JOURNAL OF BACTERIOLOGY, Sept. 1988, p. 4280–4285
0021-9193/88/094280-06$02.00/0

Vol. 170, No. 9

Methyl-Accepting Protein Associated with Bacterial Sensory Rhodopsin I

ELENA N. SPUDICH,[1] C. A. HASSELBACHER,[1] AND JOHN L. SPUDICH[1,2]*

Department of Structural Biology,[1] and Department of Physiology and Biophysics,[2] Albert Einstein College of Medicine, Bronx, New York 10461

Received 8 April 1988/Accepted 14 June 1988

In vivo radiolabeling of *Halobacterium halobium* phototaxis mutants and revertants with L-[*methyl*-^{3}H] methionine implicated seven methyl-accepting protein bands with apparent molecular masses from 65 to 150 kilodaltons (kDa) in adaptation of the organism to chemo and photo stimuli, and one of these (94 kDa) was specifically implicated in phototaxis. The lability of the radiolabeled bands to mild base treatment indicated that the methyl linkages are carboxylmethylesters, as is the case in the eubacterial chemotaxis receptor-transducers. The 94-kDa protein was present in increased amounts in an overproducer of the apoprotein of sensory rhodopsin I, one of two retinal-containing phototaxis receptors in *H. halobium*. It was absent in a strain that contained sensory rhodopsin II and that lacked sensory rhodopsin I and was also absent in a mutant that lacked both photoreceptors. Based on the role of methyl-accepting proteins in chemotaxis in other bacteria, we suggest that the 94-kDa protein is the signal transducer for sensory rhodopsin I. By [^{3}H]retinal labeling studies, we previously identified a 25-kDa retinal-binding polypeptide that was derived from photochemically reactive sensory rhodopsin I. When *H. halobium* membranes containing sensory rhodopsin I were treated by a procedure that stably reduced [^{3}H]retinal onto the 25-kDa apoprotein, a 94-kDa protein was also found to be radiolabeled. Protease digestion confirmed that the 94-kDa retinal-labeled protein was the same as the methyl-accepting protein that was suggested above to be the signal transducer for sensory rhodopsin I. Possible models are that the 25- and 94-kDa proteins are tightly interacting components of the photosensory signaling machinery or that both are forms of sensory rhodopsin I.

Spectroscopic analysis of *Halobacterium halobium* membranes has revealed two photosensory receptors which use retinal as their chromophores: sensory rhodopsins I and II (SR-I and SR-II, respectively) (for a review, see reference 28). Both pigments undergo distinct photochemical reaction cycles which can be monitored by flash-induced absorbance changes in cell membranes. SR-I (λ_{max}, 587 nm) is an attractant light receptor, and its long-lived photointermediate, S_{373} (λ_{max}, 373 nm), is a receptor for repellent light (3, 27, 33). SR-II (λ_{max}, 480 nm), also called phoborhodopsin (35), is an additional repellent light receptor (12, 25, 31, 34–36). [^{3}H]retinal labeling has been used to visualize receptor proteins on sodium dodecyl sulfate (SDS)-polyacrylamide gels, and radiolabeled bands with molecular masses of 25 and 23 kilodaltons (kDa) have been quantitatively correlated in membranes of various mutants with photocycling of SR-I and SR-II, respectively (25).

Photoexcitation of SR-I and SR-II modulates swimming behavior of cells by controlling the frequency of directional changes by swimming cells (28). The cells adapt to continuous illumination, and similarly, they respond and adapt to chemical stimuli (5, 13, 19, 29). This adaptation is essential for the cells to sense gradients, i.e., to respond to changes rather than to absolute levels of light intensity and chemical effectors (11). In chemotactic eubacteria (e.g., *Escherichia coli*), one mechanism of adaptation is by carboxylmethylation of transmembrane chemoreceptors (6, 9, 11, 20, 21).

The effects of methionine starvation and methylation inhibitors implicated a methylation system in *H. halobium* taxis as well, and protein methylation has been detected in *H. halobium* membranes (2, 17, 18; J. L. Spudich and W. Stoeckenius, Abstr. Fed. Proc. Fed. Am. Soc. Exp. Biol., 1980, abstr. 39, p. 1972). To understand the involvement of this system in *H. halobium* taxis, in vivo methyl-radiolabeling methods developed to visualize the *E. coli* chemotaxis methyl-accepting proteins have been applied. These procedures reveal altered patterns of methylation among *H. halobium* taxis mutants (M. Alam, S. Sundberg, J. Spudich, D. Oesterhelt, and G. Hazelbauer, manuscript in preparation). By analyzing protein methylation patterns and [^{3}H]retinal binding to proteins in *H. halobium* mutant membranes, we identified a 94-kDa methyl-accepting protein that was associated with SR-I.

