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Forum Review

Biliverdin Reductase: PKC Interaction at the Cross-Talk of MAPK and PI3K Signaling Pathways

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ABSTRACT

Biliverdin reductase (BVR) was characterized some 25 years ago as a unique dual-cofactor/pH-dependent enzyme that catalyzes the reduction of biliverdin-IXa. Our knowledge of functions of BVR has increased enormously in recent years. hBVR functions in the IR/IGF-1–controlled regulation of the MAPK and PI3K cascades that are linked by the PKC enzymes. The first of the two culminates in the activation of transcription factors for oxidative stress–responsive genes, including *ho-1*, where BVR functions as both a bZip (basic leucine zipper) transcription factor and a kinase. The second pathway amplifies the insulin/growth-factor signal for protein/DNA synthesis and glucose transport downstream of PI3K. hBVR is a transactivator of PKC-βII, and thus an integral component of the "activation loop" linking MAPK, PKC-βII, and PI3K to insulin/growth-factor signaling. The emergence of biliverdin and bilirubin as a newly defined category of modulators of cell signaling and kinase activity further underscores the critical input of hBVR in the response of intracellular pathways into the external environment. Structural features of BVR and recent findings relevant to its function in cell-signaling pathways are reviewed here and are intended to complement a recent commentary on the role of BVR in linking heme metabolism and cell signaling. *Antioxid. Redox Signal.* 9, 2187–2195.

INTRODUCTION

Biliverdin reductase (BVR) was first described in 1965 by Singleton (65) as the catalyst for the reduction of biliverdin IXα to bilirubin, and for nearly 35 years, this was the only function ascribed to the enzyme. BVR was overlooked for the most part by the scientific community not only because it served in a catabolic pathway, but also because it was considered specific to placental animals, wherein it was assumed to function solely in the disposal of heme-degradation products, particularly from the fetus. However, the availability of genetic methods has allowed the identification of BVR in essentially all forms of life, refuting the concept that BVR is exclusive to mammalian species, and uncovering the wide expanse of its functions in cell signaling and regulation of gene expression.

BVR is an evolutionarily conserved protein found across the spectrum of metazoans. Homologues of the reductase are found in unicellular organisms and plants (4, 29, 62). Plants use biliverdin produced by ferredoxin-dependent heme oxygenase for the synthesis of phytochromes, the sensory photoreceptors (4, 29). A considerable degree of amino acid conservation is found among higher organisms; shorter evolutionary distances correspond to higher degrees of similarity (36). In primates, a remarkable homology of the primary sequence of BVR and conservation of signaling motifs exists (Fig. 1).

Two seminal observations are considered pivotal to the recent identification of BVR as a key component of cell-signaling pathways. First, the singularly unprecedented activity profile of the enzyme having a dual pH/cofactor-dependent profile (32), which provoked much curiosity and a continued

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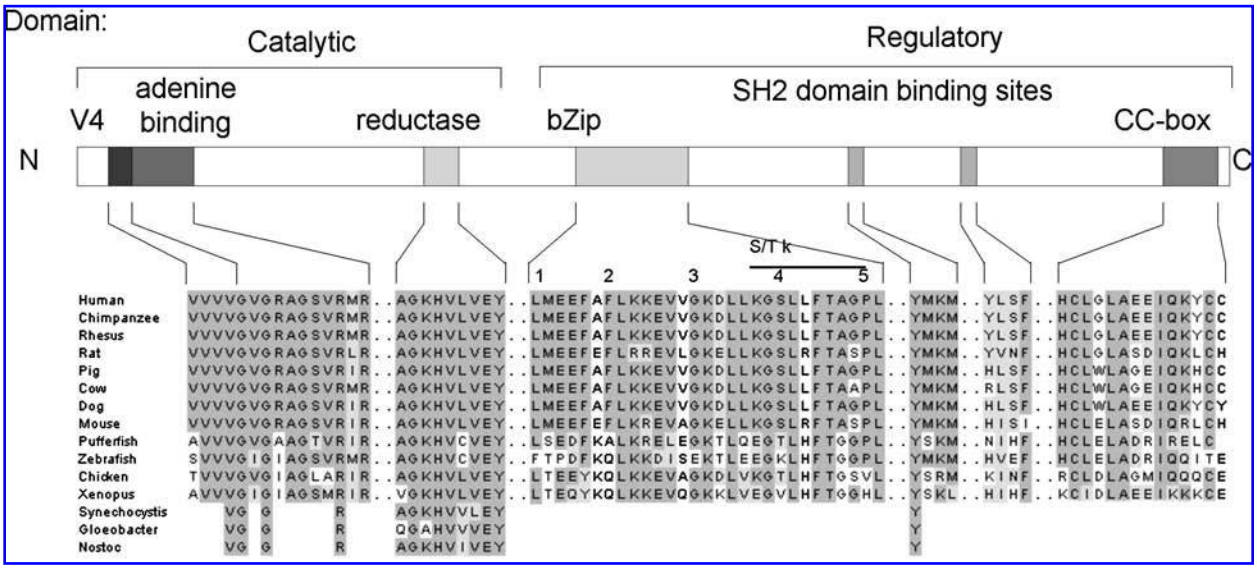

