# Rhodopsin Arginine-135 Mutants Are Phosphorylated by Rhodopsin Kinase and Bind Arrestin in the Absence of 11-*cis*-Retinal†

Wen Shi,‡,§,|| Charlene D. Sports,‡ Dayanidhi Raman,‡ Satoko Shirakawa,‡ Shoji Osawa,‡ and Ellen R. Weiss*,‡,§,⊥

*Department of Cell Biology and Anatomy, Curriculum in Genetics and Molecular Biology, and Lineberger Comprehensive Cancer Center, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7090*

*Received December 18, 1997; Revised Manuscript Received February 5, 1998*

ABSTRACT: Arginine-135, located at the border between the third transmembrane domain and the second cytoplasmic loop of rhodopsin, is one of the most highly conserved amino acids in the family of G protein-coupled receptors. The effect of mutation at Arg-135 on the ability of rhodopsin to undergo desensitization was investigated. Four mutants, R135K, R135Q, R135A, and R135L, were examined for their ability to be phosphorylated by rhodopsin kinase, to bind arrestin, and to activate the rod cell G protein, transducin ($G_t$). All of the mutants were phosphorylated, bound arrestin, and were able to activate $G_t$ when reconstituted with 11-*cis*-retinal. Surprisingly, several of the mutants could be phosphorylated by rhodopsin kinase and could bind arrestin in the absence of 11-*cis*-retinal but were not able to activate $G_t$. These observations represent the first demonstration of a mutant G protein-coupled receptor that assumes a conformation able to interact with its G protein-coupled receptor kinase and arrestin, but not with its G protein, in the absence of ligand.

Arginine-135, located at the border between the third transmembrane domain and the second cytoplasmic loop of rhodopsin (Figure 1), is the most conserved amino acid of the sequence Asp/Glu-Arg-Tyr. This motif is common to most members of the G protein-coupled receptor (GPCR) family (*1*), suggesting that it plays an important role in the regulation of G protein signaling pathways. Mutations in the codon for this arginine have profound effects on the signaling properties of several GPCRs linked to human diseases. For example, substitution of Arg-135 in rhodopsin with the amino acids glycine, leucine, or tryptophan has been identified in some patients with the retinal disease autosomal dominant retinitis pigmentosa (ADRP)[1] (*2*, *3*). In bovine rhodopsin, these mutations are reported to disrupt the activation of the rod cell G protein, transducin ($G_t$), in vitro, despite normal expression and regeneration with 11-*cis*-retinal (*4*−*6*). However, it has also been reported that R135Q activates $G_t$ normally when expressed in membranes (*7*). Therefore, the effect of mutations at Arg-135 on $G_t$ activation remains unclear. An Arg → His mutation was identified at the equivalent position (Arg-137) in the $V_2$-vasopressin receptor in patients with congenital nephrogenic diabetes insipidus, a disease in which the kidney is unable to reabsorb water from urine in response to vasopressin (*8*). In vitro expression studies suggest that this defect is due to the inability of the mutant vasopressin receptor to activate

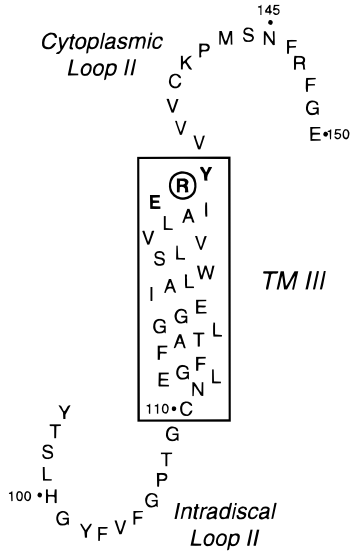


FIGURE 1: Sequence of the third transmembrane domain and the connected cytoplasmic (extradiscal) and intradiscal loops of bovine rhodopsin. The amino acids within the rectangle represent the third transmembrane domain, TM III. The amino acids shown in boldface type represent the E/D−R−Y sequence found in the majority of G protein-coupled receptors. Arg-135, the most conserved residue in this sequence, is circled.

† This work was supported by NIH Grants GM43582 (to E.R.W.) and GM47438 (to S.O.) and was performed during the tenure of an Established Investigatorship from the American Heart Association to E.R.W.

* Correspondence should be addressed to this author at the Department of Cell Biology and Anatomy, The University of North Carolina at Chapel Hill, CB 7090, 108 Taylor Hall, Chapel Hill, NC 27599-7090; (919) 966-7683 (telephone); (919) 966-1856 (Fax); erweiss@med.unc.edu (e-mail).

