Dimerization of Guanylyl Cyclase-activating Protein and a Mechanism of Photoreceptor Guanylyl Cyclase Activation*

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Ca$^{2+}$-binding guanylyl cyclase-activating proteins (GCAPs) stimulate photoreceptor membrane guanylyl cyclase (retGC) in the light when the free Ca$^{2+}$ concentrations in photoreceptors decrease from 600 to 50 nm. RetGC activated by GCAPs exhibits tight dimerization revealed by chemical cross-linking (Yu, H., Olshhevskaya, E., Duda, T., Seno, K., Hayashi, F., Sharma, R. K., Dizhoor, A. M., and Yamazaki, A. (1999) J. Biol. Chem. 274, 15547-15555). We have found that the Ca$^{2+}$-loaded GCAP-2 monomer undergoes reversible dimerization upon dissociation of Ca$^{2+}$. The ability of GCAP-2 and its several mutants to activate retGC in vitro correlates with their ability to dimerize at low free Ca$^{2+}$ concentrations. A constitutively active GCAP-2 mutant E80Q/E116Q/D158N that stimulates retGC regardless of the free Ca$^{2+}$ concentrations forms dimers both in the absence and in the presence of Ca$^{2+}$. Several GCAP-2/neurocalcin chimera proteins that cannot efficiently activate retGC in low Ca$^{2+}$ concentrations are also unable to dimerize in the absence of Ca$^{2+}$. Additional mutation that restores normal activity of the GCAP-2 chimera mutant also restores its ability to dimerize in the absence of Ca$^{2+}$. These results suggest that dimerization of GCAP-2 can be a part of the mechanism by which GCAP-2 regulates the photoreceptor guanylyl cyclase. The Ca$^{2+}$-free GCAP-1 is also capable of dimerization in the absence of Ca$^{2+}$, but unlike GCAP-2, dimerization of GCAP-1 is resistant to the presence of Ca$^{2+}$.

Photoexcitation of photoreceptors results in hydrolysis of cGMP and closure of the cGMP-gated Na$^{+}$/Ca$^{2+}$ channels, thus causing both hyperpolarization of the photoreceptor membrane and decrease in the intracellular free Ca$^{2+}$ concentrations (1–4). Lowering of the intracellular Ca$^{2+}$ concentrations caused by illumination stimulates GMP synthesis by guanylyl cyclase (retGC)$^1$ that contributes to recovery from photoexcitation and to light adaptation of photoreceptors (5, 6). Recoverin-like Ca$^{2+}$-binding proteins, GCAP-1 and GCAP-2, mediate Ca$^{2+}$ sensitivity of the cyclase in vertebrate retinas (7–10) so that GCAPs activate the cyclase when the free concentrations of Ca$^{2+}$ decrease below 100 nm (a characteristic of light-adapted photoreceptors), but they do not stimulate the cyclase in the dark when the free Ca$^{2+}$ concentrations exceed 500 nm (6, 11). GCAP-2 is highly abundant in mammalian rods, while GCAP-1 is highly expressed in cones (9, 10, 12, 13). The cDNA for the third homologue of GCAPs has been recently cloned from a human retinal cDNA library, while it was absent form retinal cDNA libraries of other vertebrate species (14).

GCAP-1 and GCAP-2 interact with the photoreceptor membrane guanylyl cyclases, retGC-1 and retGC-2 (also known as ROSGCs or GC-E and GC-F in Refs. 8 and 15–18), via cyclase intracellular domains (19–21). Several regions in GCAPs that contain amino acid sequences that are specific for GCAPs function as cyclase regulators have been identified using site-directed mutagenesis (22–24). Although the exact mechanism for retGC activation by GCAPs remains undetermined, it apparently involves dimerization of the cyclase. Consistently with other membrane guanylyl cyclases being active as dimers (25–27), retGCs in photoreceptors also form homodimers (28). Moreover, retGC subunits dimerize or at least come into closer contact when stimulated by GCAPs so that they can be chemically cross-linked (29). Here we report the evidence that GCAP-2 undergoes Ca$^{2+}$-sensitive dimerization and that GCAP-2 dimerization correlates with its ability to activate retGC. We propose that the dimer of the Ca$^{2+}$-free GCAP-2 acts as an adapter that controls dimerization of retGC. Similarly to GCAP-2 a substantial fraction of GCAP-1 also forms a dimer in the absence of Ca$^{2+}$, but unlike GCAP-2 this dimerization apparently is not reversed by Ca$^{2+}$ binding.