FIG. 1. A scale representation of the location of sequence motifs within the BVR protein sequence. Full-length sequences for eight mammalian BVRs, as well as the orthologous proteins from chicken, *Xenopus* (*X. tropicalis*), zebrafish (*Danio rerio*) pufferfish (*Tetraodon nigroviridis*) and from the prokaryotes *Synechocystis* sp., *Nostoc* sp., and *Gloeobacter violaceus* were aligned. Motifs and domains in the sequence alignment are shown and are separated by the double periods. The bar above the alignment within the bZip domain indicates the position of an S/T kinase motif. The numbers represent conserved residues in the bZip sequence $LX_6LX_6KX6LX_6L$, when X is any amino acid. The conservation of amino acid residues within each motif is indicated by the shading. The motif labeled V4 is the sequence of four consecutive valine residues found in mammalian BVRs. With ascendance in evolution, the number of valines is increased.

interest in the structure–function of the reductase; second, its microheterogeneity due to posttranslational phosphorylation (22).

DISSECTING THE STRUCTURAL FEATURES OF HBVR IN THE CONTEXT OF ITS ROLE IN CELL SIGNALING

BVR is the product of a single transcript (40) that encodes a soluble polypeptide that, in mammals, is in the range of ~300 amino acids. The human enzyme consists of 296 residues, whereas the rat enzyme is made of 295 amino acids (14, 39). Moreover, in the mammalian species, BVR shows a high degree of conservation of gene structure, which consists of seven coding exons and one noncoding exon at the 5′ end (40). Because of extensive posttranscriptional modification, the mature protein displays a substantially larger apparent molecular weight than predicted, based on amino acid composition. The reported apparent molecular weight of hBVR, as estimated by its electrophoretic mobility in SDS gel, ranges from 36 to 42 kDa. As described for the human and the rat, in the mature protein, the first methionine and the second residue, an asparagine, in the human, are deleted.

Traditionally, the presence of 12 motifs has been assigned to protein kinases (18, 19, 68). The primary structure of hBVR predicts its sharing of several consensus kinase motifs, but not all 12. hBVR is not alone in this respect, as a number of recently defined nonconventional kinases lack the complete set of 12 motifs. The catalytic domain of myosin heavy-chain kinase-A bears little resemblance to the catalytic domain of protein kinases (13).

The primary structure of hBVR exhibits a notable degree of sequence similarity to the insulin receptor kinase (IRK) domain and the insulin-receptor substrates (IRS) (33), and its organization bears a similarity to that of the serine/threonine (S/T) protein kinase C (PKC) isozymes (47, 48). The primary structure of PKCs contains a catalytic and a regulatory domain; depending on the type of PKC, the requirement for activator-divalent metals, Ca^{2+} and Mg^{2+}, and lipids is a reflection of the structure of their regulatory domain (48). hBVR kinase activity also requires a divalent metal ion that, as for all protein tyrosine kinases (PTKs) (23, 24), is Mn^{2+} (33). Metalloporphyrin complexes, Fe- and Co- protoporphyrin (Fe-PP, Co-PP), induce *ho-1* activate hBVR kinase activity (38). It is notable that Zn-PP, which does not induce *ho-1*, does not activate hBVR.

The primary structure of hBVR can be assigned two domains, "catalytic" and "regulatory," and six subdomains, V4, adenine binding, bZip, SH2 (Src homology) domain binding site, and CC-box (see Fig. 1). The N-terminus is composed of hydrophobic and charged residues that include a cluster of four valines ($V^{11\text{-}14}$) flanking the consensus Walker A homology ATP/adenine nucleotide–binding motif, $G^{15}XGXXG$ (18, 19). Both the hydrophobic chain of valines and the intact glycine motif are necessary for hBVR kinase and reductase activities; a single point mutation in the glycine motif, changing G^{17} to alanine, results in the loss of hBVR function (38).

The cluster of four valines is of primary importance for hydrophobic interactions with receptors and vesicular cell membranes, and thus far has been identified only in proteins involved in such interactions. The conservation of the cluster of

valines in all mammals may reflect a natural selection of this protein structural feature that, in addition to function, is associated with stability of the protein (16). The sequence that we refer to as the "oxidoreductase" domain is a highly conserved sequence with an invariable core sequence of GKHVXVEY.