‡ Department of Cell Biology and Anatomy.

§ Curiculum in Genetics and Molecular Biology.

|| Present address: Department of Growth and Development, University of California at San Francisco, San Francisco, CA 94143-0640.

⊥ Lineberger Comprehensive Cancer Center.

[1] Abbreviations: ADRP, autosomal dominant retinitis pigmentosa; GPCR, G protein-coupled receptor; GRK, G protein-coupled receptor kinase; GTPγS, guanosine 5′-*O*-(3-thiotriphosphate); CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]propanesulfonic acid; CHO, Chinese hamster ovary; DTT, dithiothreitol; $G_t$, transducin; HEK-293, human embryonic kidney 293; ROS, rod outer segment; SDS, sodium dodecyl sulfate.

S0006-2960(97)03110-3 CCC: $15.00 
Published on Web 03/17/1998

its G protein, $G_s$. In addition to receptors linked to diseases, other receptors expressed in vitro exhibit changes in signaling properties. For example, the $\alpha_{1B}$-adrenergic and $m_1$- and $m_2$-muscarinic acetylcholine receptors mutated at the equivalent arginine demonstrate reduced ability to regulate phospholipase C ($\alpha_{1B}$-adrenergic and $m_1$-muscarinic acetylcholine receptors) and adenylyl cyclase ($m_2$-muscarinic acetylcholine receptor) (*9*−*11*).

Although a number of studies have addressed the role of this arginine in G protein signaling, little is known about the effect of mutation at this site on the ability of G protein-coupled receptors to undergo desensitization. Our laboratory has explored this issue through the use of site-directed mutagenesis of bovine rhodopsin and in vitro reconstitution with rhodopsin kinase, arrestin, and $G_t$. We have observed that all of the Arg-135 mutants are capable of activating $G_t$, serving as substrates for phosphorylation, and binding arrestin when reconstituted with 11-*cis*-retinal. Surprisingly, several R135 mutants could be phosphorylated by rhodopsin kinase and bind arrestin in the absence of 11-*cis*-retinal, although they were not able to activate $G_t$ under these conditions.

## EXPERIMENTAL PROCEDURES

*Materials.* The cDNA for bovine arrestin (*12*) was a gift from Dr. Toshimichi Shinohara. 11-*cis*-Retinal was a gift from Hoffman-La Roche. The monoclonal antibody R2-15N, which recognizes the N-terminal 15 amino acids of bovine rhodopsin (*13*), was kindly provided by Dr. Paul Hargrave.

*Mutagenesis.* Mutations in the cDNA for bovine rhodopsin (*14*) were made as described previously (*15*). All mutants were sequenced for verification using Sequenase (Amersham) according to the manufacturer's directions.

*Expression of Rhodopsin Mutants.* Mutants were inserted into the plasmid pcDNA/Amp (Invitrogen) for transient expression in human embryonic kidney 293 (HEK-293) cells (American Type Culture Collection) (*15*). Approximately 65−70 h after transfection, membranes were prepared by sucrose density gradient centrifugation (*16*, *15*). Some mutants were analyzed for their ability to bind 11-*cis*-retinal using methods described previously (*15*, *17*).

*Western Blot Analysis.* Rhodopsin expressed in HEK-293 cells was analyzed by Western blotting using the monoclonal antibody R2-15N. Details for electrophoresis, immunoblotting, and quantification using a Molecular Dynamics PhosphorImager have been described previously (*15*, *17*, *18*). The level of rhodopsin expression was quantified using urea-stripped rod outer segment (ROS) membranes isolated from bovine retinas as a standard.

*Purification of ROS Proteins.* Urea-stripped ROS membranes were purified from frozen, dark-adapted bovine retinas (J. A. Lawson Inc., Lincoln, NB) as described previously (*19*). Rhodopsin kinase was prepared as a crude extract from light-exposed ROS membranes (*20*, *21*).

*Phosphorylation of Rhodopsin by Rhodopsin Kinase.* Phosphorylation of bovine rhodopsin expressed in HEK-293 cells was analyzed according to previously published methods (*17*). Where indicated, HEK-293 cell membranes expressing bovine rhodopsin were reconstituted with or without 28 $\mu$M 11-*cis*-retinal (14 nmol/80 $\mu$g of membrane protein) for 1 h at room temperature in the dark. The phosphorylation reaction was initiated by exposure to light and the addition of rhodopsin kinase to a reaction mixture containing 150 $\mu$M [$\gamma$-$^{32}$P]ATP (Amersham; 50 $\mu$Ci/mL) and HEK-293 cell membranes expressing 1 $\mu$g of rhodopsin using buffers and conditions as described (*17*). The amount of total membrane protein in each sample was equalized by the addition of nontransfected HEK-293 cell membranes. The samples were analyzed by immunoprecipitation using the R2-15N monoclonal antibody, followed by electrophoresis on 10% polyacrylamide gels and quantification using a Molecular Dynamics PhosphorImager as described (*17*).