EXPERIMENTAL PROCEDURES

Recombinant wild type GCAP-2 (30), its constitutively active mutant (E80Q/E116Q/D158N) (31), and GCAP-2/neurocalcin chimeras (III, IV, XIII, and XIX, Ref. 22) were expressed in E. coli and purified as described previously in detail (22, 30, 31). Recombinant myristoylated GCAP-1 was produced in E. coli and purified using chromatography on Sephacryl S-100 as described previously (23, 32). Neurocalcin was expressed in E. coli and purified using a phenyl-Sepharose chromatography column (22, 33). Based on SDS-PAGE, purity of the recombinant proteins was at least 90%. Recombinant proteins were injected in a volume of 200 μl into a Superdex 200 HR10/30 column (Amersham Pharmacia Biotech) using an automated fast protein liquid chromatography system and eluted at 0.5 ml/min in buffer A (20 mM Tris-HCl, 50 mM KCl, 10 mM NaCl, 10 mM MgCl$_2$, 1 mM dithiothreitol, pH 7.5) containing either 400 μM EGTA or 300 μM CaCl$_2$. The column was pre-equilibrated with 2 volumes of corresponding buffer between the runs. Free Ca$^{2+}$ concentrations in the samples were adjusted prior to injection by adding EGTA or CaCl$_2$, respectively. The molecular weight standards for gel filtration included blue dextran (2,000 kDa), α-amylase (200 kDa), alcohol dehydrogenase (150 kDa, Stokes radius: 45.5 Å), bovine serum albumin (66 kDa, Stokes radius: 36.1 Å), carbonic anhydrase (29 kDa, Stokes radius: 20.1 Å) and cytochrome c (12.4 kDa) (Sigma). The column was calibrated prior to the analysis and reproducing the calibration was tested between series of the experiments. It was virtually identical in the presence of either EGTA or CaCl$_2$. Activation of retGC in washed outer segment membranes reconstituted with GCAPs was assayed under infrared illumination using [α-32P]GTP as a substrate and [3H]cGMP as an internal standard and

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1 The abbreviations used are: retGC, photoreceptor membrane guanylyl cyclase; GCAP, guanylyl cyclase-activating protein.
Results and discussion

The three-dimensional structure of Ca\(^{2+}\)-bound GCAP-2 was recently determined by Ames et al. (34) using NMR spectroscopy. The main chain conformation in GCAP-2 molecule is very similar to that of neurocalcin or recoverin (35–37), although unlike Ca\(^{2+}\)-loaded neurocalcin GCAP-2, is a monomer. Consistently with the previous report by Vijay-Kumar and Kumar (37) on crystal structure of neurocalcin, in the presence of Ca\(^{2+}\), neurocalcin elutes from the Superdex 200 column as a dimer (molecular mass ~46 kDa, Fig. 1), while it becomes a monomer in the presence of EGTA (molecular mass ~22 kDa, Fig. 1). However, Ca\(^{2+}\) has an opposite effect on the chromatographic behavior of GCAP-2. We have found that in the absence of Ca\(^{2+}\), GCAP-2 can dimerize, while the presence of Ca\(^{2+}\) strongly inhibits its dimerization (Fig. 2).

GCAP-2 has a \(K_d\) for Ca\(^{2+}\) near 300 nM (34), and activation of retGC by GCAP-2 is also inhibited by Ca\(^{2+}\) (EC\(_{50}\) 200–300 nM; Refs. 8, 9, and 32). Therefore, at 300 \(\mu\)M free Ca\(^{2+}\) GCAP-2 is fully Ca\(^{2+}\)-bound and completely incapable of activating the cyclase (see also inset in Fig. 3). At the saturating free Ca\(^{2+}\) concentration myristoylated wild type GCAP-2 elutes from the column as a protein with a Stokes radius of ~22 Å and apparent molecular mass of ~31 kDa (Fig. 2), approximately 7 kDa larger than its actual molecular mass (23,808 Da) determined by electrospray mass spectrometry (30).