Located in the midsection of the protein is the bZip motif that is involved in hBVR/DNA binding, dimerization, and transcriptional activity (1, 31, 43). The consensus sequence of the motif, $L^{129}X_6LX_6K/LX_6LX_6L$, is conserved in mammalian species. The hBVR S/T kinase motif, G^{148}SLLFTAGP, closely resembles the consensus sequence of other S/T kinases (19) and partially overlaps the bZip domain. Point mutation at S^{149} leads to the loss of function of the enzyme (59). The bZip and the S/T kinase motifs are not present in unicellular species.

The regulatory domain of hBVR includes two consensus tyrosine phosphorylation motifs, YMXM and YΦSΦ; binding sites for SH2 domain–containing adaptor proteins, such as phosphatidylinositol 3 kinase (PI3K). SH2 domains are conserved sequences of ~100 amino acid residues that form binding sites for phosphorylation in specific amino acid sequences. The domain recognize short tyrosine-phosphorylated motifs (53). The sequences in hBVR are Y^{198}MKM and Y^{228}LSF. The YMXM motif is conserved among all mammalian BVRs characterized to date, with the curious exception of the zebra fish; the nonmammalian species do not have this motif. The YΦSΦ is strictly a primate hBVR feature. The restricted presence of the second SH2 binding motif to primates would suggest the evolutionary evolvement of the second Src homology site subsequent to the YMKM motif. Curiously, a single tyrosine residue corresponding to Y^{198} is present in the bacterial homologue of the protein. The phosphorylated tyrosine residue of YMXM and YΦSΦ motifs provides an optimal site for assembly of multiprotein complexes that recruit or facilitate (or both) the relocation of cell-signaling proteins. The absence of bZip, S/T kinase, and SH2 domains from the nonmammalian forms of life would suggest evolutionary evolvement of BVR signaling and transcriptional activities. This suggestion is consistent with the near-complete conservation of the reductase sequence in all forms of life.

The carboxyl-terminal segment of BVR, referred to as the "CC-box," is cysteine/histidine rich, and appears to be crucial for functions of hBVR, and binding to divalent metals (*e.g.*, zinc) (39). The CC-box consists of four cysteine/histidine residues separated by 10 amino acids ($HCX_{10}CC/H$) and is conserved in most metazoans. The conserved C-terminal domain cysteines are titratible and are involved in interactions with –SH reagents (32, 39). The potential function of this motif in the context of its role in disulfide bond formation with interactive/binding proteins, such as PKC-βII, is discussed later.

The secondary structure of kinases is often folded in interacting structures. The carboxyl half of hBVR, as predicted by the crystal structure of rat BVR (27, 71), consists of six strands that form a large β sheet; the CC-box is present in a helix after the last strand. The six-stranded β sheet extensively interacts with the N-terminal of the protein and meets the secondary requirements for an effective PH (Pleckstrin homology) domain that is a feature of receptor-docking proteins, such as IRS proteins. The large β sheet is an ideal interface for protein–protein interaction (71) and characterizes a monomer–monomer interface site. The structure is frequently found in proteins involved in intracellular trafficking of signaling molecules, including PKCs; in this case, they are referred to as "RACK," or "scaffolds" (45, 52). As is noted later, hBVR functions in the translocation of phorbol myristate acetate (PMA)-stimulated PKC-βII in the cell (38).

FUNCTION OF HBVR AS A DUAL-SPECIFIC KINASE IN THE IR/IGF-1 SIGNALING CASCADE

Protein phosphorylation by kinases and dephosphorylation by phosphatases are essential components and mechanisms of signal transduction in the cell. The plethora of signals transduced in metazoans is carried primarily by serine, threonine, and tyrosine kinases. Protein kinases constitute a rather small fraction of protein types in the cell; protein tyrosine kinases (PTKs) are particularly rare. Tyrosine kinase activity is primarily a feature of kinases that function upstream of signaling cascades; examples include IRK and PI3K. PTKs are usually, although not always, membrane bound; only a few PTKs are also S/T kinases, and as such are defined as dual-specificity kinases (18, 24). PTKs are Mn^{2+} dependent and are exclusive to higher organisms. hBVR is an S/T/Y kinase (33) and, hence, a member of the aforementioned select group of kinases. The authenticity of hBVR tyrosine kinase activity was established by the display of tyrosine phosphorylation of a PTK-specific substrate (Raytide) as well as the autophosphorylation of tyrosine residues by the recombinant protein expressed in *Escherichia coli* (33). Notably the *E. coli* genome does not encode PTKs. hBVR autophosphorylates three of its six tyrosine residues.

