Determination of in Vivo Phosphorylation Sites in Protein Kinase C*

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The primary structure of rat protein kinase C βII was probed by high pressure liquid chromatography directly coupled to an electrospray ionization mass spectrometer and by high energy collision-induced dissociation analysis to identify in vivo phosphorylation sites. The N-terminal methionine was found to be cleaved post-translationally and replaced with an acetyl group. Four phosphopeptides were identified. Two peptides, Thr500-Lys520 and Glu490-Lys520, are phosphorylated at Thr500 greater than 90%. Peptide His636-Arg649 is phosphorylated about 75% at Thr641. It is the only site that was previously identified during the in vitro autophosphorylation studies (Flint, A. J., Paladini, R. D., and Koshland, D. E., J. r. (1990) Science 249, 408-411). The fourth peptide Asn660-Lys672 is phosphorylated at Thr660. A discussion of the potential implication of these results follows.

Phosphorylation is a rapid and reversible means of regulating protein activity. Its efficiency is evident in the many signal transduction pathways that use cascades of phosphorylation to effect cellular responses (1-3). Protein kinase C plays a major role in many of these pathways (4-6). It is a serine/threonine kinase dependent on calcium and phospholipids and activated by diacylglycerols, fatty acids, or phorbol esters at physiological calcium concentrations (7). 12 members of the mammalian protein kinase C family have been identified so far (8). Regions of conservation as well as proteolysis studies indicate that protein kinase C is comprised of two domains, an N-terminal regulatory domain and a C-terminal catalytic domain (9, 10).

Protein kinase C autophosphorylates itself in vitro on both its regulatory and catalytic domains (11). Autophosphorylation is particularly intriguing in that it has been shown to be an intramolecular reaction (12), in which regions very distinct in the primary sequence have access to the active site (13). When separated from the regulatory domain by proteolysis, the catalytic domain is no longer able to autophosphorylate, even though it is still fully active against substrates (12).

Six in vitro autophosphorylation sites have been identified in the βI isozyme (13). Ser16 and Thr17 are located close to the autoinhibitory sequence in the primary structure. Thr314 and Thr324 are located in the hinge region between the catalytic and regulatory domains. Thr636 and Thr641 are in the C terminus and are the only sites conserved in all the conventional protein kinase C isozymes. These residues are outside the region conserved in most other serine/threonine kinases.

Recent studies in vitro and in vivo have elucidated a definite role for phosphorylation of protein kinase C. Phosphorylation by a second kinase is thought to be necessary in the activation of the kinase in vivo (14, 15). Mutagenesis studies of Thr497 and Thr500 in protein kinase C isozyme α and β, respectively, have proposed phosphorylation of those residues as critical for activity in vivo and/or in vitro (15-17). In addition, mutations of the in vitro autophosphorylation sites in protein kinase C β suggest a role for the C-terminal sites, Thr635 and Thr643 in protein kinase C localization, activation, and down-regulation (18).

Since previous results indicated that protein kinase C is phosphorylated in vivo and that phosphorylation is essential for activation, it is important to determine whether these phosphorylation sites are identical to in vitro autophosphorylation sites. The baculovirus expression system was chosen for protein kinase C expression because of the ease of purifying a single isozyme. Previous work has shown that the gel mobility and in vitro autophosphorylation pattern were identical between protein kinase C βI overexpressed in insect cells and purified from rat brain (13). In addition, phosphatase-treated protein kinase C from both sources exhibit similar gel shifts, suggesting identical phosphorylation patterns (19).

Mass spectrometry has been successfully used for the determination of phosphorylation sites in various proteins, such as the chemotaxis response regulator protein from Escherichia coli (20), bovine myelin basic protein (21), bovine mitochondrially activated protein kinase (22), and bleached bovine rhodopsin (23). HPLC^1 directly coupled with electrospray ionization mass spectrometry (LC/ESIMS) provides means for quick and efficient screening of entire protein digests for covalent modifications (24). LC/ESIMS analysis yields singly or multiply protonated peptide ions, and from the m/z value of these ions, the molecular mass of the corresponding peptide can be determined. Similarly, liquid secondary ionization mass spectrometry (LSIMS) analysis usually yields only molecular weight data in the form of protonated peptide ions. These ions can be activated by collision with inert gas atoms, such as helium. The

