INSIGHT INTO THE ASSOCIATION BETWEEN PHOSPHOLIPASE C-\(\gamma\)1 AND THE INSULIN RECEPTOR

Hyeung-Jin Jang, Yong-Kook Kwon, Sutapa Kole and Michel Bernier

Diabetes Section, Laboratory of Clinical Investigation, National Institute on Aging, National Institutes of Health, Baltimore.

Corresponding author: Michel Bernier. Diabetes Section, Laboratory of Clinical Investigation, National Institute on Aging, National Institutes of Health, Baltimore. MD 21224, USA. E-mail: Bernierm@mail.nih.gov.

SUMMARY

An intense investigation is underway to identify the signaling intermediates that interact directly with the insulin receptor (IR) as they appear to be associated with the transmission of insulin action. We recently reported the inducible association of phospholipase C (PLC)\(\gamma\)1 with...
the IR and its involvement in the transduction of insulin-mediated signals through activation of the Ras/extracellular-regulated kinase pathway. Akin to protein phosphorylation, redox regulation represents an important metabolic modulator of cellular functions. In this study, various mutant forms of PCLγ1 were generated to identify putative key redox-sensitive cysteines that provide sites for a number of post-translational modifications. Toward this aim, two methods were used that demonstrate the presence of oxidation-sensitive cysteine residues in PCLγ1. Using site-directed mutagenesis experiments we found that two cysteines in PCLγ1 (Cys-8 and Cys-12) were modified with hydrogen peroxide and protected against a host of reactive oxygen species through pharmacological inhibition of cellular membrane oxidases. The data suggest that the amino-terminal region of PCLγ1 contains reactive cysteine-SH groups that are exquisitely sensitive to redox regulation and which are associated with efficient interaction with the IR. The importance of these findings is already asserting themselves in studies reporting the regulatory role of cellular redox in insulin signal transduction.

**Keywords:** insulin receptor, phospholipase Cγ1, protein-protein interaction, oxidative stress, signal transduction.

**INTRODUCTION**

The physiological effects of insulin are mediated by activation of the intrinsic tyrosine kinase function of the insulin receptor (IR) and its association with a number of scaffold molecules (e.g., IRS-1, Gab-1) harboring distinct recognition domains for the binding of signaling-competent effector proteins (Saltiel and Pessin, 2002). The insulin receptor is a heterotetrameric glycoprotein consisting of two α-β dimers linked by disulfide bonds (Garant et al., 1999). We used a novel procedure to study the interaction between IR and endogenous molecules in cells by taking advantage of the fact that the homobifunctional crosslinking reagent 1,6-bismaleimidohexane (BMH) selectively reacts with nucleophilic cysteine residues to form an irreversible link between two interacting proteins (Garant et al., 2000). Immunoprecipitation-based technique coupled with mass spectrometry analysis allowed us to establish that insulin promoted the formation of a covalent complex between the IR β-subunit and phospholipase (PLC)γ1 upon addition of BMH to Chinese hamster ovary (CHO) cells stably expressing the human IR (Kwon et al., 2003) (see figure 1A, B). Western blot analysis independently confirmed the recruitment of PCLγ1 to the IR following insulin stimulation of a number of cell types. Of significance, the amino-terminal region of PCLγ1, which consists of a pleckstrin homology (PH) domain and a series of EF-hands, has been proposed to contribute to the interaction between PCLγ1 and the activated IR (Kwon et al., 2003; Bernier et al., 2004).

PCLγ1 plays an important role in the intracellular transduction of receptor and nonreceptor tyrosine kinases. It is widely distributed and exerts essential function in mammalian growth and development as evidenced by the fact that mice deficient in PCLγ1 are embryonic lethal (Ji et al. 1997). PCLγ1 is a member of the family of phosphoinositide–specific PLCs that convert phosphatidylinositol 4,5-bisphosphate (PI4,5P2) to two second messengers, inositol 1,4,5-trisphosphate (IP3) and 1,2-diacylglycerol (DAG), upon cell activation (Rhee, 2001). IP3 mobilizes Ca2+ from intracellular stores, while DAG is responsible for the activation of a subset of protein kinase C isoforms (e.g., α, β, τ, ε). Activation of a chimeric receptor containing the juxtamembrane and tyrosine kinase domains of the IR led to PCLγ1 activation and concomittant increase in calcium mobi-
Figure 1. A) Schematic representation of the covalent recruitment of PCLγ1 to the intracellular domain of the IR β-subunit in insulin-stimulated cells in the presence of BMH, a homobifunctional thiol-specific crosslinking agent. B) Immunoblot analysis in support of this process. pY783, mAb antibody specific for tyrosine phosphorylated PCLγ1. C) Identification of putative PCLγ1 nucleophilic cysteines using the thiol-modifying agent MBB. * denotes accessible cysteine(s) with low pKa at physiological pH. Panels A and B were reproduced from Bernier et al. (2004).

