Insulin Stimulates Phosphorylation of the \( \beta_2 \)-Adrenergic Receptor by the Insulin Receptor, Creating a Potent Feedback Inhibitor of Its Tyrosine Kinase*

Sergey Doronin, Hsien-yu Wang, and Craig C. Malbon‡

From the Departments of Pharmacology, Physiology & Biophysics, Diabetes & Metabolic Diseases Research Program, Health Sciences Center, State University of New York, Stony Brook, New York 11794

Insulin counterregulates catecholamine action at several levels, primarily in liver, fat, and adipose tissue. Herein we observe that expression of increased levels of \( \beta_2 \)-adrenergic receptor increasingly inhibits insulin-stimulated phosphorylation of its primary downstream substrates (IRS-1,2). In Chinese hamster ovary cells, the insulin receptor phosphorylates dominantly Tyr364 in the C-terminal cytoplasmic domain of the \( \beta \)-receptor. A Y364A mutant form of the \( \beta_2 \)-adrenergic, in contrast, loses its ability to inhibit insulin-stimulated phosphorylation of IRS-1,2. Upon phosphorylation, the C-terminal cytoplasmic domain of the \( \beta_2 \)-adrenergic receptor demonstrates a potent inhibitory feedback action that can block both insulin-stimulated autophosphorylation of the insulin receptor and phosphorylation of IRS-1,2 in NIH mouse 3T3-L1 adipocyte membranes. Studies in vitro with purified insulin receptor and the C-terminal cytoplasmic domain of the \( \beta_2 \)-adrenergic receptor demonstrate that the tyrosine-phosphorylated \( \beta \)-receptor domain is a potent counterregulatory inhibitor of the insulin receptor tyrosine kinase.

The physiological and counterregulatory effects of insulin and catecholamines on carbohydrate and lipid metabolism are well known (1, 2). Insulin counteracts the effects of catecholamines at several levels, including phosphorylation of the \( \beta_2 \)-adrenergic receptor (\( \beta_2 \)AR), which un couples \( \beta_2 \)AR from its G-protein (G\(_{\alpha} \)) and the effector adenyl cyclase (3–7). Activation of \( \beta_2 \)AR, in turn, increases intracellular cyclic AMP levels and counteracts many of the effects of insulin. Earlier studies focused on the \( \beta_2 \)AR Tyr350 residue that is phosphorylated in response to insulin and creates an SH2-binding site to which dynamin, Grb2, or 1-phosphatidylinositol 3-kinase can bind (3–7). The \( \beta_2 \)AR Tyr364 residue is also phosphorylated in response to insulin, but the nature of the role that this phosphorylation may play has remained enigmatic. In this work we report that the \( \beta_2 \)AR Tyr364, when phosphorylated in response to serum or exogenous insulin, is an potent inhibitor of the insulin receptor tyrosine kinase.

EXPERIMENTAL PROCEDURES

Materials—Recombinant tyrosine kinase domain of insulin receptor was purified from SF9 cells and activated as described (8). Expression and purification of the C-terminal cytoplasmic domain of \( \beta_2 \)AR from Escherichia coli cells were as described (9, 10). Phosphotyrosine-specific (pY69), insulin receptor substrates-1,2 (IRS-1,2)–specific, and \( \beta \)-insulin receptor antibodies were purchased from BD PharMingen.

Insulin-stimulated Protein Phosphorylation in Vitro—Chinese hamster ovary (CHO)-K cells and mouse NIH 3T3-L1 cells were propagated, transfected, and selected as previously detailed (9). The 3T3-L1 embryonic fibroblasts were induced to differentiate to adipocytes by treatment with dexamethasone and methylisobutylxanthine (11). Cultures of CHO-K cells and 3T3-L1 adipocytes were serum-starved for 18 h and treated with 100 nm insulin for 2 min or the time period indicated in figure legends. Cells were lysed, samples (0.1 mg of protein/lane) were subjected to SDS-PAGE, and the resolved protein was subjected to immunoblotting with anti-phosphotyrosine-specific antibody. Subsequently, the blots were stripped and stained again, this time with antibodies specific for the \( \beta \)-subunit of insulin receptor or anti-IRS-1,2 antibodies. Protein phosphorylation was quantified by image densitometry.