MATERIALS AND METHODS

Strains and culture conditions. The mutant phenotype abbreviations used in this report are as follows: SR-I$^+$ and SR-II$^+$, contains the apoproteins for SR-I and SR-II, respectively; Ret$^+$, synthesizes retinal; Che$^+$, forms chemotactic rings on soft agar plates (29); Pho$^+$, exhibits phototactic responses to temporal changes in visible light (29); BR$^-$ HR$^-$, deficient in bacteriorhodopsin and halorhodopsin, respectively (23).

All strains used in this study were BR$^-$ HR$^-$ (ion flux mutants) derived from *H. halobium* OD2 (23). Strains Pho81 and Pho72 were isolated from Flx15 by selection for the loss of phototaxis (32). Strain Pho72 is also deficient in chemotaxis (Che$^-$), while Pho81 is Che$^+$. Strains Pho72C1 and Pho72C2 were independently selected from Pho72 for their reversion to Che$^+$ on soft agar plates, and both regained the Pho$^+$ phenotype. Flx5R is blocked in retinal synthesis and was obtained from Flx5 by screening for white colonies, which are often Ret$^-$. Flx5R membranes contain retinal-regenerable SR-I apoprotein at approximately fourfold the concentration found in Flx3R (26) and Flx6R, which are strains with similar phenotypes that were used in earlier

* Corresponding author.

studies, and little or no SR-II apoprotein has been detected by absorbance assays or flash spectroscopy (4, 25). More sensitive behavioral analysis showed a barely detectable swimming reversal response of retinal-regenerated Flx5R cells to 450-nm light; this was probably attributable to the presence of small amounts of SR-II in this strain. Relative amounts of SR-I and SR-II appear to be sensitive to growth conditions and the time of cell harvest. Therefore, it is important to monitor both pigments by spectroscopic and behavioral assays in the particular experimental preparations. Cells were grown aerobically in peptone medium at 37°C in the dark (24).

In vivo [^{3}H]methyl labeling. A total of 2 ml at 2×10^9 cells per ml in basal salts containing 10 mM MES [2-(N-morpholino)ethanesulfonic acid; pH 6.8] was treated with 20 μl of puromycin (3 mg/ml in ethanol) for 15 min at 37°C with shaking. L-[*methyl*-^{3}H]methionine (20 μl; 13 μM, 80 Ci/mmol in ethanol; Dupont, NEN Research Products, Boston, Mass.) was added, and incubation was continued for 60 min. Incubation with puromycin for 15 min was sufficient to completely inhibit protein synthesis, as monitored by [^{35}S]methionine incorporation (data not shown). The cell suspension (200 μl) was added to 2 ml of acetone at 4°C, and the precipitated protein was processed for SDS-polyacrylamide gel electrophoresis as described previously (24). The final pellet was suspended in 120 μl of the solubilizing sample buffer, of which 50 μl was loaded onto each lane. Membrane vesicles were prepared from the methyl-labeled cell suspension by sonication and centrifugation (10), dialyzed for 2 h against 10 mM Tris hydrochloride (pH 6.8), and solubilized in sample buffer (25 μg of protein in 50 μl loaded per lane; see Fig. 3 through 5).

[^{3}H]retinal labeling of membranes. An ethanolic solution (2 μl) of all-*trans* [^{3}H]retinal (2.2 mM, 400 μCi/μmol) was added to 2 ml of Flx5R membrane vesicles suspended at 5 mg of protein per ml in basal salts. Reduction with $NaCNBH_3$, solubilization for SDS-polyacrylamide gel electrophoresis, and autofluorography were as described previously (22).

SR-I bleaching and regeneration. Membrane vesicle suspensions, at 5 mg of protein per ml in 4 M NaCl, were incubated in the presence of NH_2OH (pH 9.0) at the concentrations indicated in Fig. 6 at 37°C for 1 h while the suspensions were illuminated with orange light (2×10^6 ergs/cm^2 · s; 3-69 filter; Corning Glass Works, Corning, N.Y.). The suspensions were then pelleted by ultracentrifugation at 4°C, suspended in 4 M NaCl–25 mM Tris hydrochloride (pH 6.8), and washed 2 more times with this buffer. All-*trans* [^{3}H]retinal was added as described above. This procedure bleached and regenerated SR-I photocycling activity, which was monitored by flash-induced absorbance changes in vesicles at 5 mg of protein per ml (1-cm path length) with a flash photolysis system described previously (25). SR-I activity was defined in terms of the photocycle characteristics of SR-I determined previously (3, 4).

