Mechanism of Action of Monoclonal Antibodies That Block the Light Activation of the Guanyl Nucleotide-binding Protein, Transducin*

(Received for publication, December 5, 1986)

Heidi E. Hamm†, Dusanka Deretic‡, Klaus P. Hofmann§, Andreas Schleicher§, and Burkhard Kohl¶

From the 1Department of Physiology and Biophysics, University of Illinois College of Medicine at Chicago, Chicago, Illinois 60680 and the 2Institut für Biophysik und Strahlenbiologie, Albert-Ludwigs-Universität, D-7800 Freiburg, Federal Republic of Germany

Seven monoclonal antibodies to the α subunit (G,) of the frog photoreceptor guanyl nucleotide-binding protein (transducin or G-protein) have been characterized as to their effect on G-protein function, and this has been correlated in the accompanying paper (Deretic, D., and Hamm, H. E. (1987) J. Biol. Chem. 262, 10839–10847) with the antibody-binding sites on G,-tryptic fragments. Antibodies 4A, 7A, 7B, 7C, and 7D are members of a class of antibodies that block G-protein activation by light and therefore also block activation of the cGMP phosphodiesterase. All these blocking antibodies also block the interaction of G-protein with rhodopsin as measured by the light-scattering “binding signal,” and as measured by the stabilization of meta-rhodopsin II by bound G-protein (extra-meta-rhodopsin II). The antibodies (or Fab fragments) also solubilize G,α from the membrane in the dark under isosmotic conditions and thus interfere with G, interaction with the membrane. Antibody 4A also blocks the extra-meta-rhodopsin II generated by G-protein-rhodopsin interaction in detergent solubilized membranes. Thus, even in the absence of phospholipids, antibody 4A blocks G-protein-rhodopsin interaction. Therefore, we suggest that the antibodies recognize a region of G, involved with binding to rhodopsin. An alternative hypothesis is that this antigenic site is a region of interaction between the α and βγ subunits, disruption of this interaction leading to removal of both the α and βγ subunits from the membrane and blocking interaction with rhodopsin. This does not seem to be the case because the antibodies immunoprecipitate the αβγ complex, and not just the α subunit. Other antibodies, 4C and 4H, do not block phosphodiesterase activation, the light-scattering signal, extra-meta-rhodopsin II formation, or interaction with the membrane in the dark and therefore recognize other sites on G,.

The transduction of a variety of signals, including light and many hormones and neurotransmitters, is accomplished via a cascade of protein interactions whose essential features are very similar, while their final effects may be quite disparate. Hormone receptors, or the light receptor rhodopsin, interact with guanyl nucleotide-binding proteins which interact with effectors such as adenylyl cyclase, cGMP phosphodiesterase, or phosphoinositide phosphodiesterase. Recent evidence suggests that these receptors constitute a related class of proteins (Dixon et al., 1986; Martin et al., 1986); in the case of the guanyl nucleotide-binding proteins (G-proteins) linking hormone receptors to their effectors, it is already clear that there is a family of G-proteins with similar structure and function (Bitek et al., 1982; Manning and Gilman, 1983; Cerione et al., 1986; Lochrie et al., 1986; Medynski et al., 1985; Tanabe et al., 1986; Yatsunami and Khorana, 1985; Robishaw et al., 1986; Nakada et al., 1986a, 1986b). These G-proteins belong to a larger family of guanyl nucleotide-binding proteins which use GTP-GDP exchange as a triggering mechanism including tubulin, elongation factors (Halliday, 1984), and the ras oncogenic protein p21 (Hurley et al., 1984).

In photoreceptors, the GTP-GDP exchange reaction of the GTP-binding protein (transducin or referred to here as G-protein) is triggered by interaction with activated rhodopsin (Godchaux and Zimmerman, 1979; Fung and Stryer, 1980), and activated G-protein in turn activates a cGMP phosphodiesterase (Fung et al., 1981). G-protein has been purified and found to be a heterotrimer, αβγ (Kühn, 1980; Fung et al., 1981; Baehe et al., 1982), and several functional sites have been identified. The GTP-binding regions of the molecule are found on the α subunit and have been identified by determining homologies with nucleotide-binding sites on other proteins (Hurley et al., 1984; McCormick et al., 1985), and cholera and pertussis toxin ADP-ribosylation sites have been determined (Abood et al., 1982; West et al., 1985). Functional sites that have not yet been determined include sites of interaction with rhodopsin and phosphodiesterase. The amino acid sequence of G, has recently been deduced from the cDNA sequence by several laboratories (Tanabe et al., 1985; Medynski et al., 1985; Yatsunami and Khorana, 1985).

