

(2*R*)-[(4-Biphenyl)sulfonyl]amino]-*N*-hydroxy-3-phenylpropionamide (BiPS), a Matrix Metalloprotease Inhibitor, Is a Novel and Potent Activator of Hypoxia-Inducible Factors

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Received January 25, 2008; accepted April 16, 2008

ABSTRACT

Hypoxia-inducible factors (HIFs) are unstable heterodimeric transcription factors and decisive elements for the transcriptional regulation of genes important in the adaptation to low-oxygen conditions. Hypoxia is the ubiquitous inducer of HIFs, stabilizing the α -subunit and permitting the formation of a functional HIF complex. Here, we identify (2*R*)-[(4-biphenyl)sulfonyl]amino]-*N*-hydroxy-3-phenylpropionamide (BiPS), a commercially available metalloprotease-2 and -9 inhibitor, as a rapid and potent inducer of HIFs. We show that in different cell lines, BiPS induces the

HIF- α subunit by inhibiting its degradation through stabilization of its labile oxygen-dependent degradation domain. This is achieved through the inhibition of HIF-1 α hydroxylation. The HIF-1 complex, formed after BiPS treatment, is capable of DNA binding and activation of HIF target genes, including the expression of vascular endothelial growth factor. Because novel HIF activators have generated considerable interest in the possible treatment of different ischemic diseases, we believe that BiPS and derivative molecules could have strong therapeutic potential.

Molecular oxygen is crucial for aerobic energy metabolism and cell survival. Hypoxia-inducible transcription factors (HIFs) are decisive elements in the adaptation to low-oxygen conditions by permitting the expression of numerous genes involved in angiogenesis, anaerobic glycolysis, and cell survival (Semenza, 2003). HIFs are heterodimers composed of an oxygen-sensitive subunit, HIF-1 α , HIF-2 α , or HIF-3 α and a constitutive HIF- β subunit. HIF-1 α is expressed ubiquitously and has been highly characterized. The regulation of HIF-2 α , also named HLF, EPAS1, HRF, or MOP2, is similar to HIF-1 α , but its expression is more restricted (Ema et al., 1997; Flamme et al., 1997; Hogenesch et al., 1997; Tian et al., 1997). HIF-3 α has been less characterized and could act as a

repressor of HIF-1 and HIF-2 activities (Gu et al., 1998; Makino et al., 2001, 2002).

Under normal oxygen conditions, the α subunit is hydroxylated through the action of HIF prolyl hydroxylases (PHDs). This hydroxylation occurs on two specific proline residues (Pro402 and Pro564 on human HIF-1 α) contained in its oxygen-dependent degradation domain (ODDD). Hydroxylation of HIF- α allows the binding of pVHL, the product of the von Hippel-Lindau tumor suppressor gene. As the recognition component of an E3 ubiquitin ligase complex, pVHL allows for HIF- α polyubiquitination and subsequent proteosomal degradation (Schofield and Ratcliffe, 2004). Furthermore, HIF- α is hydroxylated on a specific asparagine residue (Asn803 on human HIF-1 α) contained in its C-terminal transactivation domain by another dioxygenase, factor-inhibiting HIF. Asn803 hydroxylation inhibits HIF transcriptional activity by preventing binding of the coactivator p300/cAMP response element-binding protein-binding protein (Mahon et al., 2001). Inactivated by hypoxia, HIF hydroxylases are dependent on oxygen

This work was supported by grants from the Canadian Institutes of Health Research (CIHR, MOP-49609) and the Heart and Stroke Foundations of Québec and Canada. D.E.R. is a recipient of a CIHR New Investigator Award. M.C.L. is a recipient of a Graduate Scholarship from the CIHR.

Article, publication date, and citation information can be found at <http://molpharm.aspetjournals.org>.
doi:10.1124/mol.108.045690.

