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SCIENCE DIRECT

Free Radical Biology & Medicine 38 (2005) 1344–1352

www.elsevier.com/locate/freeradbiomed

Original Contribution

Redox regulation of the transcriptional repressor Bach1

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Received 8 October 2004; revised 21 January 2005; accepted 24 January 2005
Available online 12 February 2005

Abstract

Bach1 is a transcriptional repressor of heme oxygenase-1, one of the most inducible phase 2 proteins. Bach1 binds in conjunction with a small Maf protein to tandem repeats of the antioxidant response element (ARE) and quenches the target gene expression. On the other hand, the transactivator Nrf2 binds and up-regulates the ARE-governed gene expression. By using a sulfhydryl oxidizing agent, diamide, here we provide evidence which indicates that the Bach1 function is regulated by the redox state. Diamide showed restricted Nrf2 nuclear translocation and ARE-driven reporter activity but reversed the ARE transcriptional activity suppressed by ectopically expressed Bach1. Substitution of the conserved cysteine residue in the DNA binding domain of Bach1 to serine (C574S mutant) caused a refractory response to the diamide-mediated reactivation of the Bach1-suppressed reporter activity. Moreover, diamide induced cytoplasmic translocation of the GFP–Bach1 fusion protein but failed to translocate the fusion protein consisting of the C574S mutant. These data suggest that redox regulation of Bach1 is an alternative mechanism to induce multiple ARE-governed genes.

Keywords: Transrepressor; HO-1; Diamide; NF-E2-related factor 2; 4-Hydroxy-2,3-nonenal; Redox signaling; Thiol modification; Free radicals

Exposure of mammalian cells to reactive oxygen species and/or electrophilic agents induces a set of phase 2 proteins, including NAD(P)H quinone oxidoreductase 1 (NQO1) [1,2], glutathione *S*-transferases (GSTs) [1,3], γ-glutamylcysteine synthetase (GCS) [4–6], and heme oxygenase-1 (HO-1). These phase 2 proteins have been shown to protect cells from oxidative insults although their functions differ extensively. Transcriptional regulation of these antioxidative genes is critically dependent on a common *cis*-acting sequence, the so-called antioxidant responsive element (ARE) located in the gene promoter or enhancer region [1,7]. The ARE consensus sequence exhibits remarkable similarity to that of the erythroid transcription factor NF-E2-binding sequence, to which homo- and heterodimeric combinations of the basic region leucine zipper (bZIP) transcription factors, such as Jun, Fos, Maf, and the Cap'n'Collar (CNC) family members, including NF-E2, bind [8–12]. Among the CNC family members, NF-E2-related factor 2 (Nrf2) has been shown to be a critical factor for the ARE-dependent transcriptional activation of genes encoding phase 2 proteins [13], as Nrf2-deficient mice failed to induce these detoxification responses to electrophiles [1]. Under unstimulated conditions, ARE transactivation by Nrf2 is inhibited by its binding to the cytoplasmic Kelch-like ECH-associated protein 1 (Keap1), which is anchored by F-actin [1,14]. When electrophiles disrupt the Keap1–Nrf2 complex, Nrf2 migrates into the nucleus and induces ARE-dependent transactivation [14].

Abbreviations: AP-1, activator protein-1; ARE, antioxidant responsive element; Bach1, BTB and CNC homology; bZIP, basic leucine zipper; CNC, Cap'n'Collar; EMSA, electrophoretic mobility shift assay; GCS, γ-glutamylcysteine synthetase; GFP, green fluorescent protein; GST, glutathione *S*-transferase; HNE, 4-hydroxy-2,3-nonenal; HO-1, heme oxygenase-1; Keap1, Kelch-like erythroid-derived CNC-homology-associated protein 1; NF-E2, nuclear factor erythroid 2; NQO1, NAD(P)H quinone oxidoreductase 1; Nrf2, NF-E2-related factor 2; TRE, 12-*O*-tetradecanoylphorbol-13-acetate responsive element.

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doi:10.1016/j.freeradbiomed.2005.01.021

On the other hand, Nrf2 has been shown to be a member of the cellular proteins that turn over most rapidly [15–20]. It has been suggested that newly synthesized Nrf2 undergoes proteasomal degradation by Keap1 sequestration [16]. Therefore, Keap1 is proposed to be a crucial regulator of Nrf2 function.

We have previously reported that 4-hydroxy-2,3-nonenal (HNE), a cytotoxic end product of lipid peroxidation, induces nuclear translocation of Nrf2 and thereby activates ARE-dependent transactivation [21]. Such an oxidative stressor induces phosphorylation of Nrf2 at Ser40 via the protein kinase C pathway, resulting in release from the Keap1 sequestration and nuclear translocation of Nrf2. On the other hand, accumulated evidence indicates that the oxidative stress conditions stabilize Nrf2 protein and hence modulate the turnover rate of the transcription factor. Moreover, oxidation of reactive cysteine residues that are rich in the Keap1 protein liberates Nrf2 from the Keap1–Nrf2 complex [22]. Considering these facts, it is suggested that Nrf2 activation is controlled by diverse mechanisms simultaneously.