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1 The abbreviations used are: HPLC, high pressure liquid chromatography; CID, collision-induced dissociation; ESIMS, electrospray ionization mass spectrometry; LC/ESIMS, HPLC directly coupled with electrospray ionization mass spectrometry; LSIMS, liquid secondary ionization mass spectrometry; rt, retention time.
Phosphorylation Sites in Protein Kinase C

Isolation of Protein Kinase C—The bII isozyme of rat protein kinase C (26) was expressed and purified according to the procedures described earlier (13). Sf9 or Sf21 insect cell lines were infected with a recombinant baculovirus, and the enzyme was purified by chromatography on DE-52 anion exchange resin, a phosphatidylserine affinity matrix, and a Mono Q column. Protein kinase C elutes from the Mono Q column in 20 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1 mM EGTA, and 1 mM dithiothreitol at 200–300 mM KCl. This mixture was dialyzed to equilibrium (19 h) against 500 ml of 0.1 mM NH₄HCO₃ (pH 7.9).

Alkylation—Approximately 750 pmol of protein kinase C were dissolved in 50–60 μl of 6 M guanidine HCl, 200 mM Tris-HCl (pH 8.0). Cysteine residues were reduced with 3 μl dithiothreitol at 60 °C for 1 h and alkylated with sodium iodoacetate (6.15 μM) at room temperature for 1.5 h in the dark. The reagent excess was removed by dialysis against approximately 2.5 liters of 100 mM NH₄HCO₃ buffer (pH 7.8).

Digestion with Trypsin—The carboxymethylated protein was incubated with about 3% (w/w) L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin (Worthington) at 37 °C for 18 h. The enzyme was added in aliquots at the beginning of the digestion and 4 h later.

Reversed-phase HPLC—The trypic peptides were separated by reverse phase HPLC (Vydac C18, 1.0 mm, inner diameter x 250-mm column) using an ABI 140A solvent delivery system. Solvent A was 0.1% trifluoroacetic acid in water; solvent B was 0.08% trifluoroacetic acid in acetonitrile. The column was equilibrated in 2% B, and the gradient was started at 5 min after the injection. A 10% solvent B concentration was reached in 5 min, and then the amount of solvent B was linearly increased to 50% over 100 min. The fractions were manually collected.

Asp-N Subdigest—Phosphopeptide-containing fractions (estimated peptide-content was about 600 pmol) were incubated with 0.2 μg of endoproteinase Asp-N (Boehringer Mannheim) in 100 μl of 50 mM sodium phosphate buffer (pH 7.2) at 37 °C for 20 h. The resulting peptides were analyzed by LC/ESIMS, LSIMS, and CID.

Chymotrypsin Subdigest—Tryptic peptides (490–520) and (650–672) were first incubated in 70 mM NH₄HCO₃ buffer (pH 7.8) with approximately 2% (w/w) chymotrypsin at 37 °C, for 1.5 h. In the following experiments, the amount of enzyme was increased to 6%, and the incubation time was 5 h. Components of the digests were separated by reversed-phase HPLC and analyzed by LSIMS.

Endoproteinase Glu-C Subdigest—Pooled fractions of the chymotryptic subdigest of tryptic peptides (490–520) and (650–672) were incubated with approximately 10% (w/w) endoproteinase Glu-C (Boehringer Mannheim) in 1.5 ml urea, 50 mM NH₄HCO₃ buffer (pH 7.8) at 37 °C for 7 h. The resulting peptides were separated by reversed-phase HPLC and analyzed by LSIMS.

HPLC/ESIMS—A dual syringe pump (Carlo Erba Fisons) was used to deliver mobile phase at a flow rate of 50 μl/min. Microbore HPLC separations were performed on an Apiezon, 300 C18 microbore column, 1.0 mm, inner diameter x 100 mm (Applied Biosystems) or on the Vyodac column mentioned above. Column effluent was monitored by a variable wavelength UV detector (Applied Biosystems) equipped with a high sensitivity capillary flow cell (LC Packings) at 215 nm. Post-column addition of 2-methoxyethanol/isopropanol (1:1) (27) was accomplished with a second syringe pump (lisc) connected to a 3.1-μl dead volume PEEX mixer (Upchurch Scientific), positioned after the UV detector. After the mixing tee, the column effluent was split at a ratio of 1:20; approximately 5% of the sample entered the mass spectrometer at a flow rate of 3–5 μl/min, while the remaining sample was manually collected for subsequent analyses. The microbore HPLC system was interfaced to a VG Biotech/Fisons Bio-Q mass spectrometer equipped with an electrospray source. Typical operating voltages were as follows: probe tip, 4200 V; counter electrode, 550 V; and sampling orifice, 40–50 V. The source temperature was maintained at 60 °C. The mass spec-
Phosphorylation Sites in Protein Kinase C