A series of monoclonal antibodies was generated to test functional roles of G-protein in phototransduction (Hamm and Bownds, 1984; Witt et al., 1984). One of these antibodies, mAb 4A, was found to block light-activated GTP-GDP exchange and cGMP phosphodiesterase, whereas other antibodies including mAb 4H did not have this effect (Hamm and Bownds, 1984). In addition, these antibodies are being used 1The abbreviations used are: G-protein, photoreceptor guanyl nucleotide-binding protein; G,α subunit of G-protein; G, and G, regulatory guanyl nucleotide-binding proteins that mediate stimulation and inhibition of adenylyl cyclase, respectively; G, GTP-binding protein of unknown function; ROS, rod outer segment; GppNHp, guanosine 5′-(3’,5’-imido)triphosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid; MOPS, 3-(N-morpholino)propanesulfonic acid; EGTA, (ethyleneglycol)tetraacetic acid; SDS, sodium dodecyl sulfate; mAb, monoclonal antibody.

10831
for elucidation of structure-function relationships of G-proteins. In this study, we examined the mechanism of action of mAb 4A blockade, as well as four independently derived monoclonal antibodies that block G-protein activation by light. All five antibodies appear to block a site on the G-protein that interacts with rhodopsin. In the accompanying paper (Deretic and Hamm, 1987), the antigenic sites of the blocking antibodies are shown to be very near the COOH terminus of the G-protein α subunit; the antigenic site of mAb 4H, which does not block activation, is on another region of the molecule.

MATERIALS AND METHODS

Preparation of Rod Outer Segments, Rod Membranes, and Proteins—Percoll-purified rod outer segments (ROS) were prepared, and fractionation of ROS proteins into soluble, peripheral, and membrane fractions was done as described by Hamm and Bovda (1986). Bovine ROS were prepared according to Papamaster and Dreyer (1974). Bovine ROS disc membranes were prepared according to Smith et al. (1975) with the modification of 2.5% Ficoll instead of 5% (Bauer and Mavromatti, 1980). Extraction of bovine soluble proteins was performed according to the method of Kühn (1980) as described by Emeis and Hofmann (1981). Bovine G-protein was prepared according to Fung (1983).

Cyclic GMP Phosphodiesterase—This was measured as described by Hamm and Bovda (1984) using the proton evolution assay described by Lieberman (1972). Purified ROS (10 mg rhodopsin) were suspended in Ringer’s solution (115 mM NaCl, 2 mM KCl, 2 mM MgCl₂, 0.1 mM CaCl₂), adjusted to 10⁻⁸ M with 0.39 mM EDTA, 10 mM HEPES, pH 7.8, with 50 kallikrein-inactivating units of Trasylol and 10 μM leupeptin), disrupted by passage through a 26-gauge needle, and heated to 70 °C in response to a flash of light bleaching rhodopsin.

Immunoprecipitation of G-protein—This was performed as follows. Purified G-protein, labeled with [35S]iodoamphetamine azide (Berkovic and Gifiler, 1978) was incubated with different antibodies to a ratio of antibodies to G-protein of 2:1. Formalin-fixed Staphylococcus aureus cells (Bethesda Research Laboratories) were centrifuged at 3000 × g for 10 min, and the cell pellet was resuspended in an equal volume of phosphate-buffered saline, pH 7.2, containing 10% (w/v) β-mercaptoethanol and 3% (w/v) SDS and boiled for 30 min to reduce the protein background. After centrifugation at 3000 × g for 10 min, the cells were washed in 150 mM NaCl, 5 mM EDTA, 50 mM Tris, pH 7.4, 0.02% sodium azide, 0.5% Nonidet P-40 (NET buffer), centrifuged, and resuspended in the same buffer at 10% (w/v). The washed cell suspension (200 μl) was then added to protein-antibody complex containing 10 μg of antibody and incubated for 1 h at room temperature. Precipitates were centrifuged for 10 min at 3000 × g and washed three times with NET buffer. The immunoprecipitated proteins were eluted from S. aureus cells by boiling for 5 min in Laemmli (1970) sample buffer and run on 12.5% SDS-polyacrylamide gel electrophoresis. Autoradiography of the dried gel was performed using Kodak X-AR2 film and an intensifying screen for 72 h at −70 °C.

