Identification of Protein Kinase C Phosphorylation Sites on Bovine Rhodopsin*

(Received for publication, January 28, 1997, and in revised form, February 20, 1997)

N. Michelle Greene, David S. Williams, and Alexandra C. Newton‡

From the Department of Pharmacology, University of California at San Diego, La Jolla, California 92037-0640

The protein kinase C phosphorylation sites on bovine rhodopsin were identified using proteolytic, phosphoamino acid, mass spectrometric, and peptide sequencing analyses. Tryptic removal of the 9 carboxyl-terminal residues of rhodopsin revealed that a major fraction of the phosphates incorporated by protein kinase C are in a region containing Ser334, Thr335, and Thr336. Phosphoamino acid analysis of the tryptic product established that Ser334 accounts for approximately 65% of the phosphorylation in this region. Analysis of the endoproteinase Asp-N-generated carboxyl terminus of rhodopsin by mass spectrometry and peptide sequencing revealed that Ser338 is also a primary phosphorylation site, with minor phosphorylation of Ser343. Quantitation of high pressure liquid chromatography-separated phosphopeptides, taken together with phosphoamino acid analysis of the tryptic product, revealed that Ser334 and Ser338 were phosphorylated equally and each accounted for approximately 35% of the total phosphorylation; Thr335/336 accounted for just under 20% of the phosphorylation, and Ser343 accounted for 10%. Thus, the primary protein kinase C sites are Ser334 and Ser338, with minor phosphorylation of Thr335/336 and Ser343. Ser334 and Ser338 have recently been identified as the primary sites of phosphorylation of rhodopsin in vivo (Ohguro, H., Van Booser, J. P., Milam, A. H., and Palczewski, K. (1995) J. Biol. Chem. 270, 14259–14262). Of these sites, only Ser338 is a significant substrate for rhodopsin kinase in vitro. Identification of Ser334 as a primary protein kinase C target in vitro is consistent with protein kinase C modulating the phosphorylation of this site in vivo.

Phosphorylation plays a pivotal role in the regulation of G protein-coupled receptors, where phosphorylation by both G protein receptor kinases (GRKs) and second messenger-regulated kinases mediates receptor desensitization (1). In photo-transduction, phosphorylation of the visual receptor, rhodopsin, is the first step in desensitization of this archetypal G protein-coupled receptor (2–4). The photoexcited conformation of rhodopsin serves as a substrate for rhodopsin kinase (also called GRK1; Ref. 5).

A number of laboratories have established that Ser338 and Ser343 on the carboxyl terminus of bovine rhodopsin are the primary phosphorylation sites of this kinase in vitro (6–8). Recently, Palczewski and co-workers showed that mouse rhodopsin is phosphorylated on Ser338 and also on a novel site, Ser334, in vivo (same numbering in mouse and bovine rhodopsin) (9). Although rhodopsin kinase has now been reported to phosphorylate Ser334 in vitro (10), this phosphorylation is minor relative to that of the primary sites and has not been consistently observed (6–8). Thus, a kinase other than rhodopsin kinase likely regulates rhodopsin at Ser334.

One candidate for modulating rhodopsin phosphorylation at the nonrhodopsin kinase site is protein kinase C. The enzyme phosphorylates the carboxyl terminus of dark-adapted rhodopsin, photoexcited rhodopsin, and opsin with equal affinity in vitro (11, 12), suggesting that its phosphorylation site(s) is equally exposed in all three receptor conformations. Evidence that this phosphorylation is physiologically relevant was first supported by the finding that phorbol esters, potent activators of protein kinase C, modulate the phosphorylation of rhodopsin in intact retinas (14, 15). Recently, the extent of protein kinase C’s involvement in rhodopsin phosphorylation in situ was established using a highly selective protein kinase C inhibitor, calphostin C; treatment of retinas with calphostin C was shown to cause a 50% reduction in the light-dependent phosphorylation of rhodopsin in intact frog retinas (16).

