# Structure and Function in Rhodopsin. Single Cysteine Substitution Mutants in the Cytoplasmic Interhelical E−F Loop Region Show Position-Specific Effects in Transducin Activation†

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ABSTRACT: The cytoplasmic interhelical E−F loop in rhodopsin is a part of the region that interacts with the G-protein transducin and rhodopsin kinase during signal transduction. In extending the previous work on systematic single cysteine substitutions of the amino acids in the cytoplasmic C−D loop, we have now replaced, one at a time, the amino acids Q225−I256 in the E−F loop region by cysteines. All the mutants formed the characteristic rhodopsin chromophore with 11-*cis*-retinal. While most of the mutants bleached normally, L226C, showed abnormal bleaching behavior. A study of the alkylation of the mutants by *N*-ethylmaleimide in dark showed low reactivity by some mutants, especially L226C. The rates of transducin activation ($G_{T(\alpha)}$−GTP$\gamma$S complex formation) were measured for all the mutants. While these were normal for the bulk of the mutants, some (L226C, T229C, V230C, A233C, A234C, T242C, T243C, and Q244C) showed strikingly reduced transducin activation. The results suggest a specific structure in the E−F loop that interacts with transducin.

In a series of experiments aimed at understanding the structural changes that occur in rhodopsin following light-activation, we are using an approach in which single cysteine residues are provided at defined positions on the cytoplasmic face (Figure 1). The cysteine residues serve as handles for attachment of probes for structural investigation by chemical and biophysical methods. Previously, we have used this approach in studies of both bacteriorhodopsin (Flitsch & Khorana, 1989; Altenbach et al., 1989, 1990; Greenhalgh et al., 1991; Steinhoff et al., 1994) and rhodopsin (Resek et al., 1993, 1994). More recently, Ridge et al. (1995) substituted, one at a time, all of the amino acids in the interhelical loop C−D region of rhodopsin (Figure 1) by cysteine residues and carried out a comparative study of the cysteine containing mutants in regard to their chemical reactivity in the dark as well as the light-dependent structural changes by the spin-labeling technique (Farahbakhsh et al., 1995). We now extend this work to the study of the cytoplasmic interhelical E−F loop region (Figure 1).

The importance of the cytoplasmic E−F loop region in metarhodopsin II interactions with transducin and rhodopsin kinase has been highlighted by a number of previous studies. Peptides with sequences corresponding to this loop inhibited transducin activation (Konig et al., 1989) as did deletions in this region (Franke et al., 1992). Results of a large number of amino acid replacements, single and multiple (Franke et al., 1990, 1992), and specific alanine substitutions (Shi et al., 1995) as well as insertion mutagenesis in this loop (Borjigin & Nathans, 1994) all supported the notion of the involvement of specific amino acid sequences in this loop in interaction with transducin. Further, these and related changes in this loop have also been demonstrated to affect phosphorylation of rhodopsin by rhodopsin kinase (Palczewski et al., 1991; Shi et al., 1995; Thurmond & Khorana, 1995). In this and the accompanying paper (Altenbach et al., 1996; following paper in this issue), we have studied rhodopsin mutants prepared by replacing one at a time every one of the amino acids 225−256 (Figure 1) by cysteine residues. The results on chemical reactivity, light-catalyzed bleaching, and transducin activation are reported in this paper. These have brought to light for the first time strong position specific effects, especially on transducin activation, in these substitution mutants, pointing to a specific structural pattern in this region of the cytoplasmic face of rhodopsin.

## MATERIALS AND METHODS

*Materials*. (GTP$\gamma$S)[1] was purchased from Boehringer Mannheim. [$^{35}$S]dATP$\alpha$S, 500 Ci/mmol), and [$^{3}$H]NEM (50 Ci/mmol) were from Dupont-New England Nuclear. NEM was from Sigma. Nitrocellulose filters (HAWP25) were from Millipore (Marlborough, MA). DM was purchased from Anatrace (Maumee, OH). Frozen bovine retinas were

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[1] Abbreviations: GTP($\gamma$S), guanosine 5′-[$\gamma$-thio]triphosphate; dATP$\alpha$S, 5′-$\alpha$-deoxyadenosinethiotriphosphate; NEM, *N*-ethylmaleimide; DM, *n*-dodecyl $\beta$-D-maltoside; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonyl fluoride; Meta II, metarhodopsin II; MES, sodium (2-(*N*-morpholino))ethanesulfonic acid; $G_T$, heterotrimeric G-protein, transducin; $G_{T(\alpha)}$, $\alpha$ subunit of transducin; WT, wild type.