In addition to the “31-kDa” monomer peak (marked “M” in Fig. 2), the Ca\(^{2+}\)-free GCAP-2 elutes as a peak that corresponds to its dimer (molecular mass ~57 ± 6 kDa, Stokes radius: ~34 Å, marked “D” in Fig. 2). The GCAP-2 dimer is relatively stable, otherwise it would have completely dissociated during the chromatography (~29 min). Dimerization of GCAP-2 in the presence of EGTA may also be one of the reasons why the Ca\(^{2+}\)-free GCAP-2 exhibits a poor NMR spectrum (34). However, the dimer is not irreversible even in the presence of EGTA, because when the peak of dimer is collected and subjected to the second round of chromatography, GCAP-2 elutes again as two peaks, a dimer and a monomer (data not shown). Also, the dimer collected in the absence of Ca\(^{2+}\) dissociates during the subsequent round of chromatography in the presence of Ca\(^{2+}\) (not shown). Conversely, the Ca\(^{2+}\)-loaded GCAP-2 monomer (as in Fig. 2A, line a) forms a dimer when rechromatographed in the presence of EGTA (Fig. 2A, line b).

Dimerization and dissociation of the GCAP-2 complex is a function of Ca\(^{2+}\) concentrations and cannot be attributed to differences in ionic strength, because concentrations of CaCl\(_2\) and EGTA are insignificant compared with the total salt concentration in the elution buffer. It can neither be attributed to a nonspecific effect of divalent cations, because both elution buffers contain 10 mM MgCl\(_2\). Importantly, we can also rule out a possibility that either of the peaks shown in Fig. 2A, line b, represents merely a misfolded inactive form of GCAP-2. When tested in retGC activation assay at low free Ca\(^{2+}\), the specific activity of GCAP-2 in each peak is similar (Fig. 2B). This demonstrates that each peak contains active GCAP-2 capable of reversible dimerization such that at low Ca\(^{2+}\) concentrations the equilibrium between the dimer and the monomer is shifted toward the formation of the dimer, and at high free Ca\(^{2+}\) it shifts toward the dissociation of the complex into Ca\(^{2+}\)-loaded monomers.

The ability of the Ca\(^{2+}\)-free GCAP-2 to dimerize gives rise to a possibility that GCAP-2 dimerization is directly involved in...
Constitutively active E80Q/E116Q/D158N GCAP-2 mutant forms dimers both in the absence and in the presence of Ca$^{2+}$. A, aliquots of 2 mg/ml myristoylated recombinant E80Q/E116Q/D158N GCAP-2 (31) were analyzed by high-resolution gel filtration as described under "Experimental Procedures" in the presence of either 300 μM CaCl$_2$ (line a) or 0.4 mM EGTA (line b). In both cases the GCAP-2 mutant elutes both as a monomer (peak M) and a dimer (peak D). Inset, Ca$^{2+}$ sensitivity of retGC in washed outer reconstituted with wild type GCAP-2 (22) or E80Q/E116Q/D158N GCAP-2 (31) assayed as described in Ref. 31. B, SDS-PAGE of the fractions eluted from the column as in A, line a. Fractions marked D and M correspond to the peaks in A. Minor upper band (+M) corresponds to the nonmyristoylated GCAP-2 (30) that comigrates with the major myristoylated form (+Myr).

Thus, our results demonstrate that the ability of these GCAP-2 mutants to activate retGC at low Ca$^{2+}$ concentrations correlates with their dimerization (Figs. 3 and 4).