Among the phase 2 proteins, the expression level of HO-1 under steady-state conditions is very low and is dramatically induced by oxidative stress [23–26]. To explain this phenomenon, transcriptional repression machinery by the BTB and CNC homolog 1 (Bach1) has been proposed. Bach1 has been identified by a yeast two-hybrid screening system using a member of the small Maf proteins, MafK, as a bait [12,27]. Bach1 belongs to the CNC-related bZIP superfamily of transcriptional factors. Unlike other CNC family members, it possesses the BTB/POZ (which stands for "Broad complex, Tramtrack, and Bric a brac/poxvirus and zinc finger") domain in its N-terminal region [12,28–30], which has been implicated in transcriptional repression by interacting with corepressors, such as N-CoR (for "nuclear hormone receptor corepressor") and SMRT (for "silencing mediator of retinoic acid and thyroid hormone receptors") [31,32]. Thus, Bach1 is considered to function as a transcriptional repressor. Because the Bach1–small Maf heterodimer binds repeated ARE sequences, the 5′-upstream region of the HO-1 gene, which possesses clusters of ARE, would be a favorite target for the repressor complex. Genes encoding other phase 2 proteins, such as NQO1, GSTs, and GCS, possess a single ARE sequence and are not governed by Bach1.

In the present study, we focused on the redox regulation of Bach1 function. A sulfhydryl oxidizing agent, diazenedicarbolic acid *bis*[*N*,*N*-dimethylamide] (diamide), reversed ectopically expressed Bach1-suppressed ARE-reporter activity despite minimal activation of Nrf2 function. We provide evidence indicating that redox-dependent modulation of the Bach1 function causes Nrf2-independent ARE activation. These observations suggest that redox control of the transcriptional repressor Bach1 serves as an alternative mechanism to induce the target gene by oxidative stress.

Materials and methods

Materials

HNE diethylacetal was obtained from Oxis International (Portland, OR, USA) and dissolved in acetonitrile as a stock solution. A portion of the stock solution was evaporated, and the residue was dissolved in 1 mM hydrochloric acid. The HNE concentration was calculated by the absorption at 220 nm before use. Diamide was purchased from ICN Biomedicals, Inc. (Aurora, OH, USA) and dissolved in water.

Cell cultures

HepG2 human hepatoma and COS-7 monkey kidney cells were obtained from Riken Cell Bank (Tsukuba, Japan). These cells were cultivated in DMEM supplemented with 10% fetal calf serum, 20 mM Hepes, and antibiotics in a humidified 5% CO_2 atmosphere at 37°C.

Western blot analysis

Cells were washed and lysed by scraping into a boiling SDS–PAGE sample buffer. The lysates were further boiled for 5 min and briefly sonicated. Samples were run on 10% SDS–PAGE and transferred to a polyvinylidene difluoride membrane (Pall BioSupport, Glen Cove, NY, USA). Blots were blocked in 0.2% I-Block (Applied Biosystems, Foster City, CA, USA), dissolved in Tris-buffered saline containing 0.2% Tween 20 for 1 h, and probed with anti-Nrf2 antibodies (H-300; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). After being washed, the blots were incubated with the secondary antibody coupled to peroxidase. Blots were developed using enhanced chemiluminescence (Amersham Bioscience, Buckinghamshire, UK) according to the supplier's instructions.

Construction of vectors

For construction of the ARE-Luc reporter plasmid used in the luciferase assay, a 120-bp fragment containing multiple AREs that reside 9 kb upstream of the transcription start site of the human HO-1 gene was produced by PCR using the primers 5′-CGCGGATCCGTGTTTTTCCTGCTGAGTCACGGTCCCGAGGTCTATTTTCGCTAAGTCACCGCCCCGAGATCTGTTTTCGCTGAG-3′ and 5′-CGCGGATCCAATCTCTAGACCGTGACTCAGCGAAAACAGACACCGGGACCGTGACTCAGCGAAAACAGATCTCGGGGCGGTGAC-3′ (possessing the underlined 30-bp overlapping sequence) digested with *Bam*HI and inserted into the *Bam*HI site upstream of the TATA-like promoter of herpes simplex virus thymidine kinase promoter of pTAL-Luc vector (BD Biosciences Clontech, Palo Alto, CA, USA). Nrf2/pcDNA3.1, Nrf2/pQBI25, and Keap1/pcDNA3.1 were constructed as