RESULTS

Phenotypes and methylation patterns of selected behavioral mutants. Strain Flx15 (Table 1) was labeled in vivo with L-[*methyl*-^{3}H]methionine under conditions in which protein synthesis is inhibited by puromycin. Several methylated proteins were observed by autofluorography of whole-cell proteins from Flx15 separated by SDS-polyacrylamide gel electrophoresis (Fig. 1). Mutant Pho72 exhibited a smooth-swimming (nonreversing) phenotype, did not form rings on soft agar plates (Che$^-$), and did not exhibit light-induced reversals (Pho$^-$) (Table 1). All but two of the methyl-labeled bands were missing in Pho72, and the Flx15 pattern returned in two independently isolated revertants with wild-type behavior (Pho72C1 and Pho72C2; Table 1 and Fig. 1). Pho72 is representative of a class of independently isolated smooth-swimming mutants defined by the loss of labeling of these bands (Alam et al., in preparation). Methylation of this set of proteins is therefore implicated in taxis.

TABLE 1. Phenotypes of selected behavioral mutants[a]

Strain	Phenotype	SR-I	SR-II	Mean run length (s)
Flx15	Pho$^+$ Che$^+$	+	+	28 ± 5
Pho72	Pho$^-$ Che$^-$	+	+	>500
Pho72C1	Pho$^+$ Che$^+$	+	+	30 ± 6
Pho72C2	Pho$^+$ Che$^+$	+	+	40 ± 6
Pho81	Pho$^-$ Che$^+$	−	−	27 ± 5

[a] Pho$^+$ and Che$^+$ were determined as described in the text. SR-I and SR-II activities were assessed by flash spectroscopy (25), and mean run length (time between reversals ± standard error of the mean for >50 cells) was determined by tracking at 37°C (13).

Methionine starvation produces smooth-swimming cells, and the normal swimming reversal frequency is restored by the addition of methionine (Spudich and Stoeckenius, Abstr. Fed. Proc. Fed. Am. Soc. Exp. Biol.). This behavior, as well as that of Pho72 and its revertants, is analogous to the behavior of *E. coli*, in which methylation promotes swimming reorientation (tumbling) so that cells are adapted to attractants (6, 9, 11, 20, 21). Undermethylation, such as that which occurs after methionine starvation or in a protein methyltransferase mutant, leads to smooth swimming in *E. coli*.

Pho81 is a photoreceptor mutant that has been isolated from Flx15 and that exhibits normal chemotaxis (32). One of

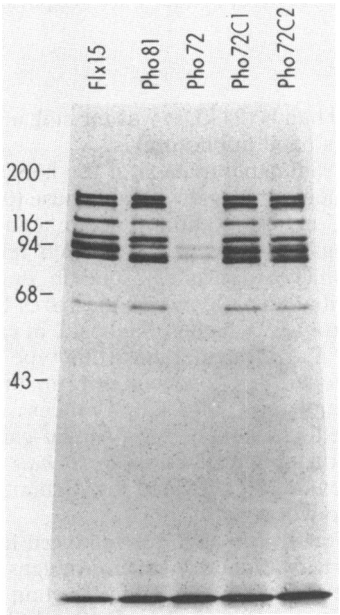

FIG. 1. Autofluorogram of SDS-polyacrylamide gels of whole-cell proteins of the strains listed in Table 1 after in vivo labeling with L-[*methyl*-^{3}H]methionine in the presence of puromycin. The following molecular weight standards (in thousands) are indicated on the left, from highest to lowest: myosin, β-galactosidase, phosphorylase *b*, bovine serum albumin, and ovalbumin.