ABBREVIATIONS: HIF, hypoxia-inducible factor; PHD, hypoxia-inducible factor prolyl hydroxylase; ODDD, oxygen-dependent degradation domain; pVHL, von Hippel Lindau protein; MMP, matrix metalloprotease; BAEC, bovine aortic endothelial cell; VSMC, vascular smooth muscle cell; 2-OG, 2-oxoglutarate; BiPS, (2*R*)-[(4-biphenyl)sulfonyl]amino]-*N*-hydroxy-3-phenylpropionamide; MG132, *N*-benzoyloxycarbonyl (Z)-Leu-Leu-leucinal; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; siRNA, small interfering RNA; NETN buffer, NaCl, EDTA, Tris, Igepal, and deferoxamine; HA, hemagglutinin; HRE, hypoxia response element; PAGE, polyacrylamide gel electrophoresis; MAPK, mitogen-activated protein kinase; DMSO, dimethyl sulfoxide; GM6001, galdardin.

and 2-oxoglutarate (2-OG) as substrates and ascorbate and iron as cofactors. Therefore, in most if not all cell types, the lack of oxygen permits the stabilization of HIF- α and the formation of a transcriptionally active HIF complex. Given the importance of HIF in response to ischemic conditions, the development of novel HIF inducers/activators has gained strong interest because of their therapeutic potential in the treatment of different ischemic disorders (Khan et al., 2003; Simons and Ware, 2003; Hewitson and Schofield, 2004).

In this study, we present (2*R*)-[(4-biphenyl)sulfonyl]-amino]-*N*-hydroxy-3-phenylpropionamide (BiPS) as a potent activator of both HIF-1 and HIF-2. This compound, derived from *N*-sulfonylamine acid, was originally designed as an inhibitor of matrix metalloproteases (MMP) -2 and -9 (Tamura et al., 1998). A number of studies using this compound have demonstrated potent effects in different in vivo and in vitro situations. These include reducing lung colonization by Lewis lung carcinoma cells in mice (Tamura et al., 1998), blocking lymphocyte migration across endothelial cells (Deem and Cook-Mills, 2004), inhibiting transforming growth factor- β -induced cataract formation in rat lens (Dwivedi et al., 2006), blocking the invasion of mouse brain microvessel endothelial cells in Matrigel (Fears et al., 2005), and impeding the migration of smooth muscle cells (Lin et al., 2007).

In addition to its well-characterized effects on MMP activity, here we show that BiPS can also induce HIFs. In the present study, we demonstrate that BiPS stabilizes HIF- α protein by blocking HIF- α hydroxylation. This increase in HIF- α protein levels leads to the formation of active HIF complexes and the expression of HIF-target genes. Therefore, our study characterizes BiPS as a novel and potent activator of HIF complexes, which could be of particular interest in the chemical pharmacology of the HIF signaling cascade.

Materials and Methods

Materials. BiPS, also known as MMP-2/MMP-9 Inhibitor II (Tamura et al., 1998), was from Calbiochem/EMD Chemicals (Gibbstown, NJ). Cobalt chloride (CoCl₂), dimethyl-2-OG, and MG132 were from Sigma (St. Louis, MO). pGL3 (R2.2) 3HRE-tk-LUC luciferase reporter vector was generated in our laboratory (Lauzier et al., 2007). GST-HIF-1 α 344–582, pVHL-HA, and CMV-Luc-ODD constructs were kindly provided by Drs Jacques Pouyssegur (Institute of Signaling, Developmental Biology and Cancer Research, Université de Nice, Nice, France), Peter Ratcliffe (University of Oxford, Oxford, England), and Richard K. Bruick (University of Texas, Austin, TX), respectively.

Cell Culture. VSMCs were isolated from thoracic aorta of 6-week-old male Wistar rats (Owens et al., 1986) and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and antibiotics (50 U/ml penicillin, 50 U/ml streptomycin). Bovine aortic endothelial cells (BAECs) were isolated from calf aortas and cultured in DMEM containing 10% FBS and antibiotics. HeLa cells were cultured in DMEM containing 10% heat-inactivated FBS and antibiotics. All cells were cultured in a humid atmosphere (5% CO₂, 95% air) and serially passaged upon reaching confluence. Hypoxic conditions were obtained by placing cells in a sealed hypoxic workstation (Ruskin, Bridgend, UK). The oxygen level in this workstation was maintained at 1% with the residual gas mixture containing 94% nitrogen and 5% carbon dioxide. All media for cell culture were from Invitrogen (Carlsbad, CA) unless otherwise indicated.