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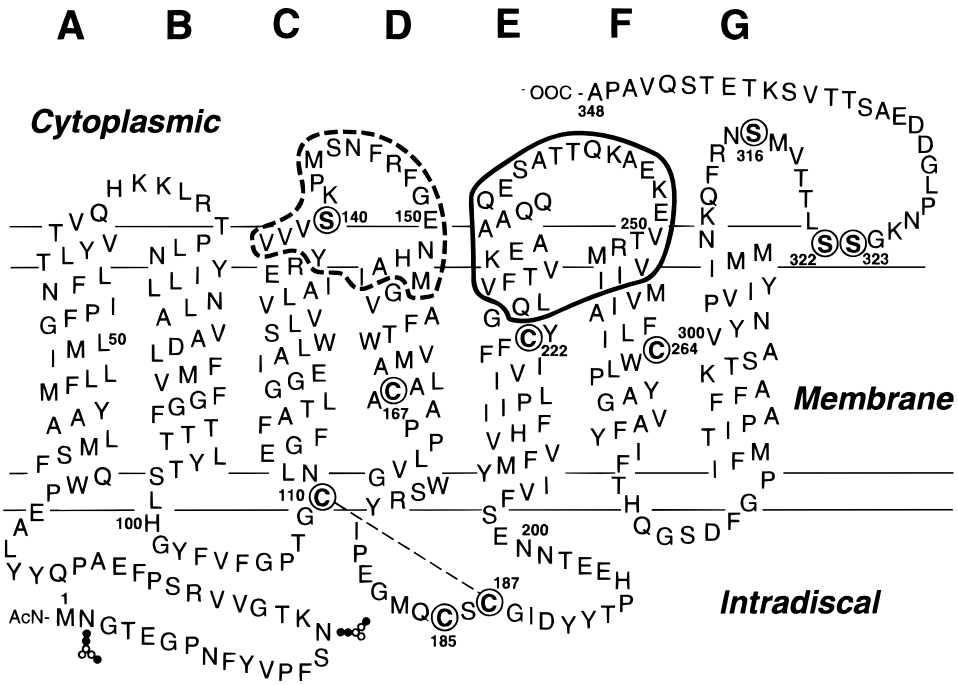


FIGURE 1: Secondary structure model of bovine rhodopsin showing regions in the cytoplasmic domain where the naturally occurring amino acids have been replaced one at a time by cysteine residues. The amino acids replaced in the present work are in the E−F loop region and are within the solid line boundary. The amino acids similarly replaced in the previous work (Ridge et al., 1995) are within the dashed line boundary. The two cysteines in native rhodopsin, C-140 and C-316, which are reactive in the dark as well as C-322 and C-323, the sites of palmitoylation, were replaced by serines. Since all the remaining cysteines in the membrane and intradiscal domains are silent, every mutant studied here contained a single reactive cysteine residue in the cytoplasmic domain.

purchased from J. A. Lawson Co. (Lincoln, NE). 11-*cis*-Retinal was a gift from Dr. Rosalie Crouch (South Carolina University and The National Eye Institute). Restriction endonucleases were from New England Biolabs (Beverly, MA). Transducin was purified from rod outer segments as described by Baer et al. (1982).

The nonapeptide corresponding to the C-terminal sequence of rhodopsin, which was used to elute rhodopsin and the mutants from the anti-rhodopsin antibody 1D4-Sepharose matrix was prepared at the MIT Biopolymers Laboratory.

*Construction of Mutants of the Synthetic Opsin Gene.* Fragment replacement mutagenesis was used to construct the mutants in the synthetic gene for the wild-type bovine opsin (Ferretti et al., 1986) and inserted in the expression vector pMT4 (Franke et al., 1988). A mutant of the wild-type opsin gene in which cysteine residues C140, C316, C322, and C323 have been replaced by serines has been described previously (Resek et al., 1993). All of the single cysteine mutants (amino acids 225−256) were derivatives of this mutant.