*Arrestin Binding.* HEK-293 cell membranes containing bovine rhodopsin were incubated with or without 14 $\mu$M 11-*cis*-retinal, as described above, and prephosphorylated by rhodopsin kinase in a buffer containing 20 mM Tris-HCl, pH 7.5, 2 mM EDTA, 2 mM ATP, and 6 mM $MgCl_2$ at 30 °C for 1 h under fluorescent light as described (*18*). Equal amounts of rhodopsin were used for each mutant. Nontransfected cell membranes were added so that the amount of total protein was also the same for each sample. After a 1 h incubation, the reaction was diluted with 1 mL of ice-cold buffer containing 20 mM Tris-HCl, pH 7.5, and 2 mM EDTA (buffer A) and washed twice in the same buffer by centrifugation at 12000*g* for 15 min.

The cDNA for bovine arrestin (*12*) was inserted into pSP73 (Promega) and transcribed and translated in vitro in the presence of [$^{35}$S]methionine using procedures described by Zhang et al. (*18*). If membranes were regenerated with 11-*cis*-retinal before being phosphorylated by rhodopsin kinase, they were also regenerated with 14 $\mu$M 11-*cis*-retinal before the arrestin binding assay. Approximately 10 fmol of radiolabeled arrestin was incubated with HEK-293 cell membranes containing 1.0 $\mu$g of phosphorylated or non-phosphorylated bovine rhodopsin. After a 5-min incubation under fluorescent room light, free arrestin was separated from bound arrestin by centrifugation through a cushion of 0.2 M sucrose as described previously (*18*). The pellets were subjected to electrophoresis on SDS−10% polyacrylamide gels and the amount of arrestin bound to rhodopsin was quantified by phosphorimage analysis.

*$G_t$ Activation.* $G_t$ activation was measured as the initial rate of [$^{35}$S]GTP$\gamma$S binding to $G_t$ in the presence of rhodopsin using a nitrocellulose filter binding assay according to published methods (*15*) with modifications as described in the figure captions. For all assays, HEK-293 cell membranes containing rhodopsin mutants were incubated with 0.5 $\mu$M $G_t$ and 1 $\mu$M GTP$\gamma$S in a 400 $\mu$L reaction volume at room temperature.

## RESULTS

*Phosphorylation of Arg-135 Mutants by Rhodopsin Kinase.* Four different mutations, R135K, R135Q, R135A, and R135L, were generated in the cDNA for bovine rhodopsin and expressed in HEK-293 cells. These substitutions represent conservative as well as nonconservative (hydrophilic, neutral, or hydrophobic) mutations. By Western analysis, the mutants appeared to be expressed at levels approximately 30−50% less than wild-type rhodopsin (data not shown). The isolated membranes were reconstituted with 11-*cis*-retinal and tested for their ability to be phosphorylated