Western Blot Analysis. Cells were lysed in 2 \times Laemmli sample buffer. Protein concentration was determined by Lowry assay. Cell

extracts (25 μ g) were loaded on SDS-polyacrylamide gels and transferred onto polyvinylidene difluoride (Immobilon-P; Millipore Corporation, Billerica, MA) or nitrocellulose membranes (Hybond C Extra; GE Healthcare Life Sciences, Piscataway, NJ). Anti-HIF-1 α and anti-HIF-2 α antisera were raised in rabbits immunized against the last 20 amino acids of the C termini of each human protein (Richard et al., 1999). Anti-hydroxylated HIF-1 α raised against hydroxylated Pro402 and hydroxylated Pro564 of the human HIF-1 α sequence were obtained as described previously (Chan et al., 2005). Monoclonal anti-HIF-1 α and anti-GST antibodies and polyclonal anti-HIF-1 β antibody were from Novus Biologicals (Littleton, CO). Monoclonal HA.11 antibody was from Convance (Emeryville, CA). Total polyclonal p42/p44 MAPK antibody was from Millipore Biotechnology (Lake Placid, NY) and used as a loading control. Horseradish peroxidase-coupled anti-mouse and anti-rabbit antibodies were from Promega (Madison, WI). Proteins were visualized with an enhanced chemiluminescence system (GE Healthcare Life Sciences) or with the Odyssey Infrared Imaging System (LI-COR, Lincoln, NE). Western blots were quantified using Odyssey quantification software or ImageJ (available at <http://rsb.info.nih.gov/ij>).

RNA Interference. To down-regulate HIF- α protein expression in HeLa cells, small interfering RNA (siRNA) duplexes targeting human HIF-1 α (accession number NM_001530; sense, 5'-AGGACAAGUCACAACAGGAUU-3') and human HIF-2 α (accession number NM_001430; sense, 5'-GGGUCAGGUAGUAAGUGGCUU-3') were obtained from Ambion (Austin, TX). As a control, *Silencer Negative Control #1 siRNA* was used (Ambion). HeLa cells were transfected with siRNA duplexes (20 nM) by calcium phosphate precipitation.

Luciferase Assay. HeLa cells, seeded in six-well plates, were transiently transfected by calcium phosphate precipitation with pGL3 (R2.2) 3HRE-tk-LUC luciferase reporter vector (1 μ g/well) or CMV-Luc-ODD (0.5 μ g/well). *Renilla reniformis* luciferase expression vector (250 ng/well) was also used as a control for transfection efficiency. At 30 h after transfection, cells were stimulated, and luciferase assays were performed using the Dual Luciferase Reporter Assay System (Promega). Results were quantified with a Luminoskan Ascent microplate reader with integrated injectors (Thermo Electron, Milford, CA). Results are expressed as a ratio of firefly luciferase activity over *R. reniformis* luciferase activity. Experiments are an average \pm S.E.M. of triplicate data and representative of three independent experiments performed on different cell cultures.

Transcription Factor Enzyme-Linked Immunoassay. Experiments were performed as described previously (Blouin et al., 2004). In brief, high-bind NeutrAvidin-coated 96-well strip plates (Pierce Biotechnology, Rockford, IL) were incubated with a 5'-biotinylated 26 base pair double-stranded DNA oligonucleotide for 1 h at room temperature. This sequence contains the wild-type or mutant (underlined) HIF-1 binding motif described previously (Semenza and Wang, 1992). Sequences used here were 5'-GATCGCCCTACGTGCTGTCTCAGATC-3' for W26 wild-type sequence and 5'-GATCGCCCTAAAAGCTGTCTCAGATC-3' for M26 mutant sequence. Nuclear extracts from HeLa cells were incubated with oligonucleotides, and HIF-1 complexes bound to DNA were detected using anti-HIF-1 α or anti-HIF- β antibodies (Novus Biologicals), horseradish peroxidase-conjugated secondary antibodies, and TMB-ONE (Promega). Experiments are an average \pm S.E.M. of triplicate data representative of three independent experiments performed on different cell cultures.