Preparation of Fab Fragments—This was performed as follows. 10 mg of Protein A-purified mAb 4A in 1.8 ml of Ringer’s solution was dialyzed against 0.1 M glycine, pH 2.0, 5.5. The solution was then added to a protein-A-Sepharose column containing 10 μg of antibody and incubated for 1 h at room temperature. Precipitates were centrifuged for 10 min at 3000 × g and washed three times with NET buffer. The immunoprecipitated proteins were eluted from S. aureus cells by boiling for 5 min in Laemmli (1970) sample buffer and run on 12.5% SDS-polyacrylamide gel electrophoresis. No residual complete antibody molecules were detected on Coomassie Blue-stained gels.

RESULTS

A series of monoclonal antibodies was generated to the α subunit of the photoreceptor G-protein and was screened to find antibodies that block functional sites on G-protein (Hamm and Bovda, 1984). Fig. 1 shows that one of these, antibody 4A, blocks light activation of cGMP phosphodiesterase, whereas another antibody of the series, 4H, has no effect. To investigate the mechanism of action of the blocking antibody, we followed the phosphodiesterase activation pathway backwards, examining the effect of the antibody on each step that could potentially be perturbed and could be measured. Antibody 4A blocks the light-activated GTP-GDP exchange reaction (Hamm and Bovda, 1984), whereas antibody 4H had no effect on this reaction (data not shown). To examine whether the antibody blocked activation of G-protein

6 Schleicher, A., Franke, O., Hofmann, K. P., Finkelman, O., and Welte, O. (1987) Biochemistry, in press.
Antibody Blockade of G-protein-Rhodopsin Interaction

by blocking interaction with rhodopsin, two complementary methods were used: light scattering (Kühn et al., 1981; Bennett et al., 1982) and extra-meta-rhodopsin II formation (Emeis and Hofmann, 1981; Emeis et al., 1982). G-protein interaction with the membrane was examined by centrifugation experiments that quantitated the amount of G-protein in the supernatant or the pellet.

The light-scattering signal measured at 704 nm in the absence of GTP is based on the observation that flash illumination causes fast changes of the light scattering of disc membranes (Hofmann et al., 1976), representing an increase in the turbidity of the ROS suspension as a consequence of G-protein binding to rhodopsin. This signal is a stoichiometric measure of the G-protein bound to rhodopsin (Kühn et al., 1981; Bennett et al., 1982). We examined the effect of several anti-G, antibodies on the light-scattering binding signal. Fig. 2A shows that antibody 4A blocks more than 80% of the binding signal in response to a flash of light bleaching 2% of the rhodopsin. In the presence of antibody 4H or nonspecific control IgG, the binding signal was undistinguishable from the control. The antibodies were present at an excess of 5:1 over G-protein in this experiment; however, in experiments where the molar ratio of antibody to G-protein was varied, we showed that the blockade was complete at a 1:1 stoichiometry between antibody 4A and G-protein (data not shown). In this experiment, the ROS were preincubated with antibody for 30 min, but varying the preincubation time showed that the antibody inhibition developed rapidly: the half-time for the inhibitory effect is less than 20 s, which was the shortest preincubation time we could reliably measure (data not shown). The lack of effect of antibody 4H is not due to a lower affinity for G-protein; Witt et al. (1984) showed that the antibody 4A and 4H affinities are very similar. Also, antibody 4H did not block the binding signal even after overnight incubation (data not shown). Although antibody 4H had no effect on the response to one flash (Fig. 2A), antibody 4H decreased the response to a second flash given to the same sample, as compared to control IgG. Fig. 2B shows the effect of four other G, antibodies on the binding signal. Antibodies 7A, 7B, 7C, and 7D all block the binding signal to a similar extent as antibody 4A at a molar ratio of 1:1 with G-protein.

