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New Concepts

A Conformational Trigger for Activation of a G Protein by a G Protein-Coupled Receptor†

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ABSTRACT: G protein-coupled receptors (GPCRs) are a family of seven transmembrane helical proteins that initiate a cellular response to an environmental signal. Once activated by an extracellular signal, GPCRs trigger the intracellular signal transduction cascade by activating a heterotrimeric G protein. The interaction between the G protein and the receptor, which triggers the signal transduction, is the focus of intense interest. Three-dimensional structures of the ground state of only one GPCR, rhodopsin, are currently available, but since the G protein cannot bind to this structure, these structures did not lead to an understanding of the activation process. The recent publication of an excited state structure for the same GPCR (and comparison to the ground state structures), in conjunction with other recent biochemical data, provides new insight into G protein activation. We find that the structure data and the biochemical data, for the first time, point to a specific mode of interaction between the G protein and the receptor. Furthermore, we find that transducin (G_t) must alter its conformation to bind to the activated receptor; the "lock and key" fit heretofore expected is likely not the correct model. We suggest that a conformational distortion, driven by the energy of binding, is induced in G_t when it binds to the activated receptor. The conformational change in turn enables the exchange of GTP for GDP and the dissociation of the subunits. This is an example of "induced fit" originally proposed by Koshland to describe enzyme−substrate interactions.

Hundreds of G protein-coupled receptors (GPCRs)[1] are known, controlling a wide variety of cellular events. The search for the molecular mechanism of signal transduction of GPCRs extends back to the initial discovery of the roles of G proteins in transmitting signals initiated through a membrane receptor (*1*). These heterotrimeric G proteins bind to activated receptors and in turn become activated so that the G protein α subunits (and sometimes the $\beta\gamma$ subunits) can subsequently modulate target enzyme activity. Understanding the molecular mechanism underlying this signaling pathway has awaited the structural information of the main protein components. Since GPCRs are integral membrane proteins, structural information has been difficult to obtain.

The first three-dimensional structural information for GPCRs has only recently become available. Structural studies on the prototypical GPCR, bovine rhodopsin, have provided three-dimensional structures for rhodopsin (inactive) (*2*, *3*) and metarhodopsin II (light-activated state) (*4*) as well as for G_t (transducin, the G protein that binds to rhodopsin) with GDP bound (*5*). These structures, along with critical, recent biochemical and biophysical data, offer a new molecular view into the process of G protein activation.

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[1] Abbreviations: $G_{t\alpha}$, α subunit of transducin; $G_{t\beta\gamma}$, β and γ subunits of transducin; GDP, guanosine diphosphate; GPCR, G protein-coupled receptor; G_t, transducin; GTP, guanosine triphosphate.

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The contact surfaces for G_t binding to metarhodopsin II, suggested by peptide inhibition studies (*4*, *6*−*8*) and fusion protein studies (*9*), include portions of the first, second, and third cytoplasmic loops and portions of the carboxyl terminus of metarhodopsin II. The C-terminus of the G_α subunit of transducin [in particular, a peptide of 11 amino acids (residues 340−350)] binds to (*10*) and stabilizes (*11*, *12*) metarhodopsin II. Mutational analysis implicated the second and third cytoplasmic loops of metarhodopsin II in binding of this peptide to the cytoplasmic face of this receptor (*13*, *14*).

Analogous experiments have suggested regions on the surface of G_t that are putative contact surfaces for binding to the receptor. These regions include the N-terminal helix and the C-terminus of the α subunit, which have been defined by peptide inhibition studies (*5*, *10*, *11*) and mutational analysis (*15*). These regions are negatively charged (*5*).