lization (Telting et al., 1999). PCLγ1-mediated PI4,5P2 hydrolysis in anti-IR immunoprecipitates has been found in insulin-stimulated 3T3-L1 adipocytes (Eichhorn et al., 2001). PCLγ1 participates in insulin-stimulated glucose uptake in adipocytes through activation of DAG-sensitive PKCζ (Lorenzo et al., 2002), whereas microinjection of neutralizing antibodies to PCLγ1 interferes with insulin’s ability to induce DNA synthesis in IR-expressing Rat-1 fibroblasts (Eichhorn et al., 2002). Finally, knockdown of PCLγ1 expression by RNA interference significantly reduces activation of extracellular-regulated kinase (ERK) pathway, but not that of Akt, in response to insulin, whereas reconstitution of PCLγ1 in PCLγ1−/− mouse embryonic fibroblasts elicits a marked increase in insulin-stimulated ERK activation (Kwon et al., 2003). These and other data provide clear indication that increased PCLγ1 activity could modulate the metabolic and mitogenic effects of insulin.

The juxtamembrane region of the human IR cytoplasmic domain contains a cysteine residue that exhibits low pKa at physiological pH (Bernier et al., 1995; Bernier et al., 2004), which makes it a chemical hot spot for a number of biochemical interactions. Indeed, the selective sensitivity of Cys-981 to thiol-modifying agents, such as maleimidobutyryl biocytin (MBB), was used to our advantage in tagging human IR in intact cells (Garant et al., 2000). However, no studies have been performed to test the presence of reactive cysteine residue(s) in PCLγ1, which could account for its ability to covalently bind the IR upon BMH treatment. On the basis of this, we set out experiments to identify oxidation-sensitive cysteine-SH groups in PCLγ1 and assess their role in the control of IR-PCLγ1 covalent interaction in the presence of BMH. While the mechanism of IR-PCLγ1 interaction remains to be fully elucidated, analysis of the cysteine residues in PCLγ1 that are potentially
susceptible to redox regulation may provide new insight into PCLγ1 function.

METHODS

Plasmid constructs. C- and N-terminal fragments and a double point-mutation (C8A/C12A) of the rat PCLγ1 (NM_013187) were generated using plasmid pRK5/HA-PCLγ1 (kindly provided by Graham Carpenter Nashville, TN, USA). The generation of HA (hemagglutinin) epitope-tagged 304N (from a.a. 1-304 of PCLγ1, which contains PH domain and EF-hands) involved a PCR-based site-directed mutagenesis approach to introduce HindIII restriction site between EF-hands and catalytic domain “X” of PCLγ1. An HindIII/HindIII fragment (2961 bp long) was excised. To generate PCLγ1Δ216C mutant (deletion of a.a. 1075-1291, which contains C2 domain), HindIII restriction site was introduced by mutating the region (3309AAGCCTTTGT3317) to AAGCTTTTG. An HindIII/HindIII fragment (660 bp long) was excised. The construction of PCLγ1Δ304N mutant was carried out as followed: Two EcoRI restriction sites were introduced by mutating the region (1001CGTCGG108) and (900ACCGGCTTC908) to GGAATTCGG and ACCGATTC, respectively, while deleting EcoRI site present in pRK5 vector’s multicloning site. An EcoRI/EcoRI fragment encompassing 304N was excised. The linearized pRK5/HA-tagged plasmids were then self-ligated. The cysteine 8 and 12 to alanine mutation in PCLγ1 (C8A/C12A) was introduced by replacing codon TGC with GCC. Similarly, several point-mutations of HA-304N (termed C8C12AA, C26A, C106A, and C247A) were constructed using the QuickChange site-directed mutagenesis kit (Stratagene). All of the mutations were verified by DNA sequence analysis.