The synthetic oligonucleotides for the duplexes corresponding to the restriction fragments to be replaced in the synthetic gene were as follows: (a) for mutants between amino acids 225 and 232, *Msc*I/*Pst*I fragment, nucleotides 673−697 in the synthetic gene; (b) for mutants between amino acid positions 233 and 234, *Pvu*II/*Mlu*I fragment, nucleotides 681−748; (c) for mutants between amino acids 235 and 250, *Mlu*I/*Pst*I fragment, nucleotides 698−748; (d) for mutants between amino acids 251 and 252, *Dde*I/*Nde*I fragment, nucleotides 729−790; (e), for mutants between amino acids 253→256, MluI/NdeI fragment, nucleotides 749−799. Checking of the mutants V227C and F228C by digestion with restriction enzymes was facilitated by changing the codon for L266 from CTG to CTT, thus removing one of the two *Pvu*II sites in vector pMT4.

All oligonucleotides were synthesized by the phosphoramidite method with an Applied Biosystems model 380B DNA synthesizer on a 40 nmol scale, and purified by gel electrophoresis (Ferretti et al., 1986). Pairs of the purified oligonucleotides were annealed to form the duplexes before the ligase joinings (Ferretti et al., 1986). The plasmid DNAs were purified by alkaline SDS extraction and CsCl density centrifugation in the presence of ethidium bromide. All DNA sequences were confirmed by the dideoxy method (Sanger et al., 1977).

*Expression and Purification of Rhodopsin Mutants*. The opsin gene mutants were expressed in COS cells after transient transfection (15 cm diameter plates) as described (Oprian et al., 1987; Karnik et al., 1993). The cells were harvested, resuspended in PBSSC, pH 7.2, and treated with 11-*cis*-retinal (5 $\mu$M) for 2 h at 4 °C in the dark. The cell suspensions were centrifuged in portions at 1000*g* for 10 min. If desired, for storage, the cell pellets were frozen to −78 °C (they could be kept at this temperature for at least 3 months). For protein purification, the cells were warmed to room temperature and the total from ten plates was treated with 10 mL of PBS (pH 7.2) containing 1% DM and 0.1 mM PMSF for 1 h at 4 °C. The suspension was centrifuged and the total supernate was mixed with 300 $\mu$L of 1D4-Sepharose beads (capacity 1 $\mu$g of rhodopsin/1 $\mu$L) at 4 °C for 3 h in the dark, in the presence of 1 M NaCl, 2 mM ATP, and 2 mM $MgCl_2$ (Ridge et al., 1995). The 1D4-Sepharose beads containing bound rhodopsin were washed twice with 10 mL each of PBSSC, pH 7.2, containing 0.1% DM. The beads were further washed five times with 10 mL each of 5 mM MES buffer (pH 6) containing 0.05% DM. Rhodopsin mutants were then eluted with 500 $\mu$L of the same buffer containing 100 $\mu$M C′-9 peptide.

*Fluorescence Measurement of Meta II Photointermediate Decay*. The Meta II decay rates of the mutants were measured as described (Farrens & Khorana, 1995). Rhodopsin mutants (2 $\mu$g) in 200 $\mu$L containing 0.05% DM and 5 mM MES (pH 6) were bleached by illumination at $\lambda > 495$ nm for 30 s at 20 °C. The samples were excited with 295 nm light, and the fluorescence increase at 330 nm was monitored.

*Derivatization of Rhodopsin Mutants with NEM*. Rhodopsin mutants (1 $\mu$M) were incubated in the dark with 100 $\mu$M [$^3$H]NEM (5 Ci/mmol) in a solution containing 50 mM Tris-HCl (pH 8), 0.05% DM, and 150 mM NaCl at 20 °C for 16 h. DTT (20-fold excess over NEM) was then added, and the reaction mixtures were kept in the dark for 1 h at 20 °C. A further addition of cold NEM (100-fold excess over initial [$^3$H]NEM) was made before carrying out SDS−PAGE. Electrophoresis was stopped immediately after the proteins had entered the resolving gel. The protein bands were then visualized by Coomassie staining, excised, and counted for radioactivity.

*Spectral Characterization of Rhodopsin Mutants*. The purified rhodopsin mutants were characterized by UV/vis spectroscopy using a Perkin Elmer $\lambda$7 UV/visible spectrophotometer. The molar extinction coefficient of each mutant was determined as described (Sakmar et al., 1989; Bhattacharya et al., 1992). The wild type rhodopsin was assumed to have a molar extinction coefficient of 40 600 (Wald & Brown, 1953). Samples were bleached for 30 s at room temperature with light from a 150 watt fiber optic illuminator passed through a >495 nm long-pass filter. For measurement of the presence of Schiff base in the MII state, the samples were acidified to pH 1.9 (Sakamoto & Khorana, 1995).