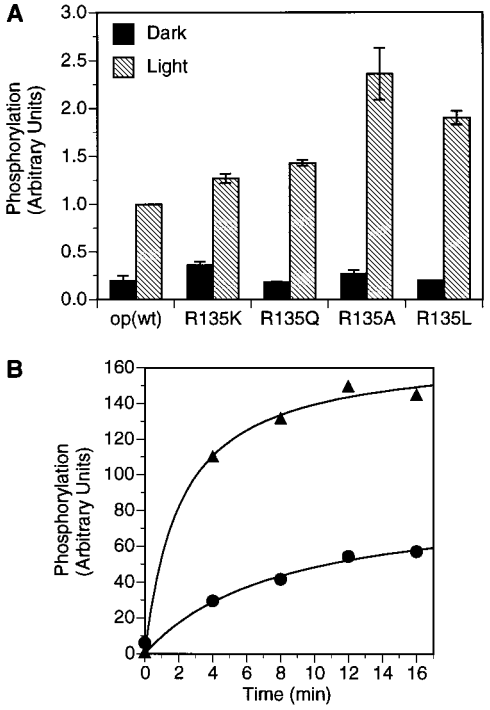


FIGURE 2: Phosphorylation of Arg-135 mutants in the presence of 11-*cis*-retinal. (Panel A) Membranes prepared from transfected HEK-293 cells expressing wild-type and mutant rhodopsins were reconstituted with 11-*cis*-retinal, phosphorylated by rhodopsin kinase for 8 min in the dark or in the light, and immunoprecipitated as described previously (*17*). The results were normalized to a value of 1.0 for wild-type rhodopsin phosphorylated in the light. The results represent the averages of duplicates from 3−7 experiments using membranes from at least two transfections. Error bars represent SE. (Panel B) Time course of phosphorylation for wild-type rhodopsin and the mutant R135A in the light. Reconstitution with 11-*cis*-retinal and phosphorylation by rhodopsin kinase were performed as described (*17*). (●) Wild-type rhodopsin [op(wt)]; (▲) R135A. The results are representative of three separate experiments.

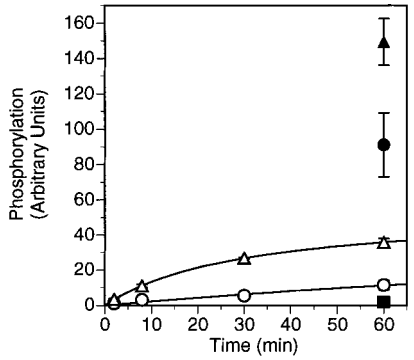


FIGURE 3: Phosphorylation of Arg-135 mutants in the presence or absence of 11-*cis*-retinal. Membranes were incubated with or without 11-*cis*-retinal as described under Experimental Procedures. A time course experiment was performed in the light for 60 min. (○,●) Wild-type rhodopsin [op(wt)]; (△,▲) R135A; (■) nontransfected HEK-293 cell membranes. Open symbols represent samples prepared in the absence of 11-*cis*-retinal. Closed symbols represent samples prepared in the presence of 11-*cis*-retinal. Error bars represent the range of duplicates from a single experiment representative of three independent experiments.

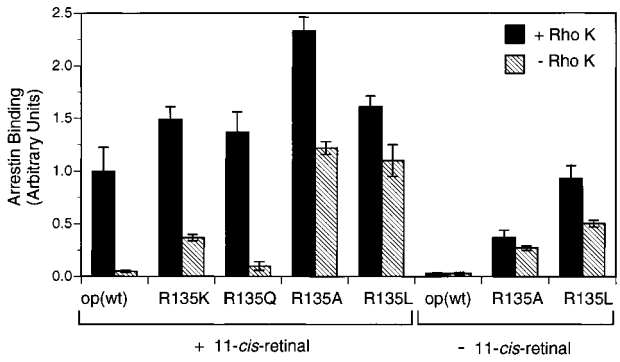


FIGURE 4: Arrestin binding to Arg-135 mutants in the presence or absence of 11-*cis*-retinal. Membranes were incubated with (+) or without (−) 11-*cis*-retinal and phosphorylated with rhodopsin kinase as described under Experimental Procedures. Samples that were treated with 11-*cis*-retinal before being phosphorylated were regenerated again with retinal before incubation with [$^{35}$S]-labeled arrestin according to methods described under Experimental Procedures and in Zhang et al. (*18*). All experiments were performed in the light. The results were quantified by phosphorimage analysis. +Rho K, phosphorylated membranes; −Rho K, nonphosphorylated membranes. Error bars represent the range of duplicates from a single experiment representative of at least three independent experiments.

by rhodopsin kinase. All four mutants showed similar levels of phosphorylation in the dark and were phosphorylated in the light to levels at least as high as for wild-type rhodopsin (Figure 2A). R135A and R135L demonstrated the highest levels of phosphorylation in the light, approximately 2.5- and 2-fold, respectively, compared to wild-type rhodopsin. A time course (Figure 2B) showed that the rate of phosphorylation for R135A in the light was elevated compared to the rate for wild-type rhodopsin. Despite these differences, absorbance studies indicated that the level of 11-*cis*-retinal binding to R135A was equivalent to that of wild-type rhodopsin (not shown). Previously, the bovine opsin mutants R135K, R135Q, R135G, R135A, R135L, and R135W were all shown to bind 11-*cis*-retinal, indicating that the structure of the transmembrane domains is intact in these mutants (*16*, *4*, *5*, *22*). However, in human opsin, of the mutants tested (R135G, R135L, and R135W), only R135G bound 11-*cis*-retinal normally (*23*, *22*), leading to speculation that human opsin is less stable (*22*). R135A was also analyzed for its ability to be phosphorylated by rhodopsin kinase in the absence of 11-*cis*-retinal (Figure 3). Under these conditions, R135A was phosphorylated to a level approximately 3-fold greater than wild-type rhodopsin in the absence of retinal and approximately 40% of the level observed for wild-type rhodopsin in the presence of 11-*cis*-retinal in the light. These data imply that R135A is functional in the absence of the rhodopsin chromophore.