Cell culture. CHO cells expressing the human IR (CHO-IR) cells were maintained and grown to near confluence in Ham’s F-12 medium supplemented with 10% FBS, glutamine and penicillin/streptomycin. Cells were then maintained in medium without serum (SFM) for 4 h, after which 100 nM insulin was added for 5 min. In some instances, cells were treated for 30 min with either vehicle (dimethylsulfoxide, DMSO), diphenyllethiodiiodonium chloride (DPI; Alexis Corp., San Diego, CA), hydrogen peroxide (H₂O₂; Fisher), pyrrolidinedithiocarbamate (PDTC; Calbiochem, La Jolla, CA), bpV(phen) (Calbiochem) or thiolic acid (oxidized form) (Sigma-Aldrich, St-Louis, MO) prior to the addition of insulin.

Transient transfection assays. CHO-IR cells were transfected by the Lipofectamine2000 method (Invitrogen). Empty expression vector and expression plasmids encoding wild-type or mutant forms of PCLγ1 were mixed with the transfection reagent and directly added into the culture plates at a ratio of 2-4 µg of each plasmid/60-mm dish. Twenty hours later, cells were serum-starved for 3 h and then subjected to treatments as described below.
INSIGHT INTO THE ASSOCIATION BETWEEN PHOSPHOLIPASE C-γ1 AND THE INSULIN RECEPTOR

A

PLCγ1

WT

C8A/C12A

Δ216C

Δ304N

304N

Number of cysteines

(24)

(22)

(21)

(19)

(5)

B

Ip: anti-HA

Blot: SA-HRP

Ip: anti-HA

Blot: anti-HA

C

Ip: PLCγ1

D

HA-304N

peDNA

B MH

Ip: HA

Blot: PLCγ1

E

Cell Lysates

HA-304N

WT

C8C12AA

peDNA

C106A

C247A

BMH

Ip: HA

Blot: IκBα
Thiol-specific biotinylation and BMH-mediated chemical crosslinking of PCLγ1 in cells. Serum-starved cells were washed twice in phosphate-buffered saline (PBS), and then incubated in Krebs Ringer Phosphate (KRP) buffer for 5-30 min at 37 °C before their transfer to thermoregulated aluminium cooling plates set at 6 °C. The thiol-biotinylation reaction was initiated by the simultaneous addition of 20 µg/ml digitonin and 100 µM MBB (Calbiochem) for 10 min, with subsequent addition of 4 mM L-cysteine to quench the reaction. Detection of biotinylated protein sulfhydryls was carried out by Western blot using HRP-conjugated streptavidin (Vector Lab, Burlingame, CA). The crosslinking reaction followed the procedure of Garant et al. (2000). After a 5-min stimulation with insulin (100 nM) in KRP buffer, cells were transferred to 6 °C and then the crosslinking reaction was initiated by the addition of 100 µM BMH (Pierce Chemical Corp., Rockford, IL) or vehicle (DMSO) and quenched 10 min later with 4 mM L-cysteine.

Immunoprecipitation and Western blot analysis. Cell lysis was performed using established procedures (Kwon et al., 2003). Following appropriate treatment of the cells, cells were washed once with PBS and then lysed in radioimmune precipitation buffer containing 20 mM Tris-HCl, pH 7.5, 137 mM NaCl, 0.1% SDS, 0.5% deoxycholate, 1% Triton X-100, 0.02% NaN3, 100 mM NaF, 1 mM orthovanadate, and protease inhibitor cocktail (Calbiochem). The clarified lysates were isolated from homogenates by centrifugation at 4 °C. An aliquot was suspended in 62.5 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, 5% β-mercaptoethanol and heated at 70 °C for 10 min. A second aliquot of the clarified lysates was incubated with mAb anti-PCLγ1 (Chemicon International; N-terminal epitope) or mAb anti-IR (clone 29B4; Calbiochem) for 16 h at 4 °C. The samples were subjected to SDS-polyacrylamide gel electrophoresis under reducing conditions, transferred onto PVDF membrane and then blots were incubated with primary antibodies in blocking buffer (mAb anti-HA antibody (Covance); rabbit anti-IR α-subunit and anti-IXβα (Santa Cruz Biotechnology); rabbit anti-phosphoPCLγ1 (pY783) antibody (BioSource International); antiphosphotyrosine (clone RC20)-linked to HRP and rabbit anti-IR β-subunit (Transduction Lab)). Bound antibody was detected using enhanced chemiluminescence according to established protocols.