Fig. 2. HPLC chromatogram of carboxymethylated protein kinase C tryptic digest. The tryptic peptides (70 pmol) were separated by reversed-phase HPLC on a Vydac C\textsubscript{18} 1.0 mm, inner diameter x 250-mm column. Solvent A was 0.1% trifluoroacetic acid in water, and solvent B was 0.08% trifluoroacetic acid in acetonitrile. The eluant was monitored at 215 nm. Phosphopeptide His\textsuperscript{636}-Arg\textsuperscript{649} started to elute in peak 1. Peak 2 contained both the non-modified and phosphorylated peptides for this sequence. Peptides corresponding to non-modified and phosphorylated Thr\textsuperscript{500}-Lys\textsuperscript{520} eluted in peak 3 (See Fig. 5). Phosphopeptide Glu\textsuperscript{490}-Lys\textsuperscript{520} eluted in peak 4. Phosphopeptide Asn\textsuperscript{650}-Lys\textsuperscript{672} eluted in peak 5. These species were not fully separated and coeluted with other tryptic peptides. Peak 6 contained peptide Asn\textsuperscript{650}-Lys\textsuperscript{672} without any covalent modification. AUFS (absorbance units full scale) gives the relative peak absorbance.

analysis (data not shown). Phosphopeptides were identified based on the 80-Da mass difference between the predicted and observed molecular masses. Addition of a phosphate group increases the molecular mass of a peptide by 80 Da. Four phosphopeptides were identified in the digest with molecular masses of 1677.4 Da (rt− 36 min), 2484.6 Da (rt− 51 min), 3630.0 Da (rt− 56.5 min), and 2771.5 Da (rt− 57.5 min), corresponding to phosphorylated sequences His\textsuperscript{636}Arg\textsuperscript{649}, Thr\textsuperscript{500}, Lys\textsuperscript{520}, Glu\textsuperscript{490}-Lys\textsuperscript{520}, and Asn\textsuperscript{650}-Lys\textsuperscript{672}, respectively (30). The expected mass values of the fragments can be calculated for peptides with known amino acid sequence.

The peptide of molecular mass of 1677.4 Da was subjected to high energy CID analysis, which confirmed the amino acid sequence as His\textsuperscript{636}Arg\textsuperscript{649} and the presence of a phosphate group at Thr\textsuperscript{641} (Fig. 3). This peptide was observed without modification as well (rt− 37 min). Based on the relative ion abundances of the phosphorylated and non-modified peptides from LC/ESIMS analysis and in vitro autophosphorylation studies (13), it is estimated that this site is phosphorylated at least 75%.

Since both peptides Glu\textsuperscript{490}-Lys\textsuperscript{520} and Thr\textsuperscript{500}-Lys\textsuperscript{520} were observed with a molecular mass increase of 80 Da, and peptide Glu\textsuperscript{490}-Lys\textsuperscript{499} was observed only without the phosphate group, it can be deduced that the modification occurs either on Thr\textsuperscript{500} or Thr\textsuperscript{504}. Phosphopeptide Glu\textsuperscript{490}-Lys\textsuperscript{520} was subjected to digestion with various enzymes to produce smaller peptides more suitable for high energy CID experiments. The peptide proved to be resistant to chymotrypsin, and endoproteinase Glu-C removed only the C-terminal nine amino acids. Digestion with endoproteinase Asp-N eventually yielded a phosphopeptide in the desired molecular weight range, D\textsuperscript{494}GVT\textsuperscript{503}TFC\textsuperscript{505}GTP\textsuperscript{505} with MH* at m/z 1364.6, which was then subject to high energy CID analysis (Fig. 4). Fragment ions with charge retention at the N terminus for the first six residues do not indicate the presence of any covalent modification. However, N-terminal fragment ion \textit{a}, (at m/z 755) that results from a cleavage between the α carbon of Thr\textsuperscript{500} and its carbonyl group exhibits an 80-Da mass shift, corresponding to a phosphate group. Similarly all the other N-terminal ions containing Thr\textsuperscript{500} display this 80-Da mass shift. C-terminal ion \textit{y}, which is formed via peptide bond cleavage between Gly\textsuperscript{504} and Thr\textsuperscript{504} with charge retention at the C terminus, was detected at m/z 217, thus indicating no covalent modification at Thr\textsuperscript{504}. Thus, the modification occurred at Thr\textsuperscript{500}. A peptide for Thr\textsuperscript{500}-Lys\textsuperscript{520} with no modification was detected as a minor component in the LC/ESIMS experiment (rt− 51 min, Fig. 5). Peptide Glu\textsuperscript{490}-Lys\textsuperscript{520} was only detected with the modification. The site occupancy for Thr\textsuperscript{500} is estimated to be higher than 90%.