*Transducin Activation by the Rhodopsin Cysteine Mutants*. $G_T$ was isolated from bovine rod outer segments as previously described (Baer et al., 1982). The ability of the cysteine mutants to activate transducin following light-activation was measured by following GDP−GTP exchange using the non-hydrolyzable GTP($\gamma$S).

The fluorescence assay procedure used was similar to that previously described (Fahmy & Sakmar, 1993). Rhodopsin (7 $\mu$L of 100 nM) in a pipette tip was bleached (20 s, $\lambda >$ 495 nm) and added to a solution of 250 nM transducin in 0.01% DM, 20 mM Tris, pH 7.4, and 100 mM NaCl in a volume of 686 $\mu$L at 20 °C. The solution was stirred for 10 min, and to it was added 7 $\mu$L of a 500 $\mu$M solution of GTP($\gamma$S) to a final concentration of 5 $\mu$M. The excitation wavelength was 295 nm (2 nm slit width), and the emission was monitored at 340 nm (12 nm slit width). To calculate the relative activation rates, the slopes of the initial fluorescence increase after GTP($\gamma$S) addition were determined by linear regressions through the data points covering the first 60 s. For plots, the value for each mutant was then divided by the activation rate for the WT rhodopsin.

## RESULTS

*Characterization of the Cysteine Mutants.* All the mutants were expressed in COS cells, isolated, and purified by elution from the rhodopsin antibody 1D4-Sepharose matrix at pH 6 in low ionic strength (Ridge et al., 1995). In general, the isolated yields of the expressed mutants were comparable to that of the wild type rhodopsin. All the mutants formed rhodopsin-like chromophore with spectral ratios ($A_{280}/A_{500}$) after purification between 1.6 and 1.8 (Table 1). Their $\lambda_{max}$ in the visible range varied between 498 and 501 nm (Table 1). The molar extinction coefficients of the mutants (Table 1) ranged between 39 500 and 46 600 compared with the value of 40 600 $M^{-1}$ $cm^{-1}$ for the wild type rhodopsin.

Table 1: Cysteine Substitution Mutant Rhodopsins (Absorption Maxima, Molar Extinction Coefficients, $t_{1/2}$ of Retinal Release Rates, and [$^3$H]-*N*-Ethylmaleimide Incorporation)

| mutants | $\lambda_{max}$ (nm) | $\epsilon_{500nm}$ ($M^{-1}$ $cm^{-1}$) | $t_{1/2}$ of retinal release[a] | [$^3$H]NEM incorporation (mol/mol of rhodopsin)[b] |
|---|---|---|---|---|
| WT(COS) | 500 | 40 600 | 13.4 | 2.0 ± 0.2 |
| Q225C | 500 | 42 100 | 14.0 | 0.7 ± 0.1 |
| L226C | 500 | 43 200 | 11.1 | 0.3 ± 0.1 |
| F227C | 500 | 42 300 | 10.4 | 0.6 ± 0.1 |
| F228C | 500 | 42 200 | 13.1 | 0.6 ± 0.1 |
| T229C | 500 | 42 000 | 10.0 | 0.6 ± 0.1 |
| V230C | 498 | 42 100 | 12.2 | 0.9 ± 0.2 |
| K231C | 500 | 44 100 | 11.6 | 1.1 ± 0.2 |
| E232C | 500 | 41 800 | 13.0 | 1.1 ± 0.1 |
| A233C | 499 | 41 200 | 12.6 | 1.2 ± 0.1 |
| A234C | 499 | 45 400 | 11.6 | 1.0 ± 0.1 |
| A235C | 500 | 41 600 | 12.2 | 1.0 ± 0.2 |
| Q236C | 499 | 44 900 | 12.3 | 1.2 ± 0.2 |
| Q237C | 500 | 41 900 | 13.5 | 0.9 ± 0.2 |
| Q238C | 500 | 40 600 | 13.8 | 1.1 ± 0.1 |
| E239C | 500 | 42 900 | 12.6 | 1.0 ± 0.1 |
| S240C | 501 | 42 700 | 11.8 | 1.1 ± 0.2 |
| A241C | 500 | 45 000 | 11.7 | 1.0 ± 0.2 |
| T242C | 498 | 44 700 | 12.2 | 1.0 ± 0.2 |
| T243C | 498 | 39 100 | 13.8 | 1.3 ± 0.2 |
| Q244C | 500 | 42 500 | 13.3 | 1.2 ± 0.2 |
| K245C | 499 | 41 100 | 12.3 | 1.2 ± 0.2 |
| A246C | 499 | 43 000 | 12.6 | 1.1 ± 0.2 |
| E247C | 500 | 46 400 | 14.6 | 1.1 ± 0.2 |
| K248C | 501 | 39 300 | 12.4 | 1.0 ± 0.1 |
| E249C | 501 | 43 500 | 13.1 | 1.1 ± 0.2 |
| V250C | 499 | 40 700 | 11.3 | 1.0 ± 0.1 |
| T251C | 500 | 39 000 | 12.8 | 1.1 ± 0.1 |
| R252C | 500 | 42 500 | 12.3 | 1.1 ± 0.1 |
| M253C | 499 | 41 500 | 12.6 | 0.9 ± 0.2 |
| V254C | 500 | 43 200 | 14.2 | 0.6 ± 0.0 |
| I255C | 499 | 42 200 | 11.8 | 1.0 ± 0.0 |
| I256C | 500 | 41 200 | 10.7 | 1.0 ± 0.1 |