A comparison of rhodopsin (inactive) and metarhodopsin II (activated) structures shows that the structure of the receptor changes upon activation. A crevice opens in a groove on the cytoplasmic surface of the receptor in the metarhodopsin II form, between cytoplasmic loops 2 and 3 (*4*). Previously, it was suggested that these two cytoplasmic loops formed a pocket for binding the C-terminus of G_α (*13*). The cytoplasmic face of the receptor contains the putative contact surface that supports G protein binding to the activated receptor. The putative contact surfaces line this groove, which is positively charged (see Figure 1). The crevice opens because ionic interactions among R135 and E134 and E247 that stabilize inactive rhodopsin are eliminated upon activation to metarhodopsin II (*4*), as had been predicted (*16*, *17*).

These data suggest that the conformational change upon activation from (dark-adapted) rhodopsin to metarhodopsin II prepares a surface for G_t binding that is partially occluded in the dark-adapted state of rhodopsin. The observation that the putative contact surface on the receptor is positively charged and that the putative contact surface on the G protein is negatively charged provides a partial basis for the binding of G_t that is observed. The observation that the putative contact surface on the receptor is partially occluded in the inactive receptor provides a partial basis for the binding of G_t only to the activated receptor.

More information is available on how G_t binds to metarhodopsin II. Recent chemical cross-linking data obtained with G_t complexed to metarhodopsin II reveal close contacts between residue 240 on metarhodopsin II (in the third cytoplasmic loop) and $G_{t\alpha}$ residues 19−28 (N-terminal helix), and between that same metarhodopsin II residue and residues 310−313 and 342−345 of G_t (carboxyl-terminal region) (*18*, *19*). These cross-links involve the third cytoplasmic loop on metarhodopsin II, which is part of the putative contact region on the cytoplasmic face of the receptor already identified by inhibition and mutation studies (see above). The N-terminus and the C-terminus of the α subunit of G_t are both part of the putative binding surface (*5*, *11*, *15*) and are partners in the cross-linking with the third cytoplasmic loop of the receptor.

Recent experiments have further linked the amino terminus of the α subunit of G_t in binding to metarhodopsin II. The data suggested that the amino terminus is ordered when the α subunit is bound to the $\beta\gamma$ subunits of G_t and transducin has GDP bound. However, after G_t binds to the receptor and GDP−GTP exchange takes place, the amino terminus is disordered (*20*). These data suggest that the amino terminus experiences a conformational change during the activation process involving the receptor such that helix-stabilizing interactions between the N-terminus and the remainder of G_t in the inactive G protein are altered in the activated G protein.

With these data, it is possible to begin to map the interactions that likely occur when G_t binds to metarhodopsin II. To simultaneously achieve the short distances between the cross-linked regions, bring the putative contact regions on both proteins (identified above) into close apposition, and avoiding severe steric problems limits the possible orientations of G_t and metarhodopsin II largely to the one identified in Figure 1. In this model, the N-terminal helix of the $G_{t\alpha}$ subunit binds to the groove that becomes more exposed on the surface when metarhodopsin II is formed. The opposite charges of these contact surfaces provide favorable binding energy. The C-terminus of $G_{t\alpha}$ is disordered in the crystal structure. However, recent NMR data have shown that the carboxyl terminus (1AQG) is bound to activated rhodopsin at an angle of ∼40° with respect to the bilayer normal and that the conformation is ordered and helical when bound (*21*). In Figure 1, this structure was added to the crystal structure of G_t by superimposing the region of overlap between the peptide and the crystal structure. In the model, the C-terminus of $G_{t\alpha}$ binds to the crevice at the bottom of the groove in the cytoplasmic surface of metarhodopsin II, between cytoplasmic loops 2 and 3. This mode of binding places residues K340 and D341 (1GOT) of $G_{t\alpha}$ close to R135 and E134 (part of the highly conserved ERY sequence) of metarhodopsin II, which are separated from E247 upon activation to metarhodopsin II. The C-terminus of G_t may substitute in part for the interactions of E134 and R135 with E247. This may explain why a small peptide from the C-terminus of $G_{t\alpha}$ stabilizes metarhodopsin II (*22*), inhibiting the re-formation of the R135−E134−E247 cluster, and why D341 of $G_{t\alpha}$ shows a strong interaction with metarhodopsin II in these experiments (*21*). It is also consistent with the observation that cytoplasmic loops 2 and 3 form a pocket for binding the C-terminus of G_α (*13*).