*Arrestin Binding to Arg-135 Mutants.* To further explore the activity of these mutants in the absence of 11-*cis*-retinal, membranes isolated from HEK-293 cells expressing R135K, R135Q, R135A, and R135L were assayed for their ability to bind arrestin (Figure 4). The membranes were incubated with or without 11-*cis*-retinal and either phosphorylated with rhodopsin kinase in the light or left untreated. All of the mutants incubated with 11-*cis*-retinal were able to bind arrestin at least as well as wild-type rhodopsin. R135A, which showed the highest levels of phosphorylation by rhodopsin kinase, demonstrated the greatest increase in light-dependent arrestin binding. Interestingly, the level of arrestin binding to R135A and R135L in the light was also elevated in samples that were not prephosphorylated by rhodopsin kinase in vitro. When reconstitution with 11-*cis*-retinal was

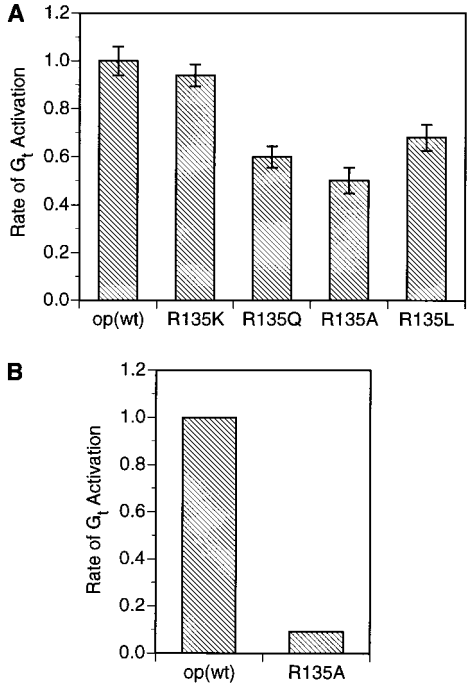


FIGURE 5: $G_t$ activation by Arg-135 mutants in the presence of 11-*cis*-retinal. (Panel A), $G_t$ activation by wild-type rhodopsin and Arg-135 mutants in membranes. Membranes from HEK-293 cells were reconstituted with 11-*cis*-retinal and assayed for the ability to catalyze the binding of [$^{35}$S]GTP$\gamma$S to $G_t$ in the light and in the dark as described (*15*). A 2.5 $\mu$g portion of membrane protein was used in each assay. Aliquots were removed from the reaction mixture at the indicated times. Initial rates of activation were calculated from the slopes of linear reaction curves. After the rates of GTP$\gamma$S binding for samples incubated in the dark were subtracted from the rates measured in light-exposed samples, light-dependent GTP$\gamma$S binding was expressed as a fraction of the level for wild-type rhodopsin normalized to a value of 1.0. No significant level of GTP$\gamma$S binding was observed in the dark (not shown). The results represent the averages of at least three experiments performed in duplicate using membranes from two transfections. Error bars represent SE. The average rate of light-dependent GTP$\gamma$S binding for wild-type rhodopsin was 0.1 mol/(s·mol). (Panel B) $G_t$ activation by wild-type rhodopsin and R135A using 0.25 $\mu$g of CHAPS-extracted membrane protein in each assay, as described (*15*). The result represents a single experiment performed in duplicate. The average rate of light-dependent GTP$\gamma$S binding for wild-type rhodopsin was 0.2 mol/(s·mol).

omitted from the experimental procedure, both phosphorylated and nonphosphorylated R135A and R135L were still able to bind arrestin. In contrast, no binding of arrestin to wild-type rhodopsin was observed in the absence of 11-*cis*-retinal.

*$G_t$ Activation.* The ability of mutations at Arg-135 to disrupt the activation of $G_t$ has been addressed by several laboratories. Although Cohen et al. (*7*) observed normal activation of $G_t$ by the R135Q mutant expressed in COS cell membranes, other laboratories, using detergent-extracted protein (*4*−*6*) or membranes (*6*), reported that mutations at Arg-135 abolished $G_t$ activation. Our results using membranes (Figure 5A) show that R135K, R135Q, R135A, and R135L are all able to activate $G_t$ when reconstituted with 11-*cis*-retinal and exposed to light. Three of these mutants, R135Q, R135A, and R135L, show partly reduced levels of light-dependent $G_t$ activation (60%, 70%, and 55% of the level for wild-type rhodopsin, respectively). In contrast, a CHAPS-extracted preparation of R135A is only 10% as active as wild-type rhodopsin in stimulating GTP$\gamma$S binding