pVHL Capture Assay. HeLa cell were grown to confluence and stimulated as indicated. Cells were washed once in phosphate-buffered saline and twice in buffer containing 20 mM Tris, pH 7.5, 5 mM KCl, 1.5 mM MgCl₂, and 1 mM dithiothreitol. Cells were then suspended and lysed using a Dounce homogenizer. Cytoplasmic extracts were cleared by centrifugation (20,000g). Extracts (250 μ g) were incubated with Sepharose-bound GST-HIF-1 α 344–582 (30 μ g) for 1 h at room temperature, washed with NETN buffer (150 mM NaCl, 0.5 mM EDTA, 20 mM Tris, pH 8.0, 0.5% Igepal, and 100 μ M

deferoxamine), and incubated overnight with in vitro translated pVHL-HA in NETN at 4°C. Samples were washed with NETN, denatured in 2× Laemmli sample buffer, resolved in SDS-polyacrylamide gels (12%), and revealed by Western blotting with anti-HA and anti-GST antibodies.

Iron Chelation Assays. Iron chelation potential was determined using a FerroZine-based iron measurement assay (Stookey, 1970; Fish, 1988). Ferrous chloride (FeCl_2 , 20 μM) was incubated with BiPS (75 μM) or deferoxamine (50 μM) for 30 min. FerroZine (20 mg/ml) and ammonium acetate (1 mg/ml) was then added to the reaction mixture and incubated at 37°C for 30 min. Iron was measured by determining sample absorbance at 562 nm by spectrophotometry. The amount of free iron in the solution was determined by comparison with a FeCl_2 /FerroZine standard curve. Experiments are an average \pm S.E.M. of triplicate data representative of three independent experiments.

Results

The effect of BiPS on HIF-1 α and HIF-2 α protein induction was evaluated in different cell lines including the established human HeLa cell line and primary cultures of BAECs and rat aortic VSMCs. As seen in Fig. 1, BiPS induced HIF- α protein levels in all cell lines tested. In HeLa cells, a treatment with 10 μM BiPS for 2 h caused a detectable increase of both HIF-1 α and HIF-2 α proteins, whereas maximal induction was observed between 75 and 100 μM BiPS (Fig. 1, top). BiPS was more potent in BAEC because 5 μM led to a detectable increase of both HIF- α proteins, whereas maximal induction was observed at 25 μM (Fig. 1, middle). In VSMCs, 10 μM BiPS led to a detectable increase of HIF-1 α protein, whereas maximal induction was observed at 50 μM BiPS (Fig. 1, bottom). However, we did not detect HIF-2 α protein levels in VSMCs treated with BiPS or under hypoxic conditions. HIF-1 α and HIF-2 α protein induction by BiPS was also observed in the macrophage-like cell line NR8383, human embryonic kidney 293 cells, the murine endothelial cell line 1G11 and mouse embryonic fibroblasts (results not shown). It is interesting to note that the maximal induction of HIF- α

after BiPS treatment was comparable with treatments under hypoxic conditions or in the presence of CoCl_2 , two main inducers of HIF- α . Finally, other MMP-2/9 inhibitors, such as GM6001 (galardin) and MMP-2/9 inhibitor I, did not induce HIF- α subunits (results not shown). These results indicate that the effect of BiPS on HIF- α induction was independent of MMP-2/9 inhibition and identify BiPS as a novel inducer of HIF- α subunits in normoxic cells.

The kinetics of HIF- α protein induction under BiPS treatment were rapid and comparable with hypoxic HIF- α induction. In HeLa cells, a detectable increase of HIF-1 α was observed by 30 min, and maximal induction was attained after 2 h of treatment with 75 μM BiPS (Fig. 2). It is interesting that although HIF-1 α levels were maintained in hypoxia for more than 6 h, treatment of cells with BiPS maintained HIF-1 α levels for up to 4 h and then subsequently decreased to basal levels. HIF-2 α protein induction by BiPS followed a similar kinetic pattern to HIF-1 α . These results suggest that like hypoxia, BiPS stabilizes HIF- α instead of exploiting transcriptional or translational mechanisms for protein induction as seen for other normoxic inducers.

