The kinase activity was enhanced about 3-fold in the presence of reconstituted fusion protein. The presence or absence of the 9-histidine tag in the fusion protein, removed by factor Xa protease, did not alter the effect on kinase activation in the ternary complex (data not shown). Accordingly the histidine-tag was not removed for measurements presented here.

SRII Photostimuli Activate CheA Phosphorylation and Phosphotransfer to CheY. Blue-green light activation of the SRII−HtrII−Tsr fusion protein in *E. coli* cells results in a repellent taxis motility response (*16*). In *E. coli* chemotaxis, repellent responses result from activation of CheA kinase activity by the receptors. The subsequent increase in phospho-CheY (CheY-P) concentration induces tumbling in *E. coli* cells, the repellent motility response. Therefore, the appropriate in vitro activity of the fusion protein, assuming correct interactions in the ternary complex, would be to mediate light-induced increases in CheA phosphorylation and CheY-P concentration. We observed such increases (Figure 3). We observed no light-induced change in phosphorylation extent in CheA or CheY in liposome suspension without the

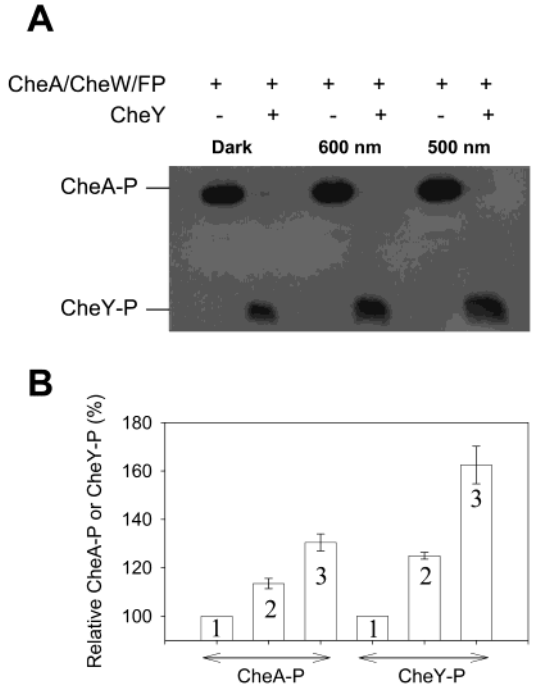

FIGURE 3: Effect of photostimuli on autophosphorylation activity in ternary complex and phosphotransfer to CheY. (A) Proteins were mixed with and without CheY as in Figure 2. The concentration of the proteins were fusion protein 5 μM, CheA 1 μM, CheW 2 μM, and CheY 10 μM. The reaction was initiated by addition of [γ-^{32}P]-ATP, and the reaction was quenched after 30 s and separated by 16.5% SDS−PAGE, followed by autoradiography. CheA-P and CheY-P levels are shown for dark and photostimulated samples at 600 and 500 nm. (B) Autoradiograph counts for bands relative to a dark sample (average of two independent data sets) for the 600- and 500-nm photostimulated samples, respectively. Bar 1 represents the average values in dark state. Bar 2 and 3 represent the values with 600 and 500 nm photostimulus, respectively.