The binding of G_t to metarhodopsin II must trigger a conformational change in G_t that favors GDP−GTP exchange and dissociation of the $G_{t\alpha}$ and $G_{t\beta\gamma}$ subunits. Once G_t is fit to the surface of metarhodopsin II according to the requirements described above, the acidic N-terminal helix of $G_{t\alpha}$ encounters the basic groove on metarhodopsin II (Figure 1), but some steric difficulties arise between the carboxyl terminus of metarhodopsin II and G_t; therefore, the two structures cannot dock without a conformational change. Movement of the N-terminal helix of $G_{t\alpha}$ would alleviate the packing problems. Data referenced above suggest a conformational change occurs at the N-terminal helix sometime in the activation of G_t. This movement would alter the interactions between the N-terminal helix and the $\beta\gamma$ subunits, consistent with the observation of a conformational change in the N-terminus when the α subunit is separated from the remainder of the protein. Interestingly, the N-terminal helix connects to a β sheet that contains the switch I region and contacts the switch II region of $G_{t\alpha}$ [previously identified as regions of conformational change in G_t activa-

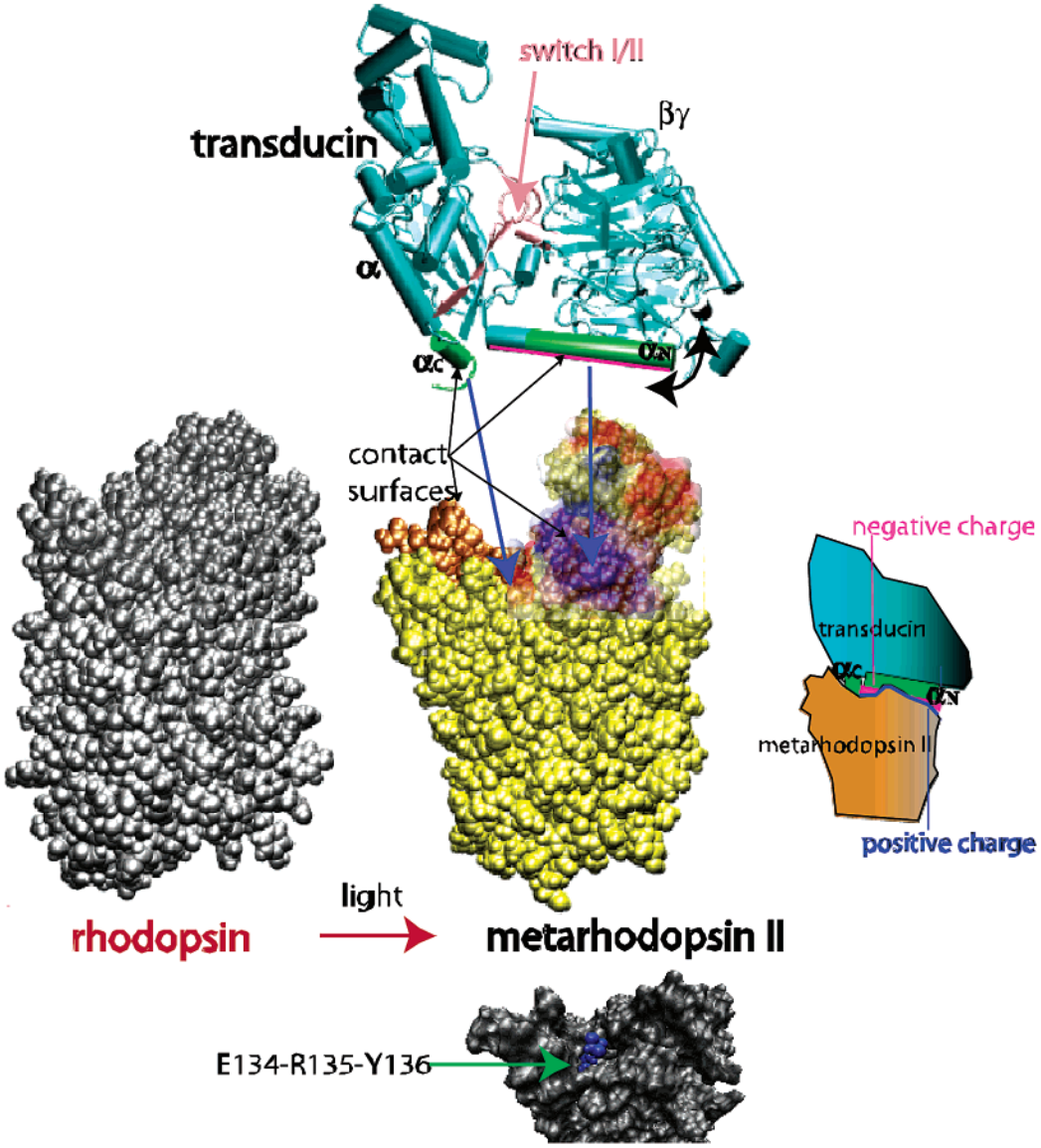

FIGURE 1: Binding of transducin to metarhodopsin II. The structure (1F88 and 1JFP) of rhodopsin (dark-adapted) is at the lower left in gray. Upon absorption of a photon of light, metarhodopsin II (1LN6) is formed (transiently), and that structure is yellow. The heterotrimer of transducin appears at the top in cyan with the α and $\beta\gamma$ subunits labeled, the switch I−switch II region colored pink [previously identified as regions of conformational change in G_t activation (*5*)], and the amino-terminal helix and C-terminus of $G_{t\alpha}$ labeled. A potential surface is overlaid on the cytoplasmic face of metarhodopsin showing positive charge in purple and negative charge in red-orange. The