Insulin Receptor-catalyzed Protein Phosphorylation in Vitro—Protein phosphorylation in vitro was conducted using either crude membranes freshly isolated from 3T3-L1 adipocytes or recombinant insulin receptor catalytic domain expressed and then purified from baculovirus-infected SF9 cells. The reaction mixture contains 0.05 mM Tris-HCl, pH 7.5, 10 mM MgCl\(_2\), 2 mM MnCl\(_2\), and 0.5 mM ATP at 30 °C. Phosphorylation studies performed with cell extracts (50 μg of protein) were initiated by addition of a mixture of insulin and ATP to create a final concentration of 0.1 μM insulin and 0.5 mM ATP. The phosphorylation products were subjected to SDS-PAGE and then to immunoblotting. Phosphorylation of rBAC1 by insulin receptor kinase (rIRK) was studied in a reaction mixture with preactivated recombinant catalytic domains of the insulin receptor. Reaction products were analyzed by SDS-PAGE and autoradiography using a PhosphorImager or by capture of phosphorylated protein via binding on PS1 filters.

RESULTS AND DISCUSSION

Increased expression of \( \beta_2 \)AR results in inhibition of the ability of insulin to stimulate tyrosine phosphorylation of IRS-1,2 (2), principle substrates in insulin signaling (Fig. 1A) (12). Transient transfection of CHO-K cells that are deficient in \( \beta_2 \)AR with increasing amounts of the \( \beta_2 \)AR expression vector (pCDNA3-\( \beta_2 \)AR) leads to increased expression of \( \beta_2 \)AR, as evidenced by increased binding of the \( \beta_2 \)AR antagonist iodocyanopindolol (Fig. 1B). Insulin-stimulated phosphorylation of IRS-1,2, in sharp contrast, was inhibited by >60% in a concentration-dependent manner by the expression of \( \beta_2 \)AR (Fig. 1B, left). The abundance of IRS-1,2 and the \( \beta \)-subunit of the insulin receptor was not influenced by the expression of \( \beta_2 \)AR (not shown). In contrast, transfections that employed

* This research was supported by U.S. Public Health Service grants from the National Institutes of Health (to C. C. M.) and a National Research Service Award (T32 to S. D.) from the National Institute of Diabetes, Digestive, and Kidney Diseases. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Pharmacology-HSC, SUNY, Stony Brook, NY 11794-8651. Tel.: 631-444-7873; Fax: 631-444-7696; E-mail: craig@pharm.sunysb.edu.

1 The abbreviations used are: \( \beta_2 \)AR, \( \beta_2 \)-adrenergic receptor; IRS, insulin receptor substrates; CHO, Chinese hamster ovary; rIRK, recombinant insulin receptor kinase.
the empty pcDNA3 vector or expression of another unrelated G-protein coupled receptor, the μ-opioid receptor, had no effect on insulin-stimulated phosphorylation of IRS-1,2 (Fig. 1B, right).

Insulin stimulated a 3-fold increase in the phosphotyrosine content of β2AR in human epidermoid carcinoma Ala431 cells (Fig. 1C) and hamster DDT1-MF2 vas deferens smooth muscle cells (3–7). Insulin stimulates tyrosine phosphorylation of the β2AR that can occur at either Tyr350 or Tyr364. The phosphorylation of Tyr364 occurs early (within 2 min) and at a site displaying a traditional tyrosine kinase substrate motif (4). Phosphorylation of the β2AR expressed by CHO-K cells was inhibited in CHO-K clones stably or transiently expressing β2AR. CHO-K cells were transiently transfected with expression vector harboring wild-type (A, B, and D) or mutant versions (C, D, and E) of β2AR. Clones were serum-starved overnight and challenged with insulin (100 μM), and phosphorylation of IRS-1,2 was measured. The amount of β2AR expressed was determined by radioligand binding assays employing iodocyanopindolol. A, phosphorylation of IRS-1,2 in response to insulin and the effects of increased expression of wild-type β2AR. B, quantification of the level of inhibition of insulin-stimulated phosphorylation of IRS-1,2 and the amount of β2AR, μ-opioid receptor, or empty vector (EV) plasmid employed in the transient transfection. C, phosphotyrosine content of β2ARs in response to a 2-min challenge with insulin (100 μM) for Ala431 wild-type cells (replete with β2ARs) and for Chinese hamster ovary clones expressing either the wild-type or Y364A mutant β2AR. Following stimulation with insulin, crude cell membranes were prepared, subjected to immunoblotting, and stained by pY69 anti-phosphotyrosine antibodies. A summation of the data from multiple experiments is provided as the mean values ± S.E. The data are reported in relative units for phosphotyrosine labeling of the β2AR, setting the level obtained in native Ala431 cells in the absence of insulin stimulation as "1." D, inhibition of insulin-stimulated phosphorylation of IRS-1,2 in β2AR-deficient (CHO-K) cells or clones stably expressing either wild-type (Tyr364) or the Y364A mutant form of the hamster β2AR. E, inhibition of insulin-stimulated phosphorylation of IRS-1,2 in β2AR-deficient (CHO-K) cells or CHO-K cells transiently transfected to express either wild-type or mutant forms of the hamster β2AR with Y364F mutation. These data are displayed as the mean values ± S.E. from at least three separate determinations.