hBVR dual-specificity kinase activity has a direct link to insulin and growth factor (GF) signaling. The insulin receptor (IR) is a PTK that consists of two α and β subunits in a heterotetrameric complex linked by disulfide bridges; the receptor also binds growth factor. The α subunits are the insulin-binding site, whereas tyrosine phosphorylation of the β subunits activates the receptor. The signaling cascade is initiated by the activation of IRK that follows ligand binding and results in the rapid tyrosine phosphorylation of docking cytoplasmic substrates (IRSs). The ensuing signaling events that involve activation of the major arms of the signaling cascade, the mitogen-activated protein kinase (MAPK) and PI3K (3), can be manifested as metabolic processes (*e.g.*, changes in carbohydrate and lipid or protein metabolism, as well as mitogenic processes, such as alteration in growth, differentiation, DNA synthesis and regulation of gene expression). A schematic presentation of the IRK/IGF-1 signal-transduction pathway is shown in Fig. 2. The balance between the strength of the two major arms of pathway determines which downstream targets are favored.

Figure 3 shows an adaptation of Pawson and Scott's depiction (54) of its interaction with the IR/IGF-1 receptor, IRS proteins, and the interaction of the docking proteins with the adaptor proteins. Docking proteins have two domains, the PH and the PTB. The PH domains are divergent in primary structure, but their secondary structure is highly conserved. The docking proteins' phosphotyrosine-binding domain (PTB) directs association with the tyrosine autophosphorylation motif, NPXY, on

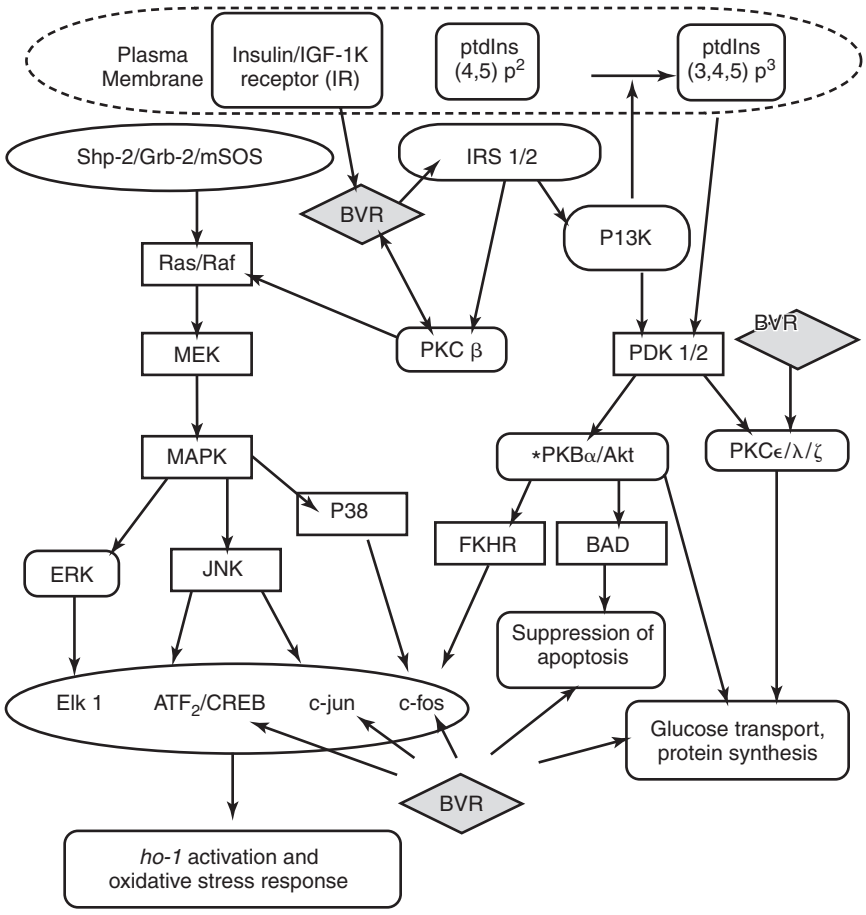

FIG. 2. The insulin/IGF response pathways. The cascades, *via* MAPK and PI3K, which transduce the signals from the insulin/IGF-1 receptor to the nucleus, are indicated. Abbreviations are defined in the following section. The points at which BVR intersects in these pathways are indicated by *red arrows*. The mechanism by which BVR modulates at each point varies; in some instances, it is the kinase function, whereas in others, it is as a transcription factor. Specific mechanisms are discussed in the text of this article or a previous article (36).