positively charged region lines part of the groove on the surface (see the text). Contact surfaces previously identified (see the text) are labeled green on G_t and orange (and the purple positively charged region in the groove which is a subset of the contact surface) on metarhodopsin II. The amino-terminal helix of $G_{t\alpha}$ is negatively charged on the contact face, and that contact face is magenta. Next to this helix is a double-headed arrow indicating that a conformational change in this region is necessary to bind G_t to metarhodopsin II. Blue single-headed arrows point from the contact regions of G_t to the likely binding sites on the surface of metarhodopsin II (see the text). The cartoon on the right schematically represents the complex of G_t with metarhodopsin II. At the bottom, a view of the groove on the cytoplasmic surface is offered from a somewhat different angle with the ERY sequence of metarhodopsin II labeled in blue. Molecular structures were drawn in part with VMD (*25*).

tion (*5*)]. Consequently, this N-terminal helix could act as a molecular trigger that, upon binding of G_t to metarhodopsin II, moves and creates a transition state of G_t. The conformational change in the C-terminus induced upon binding to the active receptor may also cooperate in the trigger mechanism. The transition state of G_t allows GDP−GTP exchange and dissociation of $G_{t\alpha}$ and $G_{t\beta\gamma}$.

These data collectively suggest a hypothesis in which the required change in the G_t conformation is driven by a favorable free energy of binding to the receptor, analogous to the initially unexpected finding that the energy source for ATP synthesis is derived from the energy of substrate binding (*23*). In the future, expectations for interactions between receptors and G proteins should include an "induced fit" at the heart of the signal transduction mechanism, analogous to that proposed by Koshland to describe enzyme−substrate interactions (*24*).