Assay of intracellular H2O2. Serum-starved CHO-IR cells were incubated with 100 µM H2O2 or 10 µM DPI for 30 min before the addition of 100 nM insulin. Five min later, 4 µM dichlorohydrofluorescein (CM-H2DCF-DA; Molecular Probes, Eugene, OR) was added in the dark for 10 min at room temperature. Fluorescence of the indicator dye was visualized using a Zeiss LSM-410 inverted confocal microscope at an excitation wavelength of 488 nm and emission at 515/540 nm.

RESULTS

PCLγ1 contains reactive thiols. MBB is a biotin-containing, thiol-specific reagent that reacts readily with proteins containing cysteine residues. When combined with streptavidin-conjugated HRP, this approach allows the detection of redox-sensitive cysteines. MBB was used successfully to identify Cys981 of the human IR as a nucleophilic thiol (Bernier et al., 1995).

Following transient expression of wild-type and mutant forms of HA-tagged PCLγ1 in CHO-IR cells (see figure 2A), MBB-mediated thiol-biotinylation reaction was carried out and anti-HA immunoprecipitates were evaluated for the content of reactive cysteines by Western blotting (see figure 2B). A significant incorporation of biotin was observed in wild-type PCLγ1 after administration of MBB to semi-permeabilized cells, which sup-
ports the notion that PCLγ1 contains reactive cysteine residues at physiological pH. Similar to wild-type PCLγ1, Δ304N and Δ216C PCLγ1 mutants showed strong reactivity to MBB, whereas the level of thiol-biotinylated C8A/C12A PCLγ1 mutant was markedly lower (see figure 2B, upper panel). Reprobing the membrane with anti-HA showed the relative expression level of each PCLγ1 construct (see figure 2B, lower panel). To further establish the presence of reactive cysteines in the N-terminal region of PCLγ1, HA-304N (which contains the PH-EF domain) was expressed in CHO-IR cells and subjected to thiol-biotinylation (see figure 2C). The results showed the conjugation of HA-304N with biotin. Moreover, the construct exhibited BMH-induced chemical crosslinking, as evidenced by the reduced signal at 43 kDa in HA immunoprecipitates (see figure 2D). As shown in see figure 2A, the N-terminal region of PCLγ1 contains 5 cysteines, some of which may be subject to oxidative modification. Various Cys to Ala point-mutants of HA-304N were generated and analyzed for their response to BMH-induced crosslinking in intact CHO-IR cells (see figure 2E, upper panel). The results clearly showed a sharp reduction in the recovery of monomeric forms of WT, C106A and C247A mutants following BMH treatment, while having no detectable effect on C8C12AA. C26A expression was very low and thus could not provide reliable information (data not shown). Reprobing the membrane with anti-IκBα antibody indicated that BMH was effective at promoting thiol modification of IκBα protein in each cell line (see figure 2E, lower panel). Taken together, our data revealed that PCLγ1 possesses reactive cysteine residues at position 8 and 12.

Modulation of PCLγ1 thiol reactivity in intact cells. Several enzymes that regulate downstream components in the insulin signaling cascade are potential targets of redox modifications that take place in cells exposed to a host of reactive oxygen and nitrogen species (Finkel, 2003). To measure the generation of intracellular H₂O₂, cells are loaded with the redox indicator dye based on dichlorohydrofluorescein (CM-H₂DCF-DA) that is trapped intracellularly after cleavage by cellular esterases. Following stimulation of CHO-IR cells with 100 nM insulin, an oxidant signal was detected by DCF fluorescence, which peaked at 5 min (see figure 3A, panel b), and began to dissipate by 10 min (not shown). Others have reported that the oxidant generated by insulin was H₂O₂, as preincubation of the cells with catalase attenuated the fluorescent signal (Mahadev et al., 2001). Exogenous addition of H₂O₂ markedly increased DCF signal (see figure 3A, panel c), whereas incubation of the cells with diphenyleneiodonium (DPI), an inhibitor of cellular NADPH oxidase activity, blocked the production of H₂O₂ in response to insulin (see figure 3A, panel d). We then investigated the effect of DPI and H₂O₂ on PCLγ1 thiol reactivity in CHO-IR cells. When cells were treated with DPI, the incorporation of MBB in wild-type PCLγ1 increased 3-fold while having no detectable effect on the C8A/C12A mutant (see figure 3B). Addition of exogenous H₂O₂ caused a 50% and full reduction in thiol biotinylation of the wild-type PCLγ1 and C8A/C12A mutant, respectively. The data suggest that PCLγ1 contains a discrete subset of cysteines that are likely to be involved in the response of PLCγ1 to redox modification. The absence of cysteines 8 and 12 in C8A/C12A mutant confers refractive-ness to DPI action.