A constitutively active mutant of GCAP-2 (E80Q/E116Q/D158N, Ref. 31) that stimulates retGC in both low and high free Ca$^{2+}$ also forms the dimer regardless of the free Ca$^{2+}$ concentrations (Fig. 3A). The elution profiles of GCAP-2 (E80Q/E116Q/D158N) in the presence of 0.4 mM EGTA and in 300 μM Ca$^{2+}$ are almost identical. Myristoylation of GCAP-2 that is apparently nonessential for regulation of retGC (22). Myristoylation of GCAP-2 (22) and or its chimera mutants (22) (lines b–e) were analyzed in the presence of 0.4 mM EGTA by high-resolution gel filtration as described under "Experimental Procedures" and in the legend to Fig. 2. Peaks corresponding to the wild type GCAP-2 (line a) and or its chimera mutants (lines b–e) were analyzed in the presence of 0.4 mM EGTA by high-resolution gel filtration as described under "Experimental Procedures" and in the legend to Fig. 2. Peaks corresponding to the wild type GCAP-2 dimer or monomer are marked D and M, respectively. Plus or minus on the right side of each protein construct indicates its ability to activate retGC at low Ca$^{2+}$ concentrations. C, unlike GCAP-2, GCAP-1 forms stable dimers both in the presence and in the absence of Ca$^{2+}$.

In our recent study we constructed several chimera mutants of GCAP-2 (22). We have found that either the substitution of a large N-terminal fragment of GCAP-2 (chimera mutant XIX, Ref. 31, see Ref. 22 for details) or the substitution of a region proximal to EF-hand 4 (chimera mutant XIII, Ref. 22) with the corresponding neurocalcin fragments interferes with activation of retGC. When tested for their ability to dimerize in the absence of Ca$^{2+}$, neither of these chimeras exhibit a distinct dimer peak in the presence of EGTA (Fig. 4B). In our experiments the elution time for GCAP-2 dimer is approximately 29 min; therefore, even if these mutants were capable of dimerization, their dimers completely dissociated during the chromatography. Thus, our results demonstrate that the ability of these GCAP-2 mutants to dimerize at low Ca$^{2+}$ concentrations is either completely lost or at least dramatically reduced compared to the wild type GCAP-2. Importantly, these GCAP-2 mutants retain their ability to bind retGC in the presence of Ca$^{2+}$, because they are both capable of inhibiting retGC at high free Ca$^{2+}$ concentrations (22).

Another GCAP-2 chimera protein whose central part was substituted with the corresponding neurocalcin fragment and
that fails to activate retGC at low free Ca\(^{2+}\) concentrations (chimera III, Ref. 22) also fails to dimerize in the presence of EGTA (Fig. 4A, line d). However, if we insert into this chimera the exiting helix of EF-2 together with the entering helix of EF-3 derived from GCAP-2 (mosaic chimera IV, Ref. 22), that simultaneously restores the ability of the mosaic GCAP-2 chimera to activate retGC at low Ca\(^{2+}\) concentrations (22) and to dimerize in the presence of EGTA similarly to the wild type GCAP-2 (Fig. 4A, line e).

We propose that dimerization of GCAP-2 is what controls dimerization of retGC and thus contributes to the cyclase regulation by Ca\(^{2+}\) (Fig. 5). This model is based on the following observations: (i) catalytic domains of guanylyl cyclases are very similar to those of membrane adenylyl cyclase (38, 39) that form dimers (40), and peptide receptor membrane guanylyl cyclases also function as dimers (25). Likewise, retGCs form homodimers in photoreceptors in vivo (28). (ii) Activation of retGC by GCAPs stimulates cyclase dimerization or at least closer contacts between the subunits in retGC dimers (29). (iii) GCAP-2 is known to bind to retGC both in the presence and in the absence of Ca\(^{2+}\) (31, 41), but only the Ca\(^{2+}\)-free GCAP-2 activates retGC, while the Ca\(^{2+}\)-loaded GCAP-2 inhibits it. (iv) The ability of GCAP-2 and its mutants to activate retGC at low free Ca\(^{2+}\) concentrations correlates with its ability to dimerize, indicating that not only GCAP-retGC interaction, but also GCAP-GCAP interaction, is important for the regulation of the cyclase.