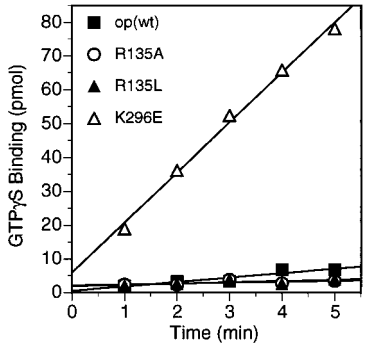


FIGURE 6: $G_t$ activation by R135A, R135L, and K296E in the absence of 11-*cis*-retinal. Membranes from HEK-293 cells expressing op(wt) (■), R135A (○), R135L (▲), and K296E (△) were assayed for the ability to catalyze the binding of [$^{35}$S]GTP$\gamma$S to $G_t$ as described (*15*) with the following modifications. HEK-293 cell membranes containing 1.4 pmol of rhodopsin were used for each sample. Nontransfected membranes were added to the assay mixtures so that the amount of total protein in each assay (5 $\mu$g) was also the same. The results represent the average of duplicates from a single experiment and are representative of two independent experiments.

to $G_t$ (Figure 5B), despite retaining the ability to bind 11-*cis*-retinal. Therefore, detergent extraction may alter the conformation of specific domains in the mutant rhodopsin that are necessary for G protein activation. We also compared the ability of R135A and R135L to activate $G_t$ in the absence of 11-*cis*-retinal with the mutant K296E, which cannot bind retinal but is known to activate $G_t$ (*24*) (Figure 6). R135A was not able to activate $G_t$ in the absence of 11-*cis*-retinal. Our results are similar to those of Cohen et al. (*7*), who found that R135Q is not constitutively active. In summary, our experiments demonstrate that Arg-135 mutants can be phosphorylated by rhodopsin kinase and can bind arrestin in the absence of 11-*cis*-retinal but do not activate $G_t$ under these conditions.

## DISCUSSION

A series of mutations at Arg-135 were analyzed for their influence on the ability of rhodopsin to be phosphorylated by rhodopsin kinase, to bind arrestin, and to activate $G_t$. All of the mutants were functional when reconstituted with 11-*cis*-retinal. R135A and R135L demonstrated the greatest increase in phosphorylation by rhodopsin kinase compared to wild-type rhodopsin, approximately 2.5-fold higher in the case of R135A. Arrestin binding was elevated for all of the mutants, particularly R135A, when reconstituted with 11-*cis*-retinal. In contrast, the level of $G_t$ activation for R135Q, R135A, and R135L was reduced by 30−45%. Surprisingly, R135A could be phosphorylated by rhodopsin kinase and could bind arrestin even in the absence of 11-*cis*-retinal but did not activate $G_t$ under these conditions. R135L was also observed to bind arrestin in the absence of 11-*cis*-retinal. These data imply that Arg-135 is critical for preventing phosphorylation and arrestin binding in wild-type rhodopsin in the absence of 11-*cis*-retinal. Mutation of this amino acid to alanine or leucine disrupts this function.

Mutation of the equivalent amino acid in the $m_1$-muscarinic acetylcholine receptor (Arg-123) results in a decrease in the number of high-affinity receptors and in the level of receptor-mediated phospholipase C signaling (*10*, *11*), suggesting that both the formation of the ternary complex (ligand−recep-

tor−G protein) and the activation of G proteins are impaired. In the $V_2$-vasopressin and $\alpha_{1B}$-adrenergic receptors, this mutation also disrupts receptor−G protein coupling (*8*, *9*). An intriguing possibility raised by our results is that the lower levels of G protein activation may be due, at least in part, to ligand-independent phosphorylation and arrestin binding rather than simply an effect on the direct interaction of the receptor with the G protein. Ligand-independent desensitization might be expected to reduce formation of the ternary complex and the activation of downstream signaling pathways. In our experiments, $G_t$ activation is only moderately reduced and arrestin binds to the R135A and R135L mutants whether or not they are prephosphorylated in vitro by rhodopsin kinase, although the levels of arrestin binding are higher after in vitro phosphorylation. Perhaps an endogenous member of the GRK family partly phosphorylates these rhodopsin mutants during their expression in HEK-293 cells, allowing that fraction of rhodopsin to bind arrestin in vitro. Phosphorylation of these mutants by an endogenous kinase would be expected to reduce the apparent level of phosphorylation in in vitro assays, due to prior phosphorylation of some of the serine or threonine residues in the cells. Therefore, although we observe an enhanced level of phosphorylation of R135A in the light compared to wild-type rhodopsin, the actual level may be even higher. The equivalent mutants of other receptors such as those mentioned above may be phosphorylated by endogenous GRKs in a similar fashion, contributing to the observed decrease in receptor−G protein coupling.