To directly evaluate the effect of BiPS on HIF- α subunit stabilization, HeLa cells were transfected with a construct encoding a luciferase protein chimera destabilized by the HIF-1 α ODDD (Salnikow et al., 2004). In these conditions, changes in luciferase activity by treatment with BiPS would indicate the specific stabilization of HIF-1 α 's ODDD. As expected, the treatment of cells with CoCl_2 (Fig. 3A) or the proteasome inhibitor MG132 (results not shown) strongly increased luciferase activity. It is noteworthy that treatment with BiPS also increased luciferase activity to levels similar to those observed during MG132 and CoCl_2 treatments. This result indicates that BiPS stabilizes the HIF-1 α ODDD.

Because decreased pVHL binding leads to increased HIF- α stabilization, we examined pVHL binding to HIF-1 α after BiPS treatment. HeLa cells were treated with BiPS or CoCl_2 followed by the preparation of cytosolic extracts and in vitro hydroxylation of GST-HIF-1 α (344–582). After incubation with in vitro translated pVHL and GST pull-down, we ob-

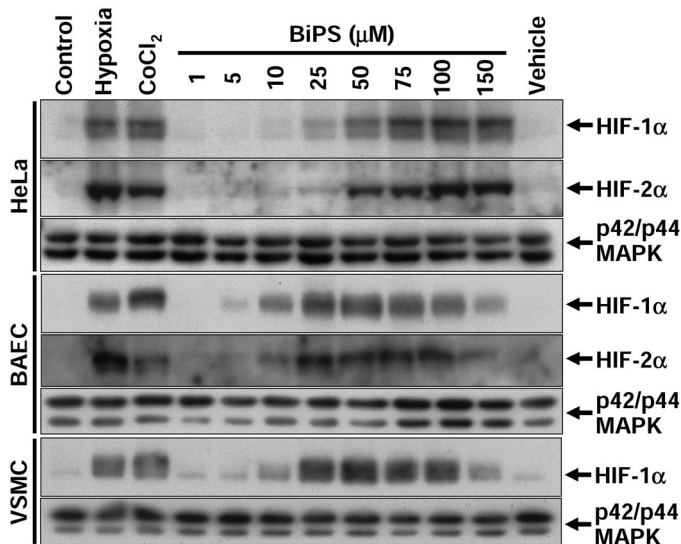


Fig. 1. HIF- α induction by BiPS. Quiescent VSMC, quiescent BAEC, or HeLa cells were maintained under control conditions, in hypoxic conditions (1% O_2), in the presence of CoCl_2 (200 μM), or various concentrations of BiPS or vehicle (DMSO 0.08%) for 2 h. Total cell extracts (25 μg) were resolved by SDS-PAGE (8%) and immunoblotted using anti-HIF-1 α , anti-HIF-2 α , or anti-p42/p44 MAPK antibodies.