Because of its complex generation mechanism, suppression of the binding signal does not necessarily indicate blocking G-protein interaction with rhodopsin. Thus, it was possible that the antibody blocks this physical consequence without blocking G-protein interaction with rhodopsin. A different monitor of G-protein-rhodopsin interaction uses the fact that the binding conformation of rhodopsin coincides with the 380 nm intermediate meta-rhodopsin II (Emeis et al., 1982; Bennett et al., 1982). meta-Rhodopsin II is in equilibrium with its tautomeric form, meta-rhodopsin I (Matthews et al., 1963; Parke and Liebman, 1984). Stabilization of meta-rhodopsin II by bound G-protein expresses itself in a shift of this equilibrium toward meta-rhodopsin II. The resulting enhanced formation of meta-rhodopsin II (so called extra-meta-rhodopsin II; Emeis and Hofmann, 1981) is easily measured because of the large spectral differences between meta-rhodopsin I (maximally absorbing at 480 nm) and meta-rhodopsin II (380 nm).

We examined the effect of the blocking antibody on extra-meta-rhodopsin II formation, a more direct measure of G-protein-rhodopsin interaction. The first two curves of Fig. 3A show the effect of light bleaching 3% rhodopsin on meta-rhodopsin II measured spectrophotometrically in the absence or presence of 100 μM GTP. In the presence of high concentrations of GTP, no extra-meta-rhodopsin II can be observed because the level of transiently formed G-protein-rhodopsin complexes is too small. Under these experimental conditions, this measurement is therefore a good control for the equilibrium level of meta-rhodopsin II without interference of G-protein. The difference between the first two curves represents the extra-meta-rhodopsin II generated by stable G-protein-rhodopsin binding. The third curve of Fig. 3A shows that in the presence of low concentrations of the nonhydro-
Antibody Blockade of G-protein-Rhodopsin Interaction

FIG. 3. Formation of extra-meta-rhodopsin II, a measure of rhodopsin-G-protein interaction in bovine disc membranes recombined with low ionic strength extract, is blocked by some antibodies. A, extra-meta-rhodopsin II is formed after a flash of light bleaching 3% rhodopsin in the absence of GTP (first curve), compared to the normal amount of meta-rhodopsin II formed in the presence of 100 μM GTP (second curve). At 20 μM GppNHp, extra-meta-rhodopsin II forms but then decays at a rate proportional to the activation of G-protein (third curve). Experiments were performed at pH 7.5 and 3 °C; all signals are from the first flash. B, two antibodies, 4H and 4C, have no effect on either extra-meta-rhodopsin II formation or its loss, whereas antibody 4A blocks the extra-meta-rhodopsin II signal as well as its decay in a dose-dependent manner. Control IgG had no effect (not shown). C, comparison of the effect of different antibodies (1:1 molar ratio) on stable extra-meta-rhodopsin II formation. Change of the absorbance difference due to the amount of extra-meta-rhodopsin II formed by the first flash, compared to the 100 μM GTP control, which gives the final absorbance level determined by the meta-rhodopsin I-meta-rhodopsin II equilibrium without interference of G-protein. WM+EX, washed disc membranes reconstituted with protein extract, control without antibody; IgG, nonspecific control antibody. Conditions were: 2 °C, pH 8.0, flash bleaching 3% rhodopsin.
 antibody 4A solubilizes G-protein from the disc membrane under isosmotic conditions in the dark, whereas antibody 4H or control IgG had no effect on G-protein localization. Frog ROS membranes were incubated in the dark for 30 min with Ringer's. A 2:1 excess of control IgG, antibody 4A, Fab 4A or 4H, and then membranes were separated from soluble proteins by centrifugation. The supernatant was run on a 12.5% SDS-polyacrylamide gel and stained with Coomassie Blue, and the density of protein stain in the supernatant even with large bleaches. The strong antibody blockade of G-protein-rhodopsin interaction does not appear to require membrane phospholipids.