[a] Retinal release rates measured for 2 $\mu$g of protein in 0.05% DM, pH 6.0. [b] Stoichiometry of [$^3$H]NEM alkylation after 16 h of reaction, representing averages from at least two independent determinations. Experimental errors are shown after ± in each result. Conditions: 100/1 [ $^3$H]NEM/rhodopsin, 0.05% DM, 150 mM NaCl, pH 7.4.

The UV/vis absorption spectral properties of selected mutants are shown in Figure 2. Spectrum 1 in each panel is in the dark. Spectrum 2 in each panel is observed after illumination for 30 s with $\lambda > 495$ nm light, while spectrum 3 is obtained after acidification of the bleached sample (formation of the 440 nm absorbing protonated retinyl Schiff base).

The majority of the mutants showed normal bleaching behavior (see, for example, F228C, Figure 2), and activated transducin normally (see below). The spectral characteristics of a number of mutants, T229C, V230C, A233C, A234C, T242C, T243C, and Q244C, are assembled in Figure 2. While, as seen, their bleaching behavior is essentially normal, these mutants showed strikingly reduced transducin activation. Only the mutant L226C showed abnormal bleaching behavior (Figure 2) which was studied further as a function of time after irradiation (Figure 3). As is seen, even 10.5 h after illumination, absorption between 450 and 500 nm