In comparison to our results with the Arg-135 mutants, Glu-113, Ala-292, and Lys-296 mutants (E113Q, A292E, K296E, K296G, and K296M), which are phosphorylated by rhodopsin kinase and bind arrestin in the absence of 11-*cis*-retinal, were also shown to constitutively activate $G_t$ (*25*−*27*), although Robinson et al. reported that K296G activated $G_t$ in the absence of 11-*cis*-retinal but was not phosphorylated (*28*). Mutants of the $\alpha_2$- and $\beta_2$-adrenergic receptors that are able to activate their G proteins in the absence of ligand were likewise shown to be phosphorylated by GRK2 under the same conditions (*29*, *30*). The majority of these studies imply that the conformation of GPCRs that interacts with the G protein is the same as the one that is phosphorylated by GRKs and binds arrestin (at least in the case of rhodopsin) and that these mutations shift the equilibrium from an inactive to an active form of rhodopsin in the absence of 11-*cis*-retinal. In contrast, mutations at Arg-135 appear to generate a conformation of rhodopsin that interacts specifically with the GRK and arrestin but not with its G protein in the absence of 11-*cis*-retinal. Therefore, transient conformations of rhodopsin may exist that interact selectively with these proteins and Arg-135 mutants may promote a shift in equilibrium in favor of such a conformation. Hofmann et al. reported that incubation of *all-trans*-retinal with opsin generates a pseudophotoproduct with spectral properties similar to Meta II that binds rhodopsin kinase and arrestin but not $G_t$ (*31*). In addition, Meta I is predicted to bind rhodopsin kinase but not $G_t$ or arrestin, whereas Meta II interacts with all three of these proteins (*32*). In our experiments, the Arg-135 mutants interact with both rhodopsin kinase and arrestin in the absence of 11-*cis*-retinal. Therefore it is unlikely that they approximate a Meta I conformation but they might be similar to the pseudophotoproduct formed by opsin plus *all-trans*-retinal.

Molecular modeling has placed the Arg-135 side chain in a polar pocket that lies between transmembrane domains I, III, VI, and VII (*33*, *9*). Upon receptor activation, this side chain is predicted to move out of the polar pocket toward the cytoplasmic surface, creating a conformation that favors G protein coupling. All mutations in spatially diverse regions of the $\alpha_{1B}$-adrenergic receptor that result in constitutive activation of its G protein are shown by molecular modeling to shift the arginine side chain out of the polar pocket (*9*). However, our results and those of Cohen et al. (*7*) suggest that this Arg is not essential for G protein coupling, since the ability to activate $G_t$ is preserved. To date, the consequence of mutagenesis of Arg-135 on the position of residues within the polar pocket has not been analyzed.

Ultimately, one of the more interesting questions concerns the effect of Arg-135 mutations on its signaling properties in vivo in patients with ADRP. As described above, K296E, a mutant that is unable to form a Schiff base with 11-*cis*-retinal, was found to constitutively activate $G_t$, to serve as a substrate for rhodopsin kinase, and to bind arrestin (*26*, *27*). Li et al. (*25*) observed that K296E was irreversibly bound to arrestin in transgenic mice. Therefore this mutant is unable to activate $G_t$ in vivo. Similar results might be expected for the Arg-135 mutants. R135A and R135L differ from K296E in that they binds 11-*cis*-retinal normally. However, once retinal is released, our studies predict that arrestin will remain bound to R135A and R135L, blocking the dephosphorylation of rhodopsin, a necessary step in its regeneration that is predicted to occur only after arrestin release (*32*). The creation of transgenic mice expressing the R135A and R135L mutants will be an important step in the investigation of the functional consequences of such mutations.

## ACKNOWLEDGMENT

We thank Drs. Paul Hargrave, Jeremy Nathans, and Toshimichi Shinohara for gifts of reagents used in our studies.