This contribution shows that Ser334 and Ser338 are the major protein kinase C phosphorylation sites on bovine rhodopsin and Ser338 and Thr335 and/or Thr336 are minor sites. The finding that protein kinase C phosphorylates Ser334, coupled with the finding that protein kinase C contributes to approximately half the phosphorylation of rhodopsin in situ (16), suggests that this is the kinase that is responsible for the physiological regulation of rhodopsin at Ser334.

EXPERIMENTAL PROCEDURES

Materials—ATP, leupeptin, phosphoserine, phosphothreonine, phosphotyrosine, trypsin (1.14 × 10⁵ benzoyl-L-arginine ethyl ester units mg⁻¹), 1.3-bis[tir][hydroxymethyl]-methylamino]propane (BTP⁺) and phorbol myristate acetate were purchased from Sigma. [γ-³²P]ATP (3000 Ci mmol⁻¹) was from DuPont NEN. Horseradish peroxidase-conjugated goat anti-rabbit IgG, Tween 80, and endoproteinase Asp-N (sequencing grade) were supplied by Calbiochem. Chemiluminescence developing reagents were from Pierce. Avicel thin layer chromatography plates were purchased from Analtech. Immobilon-P was from Millipore, and nitrocellulose was from Schleicher & Schuell. DEAR-Sephalcel resin, heparin-Sepharose Hi-Trap, and Mono Q columns were from Pharmacia Biotech Inc. Protein kinase C α was purified from the baculovirus expression system as described previously (17); protein kinase C was also purified from bovine retinas as described previously (12). The cDNA for bovine rhodopsin kinase was a generous gift from Dr. Jim Inglese. A rabbit polyclonal antibody against opsin was generated as described (15). Rod outer segment membranes were isolated from bovine retinas and urea-stripped as described (12). 11-cis retinal was kindly provided by the National Eye Institute. All other chemicals

Palczewski and co-workers recently reported that they were unable to phosphorylate rhodopsin with protein kinase C (13); this may have arisen because an irreversibly oxidized form of protein kinase C was used in their experiment.

The abbreviations used are: BTP, 1,3-bis[tir][hydroxymethyl]-methylamino]propane; PAGE, polyacrylamide gel electrophoresis; HPLC, high pressure liquid chromatography.
are reagent grade.

**Purification of Rhodopsin Kinase**—The cDNA for bovine rhodopsin kinase was subcloned from pBluescript into the baculovirus transfer vector pVL1393 (Invitrogen) and recombined with baculoviral DNA using the BaculoGold kit (Pharmingen). SF-21 cells (2 × 10^6 ml^-1, 400 ml) were infected with recombinant virus (10^7 plaque-forming unit ml^-1) and harvested 72 h post-infection. Washed cells were homogenized in buffer containing 10 mM BTP, 10 mM EGTA, 2 mM EDTA, 0.4% Tween 80, 1 mM dithiothreitol, 20 μM leupeptin, 0.2 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine. The homogenate was centrifuged at 245,000 × g for 45 min at 4 °C and the supernatant was used. Rhodopsin kinase was stored at −20 °C in buffer containing 50% glycerol, 0.5 mM EDTA, 0.5 mM EGTA, 5 mM BTP, pH 7.5, 250 mM KCl.

**Phosphorylation of Rhodopsin**—Urea-stripped membranes were phosphorylated by protein kinase Cα from bovine retinas, baculovirus-expressed protein kinase Cα, or baculovirus-expressed rhodopsin kinase under dim red light or under room light, as stated in the figure legends. Reaction mixtures (80 μl) containing 10 pmol of rhodopsin and 2 pmol of protein kinase Cα or 1 pmol of rhodopsin kinase were incubated in buffer containing 50 μM [γ-32P]ATP (3 CI mmol^-1), 1 mM MgCl₂, 1 mM dithiothreitol, and 20 mM HEPES, pH 7.5, at 30 °C unless otherwise noted. CaCl₂ (500 μM) and phosphorolystrate (2–1 μM) were included in protein kinase C reaction mixtures; 100 μM EDTA and 1 mM EGTA were included in rhodopsin kinase reaction mixtures. Reactions proceeded for 60 min at 30 °C, followed by centrifugation at 500,000 × g for 20 min at 4 °C. Membranes were resuspended in buffer and analyzed as described in appropriate sections below.