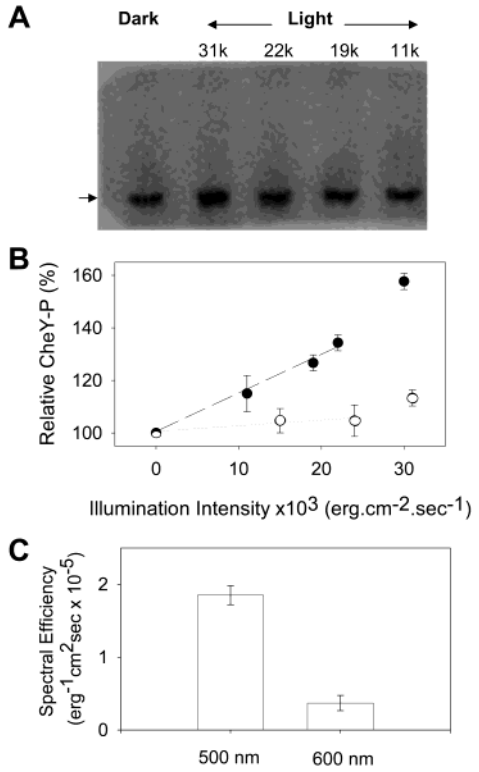

FIGURE 4: Fluence/response relationship for phosphotransfer to CheY. Phosphorylation reactions were performed as in Figure 3. (A) Effect of varying photostimulus fluence (shown in k as 10^3 erg cm^{-2} s^{-1}) on the CheY-P (position at arrow) production relative to dark state. (B) Relative CheY-P, assessed by autoradiography, dependence on illumination intensity at 500 nm (●) and 600 nm (○) Three independent measurements were used to compute the mean ± SD shown as error bars. (C) Spectral efficiency of 500 and 600 nm photostimuli. The slopes of linear fits to the data shown in Figure 4B at each wavelength were used as a measure of efficiency.

fusion protein (data not shown). The extent of increase of CheY-P from 500-nm light exceeds that of phospho-CheA (CheA-P) (Figure 3B), as expected from the amplification due to cycling of multiple CheA phosphotransfers to CheY molecules.

The fluence response curves for 500- and 600-nm stimuli show the former is ~6 times more effective in mediating the increase in CheY-P (Figure 4). A wavelength-dependence at constant (low) light fluence shows a peak in action at 500 nm in agreement with the absorption spectrum of the fusion protein (Figure 5). The small effect of 600-nm light, where the absorption by the fusion protein is negligible, may be attributable to a thermal effect on the system. No increase in temperature of the sample measured by a thermocouple was detected (sensitivity 0.1 °C), but an internal thermal increase in molecular motion may still occur.

Methylation and Demethylation of the Fusion Protein Enhance and Reduce, Respectively, CheA Kinase Activity. Methylation of the Tsr domain on fusion protein at specific glutamate residues is catalyzed by an *S*-adenosylmethionine-dependent carboxylmethyltransferase, CheR. Hydrolysis of the carboxylmethylester with subsequent production of methanol is catalyzed by the CheB protein, a methylesterase. Methylation of the enteric aspartate chemotaxis receptor in liposomes has been shown to stimulate CheA kinase activity in vitro (*19*). The effect of fusion protein methylation on kinase activity was measured by using overmethylated and

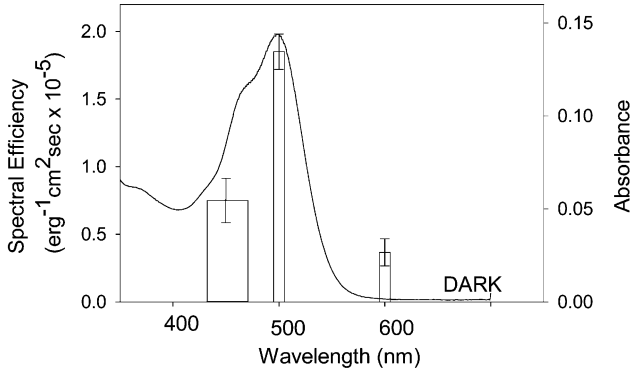

FIGURE 5: Extent of phosphotransfer to CheY at different wavelength of photostimuli. Experimental conditions were as in Figure 4 except the light intensity in each case was 12−13 K erg cm^{-2} s^{-1}. The reactions were quenched after 45 s. Narrow band (10-nm band-pass) filters were used for illumination at 500 and 600 nm, and a broad band filter (40-nm band-pass) was used at 450 nm to match the intensities of delivered stimuli. The values are relative to unphotostimulated values. Two independent measurements were used to compute the mean ± SD shown as error bars. The absorption spectrum of the SRII−HtrII−Tsr fusion chimera protein was obtained at ~3 μM concentration.

undermethylated fusion protein as compared to wild-type protein with balanced steady-state methylation. The most active methylated fusion protein showed ~5-fold stimulation of basal kinase activity as compared to <2-fold in the case