## REFERENCES

1. Probst, W. C., Synder, L. A., Schuster, D. I., Brosius, J., and Sealfon, S. C. (1992) *DNA Cell Biol. 11*, 1−20.
2. Sung, C.-H., Davenport, C. M., Hennessey, J. C., Maumenee, I. H., Jacobson, S. G., Heckenlively, J. R., Nowakowski, R., Fishman, G., Gouras, P., and Nathans, J. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 6481−6485.
3. Steele, F. R. (1994) *J. Natl. Inst. Health Res. 6*, 58−62.
4. Franke, R. R., Sakmar, T. P., Graham, R. M., and Khorana, G. H. (1992) *J. Biol. Chem. 267*, 14767−14774.
5. Min, K. C., Zvyaga, T. A., Cypess, A. M., and Sakmar, T. P. (1993) *J. Biol. Chem. 268*, 9400−9404.
6. Acharya, S., and Karnik, S. (1996) *J. Biol. Chem. 271*, 25406−25411.
7. Cohen, G. B., Yang, T., Robinson, P. R., and Oprian, D. D. (1993) *Biochemistry 32*, 6111−6115.
8. Rosenthal, W., Antaramian, A., Gilbert, S., and Birnbaumer, M. (1993) *J. Biol. Chem. 268*, 13030−13033.
9. Scheer, A., Fanelli, F., Costa, T., Debenedetti, P. G., and Cotecchia, S. (1996) *EMBO J. 15*, 3566−3578.
10. Zhu, S. Z., Wang, S. Z., Hu, J. R., and El-Fakahany, E. E. (1994) *Mol. Pharmacol. 45*, 517−523.
11. Jones, P. G., Curtis, C. A. M., and Hulme, E. C. (1995) *Eur. J. Pharmacol. Mol. Pharm. 288*, 251−257.

12. Wistow, G. J., Katial, A., Craft, C., and Shinohara, T. (1986) *FEBS Lett. 196*, 23−28.
13. Adamus, G., Zam, Z. S., Arendt, A., Palczewski, K., McDowell, J. H., and Hargrave, P. A. (1991) *Vision Res. 31*, 17−31.
14. Nathans, J., and Hogness, D. S. (1983) *Cell 34*, 807−814.
15. Weiss, E. R., Osawa, S., Shi, W., and Dickerson, C. D. (1994) *Biochemistry 33*, 7587−7593.
16. Nathans, J. (1990) *Biochemistry 29*, 937−942.
17. Shi, W., Osawa, S., Dickerson, C. D., and Weiss, E. R. (1995) *J. Biol. Chem. 270*, 2112−2119.
18. Zhang, L., Sports, C. D., Osawa, S., and Weiss, E. R. (1997) *J. Biol. Chem. 272*, 14762−14768.
19. Weiss, E. R., Kelleher, D. J., and Johnson, G. L. (1988) *J. Biol. Chem. 263*, 6150−6154.
20. Kelleher, D. J., and Johnson, G. L. (1985) *J. Cyclic Nucleotide Protein Phosphorylation Res. 10*, 579−591.
21. Kelleher, D. J., and Johnson, G. L. (1990) *J. Biol. Chem. 265*, 2632−2639.
22. Sung, C. H., Davenport, C. M., and Nathans, J. (1993) *J. Biol. Chem. 268*, 26645−26649.
23. Sung, C.-H., Schneider, B. G., Agarwal, N., Papermaster, D. S., and Nathans, J. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 8840−8844.
24. Robinson, P. R., Cohen, G. B., Zhukovsky, E. A., and Oprian, D. D. (1992) *Neuron 9*, 719−725.
25. Li, T., Franson, W. K., Gordon, J. W., Berson, E. L., and Dryja, T. P. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 3551−3555.
26. Rim, J., and Oprian, D. D. (1995) *Biochemistry 34*, 11938−11945.
27. Rim, J., Faurobert, E., Hurley, J. B., and Oprian, D. D. (1997) *Biochemistry 36*, 7064−7070.
28. Robinson, P. R., Buczylko, J., Ohguro, H., and Palczewski, K. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 5411−5415.
29. Ren, Q., Kurose, H., Lefkowitz, R. J., and Cotecchia, S. (1993) *J. Biol. Chem. 268*, 16483−16487.
30. Pei, G., Samama, P., Lohse, M., Wang, M., Codina, J., and Lefkowitz, R. J. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 2699−2702.
31. Hofmann, K. P., Pulvermüller, A., Buczylko, J., Van Hooser, P., and Palczewski, K. (1992) *J. Biol. Chem. 267*, 15701−15706.
32. Pulvermüller, A., Palczewski, K., and Hofmann, K. P. (1993) *Biochemistry 32*, 14082−14088.
33. Oliveira, L., Paiva, A. C. M., Sander, C., and Vriend, G. (1994) *Trends Pharmacol. Sci. 15*, 170−172.

BI9731100