**Time Course of Trypsin Proteolysis**—Rhodopsin (70 pmol) phosphorylated by protein kinase Cα or rhodopsin kinase was incubated in 700 μl of 20 mM HEPES, pH 7.5, containing 50 μM CaCl₂; 10 pmol (100 μl) were removed as the zero time point. Proteolysis was initiated by the addition of 145 μl of 57 units ml^-1 trypsin, and samples were incubated at 30 °C for 2–60 min. Aliquots (125 μl) were removed into SDS-PAGE buffer (40 μl; 8% SDS, 40% glycerol, 0.008% bromphenol blue, 20% β-mercaptoethanol, and 0.25 mM Tris, pH 6.8). Proteins were separated by SDS-PAGE (10% acrylamide), electrophoretically transferred to nitrocellulose, labeled with antibodies to opsin via incubation with horse-radish peroxidase-conjugated IgG, and labeling detected by chemiluminescence. Antibodies were removed by incubation of blots in 100 μM β-mercaptoethanol, 2% SDS, and 62.5 mM Tris, pH 6.8, for 60 min at 55 °C, and blots were analyzed by autoradiography or PhosphorImager analysis (Molecular Dynamics).

**Phosphoamino Acid Analysis**—Rhodopsin (70 pmol) phosphorylated by protein kinase Cα or rhodopsin kinase was proteolyzed by trypsin (17 units ml^-1) as described above. Intact rhodopsin and cleavage products were separated by SDS-PAGE (12% acrylamide) and electrophoretically transferred to Immobilon-P. Bands corresponding to intact (36 kDa) and cleavage products (35 kDa) were excised from the Immobilon-P membrane, hydrolyzed in 500 μl of 6 M HCl for 1 h at 110 °C, and subjected to phosphoamino acid analysis as described (18). Hydrolyzed sample was mixed with 1 μg each of phosphoserine, phosphothreonine, and phosphotyrosine, spotted onto a thin layer chromatography plate, and electrophoresed horizontally (2000 V for 30 min) in pH 3.5 buffer (5% acetic acid, 0.5% pyridine). Amino acid standards were visualized with 0.2% ninhydrin in acetone and 32P comigrating with peaks corresponding to amino acids were detected by autoradiography.

**Sample Preparation for Mass Spectrometry and Sequencing**—One nmol of rhodopsin was phosphorylated by protein kinase Cα as described above except the ATP concentration was 250 μM. After centrifugation, the supernatant was removed into 1 ml of 20 mM HEPES, pH 7.5, at 30 °C. The carboxyl terminus was released by the addition of 200 μl of a 20 μM solution of endoproteinase Asp-N in 10 mM HEPES, 50 mM glycerol, and incubation at 30 °C for 60 min. The solution was centrifuged again for 20 min at 500,000 × g at 4 °C to separate the 19-amino acid carboxy-terminal tail from the membrane-bound fragment of rhodopsin. The supernatant containing the carboxyl-terminal peptide was applied to a reverse phase HPLC C-18 column. Masses of peaks of interest were determined by laser desorption mass spectrometry; peptides with masses corresponding to the phosphorylated tail of rhodopsin were sequenced by automated Edman degradation or tandem mass spectrometry.