Our general hypothesis is that retGC becomes activated because GCAP dimer acts as an adapter for dimerization of the cyclase. GCAP-2 can be always bound to the cyclase through interaction with its kinase homology and/or catalytic domains regardless of the free Ca\(^{2+}\) concentrations. However, when GCAP-2 is loaded with Ca\(^{2+}\) in the dark, it is unable to promote the formation of retGC dimers. Conformational changes in GCAP-2 caused by dissociation of Ca\(^{2+}\) result in strong GCAP-GCAP interaction such that a Ca\(^{2+}\)-free GCAP-2 dimer brings the two subunits (or parts of the subunits) of retGC closer together and stabilizes the interaction between the catalytic domains in retGC subunits. Dimerization is reversed when the free Ca\(^{2+}\) concentrations increase in the dark. It cannot be excluded that cyclase subunits may also undergo reversible spontaneous dimerization in the absence of GCAPs in vitro, which may account for relatively high basal activity of retGC in washed photoreceptor membranes in the absence of GCAPs (8, 9). If Ca\(^{2+}\)-loaded GCAP-2 monomers attached to the cyclase subunits interfere with spontaneous dimerization of retGC, it would explain why a fully Ca\(^{2+}\)-loaded GCAP inhibits basal activity of retGC in vitro (31, 32).

It remains unclear whether or not such “dimer adapter” mechanism is applicable to retGC activation by GCAP-1. Similarly to GCAP-2, GCAP-1 also forms dimers in the absence of Ca\(^{2+}\) (Fig. 4C) and is apparently able to interact with similar peptide fragments derived from the cyclase sequence (42). Moreover, activation of retGC by GCAP-1 also results in tight dimerization of the cyclase or at least closer interaction between its subunits (29). Nevertheless, the ability of GCAP-1 to dimerize alone would not be sufficient to account for the activation of the cyclase. There is an obvious difference between GCAP-1 and GCAP-2 at high Ca\(^{2+}\) concentrations. The retention time for the Ca\(^{2+}\)-free dimer of GCAP-1 is slightly different from that of the Ca\(^{2+}\)-loaded dimer of GCAP-1, which suggests that Ca\(^{2+}\) affects conformation of the whole dimer, but apparently does not change the GCAP-1 dimer stability (Fig. 4C). Conformational changes in Ca\(^{2+}\)-loaded versus Ca\(^{2+}\)-free GCAP must play a critical role in the interaction between the GCAPs and the cyclase. However, only in case of GCAP-2 such changes also strongly affect stability of GCAP/GCAP dimer. That may account for the different specificity of GCAP-1 versus GCAP-2 relative to different isoforms of the cyclase (14, 20, 43) and may also contribute to the fact that similar mutations in GCAP-1 and GCAP-2 can result in different biochemical phenotypes (22–24, 31, 44).

We outline in the model described in Fig. 5 the simplest hypothesis that a dimer of GCAP activates RetGC catalytic domains by causing dimerization of the cyclase. It may be a complete dissociation/association of the cyclase subunits that regulates its activity. However, there are also indications that membrane guanylyl cyclases in photoreceptors may always exist as homodimers (28), and therefore it is possible that dimers of GCAPs can also affect the conformation of retGC dimers rather than the equilibrium between association and dissociation of the cyclase. Based on chemical cross-linking experiments (29), GCAP-activated retGC can either undergo association from two completely separate cyclase monomers or equally likely change the conformation of a pre-existing retGC dimer so that it results in a closer contacts between the two cyclase subunits. Conformational changes and dimerization of a Ca\(^{2+}\)-free GCAP-2 (or just conformational changes in GCAP-1 dimer) may provide the necessary energy to cause either association of retGC subunits or the intramolecular rearrangements within the pre-existing “flexible” dimer of the cyclase. Also, GCAP dimer does not necessarily act as an immediate “bridge” between the two catalytic domains of the cyclase. Instead, it may cause conformational changes in other parts of the cyclase subunits, and these changes could be then transduced through the structure of cyclase subunits to their catalytic domains.