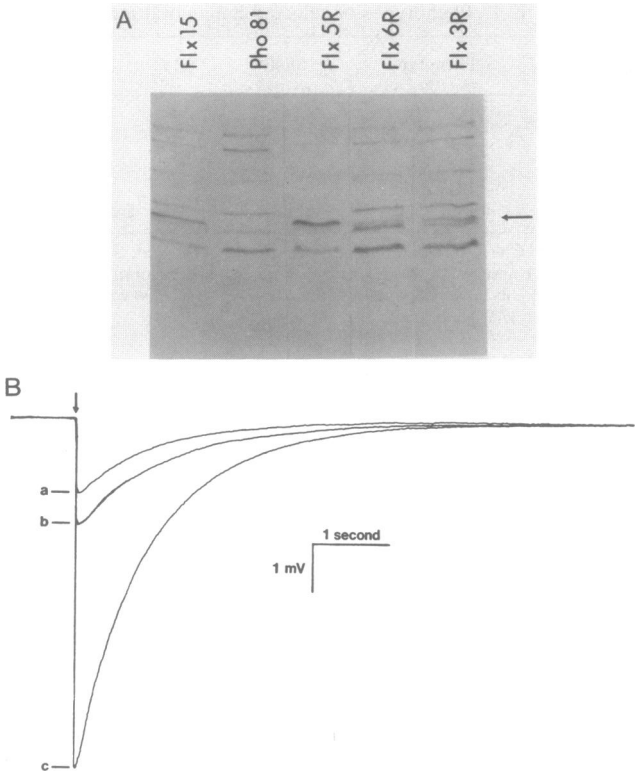

FIG. 2. (A) Autofluorogram of methylated whole-cell proteins as described in the legend to Fig. 1. A lowered concentration of bisacrylamide in the separation gel was used (see text). The arrow marks the position in this gel system of the band (94 kDa in Fig. 1) that is present in Flx15 and that is missing in Pho81. Note that overall Flx5R labeled more lightly than the other strains, except in the 94-kDa band. (B) Flash-induced changes in A_{570} in all-*trans* retinal-regenerated vesicles (actinic flash λ, ≥600 nm; duration, 1 ms). a, Flx6R; b, Flx3R; c, Flx5R ; 1 mV is equivalent to $A_{570} = 4.3 \times 10^{-3}$.

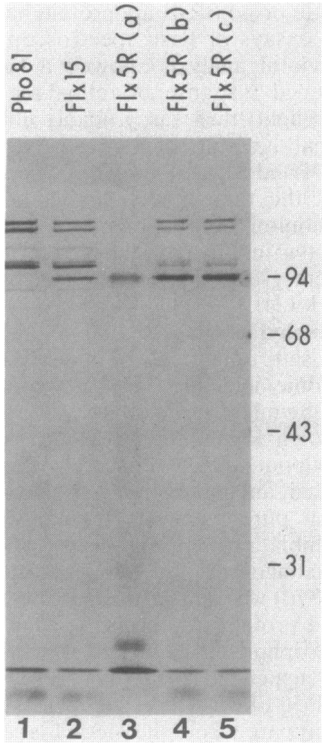

FIG. 3. Autofluorogram of SDS-polyacrylamide gel of membrane proteins. Lanes 1 and 2, membrane proteins from Pho81 and Flx15, respectively, that were [^{3}H]methyl labeled in vivo as described in the text; lanes 3 through 5, proteins from Flx5R. a, Membranes isolated and labeled with [^{3}H]retinal as described in the text; b and c, membranes labeled with [^{3}H]methyl in vivo treated as described above for lane a in the presence (lane b) and absence (lane c) of unlabeled all-*trans* retinal. Molecular weight standards on the right (in thousands) were 94, 68, and 43, as described in the legend to Fig. 1; 31 is carbonic anhydrase.

the methylated bands (94 kDa) was missing in Pho81 (Fig. 1), implicating this band in phototaxis.

The methyl radiolabel in each of the bands in Fig. 1 was labile to treatment of the gel with mild base (0.1 N NaOH for 30 min at 23°C before fixation in acetic acid). Counts from the 94-kDa region of the gel have been recovered in the volatile fraction (J. Stock and J. Spudich, unpublished data) by using a distillation assay which measures CH_3OH release after base hydrolysis, which is diagnostic of carboxylmethylesters (30). In *E. coli* membranes, this type of methylation has been demonstrated to occur only in the methylation-dependent chemotaxis adaptation process. These results provide compelling evidence for a similar carboxylmethylation-dependent adaptation system in *H. halobium* and complement the evidence provided by Schimz (17, 18) and Bibikov and co-workers (2).

The 94-kDa methylated band is enhanced in an SR-I overproducer. Flx5R is a mutant which produces approximately fourfold greater amounts of SR-I apoprotein than its parent strain and little or no SR-II, according to flash spectroscopic analysis (4, 25). Flx5R is blocked in the synthesis of retinal, and similar to the previously characterized retinal-blocked mutant Flx3R (26), its cells and membranes show regeneration of SR-I absorbance and photoreactions when all-*trans* retinal is added exogenously. Methylation patterns (Fig. 2A) and SR-I flash-induced absorbance changes (Fig. 2B) were compared for three strains that differed in their SR-I content but that otherwise had similar phenotypes. Proteins from these strains, as well as those from Flx15 and Pho81, were separated on a low bisacrylamide (25% of the usual concentration) gel (14), which allowed greater separation of the methylated bands (Fig. 2A). The 94-kDa band was labeled in greater amounts in Flx5R than in Flx3R and Flx6R strains, and it was the dominant methylated band in Flx5R (Fig. 2). This correlation with SR-I was confirmed by the presence of methylated 94-kDa protein in the SR-I$^+$ SR-II$^+$ strain Flx3, while a SR-I$^-$ SR-II$^+$ derivative Flx3b (T. Takahashi, S. Yorimitsu, N. Kamo, Y. Kobatake, and K. Tsujimoto, submitted for publication) does not show any methyl label at 94 kDa (T. Takahashi and E. N. Spudich, unpublished data).