**Fig. 1.** Insulin-dependent phosphorylation of IRS-1,2 proteins is inhibited in CHO-K clones stably or transiently expressing β2AR. CHO-K cells were transiently transfected with expression vector harboring wild-type (A, B, and D) or mutant versions (C, D, and E) of β2AR. Clones were serum-starved overnight and challenged with insulin (100 μM), and phosphorylation of IRS-1,2 was measured. The amount of β2AR expressed was determined by radioligand binding assays employing iodocyanopindolol. A, phosphorylation of IRS-1,2 in response to insulin and the effects of increased expression of wild-type β2AR. B, quantification of the level of inhibition of insulin-stimulated phosphorylation of IRS-1,2 and the amount of β2AR, μ-opioid receptor, or empty vector (EV) plasmid employed in the transient transfection. C, phosphotyrosine content of β2ARs in response to a 2-min challenge with insulin (100 μM) for Ala431 wild-type cells (replete with β2ARs) and for Chinese hamster ovary clones expressing either the wild-type or Y364A mutant β2AR. Following stimulation with insulin, crude cell membranes were prepared, subjected to immunoblotting, and stained by pY69 anti-phosphotyrosine antibodies. A summation of the data from multiple experiments is provided as the mean values ± S.E. The data are reported in relative units for phosphotyrosine labeling of the β2AR, setting the level obtained in native Ala431 cells in the absence of insulin stimulation as "1." D, inhibition of insulin-stimulated phosphorylation of IRS-1,2 in β2AR-deficient (CHO-K) cells or clones stably expressing either wild-type (Tyr364) or the Y364A mutant form of the hamster β2AR. E, inhibition of insulin-stimulated phosphorylation of IRS-1,2 in β2AR-deficient (CHO-K) cells or CHO-K cells transiently transfected to express either wild-type or mutant forms of the hamster β2AR with Y364F mutation. These data are displayed as the mean values ± S.E. from at least three separate determinations.
ation of Tyr364 residue of β2AR has been demonstrated both in vivo (in response to insulin) using either metabolic labeling or matrix-assisted laser desorption/ionization-time of flight mass spectroscopy (4–7) and in vitro in response to insulin using purified insulin receptor and synthetic peptide substrates corresponding to the C-terminal region of the β2AR (4, 10). We also examined insulin-stimulated phosphorylation of β2AR in Chinese hamster ovary cells because these cells naturally lack β2AR (Fig. 1C). The stably transfected CHO clones expressed wild-type and mutant Y364A β2AR at equivalent levels (9–10 fmol of iodocyanopindolol binding/10^6 cells). Wild-type β2AR expressed in these cells was readily phosphorylated in response to insulin, whereas the Y364A mutant lacking this site for phosphorylation displayed no phosphorylation. The loss of tyrosine phosphorylation of the Y364A mutants suggests that some hierarchy for sequential phosphorylation of β2AR by the insulin receptor may exist because the Tyr365 site is still intact in these mutants. The abilities of Y364A mutant and wild-type β2AR to inhibit IRS-1,2 phosphorylation were compared (Fig. 1D). Expression of wild-type β2AR suppressed insulin-stimulated phosphorylation of IRS-1,2. Unlike the expression of the wild-type β2AR, expression of the Y364A mutant β2AR failed to inhibit IRS-1,2 phosphorylation. The level of IRS-1,2 phosphorylation observed in the CHO clones expressing the Y364A mutant β2AR was similar to that observed in CHO-K cells that lack β2AR.