the insulin-activated kinase loop of IR (66). The tyrosine in the YMXM motif of docking proteins is recognized by the activated IRK as a substrate and is phosphorylated; the phosphotyrosine, in turn, provides a binding site for the SH2 domain of adaptor proteins. The SH2 domain, containing adaptor proteins, includes not only PI3K, the Grb family of kinases (growth factor–receptor binding protein), but others: SOS (son-of-sevenless), SOCS (suppressor of cytokine signaling), SHP-2 (Src homology phosphatase-2) (54, 61, 66). At least 12 intracellular substrates for IR have been identified, including hBVR. Six of these, IRS-1 to 6, belong to a related family of proteins. Among the six IRS proteins, IRS-1 is particularly important to insulin signaling. The PH domain is one of the membrane-targeting elements typically present in the N-terminals of docking proteins; the N-terminal myristoylation site is also a membrane-targeting element.

hBVR as a S/T/Y kinase has a central role in the control of insulin signaling; it competes with IRS-1 for phosphorylation by IRK, and in turn, functions as an S/T kinase for IRS-1 (33). S/T phosphorylation of IRS-1 terminates insulin action; thus, BVR phosphorylation of IRS-1 terminates insulin signaling, and consequently functions as a negative regulator of glucose uptake (33). The function of hBVR as a negative effector of glucose uptake was suggested by findings that cells transfected with pcDNA hBVR carrying a mutation at Y^{198}, or infected with siRNA for hBVR, showed an increase in glucose uptake (33). In addition to IRS-1, the hBVR, in strictly Mn^{2+}-dependent assay conditions, phosphorylates serine and threonine residues in known S/T substrates: myelin basic protein (MBP), casein, and, as is discussed later, PKC-βII (33, 38, 59). Several serines, and at least one threonine, T^{202}, are autophosphorylated. With respect to tyrosyl phosphorylation, it is notable that residues that are in the N-terminal kinase/catalytic domain of the protein, Y^{72}, Y^{83}, are autophosphorylated; Y^{98} is a substrate for both IRK and hBVR itself. Tyrosine residues, Y^{198}, Y^{228}, and Y^{291}, in the C-terminal regulatory segment of hBVR, are phosphorylated by IRK (33). Y^{198} and Y^{228} are in its YMKM and YLSF sequences, as noted earlier.

FUNCTION OF HBVR IN SIGNALING CASCADES ACTIVATED BY STRESS AND CYTOKINES

Stress response

In a recent review (36), the input of hBVR in the MAPK and IRS/PI3K cascades of the signal-transduction pathways was comprehensively reviewed; therefore, in this article, a rather brief account of the effector function of hBVR in those pathways is provided. As noted in Fig. 2, the two arms of the pathway cross-talk through the ATP-dependent PKC isozymes that phosphorylate serine or threonine residues or both in target pro-

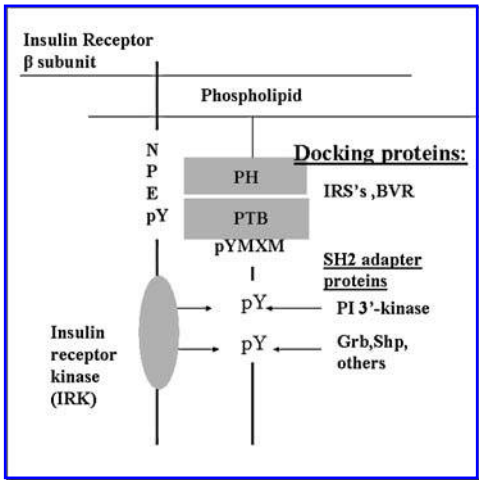

FIG. 3. Schematic presentation of insulin/IGF-1–receptor and insulin-receptor substrate interaction. The insulin-receptor substrate docking proteins contain two domains: an NH_2-terminal PH domain that mediates interaction with the membrane, and a PTB domain that binds to a specific juxtamembrane tyrosine autophosphorylation site in the NPEY sequence in the insulin-receptor kinase domain. The activated IR subsequently phosphorylates tyrosine residues in specific motifs, YMXM and YΦSΦ, in IR substrates that in turn act as docking sites for SH2-domain containing signaling proteins (52), as adapted from Pawson and Scott (53).

teins. The conventional-type PKC βII, the atypical PKC-ζ, and hBVR are all stimulated by oxidative stress, insulin, and growth factors (9, 10, 25, 33, 43, 59).

hBVR prominently figures in the cell's stress response because of its status as a member of the bZip family of transcription factors that regulates the induction of stress-regulated genes, including *ho-1* (1, 31, 43) in response to reactive oxygen species, promoters of free radical formation, such as sodium arsenite (Na_2AsO_3) (34), and insulin; BVR is activated (33, 43, 59). In addition, hBVR kinase and reductase activities are stimulated by heme and the metalloporphyrins, Co-PP (5, 38).