Methylation and [^{3}H]retinal-labeling of membrane proteins. Sonicated membrane fractions (10) were prepared by ultracentrifugation from Flx15 and Pho81 that were radiolabeled with L-[*methyl*-^{3}H]methionine in vivo. Autofluorography showed a pattern similar to that of whole-cell proteins, except for changes in the relative intensity of the bands and the loss of the 65-kDa band in the membrane preparation. The membrane pattern confirmed that the 94-kDa methylated protein was missing in Pho81 (Fig. 3, lanes 1 and 2).

[^{3}H]Retinal was added to Flx5R membranes, which were then treated with $NaCNBH_3$ by a procedure which stably links retinal to the apoproteins of bacterial sensory rhodop-

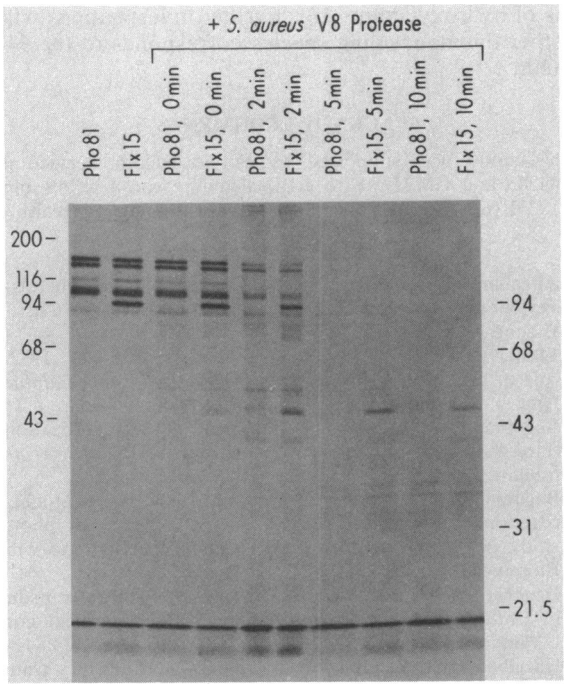

FIG. 4. Partial proteolysis of ^{3}H-methylated membrane proteins of Flx15 and Pho81. Membrane suspension (100 μl) containing 50 μg of protein in 125 mM Tris hydrochloride (pH 6.8)–10% glycerol–0.5% SDS was digested with 2 μg of protease at 37°C for the indicated times at which 10 μl of 10% β-mercaptoethanol–20% SDS was added to stop the reaction. After the solution to stop the reaction was added, the samples were immediately immersed in a 70°C water bath for 5 min. For the lanes marked Pho81 and Flx15, no protease was added. For the lanes marked 0 min, stop solution was added before protease. Numbers on the left and right of the gel indicate molecular masses (in thousands) as in Fig. 1 and 3; 21.5 is soybean trypsin inhibitor.

sins (25). Two radiolabeled bands were evident: one at 25 kDa and one at the same position as the 94-kDa methylated band (Fig. 3, lane 3). The methylation pattern of membrane proteins of Flx5R showed increased label at 94 kDa. The $NaCNBH_3$ treatment did not alter the position of the [^{3}H]methyl-labeled bands in Flx5R in the presence or absence of unlabeled retinal (Fig. 3, lanes 4 and 5).

The methyl label at 94 kDa and the retinal label at 94 kDa are on the same protein. [^{3}H]methyl-labeled membrane proteins of Flx15 and Pho81 were subjected to partial proteolysis by *Staphylococcus aureus* V8 protease. A long-lived digestion product at ~50 kDa and several lighter bands were generated from Flx15 proteins (Fig. 4). Comparison of Flx15 with Pho81 lanes shows that the ~50 kDa fragment is derived from proteolysis of the 94-kDa methylated protein. Therefore, digestion with V8 protease can be used to compare the [^{3}H]methyl-labeled and [^{3}H]retinal-labeled 94-kDa proteins of Flx5R (Fig. 5). Both labeled preparations (Fig. 5, [^{3}H]retinal-labeled, lanes a; [^{3}H]methyl-labeled, lanes b and c) produced fragments with the same apparent molecular masses (~50 kDa). From the covariation in the amounts of the methyl- and retinal-labeled bands in several mutants and the identical molecular masses of both the undigested and digested proteins that were labeled by the two methods, we conclude that the [^{3}H]methyl and [^{3}H]retinal are present on the same 94-kDa protein and that the amount of the 94-kDa protein varies among the mutants.