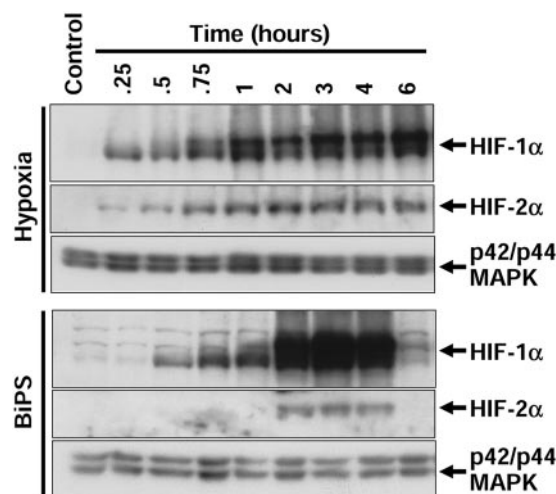


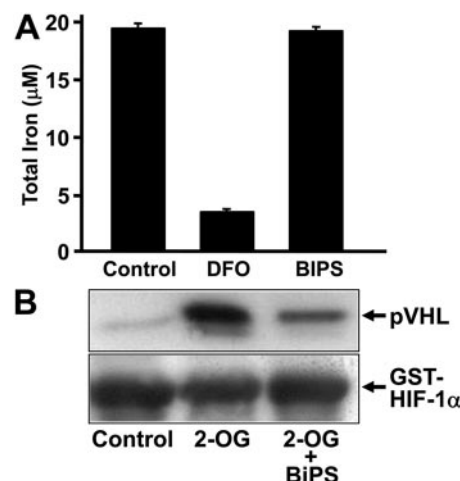
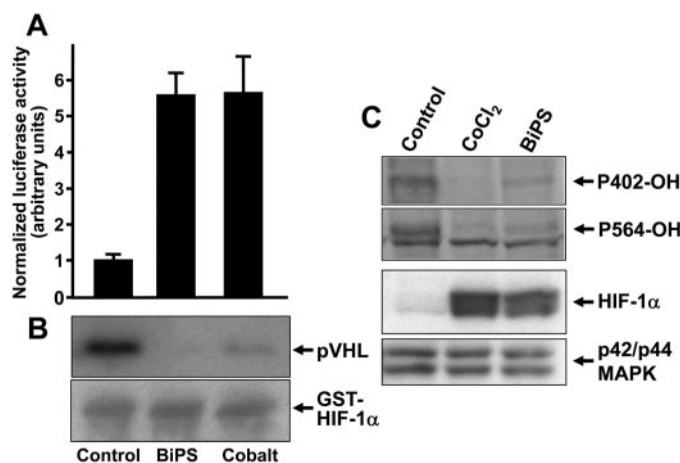
Fig. 2. Time course for HIF- α induction by BiPS. HeLa cells were maintained under control conditions, in hypoxic conditions (1% O_2), or in the presence of 75 μM BiPS or vehicle (DMSO 0.08%) for different periods of time. Total cell extracts (25 μg) were resolved by SDS-PAGE (8%) and immunoblotted using anti-HIF-1 α , anti-HIF-2 α , or anti-p42/p44 MAPK antibodies.

served that the treatment of cells with BiPS strongly prevented pVHL binding to HIF-1 α (Fig. 3B). It is interesting that the inhibition of pVHL binding with BiPS was more potent than with CoCl₂ treatment. This result suggests that BiPS inhibits PHD-mediated HIF- α hydroxylation. To confirm this, we evaluated the level of HIF-1 α hydroxylation using two specific antibodies against hydroxylated proline residues, Pro402 and Pro564 (Chan et al., 2005). For this experiment, cells were pretreated with MG132 and maintained in the presence of CoCl₂ or BiPS. As expected, the treatment of cells with CoCl₂ led to a near complete inhibition of HIF-1 α protein hydroxylation of both proline residues (Fig. 3C). The treatment of cells with BiPS also strongly decreased both HIF-1 α Pro402 and Pro564 hydroxylation. Taken together, these results indicate that BiPS is a potent inhibitor of PHD activity.

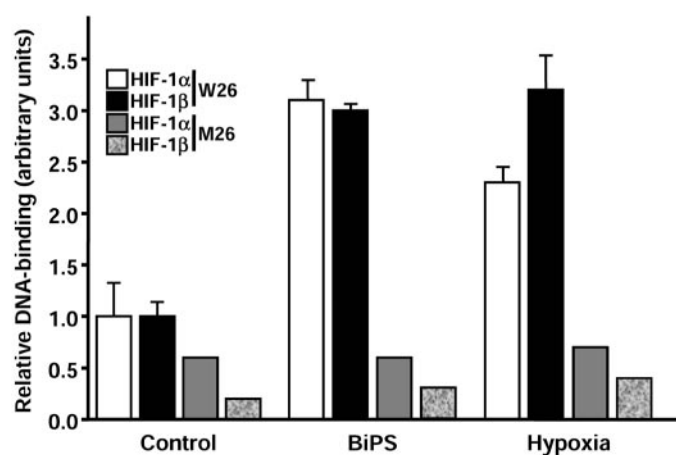
We then wanted to investigate the mechanism by which BiPS inhibits PHD activity. Because PHD requires Fe(II) to hydroxylate HIF- α subunits, we wanted to evaluate possibility that BiPS could inhibit PHD activity by acting as an iron chelating agent. In an iron chelation assay, deferoxamine, a well known iron chelator and HIF- α stabilizing agent, was able to chelate more than 80% of the iron present in our experimental conditions (Fig. 4A). However, BiPS did not act as an iron chelating agent because total free iron was completely accessible for reaction with FerroZine. This result suggests that BiPS does not inhibit PHD activity through iron chelation. PHDs belong to a large group of enzymes that use 2-oxoglutarate as a cosubstrate (Schofield and Ratcliffe, 2004). We wanted to determine whether BiPS could interfere with the binding of 2-oxoglutarate to PHD. We therefore performed a pVHL cap-