### RESULTS

In determining the protein kinase C phosphorylation site(s) on rhodopsin, we took advantage of the finding that phospho-

---

**FIG. 1.** Time course of trypsin proteolysis of rhodopsin phosphorylated by protein kinase Cα (**left**) or rhodopsin kinase (**right**). A, autoradiogram showing rhodopsin (light-adapted) phosphorylated by protein kinase Cα (**left**) or rhodopsin kinase (**right**) and then treated without trypsin (**0**) or with trypsin (11 units ml^-1) for 2–60 min at 30 °C. B, Western blot corresponding to the autoradiogram in A probed with anti-opsin antibody. C, graph of 32P associated with intact (**left**) and cleaved (**right**) rhodopsin measured by densitometric scanning of the bands on the autoradiogram in A. Data are expressed as percentages of radioactivity associated with native rhodopsin prior to trypsin treatment; the graph on the left shows rhodopsin phosphorylated by protein kinase Cα (**C**), the graph on the right shows rhodopsin phosphorylated by rhodopsin kinase (**C**). Phosphorylation on Ser^338^ prevents cleavage by trypsin at the adjacent Lys^339^ (8, 19) (see Fig. 4A). Thus, cleavage of rhodopsin at this site serves as a diagnostic for whether Ser^338^ has been phosphorylated and provides a measure for what fraction of the phosphorylation occurs amino-terminal to Ser^338^.

Fig. 1A (**left**) shows that trypsin treatment of rhodopsin phosphorylated by protein kinase Cα resulted in the appearance of a 32P-labeled peptide that migrated slightly faster (35 kDa) than native rhodopsin (36 kDa); this product corresponds to rhodopsin cleaved at Lys^339^ to release a 9-amino acid peptide. Fig. 1C (**left**) reveals that at least 40% of the radioactivity incorporated on rhodopsin was recovered in the 35-kDa cleaved product (the product is a transient intermediate, setting a lower limit on the fraction of rhodopsin modified amino-terminal to Ser^338^). Approximately 15% of the radioactivity was obtained regardless of whether light-adapted (Fig. 1) or dark-adapted (not shown) rhodopsin served as the substrate and whether protein kinase Cα (Fig. 1) or retinal protein kinase C was used.

In contrast to protein kinase C-phosphorylated rhodopsin, only one radioactive band was detected when rhodopsin kinase-
Phosphorylation of Rhodopsin by Protein Kinase C

Fig. 2. Phosphoamino acid analysis of tryptic fragment of rhodopsin phosphorylated by protein kinase C. Rhodopsin (light-adapted) was phosphorylated by protein kinase C α to a stoichiometry of 0.5 mol phosphate/mol rhodopsin and treated with trypsin, and the 35-kDa product was separated by PAGE and subjected to phosphoamino acid analysis. Shown is an autoradiogram of the thin layer chromatography plate on which the phosphoamino acids were separated. Radiolabel below the S and the T co-migrated with phosphoserine and phosphothreonine standards, respectively; the circle marks the area where the phosphotyrosine (Y) standard migrated.

Phosphorylated rhodopsin was treated with trypsin under the same conditions; this band corresponds to uncleaved rhodopsin (Fig. 1A, right). The 35-kDa product, although present (Western blot in Fig. 1B, right), was not radioactive. Fig. 1C shows that the half-time for cleavage of rhodopsin kinase-phosphorylated rhodopsin was approximately 30 min; this is at least ten times slower than the half-time for cleavage of protein kinase C-phosphorylated rhodopsin (Fig. 1C, right), consistent with phosphorylation on Ser334 preventing proteolysis at Lys 339.

To identify the residue on the tryptic fragment that is phosphorylated by protein kinase C, the 35-kDa product was subjected to phosphoamino acid analysis (Fig. 2). Compilation of data from 13 independent experiments, with stoichiometries varying from 0.2 to 1.0 mol phosphate/mol rhodopsin, revealed an average of 65 ± 9% phosphoserine on the 35-kDa tryptic product; the remaining radioactivity was on phosphothreonine with no detectable phosphotyrosine. Similar results were obtained whether rhodopsin was phosphorylated by recombinant protein kinase C α from baculovirus (Fig. 2) or protein kinase C purified from bovine retinas (not shown). Thus, Ser334 is the primary phosphorylation site on the tryptic fragment of rhodopsin, with some phosphorylation observed on one or both of the adjacent Thr residues.