The identity of phosphopeptide Asn\textsuperscript{650}-Lys\textsuperscript{672} was confirmed by Edman degradation (see Table I). The mass is increased by a single 80-Da increment, indicating one phosphate group per peptide. Since the peptide contains three possible phosphorylation sites, Ser\textsuperscript{654}, Ser\textsuperscript{660}, and Ser\textsuperscript{664}, attempts were made to produce peptides containing individual phosphorylation sites. The peptide was resistant to endoproteinase Glu-C and Asp-N; endoproteinase Asp-N was tried since it was reported to cleave at the N terminus of not only aspartic acids but also at the N terminus of other negatively charged residues such as cysteic acids (32) and glutamic acids (24). Chymotryptic digestion yielded a phosphopeptide, Asp\textsuperscript{650}-Ph\textsuperscript{666}, still containing all three serine residues. Based on the UV and LC/ESIMS data, the occupancy of on peptide Asn\textsuperscript{650}-Lys\textsuperscript{672} is estimated to be greater than 80%. Kerenan, Dutil, and Newton have informed us\textsuperscript{2} that the phosphorylation occurs at Ser\textsuperscript{660}. This correlates well with the high degree of conservation of Ser\textsuperscript{660} in comparison with Ser\textsuperscript{654} and Ser\textsuperscript{664} (Fig. 6).

DISCUSSION

Three distinct sites of phosphorylation, Thr\textsuperscript{500}, Thr\textsuperscript{641}, and one of the serines on phosphopeptide, Asn\textsuperscript{650}-Lys\textsuperscript{672}, were determined by mass spectrometry (Fig. 7). Each site was phosphorylated greater than 75%. Thr\textsuperscript{500} lies in the conserved serine/threonine kinase catalytic region. Thr\textsuperscript{641} lies outside that conserved region, but the residue itself is conserved in the protein kinase C family. Phosphopeptide Asn\textsuperscript{650}-Lys\textsuperscript{672} is at the C terminus and lies within a region defined as variable among the major members of the protein kinase C family.

Of the autophosphorylation sites previously identified in vitro (13), only Thr\textsuperscript{641} is detected in this analysis of unstimulated sample. The fact that 75% of the sample is already phosphorylated at this residue explains the apparent low labeling level detected in the in vitro autophosphorylation studies. A single mutation to alanine at the corresponding residue in the β isozyme decreases activity in vivo, and the mutant is no longer able to autophosphorylate (33). Recent work using phosphatase treatment and subsequent autophosphorylation of protein kinase C βII has suggested that protein kinase C is solely

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\textsuperscript{2} L. M. Kerenan, E. M. Dutil, and A. C. Newton, personal communication.
responsible for phosphorylation of this residue (19).

It is notable that only one of six in vitro autophosphorylation sites was found to be phosphorylated in this study. The difference between the level of protein kinase C stimulation in vitro (activation by diacylglycerol, Ca\(^{2+}\), and phosphatidylserine) (13) and in vivo (no artificial stimulation) could explain a lack of autophosphorylation. However, one of the sites, Thr\(^{641}\), is phosphorylated, which suggests that protein kinase C was activated in vivo. If protein kinase C autophosphorylates itself at Thr\(^{641}\), why are the other autophosphorylation sites also not phosphorylated? Possible explanations are (a) degree of accessibility of Thr\(^{641}\) relative to the other sites, (b) sensitivity or resistance to phosphatases, (c) specific post-translational processing of protein kinase C (19), (d) a second kinase phosphorylating only Thr\(^{641}\) (33), or (e) discrepancies between the plasma membrane in vivo and detergent micelles in vitro. Further work will be needed to clarify this issue.