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REFERENCES

1. Freissmuth, M., Casey, P. J., and Gilman, A. G. (1989) *FASEB J. 3*, 2125−2131.
2. Palczewski, K., Kumasaka, T., Hori, T., Behnke, C. A., Motoshima, H., Fox, B. A., Le Trong, I., Teller, D. C., Okada, T., Stenkamp, R. E., Yamamoto, M., and Miyano, M. (2000) *Science 289*, 739−745.
3. Yeagle, P. L., Choi, G., and Albert, A. D. (2001) *Biochemistry 40*, 11932−11937.
4. Choi, G., Landin, J., Galan, J. F., Birge, R. R., Albert, A. D., and Yeagle, P. L. (2002) *Biochemistry 41*, 7318−7324.
5. Lambright, D. G., Sonked, J., Bohm, A., Skiba, N. P., Hamm, H. E., and Sigler, P. B. (1996) *Nature 379*, 311−319.
6. Marin, E. P., Krishna, A. G., Zvyaga, T. A., Isele, J., Siebert, F., and Sakmar, T. P. (2000) *J. Biol. Chem. 275*, 1930−1936.
7. Ernst, O. P., Meyer, C. K., Marin, E. P., Henklein, P., Fu, W.-Y., Sakmar, T. P., and Hofmann, K. P. (2000) *J. Biol. Chem. 275*, 1937−1943.
8. Konig, B., Arendt, A., McDowell, J. H., Kahlert, M., Hargrave, P. A., and Hofmann, K. P. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 6878−6882.
9. Abdulaev, N. G., Ngo, T., Chen, R., Lu, Z., and Ridge, K. D. (2000) *J. Biol. Chem. 275*, 39354−39363.
10. Natochin, M., Muradov, K. G., McEntaffer, R. L., and Artemyev, N. O. (2000) *J. Biol. Chem. 275*, 2669−2675.
11. Hamm, H. E., Deretic, D., Arendt, A., Hargrave, P. A., Koenig, B., and Hofmann, K. P. (1988) *Science 241*, 832−835.
12. Acharya, S., and Karnik, S. S. (1996) *J. Biol. Chem. 271*, 25406−25411.
13. Acharya, S., Saad, Y., and Karnik, S. S. (1997) *J. Biol. Chem. 272*, 6519−6524.
14. Yamashita, T., Terakita, A., and Shichida, Y. (2000) *J. Biol. Chem. 275*, 34272−34279.
15. Onrust, R., Herzmark, P., Chi, P., Garcia, P. D., Lichtarge, O., Kingsley, C., and Bourne, H. R. (1997) *Science 275*, 381−384.
16. Ballesteros, J. A., Jensen, A. D., Liapakis, G., Rasmussen, S. G. F., Shi, L., Gether, U., and Javitch, J. A. (2001) *J. Biol. Chem. 276*, 29171−29177.
17. Shapiro, D. A., Kristiansen, K., Weiner, D. M., Kroeze, W. K., and Roth, B. L. (2002) *J. Biol. Chem. 277*, 11441−11449.
18. Itoh, Y., Cai, K., and Khorana, H. B. (2001) *Proc. Natl. Acad. Sci. U.S.A.* 98, 4883−4887.
19. Cai, K., Itoh, Y., and Khorana, H. B. (2001) *Proc. Natl. Acad. Sci. U.S.A. 98*, 4877−4882.
20. Medkova, M., Preininger, A. M., Yu, N. J., Hubbell, W. L., and Hamm, H. E. (2002) *Biochemistry 41*, 9962−9972.
21. Koenig, B., Kontaxis, G., Mitchell, D., Louis, J., Litman, B., and Bax, A. (2002) *J. Mol. Biol. 322*, 441.
22. Koenig, B. W., Mitchell, D. C., Konig, S., Grzesiek, S., Litman, B. J., and Bax, A. (2000) *J. Biomol. NMR 16*, 121−125.
23. Boyer, P. D. (2000) *Biochim. Biophys. Acta 1458*, 252−262.
24. Koshland, D. E. (1958) *Proc. Natl. Acad. Sci. U.S.A. 44*, 98.
25. Humphrey, W., Dalke, A., and Schulten, K. (1996) *J. Mol. Graphics 14*, 33−38.

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