An alternative hypothesis is that this antigenic site is a region of interaction between the α and β subunits. Fung (1983) showed that the subunits of the G-protein must be together in an αβγ complex for effective interaction with the ROS membrane. Interruption of interaction between the α and βγ subunits would result in all the functional effects shown in Figs. 1–5, resulting from an inability of the individual subunits to bind to the membrane. If antibody 4A interrupted αβγ interaction, one would expect it would immunoprecipitate only the α subunit, with the βγ subunit left behind in the supernatant. Immunoprecipitation experiments attempting to test this hypothesis are shown in Fig. 6. Purified G-protein radiolabeled with \(^{125}\)Iodonaphthyl azide was incubated in the presence of antibodies 4A, 4H, or IgG, and then formalin-fixed S. aureus cells were used to precipitate G-protein-antibody complexes. The first lane shows the start of interaction between the

\[\frac{\Delta t}{t} = 1 \times 10^{-3}\]

antibody blockade of G-protein-rhodopsin interaction does not appear to require membrane phospholipids.

An alternative hypothesis is that this antigenic site is a region of interaction between the α and β subunits. Fung (1983) showed that the subunits of the G-protein must be together in an αβγ complex for effective interaction with the ROS membrane. Interruption of interaction between the α and βγ subunits would result in all the functional effects shown in Figs. 1–5, resulting from an inability of the individual subunits to bind to the membrane. If antibody 4A interrupted αβγ interaction, one would expect it would immunoprecipitate only the α subunit, with the βγ subunit left behind in the supernatant. Immunoprecipitation experiments attempting to test this hypothesis are shown in Fig. 6. Purified G-protein radiolabeled with \(^{125}\)Iodonaphthyl azide was incubated in the presence of antibodies 4A, 4H, or IgG, and then formalin-fixed S. aureus cells were used to precipitate G-protein-antibody complexes. The first lane shows the start of interaction between the

\[\frac{\Delta t}{t} = 1 \times 10^{-3}\]

antibody blockade of G-protein-rhodopsin interaction does not appear to require membrane phospholipids.

An alternative hypothesis is that this antigenic site is a region of interaction between the α and β subunits. Fung (1983) showed that the subunits of the G-protein must be together in an αβγ complex for effective interaction with the ROS membrane. Interruption of interaction between the α and βγ subunits would result in all the functional effects shown in Figs. 1–5, resulting from an inability of the individual subunits to bind to the membrane. If antibody 4A interrupted αβγ interaction, one would expect it would immunoprecipitate only the α subunit, with the βγ subunit left behind in the supernatant. Immunoprecipitation experiments attempting to test this hypothesis are shown in Fig. 6. Purified G-protein radiolabeled with \(^{125}\)Iodonaphthyl azide was incubated in the presence of antibodies 4A, 4H, or IgG, and then formalin-fixed S. aureus cells were used to precipitate G-protein-antibody complexes. The first lane shows the start of interaction between the

\[\frac{\Delta t}{t} = 1 \times 10^{-3}\]

antibody blockade of G-protein-rhodopsin interaction does not appear to require membrane phospholipids.

An alternative hypothesis is that this antigenic site is a region of interaction between the α and β subunits. Fung (1983) showed that the subunits of the G-protein must be together in an αβγ complex for effective interaction with the ROS membrane. Interruption of interaction between the α and βγ subunits would result in all the functional effects shown in Figs. 1–5, resulting from an inability of the individual subunits to bind to the membrane. If antibody 4A interrupted αβγ interaction, one would expect it would immunoprecipitate only the α subunit, with the βγ subunit left behind in the supernatant. Immunoprecipitation experiments attempting to test this hypothesis are shown in Fig. 6. Purified G-protein radiolabeled with \(^{125}\)Iodonaphthyl azide was incubated in the presence of antibodies 4A, 4H, or IgG, and then formalin-fixed S. aureus cells were used to precipitate G-protein-antibody complexes. The first lane shows the start of interaction between the

\[\frac{\Delta t}{t} = 1 \times 10^{-3}\]
inhibit that interaction. Since Fab fragments of mAb 4A also solubilize G-protein and block its interaction with rhodopsin, the antigenic site should be relatively close to the G-protein-rhodopsin interaction site (a Fab interaction site is approximately 20 Å in diameter; Amit et al., 1986). The fact that Fab fragments of antibody 4A are as effective as the bivalent antibody also indicates that cross-linking of G-protein is not necessary for solubilizing G-protein from the membrane.