hBVR and PKCs cross-talk in their capacity as kinases in the MAPK pathway, wherein hBVR is involved in the activation of AP-1 and CRE binding and induction of stress and cAMP-regulated genes, such as oncogenes *c-jun*, *c-fos*, *and creb/atf-2* (1, 31, 38, 43). The AP-1 binding site and CRE are, respectively, 7- and 8-bp DNA recognition sequences (TGACTCA, TGACNTCA) for the bZip transcription factors that regulate *ho-1* stress response. In cells transfected with adenovirus hBVR expression construct, a time-dependent increase in the CRE binding protein, CREB/ATF-2, at transcription, protein and phosphorylation levels, and in HO-1 mRNA levels, has been observed (31). Transcriptional activation of *c-fos* and *c-jun* in cells transfected with pcDNA; hBVR expression construct is detected (38, 43). Because CREB/ATF-2 acts downstream of PKC-βII (63, 67), activation of PKC-βII by hBVR (discussed in the following section), is likely a constituent of the events that trigger AP-1/CRE-regulated gene expression in the MAPK pathway. The transcription factors downstream of the MAPK pathway influence cell growth, transformation, and survival. Notably, activation of the MAPK pathway is associated mostly with the transcriptional and mitogenetic effects of insulin/IGF. The CREB is a suspected target of the atypical PKC-ζ (46); the same signaling pathway that controls insulin/IGF mitogenic effects is also targeted by PKC-ζ and atypical PKCs (6, 72).

Inflammatory/cytokine response

The input of hBVR in the regulation of stress-response gene expression extends to the effector function of cytokines in mediating inflammatory response and activation of the Toll-like receptors (TLRs). These receptors include at least 11 type-I membrane glycoproteins that are the key sensors of bacterial lipopolysaccharide (LPS), as well as virus-like bacterial nucleic acids, RNAs and DNAs, which are recognized by the immune cells (55). The same signal, LPS, also activates hBVR, facilitates its translocation from the cytoplasm to the nucleus, and includes *ho-1* expression in intact LPS-treated rat kidneys (37). TLRs can activate kinases in the MAPK and NF-κB signaling pathways, producing different profiles of induced genes. The resulting profile depends on the activation of specific TLRs and the adaptor proteins that they recruit (50). Clearly, an interaction between hBVR and TLRs must occur for the activation of hBVR by LPS; however, the identity of individual TLRs involved in the activation remains to be established. The likelihood of the existence of an intimate link between the activation of TLRs by hBVR and *ho-1* induction in response to LPS treatment extends to the induction of *ho-1* by cytokines and hBVR, influencing the multitude of interactions between cytokines and the MAPK signaling pathway. To elaborate, interactions between cytokines, such as interleukin-6 (IL-6), interferon-γ (IFN-γ), and tumor necrosis factor-a (TNF-α) with intracellular adapter proteins lead to the activation of JNK (c-Jun NH2-terminal kinase) and p38 (2, 11); JNK and p38 phosphorylate the transcriptional regulators of *ho-1* expression, *c-jun*, and CREB/ATF-2, respectively (20, 42). Cytokines, including TNF-α, activate PKC-β and PKC-ζ, increase AP-1 binding (56, 73), and activate hBVR (unpublished observation). Moreover, the induction of transient expression of MAPK phosphatase-1 (MKP-1) in macrophages involves the novel PKC form, PKC-ζ (69). PKC-ζ additionally interacts with and enhances the activation of MAPK by enhancing the activation of Raf kinase and through ERK interaction (7, 8). Accordingly, it is reasonable to speculate on a role for BVR in the activation of a novel PKC isoform that includes δ, ϵ, θ, η, and μ in addition to ζ. Together, these considerations strengthen the prospect of the existence of a link between hBVR and cytokine-activated stress signaling for increased expression of *ho-1*.

hBVR–PKC ENZYME INTERACTIONS

The phosphotransferase activity of protein kinases, including PKCs, can be modulated by a binding protein partner. Protein–protein interactions can act to aid substrate/cofactor presentation, to phosphorylate residues essential for initiation of autophosphorylation, or to cause a required change in the secondary structure of the kinase. Moreover, spatial localization

within the cell is a component of the biologic function of many protein kinases, whose catalytic competence and localization to subcellular targets are regulated by S/T phosphorylation (44, 47, 57, 64). For instance, in the cell, the interaction with binding partner(s) confers specificity to individual PKCs.

Most recently, hBVR was identified (38) as a transactivator of a conventional type of PKC, PKC-βII; the other members of this group of PKCs are α, βI, βII, and γ isoforms (47, 48). The PKC family of kinases controls a multitude of biologic functions, including cell differentiation and proliferation, as well as carcinogenesis and tumor promotion. PKC-β isozymes promote cell division and differentiation in response to insulin stimulation by regulating the expression of several oncogenes, including *c-fos* (41). hBVR, conversely, is essential for a robust induction of *c-fos* mRNA by PMA-activated PKC-βII (38). Furthermore, hBVR potentiates PKC-βII kinase activity, as well as shuttles PKC-βII from the cytosol to the cell membrane in PMA-stimulated cells. Translocation of PKC-βII to the plasma membrane from the cytoplasm takes place only when the kinase is activated (15).