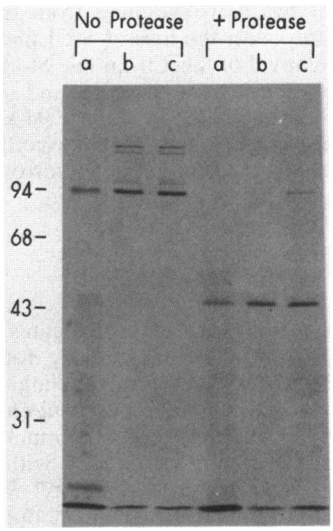

FIG. 5. V8 protease digestion of Flx5R membrane proteins labeled with either [^{3}H]methyl or [^{3}H]retinal. Lanes a, labeled with [^{3}H]retinal, as described in the text; lanes b and c, labeled with [^{3}H]methyl, as described in the legend to Fig. 3 in the presence (lanes b) or absence (lanes c) of unlabeled retinal. All of the samples were reduced with $NaCNBH_3$ prior to digestion. Membrane proteins were digested for 10 min as described in the legend to Fig. 4. Numbers on the left of the gel indicate molecular masses (in thousands).

Differential bleaching with hydroxylamine correlates SR-I photocycling activity with the 25-kDa and not the 94-kDa protein. Flx5R membranes were reconstituted with [^{3}H]retinal and treated with various concentrations of hydroxylamine, which is known to inactivate SR-I photocycling (16, 25), presumably by cleavage of the retinal Schiff base linkage as in bacteriorhodopsin (15). After extensive washing, the treated membranes were assayed by flash photolysis and the amount of photocycling SR-I was determined (Fig. 6). Following reduction with $NaCNBH_3$, the amount of [^{3}H]retinal labeling of the 25- and 94-kDa proteins was assessed by densitometry of the autofluorograms. Re-

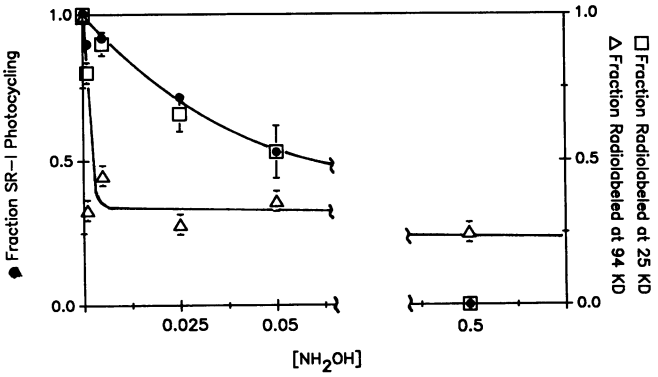

FIG. 6. Hydroxylamine concentration dependence of SR-I photocycling activity and [^{3}H]retinal-labeled proteins in Flx5R membranes. Photocycling SR-I was determined by flash photolysis. Amounts of label remaining at 25 and 94 kDa after hydroxylamine treatment were determined by densitometric scans of autofluorograms with a laser densitometer (Ultroscan XL; Pharmacia LKB Biotechnology, Inc., Piscataway, N.J.). Error bars are drawn between values that were obtained from two independent labeling experiments.

moval of label by hydroxylamine from the 25-kDa band correlated closely with the loss of SR-I photocycling activity, whereas removal of label from the 94-kDa band demonstrated heterogeneity in the labeling and did not correlate with SR-I photocycling (Fig. 6). In the 94-kDa band, ~70% of the label was removed by 1 mM hydroxylamine, while the remaining label was resistant to high hydroxylamine concentrations.

DISCUSSION

The evidence presented here indicates that a 94-kDa methyl-accepting protein is necessary for SR-I-mediated phototaxis in *H. halobium*. The methyl linkage appears to be a carboxylmethylester, as in eubacterial chemotaxis receptor-transducers. Data from analysis of mutants indicate that this protein is associated specifically with the phototaxis receptor SR-I. Given the role of methyl-accepting chemotaxis proteins as transducers which modulate the flagellar motor in other bacteria, we suggest that the 94-kDa protein is the SR-I signal transducer, i.e., the protein which communicates the state of SR-I to the flagellar motor switch. The mutant data indicate that the other bands are also involved in adaptation to taxis stimuli, but we cannot yet assign them to specific receptors.

Previous [^{3}H]retinal-labeling studies have revealed radiolabeled bands at 25 and 23 kDa, which are quantitatively correlated in various mutants with SR-I and SR-II, respectively (25). Bleaching by hydroxylamine and regeneration with [^{3}H]retinal also correlates proteins of similar sizes with SR-I and SR-II (16). In this study we confirmed that the 25-kDa protein is derived from photochemically reactive SR-I by showing that removal of the retinal label from the 25-kDa protein exhibits a hydroxylamine concentration dependence which corresponds closely to the loss of photocycling SR-I.