The overall three-dimensional structure of the Ca\(^{2+}\)-bound monomer of GCAP-2 is similar to that of recoverin or neurocalcin (34–38). Neurocalcin dimers formation results from close
interactions between EF-hands 2, 3, and 4 in its subunits. Hence, one could assume that the corresponding residues on the surface of GCAP-2 may also be involved in dimerization. However, there is an obvious difference between GCAP-2 and neurocalcin, since GCAP-2 forms relatively stable dimers only in the absence of Ca\(^{2+}\). Besides, the dimers of Ca\(^{2+}\)-free GCAP-2 are less stable compared with Ca\(^{2+}\)-loaded neurocalcin, because they partially dissociate during the time of chromatography (compare Figs. 1 and 2). Further study will have to determine what sites in GCAP-2 may play critical role in its dimerization and what sites are directly involved in interaction with the cyclase. It will also require additional study to determine how Ca\(^{2+}\) binding changes the kinetic parameters of the GCAP-2 dimerization.

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REFERENCES
1. Stryer, L. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 557–559
2. Baylor, D. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 560–565
3. Molday, R. S. (1998) Invest. Ophthalmol. Vis. Sci. 39, 2483–2509
4. Koutalos, Y., and Yau, K.-W. (1996) Trends Neurosci. 19, 73–81
5. Koch, K.-W., and Stryer, L. (1988) Nature 334, 64–66
6. Pugh, E. N., Jr., Duda, T., Sitaramayya, A., and Sharma, R. K. (1997) Biosci. Rep. 17, 429–472
7. Palczewski, K., Subbaraya, I., Gorczyca, W. A., Helekak, B. S., Ruiz, C. C., Oshuro, H., Huang, J., Zhao, X., Crabb, J. W., Johnson, R. S., Walsh, K. A., Gray-Keller, M. P., Dettwiler, P. B., and Baehr, W. (1994) Neuron 13, 395–404
8. Dizhoor, A. M., Lowe, D. G., Olshesvksaya, E. V., Laura, R. P., and Hurley, J. B. (1994) Neuron 12, 1345–1352
9. Dizhoor, A. M., Olshesvksaya, E. V., Henzel, W. J., Weng, S. C., Stults, J. T., Ankoudinova, I., and Hurley, J. B. (1995) J. Biol. Chem. 270, 25200–25206
10. Gorczyca, W. A., Polans, A. S., Surgucheva, I. G., Subbaraya, I., Baehr, W., and Palczewski, K. (1995) J. Biol. Chem. 270, 22025–22036
11. Sampath, A. P., Matthews, H. R., Cornwell, M. C., and Fain, G. L. (1998) J. Gen. Physiol 111, 53–64
12. Howes, K., Bronson, J. D., Dong, Y. L., Li, N., Zhang, K., Rouz, C., Helekak, B., Lee, M., Subbaraya, I., Kelb, H., Chen, J., and Baehr, W. (1998) Invest. Ophthalmol. Vis. Sci. 39, 867–875
13. Kashi, S., Olshesvksaya, E. V., Nishizawa, Y., Watanabe, N., Yamazaki, A., Dizhoor, A., and Ueukura, J. (1999) Exp. Eye Res. 68, 465–473
14. Haeseleer, F., Sokal, I., Li, N., Pettenati, M., Rao, N., Bronson, D., Wechter, R., Baehr, W., and Palczewski, K. (1999) J. Biol. Chem. 274, 6526–6535
15. Lowe, D. G., Dizhoor, A. M., Liu, K., Fu, O., Laura, R., Lu, L., and Hurley, J. B. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 5535–5539
16. Yang, R. B., Foster, D. C., Garbers, D. L., and Fulle, H. J. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 602–606