The finding that G-protein cannot bind to pure phospholipid vesicles but binds well to phospholipid vesicles containing pure rhodopsin, both in the dark and the light (Fung, 1983), suggests that the major site of interaction of G-protein with the membrane is rhodopsin and not an interaction with phospholipids, although phospholipids may play some role. mAb A4 removes G-protein from the ROS membrane in the dark (Fig. 4) and thus disrupts the major site of G-protein binding to the membrane. mAb 4A can also block the light-activated G-rhodopsin binding measured by extra-meta-rhodopsin II formation in reconstituted membranes (Fig. 3) as well as in purified rhodopsin in the absence of phospholipids (Fig. 5), suggesting that mAb 4A directly blocks the interaction between G-protein and rhodopsin. These findings are consistent with the notion that in the dark, the membrane-binding site for G-protein is rhodopsin and that the mAb 4A antigenic site is at or near the site of G-protein-rhodopsin interaction.

The affinity of the putative dark G-protein-rhodopsin site must be relatively low because Kuhn (1984) showed that in the dark, G-protein does not bind to columns of purified rhodopsin bound to concanavalin A-Sepharose, but that in the light, it does bind to the column. The dissociation constant has been estimated as $K_d = 10^{-10}$ to $10^{-9}$ M (depending on the preparation) from membrane mixing experiments (Liebman and Sitaramayya, 1984) and $10^{-5}$ M from light scattering measurements. The dark membrane-binding site saturates at 25% rhodopsin, i.e. 2.5-fold more G-protein can bind than is bound in native membranes (Liebman and Sitaramayya, 1984). The only protein present at such abundance in washed membranes is rhodopsin (Hamm and Bownds, 1986). There is known to be a considerably increased affinity of G-protein for bleached rhodopsin ($K_d = 2 \times 10^{-7}$ to $10^{-6}$ M, depending on GDP binding (Bennett and Dupont, 1985)). Whether the dark binding site is similar or different from the light binding site between photoactivated rhodopsin and G-protein is not known; however, it could be the same site whose affinity increases upon the light-induced conformational change in rhodopsin or a neighboring region. A reactive cysteine is located in the vicinity of the light binding site of $G_{\alpha}$ for rhodopsin (Hofmann and Reichert, 1985). Modification of one SH group at $G_{\alpha}$ by N-ethylmaleimide does not disturb light binding but blocks light binding completely; however, the smaller cysteine group does not block light binding.

If this is so, monoclonal antibodies against rhodopsin at the site of interaction with G-protein should disrupt G-protein binding in the dark. Preliminary experiments have been done using a monoclonal antibody against the COOH-terminal region of rhodopsin, antibody 1D4. G-protein is indeed eluted from the membrane by preincubation with this antibody, although the release is not quantitative. 

Protolytic digest studies have shown that the first cytoplasmic loop near the COOH-terminus of rhodopsin is an important region of G-
protein binding on rhodopsin (Kühn and Hargrave, 1981). Monoclonal antibodies are currently being generated to this region, and their effects on G-protein binding will be tested.

The question arises whether such a G-protein-rhodopsin interaction in the dark would result in a relatively long-lived preformed complex between G-protein and rhodopsin so that 10% of the rhodopsin might be activated more rapidly than freely diffusing components. There is evidence in other systems of a complex between receptors and CTP-binding proteins. In a purification of the α2-adrenergic receptor, the inhibitory guanyl nucleotide regulatory protein, G,, copurified through several steps with the receptor (Cerione et al., 1986). In hormone-sensitive membranes, which contain only a few thousand hormone receptors, a preformed complex of receptor and G-protein could have a major effect on the kinetics of hormone activation of intracellular events. In the photoreceptor membrane, the implications of such a preformed complex on the kinetics of G-protein activation are not clear. In the typical operating range of this receptor (~1 photoactivated rhodopsin/disc membrane), the probability of hitting a precomplexed rhodopsin would only be 10%. On the other hand, stimulation of one of the preformed complexes would activate only one G-protein with the reaction mode of the complex. A sufficiently fast exchange of photoactivated rhodopsin and G-protein therefore appears to be required for efficient excitation of the photoreceptor.