The observation that BVR is able to translocate in the cell in response to external stimuli is in keeping with a recent report that BVR is detected in association with the inner mitochondrial membrane, together with HO-1 (12); a binding site for one human *ho-1* BVR has been identified (70). The mitochondrial location of both proteins is likely an element in the cellular defense offered by bilirubin, the end product of HO-1 and BVR reductase activities, against oxidative and nitrosative damage. The functions of HO- activity products in cellular defense mechanisms are discussed in depth in other articles in this issue of the journal. The influence of BVR on the mitochondrial functions that are involved in cell death/survival of hBVR has been demonstrated (43) in 293A cells (a human embryonic kidney cell line) transfected with pcDNA hBVR expression construct and treated with sodium arsenite. In those cells, the release of mitochondrial cytochrome *c*, increases in TRAIL (tumor necrosis factor–related apoptosis-inducing ligand) mRNA levels, PARP (polyADP-ribose polymerase) fragmentation, and DR-5 (death receptor-5) transcription were attenuated (43). Cytochrome c release is one of the initial events in the onset of apoptosis.

A number of potential sites of interaction between hBVR and PKC enzymes are present across the former polypeptide; this includes both the N- and the C-termini and the mid region of the protein. Interaction at the N-terminals would likely involve the charged and the hydrophobic segments of the protein, notably six valines (four of them appearing as a cluster; see Fig. 1) are present in the N-terminal 20 amino acids of the mature protein; a preponderance of charged residues precedes the hydrophobic sequence (35, 36).

The activation of PKCs largely relates to their structural features; specifically, the PKC isozymes contain a conserved sequence in their regulatory domain, referred to as pseudosubstrate (21, 47, 49), and a RACK-binding site (57). The pseudosubstrate domain, as predicted by the secondary structural analysis, interacts with the substrate-binding site in the catalytic domain and maintains the kinase in an inactive form (21). PKC activators, such as membrane phospholipids and phorbol esters, do so by relieving this constraint and allowing exposure of the active site (58). Mochly-Rosen *et al*. (45) identified RACK-1–like sequences in conventional PKC isozymes that are located within the cysteine-rich C_2 region in their regulatory domain. The RACK 1–like conserved six-residue-long sequence in PKC-β, SVEIWD, pseudo-RACK, has structural requirements that include a conserved tryptophan at position 5 and a negatively charged residue at position 3. This sequence is similar to the AQELWE sequence in the hBVR, aa 107-112, which is also very similar to that of the PKC pseudosubstrate that, like hBVR, has serine substituting for alanine at position 1.

Depending on the type of PKC, one or two cysteine-rich regions (C1 or C2 domains) occur in their regulatory domain and play a dynamic role in their functions (47). Within the cysteine-rich regions of PKCs, as well as proximal to the cysteine-rich region of hBVR, are four of each of the Asn and Gln residues, with reactive carbonyl groups. Activators of PKCs are hydrogen-bonded to sulfhydryl groups of cysteine residues and to the carbonyl of the Asn in the cysteine-rich region of PKC (17). We reason that the cysteine residues in the CC-box in the carboxyl-terminal segment of hBVR (see Fig. 1) are likely involved in interaction with their counterpart(s) in PKCs. The cysteine-rich segments of hBVR and PKC proteins are likely to interact through either disulfide bond formation or coordination of divalent metals (28). Notably, Zn release from PKC-α is a mechanism for its activation (30).

The required change in secondary conformation of PKC-βII precedes phosphorylation of three key residues: a threonine in the activation loop that must be phosphorylated by another kinase, and two carboxyl-terminal residues, a serine and a threonine that are autophosphorylated. The secondary conformation is controlled intramolecularly through the interaction of the regulatory and catalytic domains with the kinase. The detection of hBVR-mediated transfer of a phosphate group to a specific peptide, Thr^{500} peptide (38), which was designed to correspond to the activation domain of PKC-βII (47), suggests that hBVR is the elusive kinase (or one of the kinases) that activates the PKC. Phosphorylation of Thr^{500} is the key to the function of PKC-βII in the cell. The initial phosphorylation of this residue is followed by autophosphorylation of the other two residues, Thr^{641} and Ser^{660}, and the resulting kinase-competent enzyme (25, 26).