The Y364F mutation, in contrast to Y364A, failed to abolish β2AR-mediated inhibition of insulin signaling to IRS-1,2 (Fig. 1, compare D and E). The phenylalanine-substituted mutant (Y364F) of the β2AR was not phosphorylated in response to insulin, and its expression enhanced the ability of the β2AR to inhibit insulin-stimulated phosphorylation of IRS-1,2 (Fig. 1E). This phenylalanine residue (Y364F) may well contact the active site of insulin receptor tyrosine kinase, enabling inhibition. These data suggest that the C-terminal domain of the β2AR and phosphorylation of β2AR Tyr364 are essential for inhibition of the insulin receptor tyrosine kinase by the phospho-β2AR.

We investigated whether the inhibition of insulin-stimulated IRS-1,2 by β2AR could be influenced by changes in intracellular cyclic AMP (Fig. 2). To address this question, cells were challenged with and without the β-adrenergic agonist isoproterenol (to elevate intracellular cyclic AMP levels), and insulin-stimulated phosphorylation of IRS-1,2 was measured in cells treated with or without KT5720, a selective inhibitor of protein kinase A. As shown in the immunoblot (Fig. 2A) as well as by the summation of data from several independent experiments (Fig. 2B), inhibition of insulin-stimulated phosphorylation of IRS-1,2 mediated by the β2AR was not influenced by the presence of the protein kinase A inhibitor.

We tested further the role of the β2AR C-terminal domain as an inhibitor of insulin action using mouse NIH 3T3-L1 adipocytes, an important and established model of insulin action (Fig. 3). Insulin action was studied in wild-type adipocyte clones and clones expressing the C-terminal cytoplasmic domain of β2AR (Pro323-Leu418, BAC1) harboring the Tyr364 residue. Wild-type adipocytes treated with insulin show a robust increase in insulin receptor autophosphorylation and phosphorylation of IRS-1,2. For the clones stably expressing the C-terminal domain of the β2AR in vivo, however, both autophosphorylation of the insulin receptor (Fig. 3, A and B) and phosphorylation of IRS-1,2 (Fig. 3, A and C) in response to insulin stimulation were suppressed. We further examined the effects of expression of the β2AR C-terminal domain on the ability of insulin to stimulate phosphorylation of IRS-1,2 in adipocyte cultures (assayed at day 6) that were transfected as preconfluent cultures (day 0) and treated with dexamethasone and methylisobutylxanthine (at day 2) to induce terminal differentiation to adipocytes (day 6). Expression of the β2AR C-terminal domain in these cultures displayed the same ability to block insulin-stimulated phosphorylation of IRS-1,2 as did stably transfected clones expressing BAC1, demonstrating that the inhibitor effects of BAC1 observed are not caused by clonal variability (Fig. 3D). Expression of the Y364A mutant form of BAC1, in contrast to expression of the wild-type BAC1, displayed a marked reduction in the ability to inhibit insulin-stimulated phosphorylation of IRS-1,2. Previously, we have shown both by metabolic labeling followed either by mapping of tryptic digests (4, 5) or by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (6) of the β2AR fragments that the Tyr364 residue is phosphorylated in response to insulin both in vivo (4, 10) and in vivo (7). Thus, expression of the C-terminal domain of the β2AR in 3T3-L1 adipocyte cultures or stable transfectants is sufficient to mediate an inhibition of insulin action with respect to the two most proximal steps, insulin receptor and IRS-1,2 autophosphorylation. The inhibitory nature of the C-terminal domain of the β2AR requires the integrity and phosphorylation of the Tyr364 residue; mutation of this site abolishes the inhibitory capacity of this domain.

We performed complementary studies in vitro making use of the rBAC1 domain and activated insulin receptors. Purified, activated recombinant catalytic domain of rIRK phosphorylates rBAC1 (Fig. 4, A and B), much like the partially purified intact insulin receptor (4). The products of phosphorylation are rBAC1 and the rIRK itself, i.e. autophosphorylation (Fig. 4B). When corrected for the autophosphorylation component, the phosphorylation of rBAC1 by rIRK is shown to plateau at ~1 mol of phosphate in rBAC1 per mol of activated rIRK (Fig. 4A), providing additional compelling evidence that the phosphorylated form of rBAC1 is an inhibitor of rIRK. The Y364A mutant form of rBAC1, in contrast to wild-type rBAC1, was not a substrate for phosphorylation by
Histone H2B is a well known substrate for rIRK, and so we compared the phosphorylation of histone 2B to that of rBAC1.