The transient formation and then decay of extra-meta-rhodopsin II in the presence of 20 μM GppNHp (Fig. 3A, third curve) provide a kinetic measure of the guanyl nucleotide exchange rate, leading to the release of G-protein from rhodopsin, with the consequent decay of meta-rhodopsin II (Hofmann, 1985). GppNHp exchanges into the guanyl nucleotide-binding site more slowly than does GTP; therefore, under these conditions, the rate is slow enough to be easily measurable. If the G-protein diffused to an activated rhodopsin, the bound antibody could be expected to decrease its diffusion rate, with a consequent slowing of activation. It is striking that antibodies 4H and 4C bind to G-protein but do not change the kinetics of its activation. Under the conditions of these experiments, the G-protein can carry a large load (IgG, M, 150,000) without a visible effect on its activation kinetics. However, the actual G-protein-rhodopsin binding reaction is kinetically buried in the rate-limiting meta-rhodopsin II formation under the conditions of the measurements (Eimeis et al., 1982).

It is of interest to know whether this antigenic site is conserved in other GTP-binding proteins. Dot-blotting experiments show that all the antibodies described here do cross-react to some degree with purified G,, G,, and G,, although cross-reactivity is substantially lower than with photoreceptor G-protein. Functional experiments also show that antibodies that block photoreceptor G-protein activation also block stimulation and inhibition of adenylyl cyclase by hormones and guanyl nucleotides in pineal, brain, and S49 ccc membranes. Thus, the site of interaction between receptors and GTP-binding proteins may be conserved.

Acknowledgments—We thank Nelly Bennett for her help in measuring light-scattering signals and for helpful discussions, Roland Franke for providing us with the biphenyl detergent, and I. Baemuller for technical assistance. [125I]-Labeled G-protein was kindly provided to us by Y. Salomon (Department of Hormone Research, Weizmann Institute of Science).

REFERENCES

Abood, M. E., Hurley, J. B., Pappone, M. C., Bourne, H. R., and Streyer, L. (1982) J. Biol. Chem. 257, 10540–10543
Amir, A. G., Mariuzza, R. A., Phillips, S. E. V., and Poljak, R. J. (1986) Science 233, 747–748
Baehr, W., Morita, E. A., Swanson, R. J., and Applebury, M. L. (1982) J. Biol. Chem. 257, 6452–6460
Bauer, P. J. and Mavromatti, E. (1960) Biophys. Struct. Mech. 6, (suppl.) 116
Bennett, N. and Dupont, Y. (1985) J. Biol. Chem. 260, 4156–4168
Bennett, N., Michel-Villaz, M., and Kühn, H. (1982) Eur. J. Biochem. 127, 97–103
Bercovici, T. and Gitler, C. (1978) Biochemistry 17, 1484–1489
Bentsky, M. W., Wheeler, M. A., Rassenick, M. M., Yamazaki, A., Stein, P. J., Halliday, K. R., and Wheeler, G. L. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 3408–3412
Cerione, R. A., Regan, J. W., Nakata, H., Codina, J., Benovic, J. L., Gierschik, P., Somers, R. L., Spiegel, A. M., Birnbaumer, L., Leffowitz, R. J., and Caron, M. G. (1986) J. Biol. Chem. 261, 3901–3909
De Grip, W. J. (1982) Methods Enzymol. 81, 197–207
Deretic, D. and Hamm, H. E. (1987) J. Biol. Chem. 262, 10389–10397
Dixit, R. A. F., Kohilka, B. K., Strader, D. J., Benovic, J. L., Doholm, H. G., Frielle, T., Bolarowski, M. A., Bennett, C. D., Rands, E., Diehl, R. E., Mumford, R. A., Slater, E. E., Sigal, I. S., Caron, M. G., Leffowitz, R. J., and Strader, C. D. (1986) Nature 321, 75–79
Dreyfus-Manance, L., Friguet, B., and Goldberg, M. E. (1984) Biochemistry 23, 97–104
Eimeis, D. and Hofmann, K. P. (1981) FEBS Lett. 136, 201–207
Eimeis, D., Kühn, H., Reichert, J., and Hofmann, K. P. (1982) FEBS Lett. 143, 29–34
Fong, B. K.-K. (1983) J. Biol. Chem. 258, 10495–10502
Fong, B. K.-K. and Stryer, L. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 2500–2504
Fong, B. K.-K., Hurley, J. B., and Stryer, L. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 152–156
Godchaux, W. J., III, and Zimmerman, W. F. (1979) J. Biol. Chem. 254, 7874–7884
Halliday, K. R. (1984) J. Cyclic Nucleotide Protein Phosphorylation Res. 9, 435–448
Hamm, H. E., and Bownds, M. D. (1984) J. Gen. Physiol. 84, 265–280
Hamm, H. E., and Bownds, M. D. (1986) Biochemistry 25, 4512–4523
Hofmann, K. P. (1985) Biochim. Biophys. Acta 810, 278–281
Hofmann, K. P., and Eimeis, D. (1981) Biochim. Biophys. Acta 650, 23–34
Hofmann, K. P., and Reichert, J. (1985) J. Biol. Chem. 260, 7990–7999
Hofmann, K. P., Uhl, R., Hoffmann, W., and Kreutz, W. (1976) Biophys. Struct. Mech. 2, 61–77
Hurley, J. B., Simon, M. I., Teplow, D. B., Robishaw, J. D., and Gilman, A. G. (1984) Science 224, 560–562
Kühn, H. (1984) Prog. Retinal Res. 3, 123–156
Kühn, H., and Hargrave, P. (1981) Biochemistry 20, 2410–2417
Kühn, H., Bennett, N., Michel-Villaz, M., and Chabre, M. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 6873–6877
Laemmli, U. K. (1970) Nature 227, 680–688
Liebermeister, W. P., and Sitarayamsa, A. (1984) Adv. Cyclic Nucleotide Phosphorylation Res. 17, 215–225
Lochrie, M. A., Hurley, J. B., and Simon, M. I. (1985) Science 228, 96–99
Manning, D. R., and Gilman, A. G. (1983) J. Biol. Chem. 258, 7059–7064
Martin, R. L., Wood, C., Baehr, W., and Applebury, M. L. (1986) Science 232, 1566–1569
Matthew, W. D., and Patterson, P. H. (1983) Cold Spring Harbor Symp. Quant. Biol. 48, 625–631
Matthews, R. G., Hubbard, R., Brown, P. K., and Wald, G. (1963) J. Gen. Physiol. 47, 215–240
McCormick, F., Clark, B., LaCour, T., and Kjeldgaard, M. (1985) Science 230, 78–82
Medynski, D. C., Sullivan, K., Douglas, S., VanDop, C., Chang, F.-H., Fung, B. K.-K., Seeburg, P. H., and Bourne, H. R. (1985)
Antibody Blockade of G-protein-Rhodopsin Interaction