The observed phosphorylation of protein kinase C at Thr\(^{600}\) is interesting in view of known serine/threonine kinase structures. In protein kinase A (34), a phosphorylated residue at this position is necessary for the integrity of the active site structure. In the modeled protein kinase C structure, a phosphorylated threonine would be able to interact with surrounding residues in a manner very reminiscent to protein kinase A (17).

Fig. 3. High energy CID spectrum of phosphorylated peptide His\(^{636}\)-Arg\(^{649}\). MH\(^{+}\) = 1677.8. Fragment ions are labeled according to the accepted nomenclature (40).

Fig. 4. High energy CID spectrum of phosphorylated peptide Asp\(^{494}\)-Pro\(^{505}\). MH\(^{+}\) = 1364.6. N-terminal sequence ions starting from the amino acid at position 7 (Thr\(^{500}\)) show the 80-Da mass shift. The cysteine marked with an asterisk is carbamylated. Ions labeled with asterisks are matrix-related background ions (41).
These residues are conserved in many other serine/threonine kinases (35). Complete in vivo phosphorylation at Thr500 supports the conclusion that it is the activating phosphorylation site conserved in many kinases (36). Phosphorylation at Thr500 agrees with biochemical evidence that this residue is critical for activity. Mutagenesis studies demonstrated that Thr497 and Thr500 in the α and βII isozymes, respectively, were essential for activity (16, 17). The authors suggested that a second kinase must be phosphorylating and thus activating protein kinase C. In fact, replacement of Thr500 in the βII isozyme with glutamate restored complete activity and suggests that it is the phosphorylation of the residue that is critical (17). Thorsness and Koshland (37) have shown that an aspartate can mimic the presence and an alanine the absence of an inhibitory phosphorylation in isocitrate dehydrogenase. In protein kinase C, it appears that the larger glutamate is better able to maintain the integrity of the active site.

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These studies are in agreement with our identification of greater than 90% in vivo phosphorylation at Thr500.

Deletion studies of protein kinase C α (38) suggest that phosphorylation near the C terminus is critical for protein kinase C activity. Truncation of 23 amino acids from the C terminus fully inactivates the kinase (38); Ser660 corresponds to the 16th residue from the C terminus in the α isozyme (Fig. 6). In addition, the high degree of conservation of Ser660 suggests a potential family-wide regulation (Fig. 6).

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We have determined in vivo phosphorylation sites of unstimulated protein kinase C βII. All three of these regions appear to play a strong role in protein kinase C function. Phosphorylation at a particular residue such as Thr500 may be of structural importance. The other phosphorylated sites may be involved in substrate recognition or activator affinity. The fact that activators of protein kinase C increase the phosphorylation state while epidermal growth factor decreases the...
phosphorylation state (39) suggests that phosphorylation is an important means of regulating protein kinase C activity.