**FIG. 3.** Insulin-stimulated phosphorylation of IRS-1,2 and autophosphorylation of the insulin receptor are inhibited in 3T3-L1 adipocytes by expression of the C-terminal cytosolic domain of β2AR. 3T3-L1 adipocytes were serum-starved overnight, stimulated with 100 nM insulin for periods up to 15 min, and used to prepare whole-cell lysates. A, insulin-stimulated phosphorylation of IRS-1,2 and autophosphorylation of the β-subunit of the insulin receptor is inhibited in clones expressing the C-terminal cytoplasmic domain of β2AR (BAC1). B and C, time-course of autophosphorylation of the insulin receptor (B) and of IRS-1,2 phosphorylation (C) in response to insulin stimulation in clones expressing either empty vector (−BAC1) or vector harboring the BAC1 cDNA (+BAC1). The data for protein phosphorylation are displayed in arbitrary units obtained from scanning densitometry of the blots. D, insulin-stimulated phosphorylation of IRS-1,2 in 3T3-L1 cultures transfected with an expression vector harboring either wild-type BAC1 (wtBAC1) or the mutant form of BAC1 with a Y364A substitution (BAC1Y364A). Preconfluent cultures of 3T3-L1 embryonic fibroblasts were transfected with an expression vector harboring wtBAC1, BAC1Y364A, or the empty vector (EV) on day zero. At day 2 following transfection the cultures reached confluence and were treated with dexamethasone and methylisobutylxanthine to induce terminal differentiation to adipocytes (by day 6). At day 6, cultures were stimulated with or without insulin, and IRS-1,2 phosphorylation was assayed. The cultures were assayed in triplicate. The immunoblots of IRS-1,2 from the insulin-treated and untreated cultures were stained with anti-phosphotyrosine antibodies (pY69). The results from replicate determinations are displayed as mean values ± S.E.

**FIG. 4.** The C-terminal domain of β2AR is a substrate and feedback inhibitor of the insulin receptor tyrosine kinase. Insulin receptor-catalyzed phosphorylation of rBAC1 was studied in a mixture containing rBAR1 and rIRK. A, time-course of rBAC1 phosphorylation by rIRK displayed as the ratio between phosphorylated rBAC1 and phosphorylated rBAC1 plus rIRK. B, SDS-PAGE analysis and autoradiography of rBAC1 (10 μM) phosphorylation by preactivated rIRK (1 μM). Phosphorylation reaction of activated rIRK either alone (lane 1) or with the addition of rBAC1 (lane 2). rBAC1 phosphorylation corresponds to 30% of that observed for rIRK, which is phosphorylated to a level of 3 mol of phosphate/mol of receptor during activation. C, phosphorylation of histone H2B (10 μM) by preactivated rIRK (0.1 μM) is compared with that of rBAC1 (10 μM), both assayed at 2 min at 30 °C. D, rBAC1 but not the random copolymer (DY) substrate for insulin receptor kinase inhibits insulin receptor- (rIRK, 0.1 μM) catalyzed phosphorylation of histone H2B (10 μM); dose-response is in increasing amounts of either rBAC1 of the random copolymer (DY), substrate. The phosphorylation reaction was conducted for 2 min at 30 °C, and protein phosphorylation was assayed by binding on P81 filters.
Histone H2B was phosphorylated readily in the in vitro assay although rBAC1 was much less phosphorylated (Fig. 4C). More telling, the addition of rBAC1 (10 μM) in the phosphorylation reaction mixture with histone H2B yielded a dose-dependent inhibition of rIRK-catalyzed phosphorylation (Fig. 4D). The phosphorylation of another insulin receptor substrate random copolymer (DY)$_n$, unlike rBAC1, failed to inhibit rIRK-catalyzed phosphorylation of histone H2B. The IC$_{50}$ for rBAC1-mediated inhibition of insulin receptor-catalyzed phosphorylation of histone is 1–2 μM (Fig. 4D). These studies provide additional in vitro data that the C-terminal domain of the β$_2$AR indeed can act as a potent inhibitor of the insulin receptor tyrosine kinase.