17. Gorcznaizniak, R. M., Duda, T., Sitaramayya, A., and Sharma, R. K. (1994) Biochem. J. 302, 455–461
18. Sitaramayya, A., Dutta, T., and Sharma, R. K. (1995) Mol. Cell. Biochem. 148, 139–145
19. Laura, R. P., Dizhoor, A. M., and Hurley, J. B. (1996) J. Biol. Chem. 271, 11646–11651
20. Duda, T., Goraczniak, R., Surgucheva, I., Rudnica-Nawrot, M., Gorczyca, W. A., Palczewski, K., Sitaramayya, A., Baehr, W., and Sharma, R. K. (1996) Biochemistry 35, 8478–8482
21. Krishnan, A., Gorcznaizniak, R. M., Duda, T., and Sharma, R. K. (1998) Mol. Cell. Biochem. 178, 251–259
22. Olshesvksaya, E. V., Boikov, S., Ermiolov, A., Krylov, D., Hurley, J. B., and Dizhoor, A. M. (1999) J. Biol. Chem. 274, 10823–10832
23. Krylov, D. M., Niemi, G. A., Dizhoor, A. M., and Hurley, J. B. (1999) J. Biol. Chem. 274, 10833–10839
24. Otto-Bruc, A., Buczylko, J., Surgucheva, I., Subbaraya, I., Rudnica-Nawrot, M., Crabb, J. W., Arendt, A., Harrgrade, P. A., Baehr, W., and Palczewski, K. (1997) Biochemistry 36, 4295–4302
25. Garbers, D. L., and Lowe, D. G. (1994) J. Biol. Chem. 269, 30741–30744
26. Lowe, D. G. (1992) Biochemistry 31, 10421–10425
27. Wilson, E. M., and Chinkers, M. (1995) Biochemistry 34, 4696–4701
28. Yang, R.-B., and Garbers, D. L. (1997) J. Biol. Chem. 272, 13738–13742
29. Yu, H., Olshesvksaya, E., Duda, T., Seno, K., Hayashi, F., Sharma, R. K., Dizhoor, A. M., and Yamazaki, A. (1999) J. Biol. Chem. 274, 15547–15555
30. Olshesvksaya, E. V., Hughes, R. E., Hurley, J. J., and Dizhoor, A. M. (1997) J. Biol. Chem. 272, 14327–14333
31. Dizhoor, A. M., and Hurley, J. B. (1996) J. Biol. Chem. 271, 19346–19350
32. Dizhoor, A. M., Boikov, S. G., and Olshesvksaya, E. V. (1998) J. Biol. Chem. 273, 17311–17314
33. Ladant, D. (1995) J. Biol. Chem. 270, 3179–3185
34. Ames, J. B., Dizhoor, A. M., Ikura, M., Palczewski, K., and Stryer, L. (1999) J. Biol. Chem. 274, 19329–19337
35. Flaherty, K. M., Zozulya, S., Stryer, L., and McKay, D. B. (1993) Cell 75, 709–716
36. Ames, J. B., Ishina, E., Tanaka, T., Gordon, J. I., Stryer, L., and Ikura, M. (1997) Nature 389, 198–202
37. Vijay-Kumar, S., and Kumar, V. D. (1999) Nat. Struct. Biol. 6, 80–87
38. Tucker, C. L., Hurley, J. H., Miller, T. R., and Hurley, J. B. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 5993–5997
39. Sunahara, R. K., Beuee, A., Tesmer, J. J., Sprang, S. R., Garbers, D. L., and Gilman, A. G. (1998) J. Biol. Chem. 273, 16332–16338
40. Zhang, G., Liu, Y., Rusto, A. E., and Hurley, J. H. (1997) Nature 386, 247–253
41. Laura, R. P., and Hurley, J. B. (1998) Biochemistry 37, 11264–11271
42. Sokal, I., Haeseleer, F., Arendt, A., Adman, E. T., Hurgrade, P. A., and Palczewski, K. (1999) Biochemistry 38, 1387–1393
43. Surgucheva, I., Dizhoor, A. M., Hurley, J. B., Palczewski, K., and Baehr, W. (1997) Invest. Ophthalmol. Vis. Sci. 38, 4577
44. Rudnica-Nawrot, M., Surgucheva, I., Bulmes, J. D., Haeseleer, F., Sokal, I., Crabb, J. W., Baehr, W., and Palczewski, K. (1998) Biochemistry 37, 248–257