Insulin facilitates the recruitment of PCLγ1 to the activated IR, enabling both proteins to be covalently linked using BMH, a selective homobifunctional thiol-specific crosslinker (Kwon et al., 2003). To test whether alteration in cellular redox could affect this process, CHO-IR cells were preincubated with DPI or H₂O₂ followed by the addition of insulin and subsequent treatment with BMH (see figure 3C). A dose-dependent enhancement in IR-PCLγ1 covalent association was
achieved with DPI with concomitant reduction in unconjugated IR β-subunit (see figure 3C, left panel). Conversely, \( \text{H}_2\text{O}_2 \), which increases cellular thiol oxidation (see figure 3B), dose-dependently reduced covalent binding of PCL\( \gamma \)1 to the IR (see figure 3C, right panel) while promoting the recovery of tyrosine phosphorylated IR β-subunit and PCL\( \gamma \)1 (see figure 3D).

BpV(phen) is a stable peroxovanadium compound with insulinomimetic properties by virtue of its ability to activate the IR kinase and subsequent stimulation of downstream signaling (Bevan et al., 1995). Previous studies indicated that bpV(phen) induces the production of intracellular superoxide anion through activation of \( \text{NADPH} \) oxidase at the plasma membrane (Yamaguchi et al., 1995), which leads to transient cytosolic acidification (Bianchini et al., 1994). We next ascertain the effect of bpV(phen) on BMH-dependent IR-PCL\( \gamma \)1 crosslinking. When stimulated with insulin and challenged with 20 \( \mu \)M bpV(phen), CHO-IR cells exhibited a marked attenuation in IR-PCL\( \gamma \)1 complex formation (see figure 3E, lane 2 vs. 1). Because antioxidants can offer cellular defense against oxidative stress (Roy et al., 1997), we tested the effect of three structurally unrelated antioxidants on the cellular response to bpV(phen). While thiotic acid (α-lipoic acid) and the metal chelator PDTC were mostly ineffective at counteracting bpV(phen) signaling, pretreatment with DPI for 30 min led to a prominent retention of the IR-PCL\( \gamma \)1 complex despite the presence of bpV(phen) (see figures 3E and 3F). The data support the model whereby plasma membrane \( \text{NADPH} \) oxidase activity plays a key role in the regulation of protein-protein interaction of redox-sensitive signaling molecules, such as PCL\( \gamma \)1 and the IR.

**DISCUSSION**

In our previous work, we have shown that PCL\( \gamma \)1 covalently binds to the activated IR upon addition of BMH to insulin-stimulated CHO-IR cells (Kwon et al., 2003). Deletion of the juxtamembrane NPEY motif or truncation of the carboxyl 43 amino acids of the IR did not inhibit this process (Bernier et al., 2004), suggesting that the association of PCL\( \gamma \)1 with the IR occurs at a region/motif that differs from that required by the well-known effectors of insulin action (Kharitonenkov et al., 1995; Sawka-Vervelle et al., 1996; O’Neill et al., 1996; Kasus-Jacobi et al., 1998). It is generally accepted that intracellular redox plays an important role in the modulation of insulin action. The results of our study document an
active role of a discrete population of Cys-SH moieties in mediating IR-PCLγ1 covalent association in response to BMH, in particular those present in the NH$_2$-terminus of PCLγ1. We approached their characterization by ectopically expressing wild-type, truncated versions and point-mutant forms of PCLγ1 proteins in CHO-IR cells.