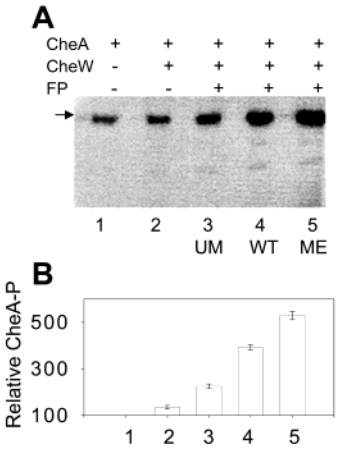

FIGURE 6: Effect of methylation of the Tsr domain of chimeric fusion protein on kinase stimulation. CheA alone (lane1), with CheW (lane2), and CheA and CheW incubated with undermethylated (lane 3), overmethylated (lane 4), and wild-type fusion protein (lane 5). The final concentrations of each component were 2 μM Tsr fusion protein, 0.4 μM CheA, and 1 μM CheW. Liposomes were prepared with or without Tsr-fusion proteins. The autophosphorylation reaction was initiated by addition of [γ-^{32}P]ATP, quenched after 2 min and analyzed for phosphoCheA (CheA-P). CheA-P is positioned by arrow. (B) Extent of relative CheA-P in the presence and absence of various modified fusion proteins was computed from two individual experiment data points and standard error of mean (±SEM) shown as error bars.

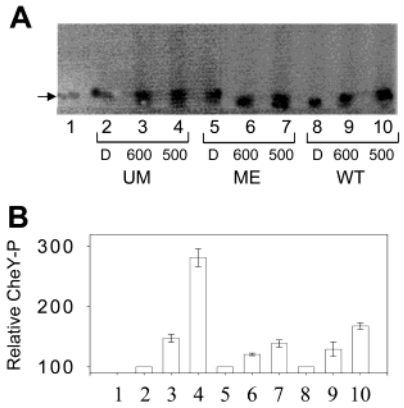

FIGURE 7: Photostimulated phosphotransfer to CheY with different methylated fusion proteins. Proteins were mixed as in Figure 2. The concentration of the proteins were reconstituted fusion protein (UM/ME/WT) 5 μM, CheA 1 μM, CheW 2 μM, and CheY 10 μM. Control liposomes were used in fusion protein free sample (lane 1). The reaction was initiated by addition of [γ-^{32}P]ATP, quenched after 30 s, and separated by 16.5% SDS−PAGE, followed by autoradiography. Arrow indicates the position of phosphoCheY CheY-P). Fusion proteins used for photoinduced phosphotransfer were lanes 2−4 (UM), lanes 5−7 (ME), and lanes 8−10 (WT). In each case D represents the dark state and 600 and 500 indicate the photoilluminated states at 600 and 500 nm. Arrow indicates the position of CheY-P. (B) Relative phosphoCheY (100 for dark state) in individual sets was used to compute the extent of photostimulated phosphoCheY transfer. Values for three independent experiments were used to compute the standard error of mean (± SEM) shown as error bars.

of the undermethylated protein (Figure 6). Basal kinase stimulation by WT fusion protein was intermediate (Figures 2 and 6). The results of increased kinase stimulation with an increase in extent of methylation are consistent with those observed with *E. coli* chemotaxis receptors (*19*, *29*).

Effect of State of Fusion Protein Methylation on Photostimuli-Induced Phosphotransfer. We examined the effect of three states of fusion protein methylation, i.e., undermethylated (UM), overmethylated (ME), and wild type (WT) on photostimuli-induced phosphotransfer (Figure 7). Less basal activation was observed with undermethylated fusion protein compared to the control fusion protein in the dark (lane 2 versus lane 1). Wild-type levels of methylation resulted in moderate basal activation (lane 8), while overmethylation yielded the highest basal activation (lane 5). 500-nm photosimuli further activated the CheY phosphotranser in each case compared to their respective dark state and to a 600 nm photostimulus. The small effect of the 600-nm stimulus is consistent with the low absorption of SRII at this wavelength, but we cannot rule out a small internal thermal effect. The activation of SRII by the 500-nm stimulus gave the largest fold change (3-fold) in phosphotransfer for the least methylated (UM) fusion chimera with the lowest background signal in the dark state.