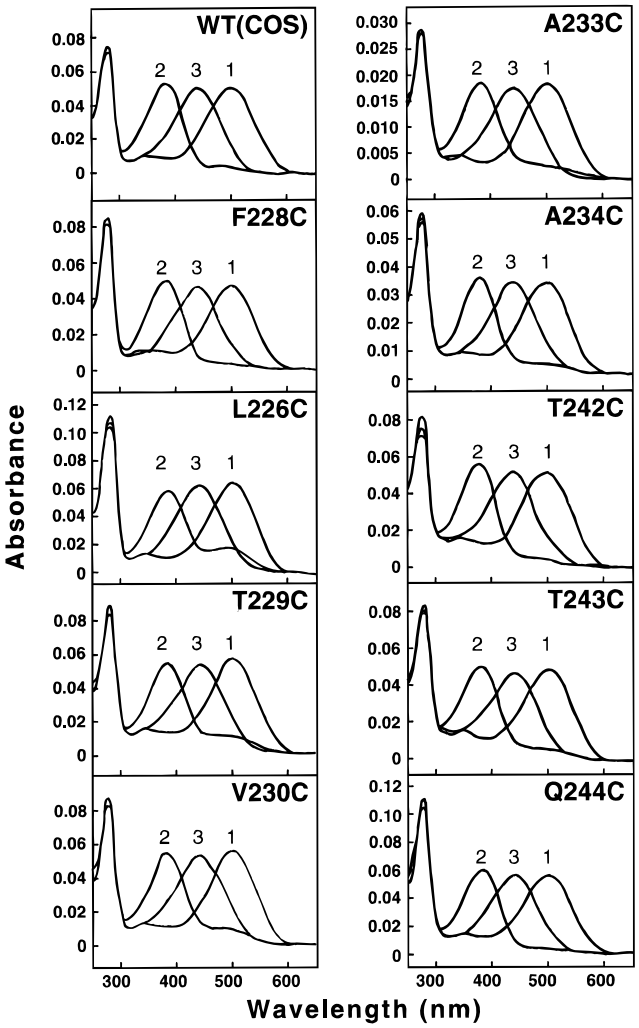


FIGURE 2: UV/Vis spectral properties of wild type COS cell rhodopsin and of selected single cysteine substitution mutants. In each panel, curve 1 shows the UV/vis spectrum in the dark; curve 2 shows the spectrum after illumination ($\lambda$ > 495 nm) for 30 s; and curve 3 shows the spectrum on acidification to pH 1.9 after photobleaching. Spectra 1 and 2 were taken in 5 mM MES (pH 6) in the presence of 0.05% DM at 20 °C. The mutants, whose spectra are shown, were selected on the basis of their defective transducin activation (Figure 4) although, as seen, except for L226C, their bleaching properties were essentially normal.

persisted. This mutant showed no detectable activation of transducin (see below).

*Meta II Decay Rates of the Cysteine Mutants*. Under the conditions used for bleaching, the $t_{1/2}$ for retinal release from WT rhodopsin was 13.4 min. The corresponding $t_{1/2}$ for the cysteine mutants ranged from 10.0 to 14.0 min (Table 1).

*Reactivity of Sulfhydryl Groups in the Cysteine Mutants toward NEM*. Every mutant was reacted with [$^3$H]NEM as described in Methods, and the incorporation of the radioactivity was followed. The normalized results (mole of [$^3$H]-NEM/mole of protein) are shown in Table 1. As expected, under the conditions used, wild type (COS) rhodopsin showed incorporation of 2 mol of the reagent [cf. Ridge et al. (1995)]. While the majority of the mutants showed essentially mol/mol incorporation of the reagent, some showed reduced incorporation. L226C incorporated the smallest amount (0.3 mol/mol) while the mutants, V254C, Q225C, V227C, F228C, and T229C, showed incorporations of 0.5−0.7 mol/mol. The NEM incorporation in the "base" mutant lacking any reactive cysteine on the cytoplasmic face is about 0.3 mole/mole [cf. Resek et al. (1994)].

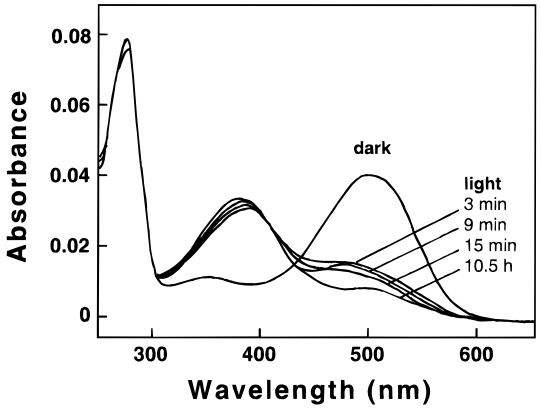


FIGURE 3: Spectral property of the mutant L226C at different times after illumination (further study of the abnormal photobleaching shown for this mutant in Figure 2). The origin of the ~480 nm absorbing residual species is not known.

*Transducin Activation by the Cysteine Mutants*. The majority of the mutants showed rates of fluorescence increase that were similar to that of WT rhodopsin. However, some showed much diminished rates of increase while a few showed essentially no fluorescence increase. The data for selected mutants are shown in Figure 4. As is seen in two cases, L226C and T243C, no increase in fluorescence was observed at all. As an internal control, subsequent addition of bleached WT rhodopsin to their reaction mixtures resulted in the expected fluorescence increase. The initial activation rates on the basis of fluorescence assay for wild type and all the mutants are shown by bars in Figure 5.

## DISCUSSION

Little precise information exists regarding the dark state structure of the cytoplasmic domain in rhodopsin and the structural change that occurs on light-activation. One general approach is the attachment of suitable reporter groups at different sites in the cytoplasmic domain and study of how the signals from the reporter groups change as a consequence of changes in their environment following light-activation. Resek et al. (1993, 1994) carried out such a study by attaching an EPR probe to the single reactive cysteine residues provided on the cytoplasmic face of rhodopsin. More recently, every one of the amino acids in the cytoplasmic loop C−D was replaced by a cysteine residue one at a time and systematic studies of the reactivity of the cysteine mutants (Ridge et al., 1995) and of the EPR spectra, both in dark and in light, of their spin-labeled derivatives were reported (Farahbakhsh et al., 1995).