Proc. Natl. Acad. Sci. U. S. A. 82, 4311-4315
Nukada, T., Tanabe, T., Takahashi, H., Noda, M., Hirose, T., Inayama, S., and Numa, S. (1986a) FEBS Lett. 195, 220–224
Nukada, T., Tanabe, T., Takahashi, H., Noda, M., Haga, K., Haga, T., Ichiyama, A., Kangawa, K., Hiranaga, M., Matsuo, H., and Numa, S. (1986b) FEBS Lett. 197, 305–310
Papermaster, D. S., and Dreyer, W. J. (1974) Biochemistry 13, 2438–2444
Parkes, J. H., and Liebman, P. A. (1984) Biochemistry 23, 5054–5061
Robishaw, J. D., Russell, D. W., Harris, B. A., Smigel, M. D., and Gilman, A. G. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 1251–1255
Smith, H. G., Stubbs, G. W., and Litman, B. J. (1975) Exp. Eye Res. 20, 211–217
Tanabe, T., Nukada, T., Nishikawa, Y., Sugimoto, K., Suzuki, H., Takahashi, H., Noda, M., Haga, T., Ichiyama, A., Kangawa, K., Minamino, N., Matsuo, H., and Numa, S. (1985) Nature 315, 242–245
West, R. E., Jr., Moss, J., Vaughan, M., Liu, T., and Liu, T.-Y. (1985) J. Biol. Chem. 260, 14428–14430
Witt, P. L., Hamm, H. E., and Bownds, M. D. (1984) J. Gen. Physiol. 84, 251–263
Yatsunami, K., and Khorana, G. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 4316–4320
Yee, R., and Liebman, P. A. (1978) J. Biol. Chem. 253, 8902–8909