Additional phosphorylation sites (i.e. not present in the tryptic product) were identified by analysis of a carboxyl-terminal peptide, released by endoproteinase Asp-N (20), which contains all the in vitro phosphorylation sites of rhodopsin kinase (RK) (6–8) and protein kinase C (PKC) (7). "In vivo phosphorylation sites determined for mouse rhodopsin are circled (9). Endoproteinase Asp-N and trypsin sites are indicated. B, ribbon diagram of solution structure of peptide comprising 40 carboxyl-terminal residues of bovine rhodopsin elucidated by Yeagle and co-workers (21). In this orientation, the membrane plane would be above the structure (the beginning of transmembrane helix 7 is apparent at the amino terminus of the peptide, marked N). The protein kinase C phosphorylation sites determined in vitro, Ser343 and Ser338, are represented in yellow and green, respectively. These two sites are the major in vitro phosphorylation sites in mouse (9); of these, only Ser334 is a major rhodopsin kinase site in vitro. The other major in vitro rhodopsin kinase site, Ser343, is indicated in blue.

**Fig. 3. Analysis of carboxyl terminus of rhodopsin after phosphorylation by protein kinase C.** Chromatogram of endoproteinase Asp-N-generated tail of rhodopsin (dark-adapted) phosphorylated by retinal protein kinase C. Indicated peaks correspond to peptides containing phosphorylated Ser343 (Peak 1), phosphorylated Ser338 (Peak 2), and phosphorylated 334–336 (left shoulder of Peak 3). The trace represents the absorbance at 209 nm.

**Fig. 4. Location of phosphorylation sites on bovine rhodopsin.** A, sequence of carboxyl-terminal tail of bovine rhodopsin (24) showing primary in vitro phosphorylation sites of rhodopsin kinase (RK) (6–8) and protein kinase C (PKC). In vivo phosphorylation sites determined for mouse rhodopsin are circled (9). Endoproteinase Asp-N and trypsin sites are indicated. B, ribbon diagram of solution structure of peptide comprising 43 carboxyl-terminal residues of bovine rhodopsin elucidated by Yeagle and co-workers (21). In this orientation, the membrane plane would be above the structure (the beginning of transmembrane helix 7 is apparent at the amino terminus of the peptide, marked N). The protein kinase C phosphorylation sites determined in vitro, Ser343 and Ser338, are represented in yellow and green, respectively. These two sites are the major in vitro phosphorylation sites in mouse (9); of these, only Ser334 is a major rhodopsin kinase site in vitro. The other major in vitro rhodopsin kinase site, Ser343, is indicated in blue.

**TABLE I**

| Peak | Mass* | Phosphorylated residue | Method |
|------|-------|------------------------|--------|
| 1    | 2015.5| Ser343                 | Edman  |
| 2    | 2015.4| Ser338                 | Edman  |
| 3 shoulder | 2017.1 | Ser334 or Thr335 or Thr336 | ES-MS |
| 1937.0 | None |                        |        |

*Masses for Peaks 1 and 2 were determined by laser desorption mass spectrometry; masses for Peak 3 shoulder were determined by electrospray mass spectrometry (ES-MS).
Phosphorylation of Rhodopsin by Protein Kinase C

Multiply phosphorylated species, which elute earlier than monophosphorylated species (6), were not detected and could account for no more than 3% of the sample based on the sensitivity of the detection. Similar results were obtained whether dark-adapted (Fig. 3) or light-adapted (not shown) rhodopsin was phosphorylated by protein kinase C. The carboxyl-terminal fragment of unphosphorylated rhodopsin eluted as a single peak with the same retention time as Peak 3 above; it had a mass of 1938.1, establishing no phosphorylation prior to protein kinase C treatment (not shown).