In the present paper, we have extended the cysteine substitution approach to the cytoplasmic E−F loop region by replacing every one of the amino acids at positions 225−256. As in earlier work, the two WT cysteines, Cys-140 and Cys-316, which show reactivity in the dark state, as well as Cys-322 and Cys-323, the palmitoylation sites, were all replaced by serines in the base rhodopsin mutant that served as the starting point for introduction of single reactive cysteine residues (Resek et al., 1993). We found that all the cysteine substitution mutant rhodopsin genes were expressed normally in COS-1 cells and all the mutant proteins bound 11-*cis*-retinal to form the characteristic rhodopsin

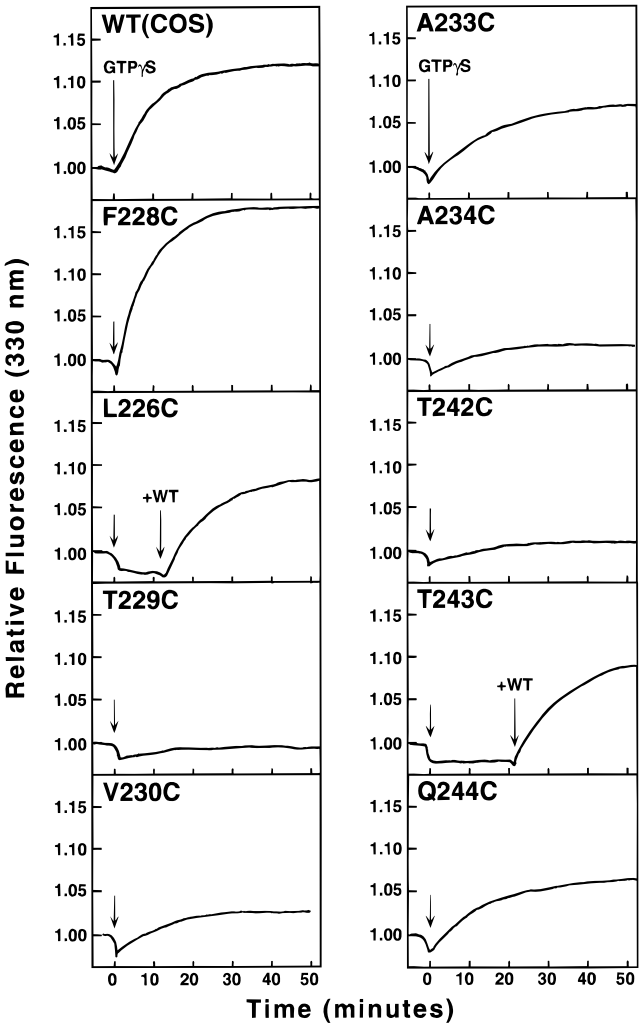


FIGURE 4: Transducin activation by the single cysteine rhodopsin mutants as measured by the fluorescence assay. The assay directly monitors transducin activation by measuring the increase in $G_{T(\alpha)}$ fluorescence which occurs upon formation of the $G_{T(\alpha)}$−GTP($\gamma$S) complex. Only the results with selected mutants showing differences from the WT (COS) rhodopsin are shown. Assay conditions were 1 nM rhodopsin, 250 nM transducin, 5 $\mu$M GTP($\gamma$S) in 0.01% DM, 20 mM Tris, pH 7.4, 100 mM NaCl at 20 °C. The addition of GTP($\gamma$S) (indicated by arrow) is defined as the start of the reaction ($t$ = 0). As shown, an aliquot of WT rhodopsin was later added, as an internal control, to mutant samples (L226C and T243C) which showed no transducin activation. For further details see Materials and Methods.