ture assay in which 2-OG was added to the cellular extracts. In these conditions, the addition of 2-OG increased pVHL binding to HIF-1 α (Fig. 4B). It is interesting that the treatment of cellular extracts with BiPS blocked 2-OG-induced pVHL binding to HIF-1 α . These results indicate that BiPS decreases PHD activity by interfering with 2-OG binding.

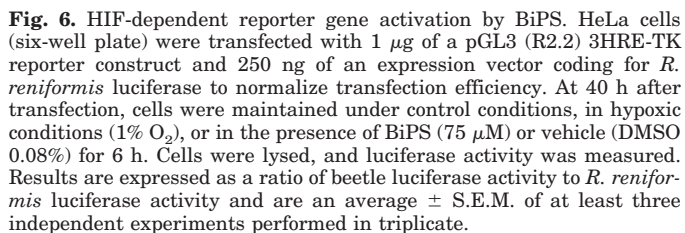
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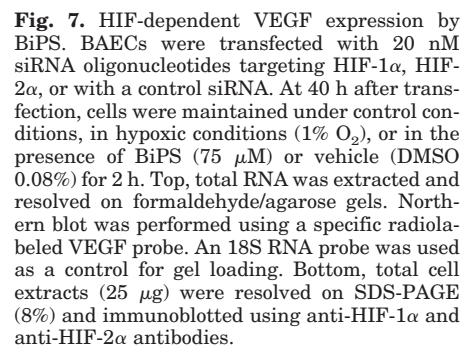


We next attempted to determine whether BiPS treatment led to HIF-mediated transcriptional activity using a HRE-controlled reporter assay. HeLa cells were transiently transfected with a 3HRE-tk-LUC reporter vector followed by treatment of cells with BiPS or in hypoxic conditions for 6 h. Under these conditions, hypoxia led to an 11-fold induction in luciferase activity (Fig. 6). It is interesting that BiPS was more potent than hypoxia for activating HIF and increased reporter activity by 15.4-fold. We then studied the effect of BiPS treatment on the expression of a well known HIF-1-activated target gene, vascular endothelial growth factor (Ebert et al., 1995; Liu et al., 1995; Forsythe et al., 1996; Mazure et al., 1996). As seen in Fig. 7, the treatment of HeLa cells with BiPS strikingly increased VEGF expression. As in



Discussion

HIFs are decisive elements in the transcriptional regulation of genes implicated in cellular and physiological adaptation to hypoxic stress. The products of these genes are involved in several adaptive responses, including angiogenesis and erythropoiesis. A complex process, angiogenesis is regulated by several endogenous proteins and can be deregulated in tumor cell progression and during ocular and inflammatory pathologies. Angiogenesis may also be ineffective during ischemic stress, leading to tissue destruction. In this regard, the first therapeutic attempts to promote angiogenesis used the delivery of VEGF alone. This led to the production of leaky blood vessels and was proven to be ineffective in clinical trials (Ferrara, 2004). As a powerful regulator of many endogenous proangiogenic molecules, HIFs are considered appealing pharmacological targets for therapeutic angiogenesis. Different groups have shown the strong potential of HIF complexes in promoting vascularization in ischemic tissues (Li et al., 2000; Vincent et al., 2000; Elson et al., 2001; Shyu et al., 2002; Willam et al., 2002). In addition, HIFs are strong regulators of erythropoiesis by inducing erythropoietin (Epo) production. A recombinant form of this specific hormone is commonly used for its ability to treat anemia. However, the high cost of this therapy, combined with the need for parenteral administration and the development of anti-Epo antibodies, has triggered the development of different approaches to stimulate erythropoiesis. Therefore, the discovery of novel HIF-inducing compounds should lead to new strategies to promote angiogenesis and treat anemia. It is interesting that specific PHD inhibitors have been shown to successfully increase the hemoglobin levels in rhesus macaques (Hsieh et al., 2007) and Epo levels in mice (Kasiganesan et al., 2007). Here, we have identified BiPS, an MMP-2/9