Sequencing by Edman degradation identified the phosphorylated residues as Ser334 and Ser338 in Peaks 1 and 2, respectively. The order of elution of these peptides relative to unphosphorylated peptide was as reported previously (6). The presence of unphosphorylated peptide in Peak 3 should confound the unambiguous identification of the phosphorylation site on the monophosphorylated peptide; the signal for detection of released derivatized amino acid dropped from 3.2 to 0.2 pmol in the cycle from residues 333 to 334, consistent with modification at Ser334. Further analysis of Peak 3 shoulder by tandem mass spectroscopy confirmed that phosphate was present at one of the 3 adjacent residues Ser334-Thr335-Thr336. The stoichiometry of the analyzed phosphorhodopsin was 0.6 mol phosphate/mol rhodopsin. Based on this and the areas of the three peaks, we calculated the relative distribution of phosphates on the identified phosphorylation sites. Peaks 1, 2, and 3 represented 6, 23, and 71%, respectively, of the total peak area in the chromatogram in Fig. 3. Given that 60% of the sample was phosphorylated, we calculated that the phosphopeptide component in Peak 3 (Ser334-Thr335-Thr336) accounted for 31% of the total peak area. Expressed relative to the total phosphorylation, Ser334 (Peak 1) incorporated 10% of the phosphate in the sample, Ser338 (Peak 2) incorporated 38% of the phosphate, and Ser334-Thr335-Thr336 (phosphorylated component of Peak 3) accounted for 52% of the phosphorylation. Phosphoamino acid analysis revealed that 65 ± 9% of the phosphate in the Ser334-Thr336 region was on Ser334 (Fig. 2), so that phosphate on Ser334 accounted for 34% of the total phosphate and phosphate on Thr335 and/or Thr336 accounted for 18%. Thus, the phosphorylation preference was: Ser334 ≫ Ser338 > Thr335/336 > Ser343.

DISCUSSION

The foregoing results reveal that 1) the primary in vitro protein kinase C phosphorylation sites of bovine rhodopsin are Ser334 and Ser338 (Fig. 4A), with minor phosphorylation on Ser334 and Thr335 and/or Thr336, 2) a single phosphate is incorporated per molecule of rhodopsin, and 3) light has no significant effect on the sites phosphorylated. Importantly, Ser334 and Ser338 are the major in vivo phosphorylation sites of rhodopsin (9). Furthermore, of these two sites, only Ser338 is phosphorylated significantly by rhodopsin kinase in vitro (6–8). This suggests that protein kinase C is the primary kinase that modulates Ser334 in vivo. This possibility is also supported by kinetic data; the contribution of protein kinase C to the phosphorylation of rhodopsin in intact retinas is slower than that of rhodopsin kinase (16), and Ser334 is phosphorylated more slowly than Ser338 in vivo (9).

Recent elucidation of the solution structure of a domain of rhodopsin encompassing the seventh transmembrane span and carboxyl terminus (21) reveals that Ser334 and Ser338 are surface exposed (Fig. 4B). Furthermore, the secondary protein kinase C sites Thr335 and Thr336 are less exposed; and the least favored site, Ser334 is on the opposite face of the surface containing the primary sites. Because the protein kinase C phosphorylation is unaffected by light (12), Ser334 is likely to be equally exposed in all conformations of the receptor. Although protein kinase C has a preference for basic residues near the phosphoacceptor site (22), accessibility, rather than a defined consensus sequence, appears to determine substrate specificity (23). Thus, orientation and proximity to the membrane interface, where protein kinase C binds (23), may be the driving force in promoting rhodopsin phosphorylation.