One model for signal transduction by SR-I is suggested by the triggering of transducers by binding of ligand-activated receptors in the maltose and ribose-galactose chemotaxis systems in *E. coli* (6, 8). In these systems the transducer recognizes the ligand-occupied receptor by protein-protein interaction. In an analogous type of mechanism for SR-I, the light-activated 25-kDa receptor would trigger the 94-kDa transducer by direct protein-protein interaction. In this case, it is possible that some retinal attaches to the 94-kDa protein during the reduction procedure in a domain that is in close proximity to the retinal linkage site in the chromophoric 25-kDa polypeptide. It is known that migration of retinal to the neighboring lysines can occur under reduction conditions (1, 7). Some of the label at 94 kDa may result from such a transfer. However the presence of more label at 94 kDa than at 25 kDa after the addition of 0.5 M NH_2OH (Fig. 6) is difficult to interpret in terms of retinal transfer alone.

An important question is whether the attachment of retinal to the 94-kDa protein is due to physiologically relevant binding sites on this protein. One possibility is that the 25- and 94-kDa proteins are both forms of SR-I which contain its retinal-binding domain, and methylation modulates the amount of SR-I that is present in the 25- and 94-kDa forms. The 94-kDa form could result from the association of the 25-kDa polypeptide with itself as a tetramer or with another component to form an SDS-resistant complex. A noncycling pigment with λ_{max} near 590 nm has been detected spectroscopically in *H. halobium* membranes, and the pigment, like the 94-kDa protein (Fig. 6), is bleached by low concentrations of hydroxylamine (16). Further investigations will test whether this noncycling species corresponds to the 94-kDa protein.

ACKNOWLEDGMENTS

This study was supported by Public Health Service grants GM34283 and GM27750 (to J.L.S.) and research fellowship F32 GM12231 (to C.A.H.) from the National Institutes of Health.

LITERATURE CITED

1. **Abrahamson, E. W., and R. S. Fager.** 1973. The chemistry of vertebrate and invertebrate visual photoreceptors. Curr. Top. Bioenerg. **5**:125–200.
2. **Bibikov, S. I., V. A. Baryshev, and A. N. Glagolev.** 1982. The role of methylation in the taxis of *Halobacterium halobium* to light and chemo-effectors. FEBS Lett. **146**:255–258.
3. **Bogomolni, R. A., and J. L. Spudich.** 1982. Identification of a third rhodopsin-like pigment in phototactic *Halobacterium halobium*. Proc. Natl. Acad. Sci. USA **79**:6250–6254.
4. **Bogomolni, R. A., and J. L. Spudich.** 1987. The photochemical reactions of bacterial sensory rhodopsin-I: flash photolysis study in the one microsecond to eight second time window. Biophys. J. **52**:1071–1075.
5. **Dencher, N. A., and E. Hildebrand.** 1979. Sensory transduction in *Halobacterium halobium*: retinal protein pigment controls UV-induced behavioral response. Z. Naturforsch. **34**:841–847.
6. **Hazelbauer, G. L., and S. Harayama.** 1983. Sensory transduction in bacterial chemotaxis. Int. Rev. Cytol. **81**:33–70.
7. **Katre, N. V., P. K. Wolber, W. Stoeckenius, and R. M. Stroud.** 1981. Attachment site(s) of retinal in bacteriorhodopsin. Proc. Natl. Acad. Sci. USA **78**:4068–4072.
8. **Koiwai, O., and H. Hayashi.** 1979. Studies on bacterial chemotaxis. IV. Interaction of maltose receptor with a membrane-bound chemosensing component. J. Biochem. **86**:27–34.
9. **Koshland, D. E., Jr.** 1981. Biochemistry of sensing and adaptation in a simple bacterial system. Annu. Rev. Biochem. **50**:765–782.
10. **Lanyi, J. K., and R. E. MacDonald.** 1979. Light-induced transport in *Halobacterium halobium*. Methods Enzymol. **56**:398–407.
11. **Macnab, R. M.** 1987. Motility and chemotaxis, p. 732–759. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*. American Society for Microbiology, Washington, D.C.
12. **Marwan, W., and D. Oesterhelt.** 1987. Signal formation in the halobacterial photophobic response mediated by a fourth retinal protein (P_{480}). J. Mol. Biol. **195**:333–342.
13. **McCain, D. A., L. A. Amici, and J. L. Spudich.** 1987. Kinetically resolved states of the *Halobacterium halobium* flagellar motor switch and modulation of the switch by sensory rhodopsin I. J. Bacteriol. **169**:4750–4758.
14. **Nowlin, D. M., D. O. Nettleton, G. W. Ordal, and G. L. Hazelbauer.** 1985. Chemotactic transducer proteins of *Escherichia coli* exhibit homology with methyl-accepting proteins from distantly related bacteria. J. Bacteriol. **163**:262–266.
15. **Oesterhelt, D., L. Schuhmann, and H. Gruber.** 1974. Light dependent reaction of bacteriorhodopsin with hydroxylamine in cell suspensions of *Halobacterium halobium*: demonstration of an apomembrane. FEBS Lett. **44**:257–261.
16. **Scherrer, P., K. McGinnis, and R. A. Bogomolni.** 1987. Biochemical and spectroscopic characterization of the blue-green photoreceptor in *Halobacterium halobium*. Proc. Natl. Acad. Sci. USA **84**:402–406.
17. **Schimz, A.** 1981. Methylation of membrane proteins is involved in chemosensory and photosensory behavior of *Halobacterium halobium*. FEBS Lett. **125**:205–207.
18. **Schimz, A.** 1982. Localization of the methylation system involved in sensory behavior of *Halobacterium halobium* and its dependence on calcium. FEBS Lett. **139**:283–286.
19. **Schimz, A., and E. Hildebrand.** 1979. Chemosensory responses of *Halobacterium halobium*. J. Bacteriol. **140**:749–753.