Because phosphorylation of Thr^{500} peptide is further enhanced by Co-PP–activated hBVR, it is reasonable to consider that BVR activation precedes the rendition of PKC-βII as a kinase-competent enzyme. Moreover, increased autophosphorylation of PKC-βII in the presence of activated hBVR is consistent with the possibility that the protein–protein interaction induces conformational change in the PKC-βII secondary structure that allows exposure of the three key phosphorylation sites.

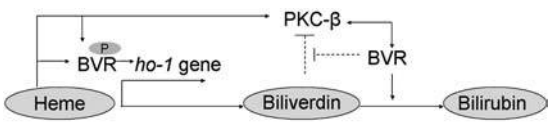

FIG. 4. Known interactions between hBVR and PKCs in *ho-1* induction. *Solid lines*, Activation events. *Dashed lines*, Inhibition. The scheme is formulated based on published reports (35, 38, 43).

Notably, the equivalent residue to Thr^{500} is conserved in other conventional PKCs (51).

Some examples exist of factors that cause enhancement of PKC activation over that achieved by phorbol esters, diacylglycerol (DAG), and phosphatidyl serine (PS) (64). However, because activation of PKC-βII by hBVR occurs in the *absence* of PKC allosteric activator phospholipids, is independent of their concentration, and changes both the V_{max} and K_m of PKC-βII for MBP, in our view, the process is considered to involve multiple components that reflect the primary and secondary structural features of hBVR discussed earlier and characterize this unusual protein. Moreover, because hBVR does not phosphorylate substrate under conditions that support PKC kinase activity, and, compared with MBP, is a relatively poor substrate for PKC-βII, the hBVR-mediated enhancement of substrate phosphorylation by PKC-βII provides additional confirmation that the activation of PKC-βII by hBVR is not merely an additive effect of two S/T kinase activators or two phospho-acceptors.

The expression of PKC isozymes and BVR is not restricted to a specific tissue, albeit the level varies from one tissue to another. On the whole, in the normal tissue, BVR levels far exceed those of heme-oxidizing enzymes, HO-1 and HO-2. Therefore, transactivation of hBVR and PKC-βII, and potentially hBVR and other PKC enzymes, is considered significant in the amplification and propagation of signals in the cell and response to free radicals oxidative stress and insulin. It follows that identification of PKC-βII as the kinase for hBVR would raise the prospect that the link between PKC enzymes and insulin/growth factor signaling for the regulation of *ho-1* is indeed BVR.

CROSS-TALK BETWEEN hBVR AND PKCs IN *ho-1* INDUCTION

Data supporting the occurrence of a regulatory loop between hBVR activity and *ho-1* gene expression response to oxidative stress has been addressed in length in previous reviews (35, 36). Of course, other types of kinase that function in the insulin/growth-factor cascade are also involved in the induction of *ho-1*. For instance, activation of PKB/Akt has been linked to the phosphorylation of HO-1 (60); HO-1 is a phosphoprotein (59). At this time, whether BVR influences PKB/Ak activity is not known; nonetheless, the newly uncovered transactivation loop between hBVR and PKC-βII corroborates and extends the intricacies of signaling interactions that govern *ho-1* induction through the activation of BVR. Figure 4 depicts the current understanding of the PKC-βII–hBVR interaction in the regulation of *ho-1* gene expression.

Collectively, the recent findings and past reports define hBVR not only as an enzyme with a unique activity profile, but also as having the potential to input at multiple stages in cell-signaling pathways.

ABBREVIATIONS

AP-1, activator protein-1; ATF-2, activating transcription factor-2; BAD, bcl-2–related antagonist of cell death; bZip, basic leucine zipper protein; Co-PP, cobalt protoporphyrin; CRE, cAMP regulatory element; CREB, cAMP regulatory element binding protein; DAG, diacylglycerol; FKHR, forkhead transcription factor; Grb, growth factor–receptor binding protein; hBVR, human biliverdin reductase; HO, heme oxygenase; IGF-1, insulin-like growth factor-1; IL-6, interleukin-6; IRK, insulin receptor kinase; IRS, insulin-receptor substrate; JNK, c-Jun NH2-terminal kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; MBP, myelin basic protein; PtdIns, phosphatidyl inositol; PH domain, Pleckstrin homology domain; PI3K, phosphatidyl inositol 3 kinase; PMA, phorbol myristate acetate; PS, phosphatidyl serine; PTB domain, phosphotyrosine-binding domain; PTK, protein tyrosine kinase; RACK, receptor for activated C–kinase; Raf, Ras-activated factor; SH2 domain, Src homology-2 domain; SHP-2, Src homology phosphatase-2; siRNA, small interfering RNA; SOCS, suppressor of cytokine signaling; SOS, son-of-sevenless; TNF-α, tumor necrosis factor-α.

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