We returned to the 3T3-L1 adipocyte model to test in vitro the ability of BAC1 peptide to act as a feedback inhibitor of insulin action. Insulin receptor catalyzed both autophosphorylation and IRS-1,2 phosphorylation in vitro in crude adipocyte membranes in response to insulin stimulation (Fig. 5). Autophosphorylation of the insulin receptor (Fig. 5, A and B) as well as phosphorylation of IRS-1,2 (Fig. 5, C and D) of adipocyte membranes in response to insulin stimulation were nearly abolished by the addition of the BAC1 peptide. We investigated whether the ability of the BAC1 domain of the β$_2$AR to inhibit insulin action in the adipocyte membranes also was dependent upon the availability of Tyr$_{364}$ for insulin receptor-catalyzed phosphorylation, as observed in study of the Y364A mutated receptor in vivo. rBAC1 suppressed, in a dose-dependent manner, insulin-stimulated phosphorylation of IRS-1,2 in the 3T3-L1 adipocyte membrane preparation (Fig. 5C). The addition of rBAC1 harboring a Y364A mutation, in sharp contrast to wild-type rBAC1, failed to suppress the ability of the insulin to act upon IRS-1,2 (Fig. 5C). Taken together with the results from studies of expression of wild-type versus Y364A mutant β$_2$AR in intact cells and of expression of wild-type versus Y364A mutant BAC1 in intact cells, data comparing the ability of the purified wild-type versus Y364A rBAC1 to block insulin-stimulated phosphorylation of IRS-1,2 provide compelling evidence that the nature of the phospho-Tyr$_{364}$ β$_2$AR is as an inhibitor of insulin receptor kinase signaling. The ability of rBAC1 to block the phosphorylation of histone H4B extends the analysis to suggest that the phospho-Tyr$_{364}$ β$_2$AR generated by stimulated insulin may function as a feedback inhibitor in insulin action. The results offered by several complementary strategies are consistent with and give support to this possibility.

The observation that the β$_2$AR can act as a feedback inhibitor of insulin action is a novel discovery. Herein we showed that the insulin receptor signaling pathway can be counter-regulated by the β$_2$AR at the most proximal point, i.e. by direct inhibition of the receptor tyrosine kinase by phospho-β$_2$AR. The ability of a tyrosine-phosphorylated β$_2$AR to inhibit the tyrosine kinase forms the basis for a negative feedback loop in which insulin catalyzes phosphorylation and inactivation of the β$_2$AR that blocks catecholamine signaling while creating a feedback inhibitor of insulin signaling.

![Fig. 5](image-url). The C-terminal domain of β$_2$AR receptor is the substrate for insulin receptor-catalyzed phosphorylation and inhibits autophosphorylation of the insulin receptor and IRS-1,2 phosphorylation in subcellular extracts prepared from 3T3-L1 adipocytes. Insulin-stimulated phosphorylation was studied in crude membranes prepared from 3T3-L1 adipocytes performed in the absence and presence of the C-terminal β$_2$AR fragment, rBAC1. A and B, the time-course (top) and quantification (bottom) of insulin-stimulated autophosphorylation of the insulin receptor in the absence and presence of rBAC1 (10 μM). C, the time-course (top) and quantification (bottom) of insulin-stimulated phosphorylation of IRS-1,2 in the absence and presence of rBAC1 (10 μM). The data for protein phosphorylation are displayed in arbitrary units obtained from scanning densitometry of the blots.
REFERENCES
1. Morris, A. J., and Malbon, C. C. (1999) Physiol. Rev. 79, 1373–1430
2. White, M. F., and Kahn, C. R. (1994) J. Biol. Chem. 269, 1–4
3. Hadcock, J. R., Port, J. D., Gelman, M. S., and Malbon, C. C. (1992) J. Biol. Chem. 267, 26017–26022
4. Baltensperger, K., Karoor, V., Paul, H., Rushe, A., Czech, M. P., and Malbon, C. C. (1996) J. Biol. Chem. 271, 1061–1064
5. Karoor, V., Baltensperger, K., Paul, H., Czech, M. P., and Malbon, C. C. (1995) J. Biol. Chem. 270, 25305–25308
6. Karoor, V., and Malbon, C. C. (1996) J. Biol. Chem. 271, 29347–29352
7. Karoor, V., and Malbon, C. C. (1998) Adv. Pharmacol. 42, 425–428
8. Wei, L., Hubbard, S. R., Hendrickson, W. A., and Ellis, L. (1995) J. Biol. Chem. 270, 8122–8130
9. Wang, H., Doronin, S., and Malbon, C. C. (2000) J. Biol. Chem. 275, 36686–36693
10. Doronin, S., Lin, F., Wang, H., and Malbon, C. C. (2000) Protein Expr. Purif. 20, 451–461
11. Guest, S. J., Hadcock, J. R., Watkins, D. C., and Malbon, C. C. (1990) J. Biol. Chem. 265, 5370–5375
12. Virkamaki, A., Ueki, K., and Kahn, C. R. (1999) J. Clin. Invest. 103, 931–943