The identification of the two in vivo phosphorylation sites on rhodopsin as protein kinase C phosphorylation sites underscores the importance of this second messenger-regulated kinase in regulation of rhodopsin function. The phosphorylation by rhodopsin kinase and protein kinase C at distinct sites, in addition to the shared phosphorylation of Ser338, should allow mechanistic insight into what promotes the activity of each kinase under specific conditions.

REFERENCES

1. Lefkowitz, R. J. (1993) Cell 74, 409–412
2. Hargrave, P. A., and McDowell, J. H. (1992) FASEB J. 6, 2323–2331
3. Stryer, L. (1991) J. Biol. Chem. 266, 10711–10714
4. Yarfitz, S., and Hurley, J. B. (1994) J. Biol. Chem. 269, 14329–14332
5. Inglese, J., Freedman, N. J., Koch, W. J., and Lefkowitz, R. J. (1993) J. Biol. Chem. 268, 23735–23738
6. Papac, D. I., Oatis, J. E., Jr., Crouch, R. K., and Knapp, D. R. (1993) Biochemistry 32, 5930–5934
7. McDowell, J. H., Nawrocki, J. P., and Hargrave, P. A. (1993) Biochemistry 32, 4968–4974
8. Ohguro, H., Palczewski, K., Ericsson, L. E., Walsh, K. A., and Johnson, R. S. (1993) Biochemistry 32, 5718–5724
9. Ohguro, H., Van Hoozer, J. P., Milam, A. H., and Palczewski, K. (1995) J. Biol. Chem. 270, 14259–14262
10. Ohguro, H., Johnson, R. S., Ericsson, L. E., Walsh, K. A., and Palczewski, K. (1994) Biochemistry 33, 1023–1028
11. Kellerer, D. J., and Johnson, G. L. (1986) J. Biol. Chem. 261, 4749–4757
12. Greene, N. M., Williams, D. S., and Newton, A. C. (1995) J. Biol. Chem. 270, 6713–6717
13. Ohguro, H., Rudnicka-Nawrot, M., Buczylko, J., Zhao, X., Taylor, J. A., Walsh, K. A., and Palczewski, K. (1996) J. Biol. Chem. 271, 5215–5224
14. Newton, A. C., and Williams, D. S. (1993) J. Biol. Chem. 268, 17725–17728
15. Newton, A. C., and Williams, D. S. (1993) J. Biol. Chem. 268, 18181–18186
16. Udovicchenko, I. P., Newton, A. C., and Williams, D. S. (1997) J. Biol. Chem., in press
17. Orr, J. W., Kerranen, L. M., and Newton, A. C. (1992) J. Biol. Chem. 267, 15683–15696
18. Boyle, W. J., van der Geer, P., and Hunter, T. (1991) Methods Enzymol. 201, 110–140
19. Molloy, R. S., and MacKenzie, D. (1985) Biochemistry 24, 776–781
20. Palczewski, K., Buczylko, J., Kaplan, M. W., Polans, A. S., and Crabb, J. W. (1991) J. Biol. Chem. 266, 12949–12955
21. Yeagle, P. L., Alderfer, J. L., and Albert, A. D. (1996) Mol. Vis. 2, 12
22. Nishikawa, K., Toker, A., Johannes, F.-J., Songyang, Z., and Cantley, L. C. (1997) J. Biol. Chem. 272, 952–960
23. Newton, A. C. (1995) J. Biol. Chem. 270, 28485–28498
24. Applebury, M. L., and Hargrave, P. A. (1986) Vision Res. 26, 1881–1895
Identification of Protein Kinase C Phosphorylation Sites on Bovine Rhodopsin
N. Michelle Greene, David S. Williams and Alexandra C. Newton

J. Biol. Chem. 1997, 272:10341-10344.
doi: 10.1074/jbc.272.16.10341

Access the most updated version of this article at http://www.jbc.org/content/272/16/10341

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 23 references, 13 of which can be accessed free at
http://www.jbc.org/content/272/16/10341.full.html#ref-list-1