DISCUSSION

The results above establish that the SRII−HtrII photoactive module generates signals resulting in CheA kinase activation, as predicted by models of the haloarchaeal phototaxis signaling pathway. The activity of the SRII−HtrII−Tsr fusion chimera provides a striking example of the modular function of protein domains in taxis signaling systems (*30*), and provides the first tool for in vitro measurement of photosignaling activity of SRII−HtrII molecular complexes. In vitro assays for protein conformational changes in SRII−HtrII molecular complexes based on EPR of introduced spin-labels (*31*, *32*), cross-linking of site-specifically introduced cysteines (*33*), and fluorescent probes (Yang & Spudich, unpublished data) have begun to clarify their conformationally active regions. An in vitro assay for light-modulation of the kinase such as reported here provides a useful tool for these approaches which entail modifying the complex with bulky substituents which may affect signaling complex activity.

The SRII−HtrII−Tsr fusion protein stimulates CheA kinase activity ∼2−5-fold depending on the extent of methylation of the Tsr domain (Figure 6). The basal stimulation of CheY-P production in the prestimulus (dark) state due to the fusion protein varied from ∼3- to 7-fold. As expected in the presence of excess CheY from cycling of the phosphotransfer from CheA to CheY, these values are higher than those of the basal kinase activity stimulation by fusion addition in the dark. This value is lower than that reported for purified Tar, which stimulated the CheY-P production by 10-fold in the presence of CheA/CheW in the prestimulus state (*19*). The lesser basal activation by the fusion protein may be due to differing preparation or reaction conditions or to conformational differences between the fusion-Tsr construct and Tar.

The maximum activation of phosphotransfer by photoactivation of SRII that we observe is a 3-fold change over prestimulus values. The effect is small but reproducible, and is comparable to that observed for in vitro CheA kinase modulation by aspartate-binding to the aspartate chemotaxis receptor, Tar, in a completely purified liposomal system (a 4-fold decrease in kinase activity, *19*). Greater changes in stimulus-induced modulation of phosphotransfer have been observed when *E. coli* membranes are used instead of

proteoliposomes (*28*, *29*, *34*, *35*). The reason for the greater effectiveness of natural membranes is not clear. One possibility is that *E. coli* membranes have been indicated to contain aggregate arrays of the receptors which may provide additional amplification of the phosphorylation cascade (*26*). The purified in vitro systems for the Tar chemotaxis receptor (*19*) and the light-activated fusion chimera, as reported here, provide tools for investigating the unknown amplication factors in the natural membrane not yet reproduced in vitro.

Signaling Mechanism. The homology of Htr transducers to chemotaxis receptors (*10*−*12*) and the requirement for *che* genes in *H. salinarum* phototaxis (*15*, *36*) supported a similar pathway in both haloarchaeal phototaxis and bacterial chemotaxis. Photoactivation of SRII produces long-lived photointermediates implicated as signaling states (*37*). The atomic structure of the dark form of SRII is known from X-ray crystallography (*2*, *3*) and a recent structure of SRII cocrystallized with a membrane-bound fragment of its HtrII transducer reveals a tight packing of receptor F and G helices and transducer TM1 and TM2 helices within the membrane (*4*). Electron paramagnetic resonance (EPR) spectroscopy of spin-labeled SRII (*31*, *32*) and effects of HtrII on SRII photoactive site accessibility to protons (*38*−*40*) strongly support that sensory rhodopsin activation entails an outward tilting of helix F similar to that occurring in the proton pumping cycle of bacteriorhodopsin (*41*). The current view is that the movement of receptor helix F results in a displacement of transducer helix TM2, although the nature of this displacement is not yet clear (*4*, *42*). The motion of TM2 is likely to be the common determinant of CheA kinase activity modulation in haloarchaeal phototaxis receptors and bacterial chemotaxis receptors. Further support for this common step is that chimeric fusion proteins containing the SRII receptor fused to the N-terminal portion of HtrII, in which post-TM2 C-terminal domains are replaced with cytoplasmic signaling and adaptation domains of *E. coli* chemotaxis receptors, mediate phototaxis responses in *E. coli* cells (*16*).

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