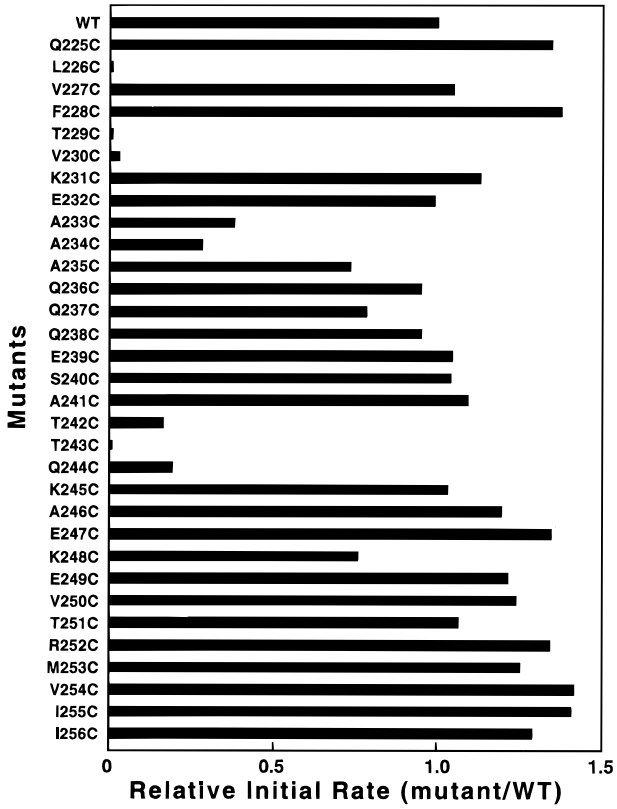


FIGURE 5: Comparison of the initial rates for activation of transducin by the cysteine substitution mutants. The rates of GTP($\gamma$S) fluorescence increase (formation of $G_{T(\alpha)}$−GTP($\gamma$S) complex) for each mutant were calculated from fluorescence data such as shown in Figure 4. This rate in the first minute after GTP($\gamma$S) addition relative to wild type rhodopsin is shown for each mutant.

chromophore. These results (Table 1) confirm what has been evident from the previous work (Franke et al., 1992; Resek et al., 1993; Ridge et al., 1995) that amino acid substitutions in the cytoplasmic face do not affect the rhodopsin folding.

Study of the dark state reactivity of the cysteine-containing mutants toward *N*-ethylmaleimide showed markedly low reactivity at certain positions. Thus, as seen in Table 1, Q225C−V230C as well as V254C showed reduced alkylation. In particular, the mutant Q226C showed uniquely low reactivity. Clearly, these amino acids are embedded and therefore inaccessible even to the membrane-permeant *N*-ethylmaleimide.

On illumination, most of the mutants formed the 380 nm-absorbing metarhodopsin II intermediates, and the latter decayed normally as measured by the fluorescence increase (Table 1). While some mutants showed essentially normal bleaching behavior, as shown in Figure 2, they were defective in transducin activation (see below). The behavior of L226C was uniquely abnormal in that after illumination, significant absorption in the 450−500 nm region persisted (Figure 3). The reason for this is not known.

While the bulk of the mutants upon illumination activated transducin, some mutants showed striking reductions in transducin activation. The initial rates of $G_{T(\alpha)}$−GTP($\gamma$S) formation (fluorescence assay) for these mutants are assembled in Figure 4. Thus, the mutants L226C, T229C, V230C, A233C, A234C, T242C, T243C, and Q244C all showed substantial to striking reductions in activation, and, further, L226C and T243C did not activate transducin at all. The total results on transducin activation by the cysteine mutants are shown in the bar graph in Figure 5.

As mentioned above, extensive mutagenesis, largely sporadic, has previously been carried out in the E−F loop region (Franke et al., 1992; Borjigin & Nathans, 1994; Shi et al., 1995), and the results have all suggested the involvement of this region in transducin interaction. However, the present work involving systematic and comprehensive cysteine substitutions has provided the most incisive structural information in this region. A highly likely interpretation of the periodic pattern observed (Figure 5) is that metarhodopsin II contains an α-helical structure in this region and that one face of the helix makes important contacts with transducin. Cysteine substitutions of amino acids on this face affect the binding and/or activation of transducin. It is also of interest that the N-terminal half of the E−F loop is more important in these contacts than the bulk of the C-terminal half of the loop, which does not seem to be sensitive to cysteine

substitutions. Of further interest are the observations that, firstly, the amino acids in the N-terminal half of the loop whose replacement by cysteine residues affects the interaction are mainly hydrophobic and polar but not charged amino acids. Secondly, replacement of several of the charged amino acids in the C-terminal part of the loop evidently does not seem to affect significantly the transducin−rhodopsin interaction. The structural aspects of this loop region, especially the changes on light-activation, are further discussed in the accompanying paper (Altenbach et al., 1996).

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