REFERENCES

1. Fisher, E. H., and Krebs, E. G. (1955) J. Biol. Chem. 216, 121–132
2. Peled, S. L., Sanghera, J. S., and Daya-Makin, M. (1991) Biochem. Cell. Biol. 69, 1297–1330
3. Sturgill, T. W., and Wu, J. (1991) Biochim. Biophys. Acta 1092, 350–357
4. Nishizuka, Y. (1986) Science 233, 305–312
5. Azzi, A., Boscoboinik, D., and Hensey, C. (1992) Eur. J. Biochem. 208, 1297–1330
6. Sturgill, T. W., and Wu, J. (1991) Biochim. Biophys. Acta 1092, 350–357
7. Nishizuka, Y. (1992) Science 258, 607–614
8. Dekker, L. V., and Parker, P. J. (1994) Trends. Biochem. Sci. 19, 73–77
9. Coussens, L. M., Takai, Y., Minakuchi, R., Inohara, S., and Nishizuka, Y. (1982) J. Biol. Chem. 257, 13341–13348
10. Kikkawa, U., Takai, Y., Minakuchi, R., Inohara, S., and Nishizuka, Y. (1982) J. Biol. Chem. 257, 13341–13348
11. Mochly-Rosen, D., and Koshland, D. E., Jr. (1987) J. Biol. Chem. 262, 2291–2297
12. Newton, A. C., and Koshland, D. E., Jr. (1987) J. Biol. Chem. 262, 10185–10188
13. Flint, A. J., Paladini, R. D., and Koshland, D. E., Jr. (1990) Science 240, 408–411
14. Peers, C., Stabel, S., Cazaubon, S., and Parker, P. J. (1992) Biochemistry 283, 515–518
15. Cazaubon, S. M., and Parker, P. J. (1993) J. Biol. Chem. 268, 17559–17563
16. Cazaubon, S., Bornancin, F., and Parker, P. J. (1994) Biochem. J. 301, 443–448
17. Orr, J. W., and Newton, A. C. (1994) J. Biol. Chem. 269, 27715–27718
18. Zhang, J., Wang, L., Petrin, J., Bishop, W. R., and Bond, R. W. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 6130–6134
19. Drapeau, G. R. (1980) J. Biol. Chem. 255, 839–840
20. Zhang, J., Wang, L., Schwartz, J., Bond, R. W., and Bishop, W. R. (1994) J. Biol. Chem. 269, 19578–19584
21. Knighton, D. R., Zheng, J. H., Ten Eyck, L. F., Ashford, V. A., Xuong, N. H., Taylor, S. S., and Sowadski, J. M. (1991) Science 253, 407–414
22. Hanks, S. K., Quinn, A. M., and Hunter, T. (1988) Science 241, 42–52
23. Taylor, S. S., and Radzio-Andzelm, E. (1994) Structure 2, 345–355
24. Thorsness, P. E., and Koshland, D. E., Jr. (1987) J. Biol. Chem. 262, 10422–10425
25. Su, L., Parissenti, A. M., and Riedel, H. (1993) Receptors Channels 1, 1–9
26. Mitchell, E. F., Marais, R. M., and Parker, P. J. (1989) Biochemistry 28, 3131–3136
27. Biemann, K. (1990) Methods Enzymol. 193, 866–887
28. Falick, A. M., Medzihradszky, K. F., and Walls, F. C. (1995) Anal. Chem. 68, 1308–1311
29. Walls, F. C., Hall, S. C., Medzihradszky, K. F., Yu, Z., and Burlingame, A. L. (1993) Proceedings of the 41st ASMS Conference on Mass Spectrometry and Allied Topics (Abstr. 937)
30. Medzihradszky, K. F., and Burlingame, A. L. (1994) Methods Companion Methods Enzymol. 6, 284–303
31. Johnson, R. S., Martin, S. A., and Biemann, K. (1988) Int. J. Mass Spectrom. Ion Proc. 86, 137–154
32. Drapeau, G. R. (1980) J. Biol. Chem. 255, 839–840
33. Zhang, J., Wang, L., Schwartz, J., Bond, R. W., and Bishop, W. R. (1994) J. Biol. Chem. 269, 19578–19584
34. Knighton, D. R., Zheng, J. H., Ten Eyck, L. F., Ashford, V. A., Xuong, N. H., Taylor, S. S., and Sowadski, J. M. (1991) Science 253, 407–414
35. Hanks, S. K., Quinn, A. M., and Hunter, T. (1988) Science 241, 42–52
36. Taylor, S. S., and Radzio-Andzelm, E. (1994) Structure 2, 345–355
37. Thorsness, P. E., and Koshland, D. E., Jr. (1987) J. Biol. Chem. 262, 10422–10425
38. Su, L., Parissenti, A. M., and Riedel, H. (1993) Receptors Channels 1, 1–9
39. Mitchell, E. F., Marais, R. M., and Parker, P. J. (1989) Biochemistry 28, 3131–3136
40. Biemann, K. (1990) Methods Enzymol. 193, 866–887
41. Falick, A. M., Medzihradszky, K. F., and Walls, F. C. (1990) Rapid Commun. Mass Spectrom. 4, 318–322
42. Orr, J. W., and Newton, A. C. (1994) J. Biol. Chem. 269, 8383–8387