Ectopic expression of PCLγ1 N-terminal fragment, 304N (which contains PH domain and EF-hands), was described as having great potency in interfering with endogenous IR-
PCLγ1 interaction (Kwon et al., 2003). Our present data show that 304N contains cysteines that are reactive to cell redox modifiers. Substitution of PCLγ1 at Cys-8 and -12, but not those present in the PH-EF-hands, abrogated responsiveness to BMH while reducing thiol-alkylation with MBB by only 60%, which could indicate the presence of additional reactive Cys-SH moieties at a site (or sites) distant from the N-terminus of PCLγ1. By extension, we propose that these distant Cys residues do not play a role in BMH-mediated covalent binding of PCLγ1 to the IR, which relies instead on the close proximity of the N-terminus PCLγ1 thiol(s) with Cys981 of the human IR. We have recently observed that cell stimulation with insulin led to the detection of both the IR and activated, tyrosine-phosphorylated form of PCLγ1 in subdomains of the plasma membrane known as lipid rafts (Kwon et al., in preparation). Rafts are enriched in actin filaments and have been proposed to serve several important functions, including the preassembly of signaling proteins onto a cytoskeletal scaffold (Whitehead et al., 2000). Interestingly, reactive cysteine moieties are frequent sites for the posttranslational addition of lipids to integral membrane and membrane-associated proteins. These acylation reactions have been shown to be required for optimal targeting and proper function of several families of signaling molecules at the plasma membrane (Resh, 2004). Our present data are the first to document the presence of oxidation-sensitive nucleophilic thiols in PCLγ1; whether lipid modification of PCLγ1 is required for its targeting to rafts microdomains and tyrosine phosphorylation following cell activation by insulin is unclear. Such thioester bond may be transitory and have transient regulatory effects on the protein function. Experiments are currently underway to fully characterize the importance of a cell’s redox system in controlling the targeting and cellular function of PCLγ1.

We found that the inhibition of cellular NADPH oxidases by DPI was the most effective approach against bpV(phen)-mediated decrease in BMH-dependant IR-PCLγ1 cross-linking. It is thought that bpV(phen) can induce superoxide anion production as a result of translocation and phosphorylation of the 47-kDa protein component of the NADPH oxidase at the plasma membrane where the catalytic flavocytochrome b resides (Yaname et al., 1999). Herein, the increase in intracellular H2O2 production with bpV(phen) in CHO-IR cells (data not shown) is likely to promote oxidative thiol modification on either the IR, PCLγ1 or both proteins, thus impacting on their reactivity toward BMH. Components of the NADPH oxidase are known to be localized on the cytoplasmic surface of the plasma membrane through association with the actin cytoskeleton (Woodman et al., 1991; el Benna et al., 1994; Buul et al., 2005). Similar to the components of this oxidase, PCLγ1 binds to elements of the cytoskeleton that directly appose the plasma membrane of the cell (Chou et al., 2002). These results support the view that insulin-mediated PCLγ1 signal transduction involves an oxidation-sensitive pathway through activation of NADPH oxidase at the plasma membrane.

Changes in cellular redox are known to occur with aging, diabetes and other metabolic conditions (Browlee, 2001). The consequences of oxidative damage include altered cell signaling, proliferation and apoptosis. We believe that PCLγ1 is target of such oxidative attack due to the presence of reactive cysteine residues in its N-terminal domain. PCLγ1 binding to the IR has been associated with upregulation of the Ras/ERK pathway in response to acute insulin stimulation (Kwon et al., 2003). Better knowledge of the implications of redox alterations will open new opportunities into the understanding of PCLγ1 signaling and provide a new insight into the ways this enzyme exerts the pleiotropic ac-
tions of insulin in normal and pathophysiological states.

REFERENCES


Sawka-Verhelle, D.; Tartare-Decker, S.; White, M. F.; Obberghen, E. van (1996). «Insulin receptor substrate-2 binds to the insulin receptor through its phosphotyrosine-binding domain and through a newly identified
domain comprising amino acids 591-786». J. Biol. Chem.,
271: 5980-5983.
Telting, D.; Smeets, R. L.; Willems, P. H.; Zon, G. C.
van der; Frankhuizen, W. S.; Maassen, J. A. (1999).
«The insulin receptor tyrosine kinase domain in a chi-
maeric epidermal growth factor-insulin receptor gener-
ates Ca$^{2+}$ signals through the PLC-$\gamma$1 pathway». Biochim.
Whitehead, J. P.; Clark, S. F.; Urso, B.; James, D. E.
Woodman, R. C.; Ruedi, J. M.; Jesaitis, A. J.; Okam-
ura, N.; Quinn, M. T.; Smith, R. M.; Curnutte, J.
three of four oxidase-related polypeptides are associated
with the cytoskeleton of human neutrophils». J. Clin. In-
vest., 87: 1345-1351.
Yamaguchi, M.; Oishi, H.; Araki, S.; Saeke, S.; Yamane,
burst and tyrosine phosphorylation by vanadate». Arch.
Yaname, H.; Fukunaga, T.; Nigorikawa, K.; Okam-
NADPH oxidase via protein kinase C-independent phos-
phorylation of p47-phox». Arch. Biochem. Biophys., 361:
1-6.