20. **Simon, M. I., A. Krikos, N. Mutoh, and A. Boyd.** 1985. Sensory transduction in bacteria. Curr. Top. Membr. Transp. **23**:3–16.
21. **Springer, M. S., M. F. Goy, and J. Adler.** 1979. Protein methylation in behavioral control mechanisms and in signal transduction. Nature (London) **280**:279–284.
22. **Spudich, E. N., R. A. Bogomolni, and J. L. Spudich.** 1983. Genetic and biochemical resolution of the chromophoric polypeptide of halorhodopsin. Biochem. Biophys. Res. Commun. **112**:332–338.
23. **Spudich, E. N., and J. L. Spudich.** 1982. Control of transmembrane ion fluxes to select halorhodopsin-deficient and other energy transduction mutants of *Halobacterium halobium*. Proc. Natl. Acad. Sci. USA **79**:4308–4312.
24. **Spudich, E. N., and J. L. Spudich.** 1982. Measurement of light-regulated phosphoproteins of *Halobacterium halobium*. Methods Enzymol. **88**:213–216.
25. **Spudich, E. N., S. A. Sundberg, D. Manor, and J. L. Spudich.** 1986. Properties of a second sensory receptor protein in *Halobacterium halobium* phototaxis. Proteins **1**:239–246.
26. **Spudich, J. L., and R. A. Bogomolni.** 1983. Spectroscopic discrimination of the three rhodopsinlike pigments in *Halobacterium halobium* membranes. Biophys. J. **43**:243–246.
27. **Spudich, J. L., and R. A. Bogomolni.** 1984. Mechanism of colour discrimination by a bacterial sensory rhodopsin. Nature (London) **312**:509–513.
28. **Spudich, J. L., and R. A. Bogomolni.** 1988. Sensory rhodopsins of halobacteria. Annu. Rev. Biophys. Biophys. Chem. **17**:193–215.
29. **Spudich, J. L., and W. Stoeckenius.** 1979. Photosensory and chemosensory behavior of *Halobacterium halobium*. Photobiochem. Photobiophys. **1**:43–53.
30. **Stock, J. B., S. Clarke, and D. E. Koshland, Jr.** 1984. The protein carboxylmethyltransferase involved in *Escherichia coli* and *Salmonella typhimurium* chemotaxis. Methods Enzymol. **106**:310–321.
31. **Sundberg, S. A., M. Alam, and J. L. Spudich.** 1986. Excitation signal processing times in *Halobacterium halobium* phototaxis. Biophys. J. **50**:895–900.
32. **Sundberg, S. A., R. A. Bogomolni, and J. L. Spudich.** 1985. Selection and properties of phototaxis-deficient mutants of *Halobacterium halobium*. J. Bacteriol. **164**:282–287.
33. **Takahashi, T., Y. Mochizuki, N. Kamo, and Y. Kobatake.** 1985. Evidence that the long-lifetime photointermediate of s-rhodopsin is a receptor for negative phototaxis in *Halobacterium halobium*. Biochem. Biophys. Res. Commun. **127**:99–105.
34. **Takahashi, T., H. Tomioka, N. Kamo, and Y. Kobatake.** 1985. A photosystem other than PS370 also mediates the negative phototaxis of *Halobacterium halobium*. FEMS Microbiol. Lett. **28**: 161–164.
35. **Tomioka, H., T. Takahashi, N. Kamo, and Y. Kobatake.** 1986. Flash spectrophotometric identification of a fourth rhodopsin-like pigment in *Halobacterium halobium*. Biochem. Biophys. Res. Commun. **139**:389–395.
36. **Wolff, E. K., R. A. Bogomolni, P. Scherrer, B. Hess, and W. Stoeckenius.** 1986. Color discrimination in halobacteria: spectroscopic characterization of a second sensory receptor covering the blue-green region of the spectrum. Proc. Natl. Acad. Sci. USA **83**:7272–7276.