inhibitor, as a novel and potent activator of HIFs. BiPS stabilizes HIF- α protein by blocking HIF α hydroxylation. This increase in HIF- α protein levels leads to the formation of active HIF complexes and the expression of HIF target genes. Therefore, BiPS or derivative molecules could have interesting therapeutic options for HIF-regulated responses.

To our knowledge, the only compounds currently used to stabilize and activate HIFs are inhibitors of PHD activity. The hydroxylase activity of these enzymes is modulated by their cofactors, namely O₂, 2-OG, and Fe(II). The identification of PHD cofactors has led to the identification/design of compounds able to increase HIFs, including compounds that chelate Fe(II) (deferrioxamine) or compete with 2-OG binding to PHD (Hewitson and Schofield, 2004). Because BiPS can efficiently inhibit HIF-1 α hydroxylation and pVHL binding without chelating iron, we believe that BiPS functions by interfering with the binding of 2-OG to PHD. This is supported by the demonstration that BiPS blocks the potentiation of pVHL binding by 2-OG. In addition, using a docking simulation software (AutoDock, available at <http://autodock.scripps.edu/>), we performed a binding simulation of 2-OG and BiPS to PHD2, which corresponds to the current understanding of the 2-OG/PHD2 binding model (McDonough et al., 2006). In this simulation, the most probable position for BiPS binding to PHD2 was the 2-OG binding site in the cavity containing the Fe(II) atom. The biphenyl group of BiPS was positioned inside the cavity, whereas the two oxygen atoms of the sulfur group interacted with the Fe(II) atom. Furthermore, this model predicted that the PHD2 binding energy of BiPS is 1.65 times higher than 2-OG (mean of -7.009 ± 0.3 kcal/mol for BiPS and -4.245 ± 0.04 kcal/mol for 2-OG). Taken together, experimental and in silico evidence strongly suggests that BiPS induces and activates HIF by interfering with the binding of the PHD cofactor, 2-OG, leading to PHD inhibition.

We have successfully used BiPS for HIF induction in a variety of cell types. These characteristics reveal BiPS as an interesting compound for researchers interested in studying the HIF system. As a potent inhibitor of MMP-2/9, BiPS was used by many different groups that were unaware of its effect on HIF activity. BiPS was used to study migration and invasion properties of cells (Deem and Cook-Mills, 2004; Fears et al., 2005; Lin et al., 2007), to study the colonization of cancer cells in mice (Tamura et al., 1998), and to evaluate the remodeling and inflammation of airways during asthma (Lee et al., 2004). Depending on tissues targeted by BiPS the concentrations used, HIF activation could play an important role in the responses obtained using BiPS as an MMP-2/9 inhibitor. We believe that researchers considering the use of BiPS in their studies should strongly consider this possibility.

In this study, we present evidence that BiPS is a novel and potent PHD inhibitor in addition to its known role as an MMP-2/9 inhibitor. A PHD inhibitor, BiPS prevents pVHL binding to HIF- α and its subsequent degradation. In addition, BiPS permits the transcriptional activity of HIFs and the expression of target genes. Because PHD inhibitors are now recognized as potential therapeutic drugs in the treatment of anemia and ischemic diseases, we strongly believe that BiPS and derivative molecules could have strong therapeutic potential.

Acknowledgments

We are grateful to Guylaine Soucy for technical assistance, Marc-André Déry for help with plasmid constructs, Éric Paquet for AutoDock expertise, and Elisabeth Pagé and David Patten for helpful comments.

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