The binding site for the transcription factor, NF-κB, on the cystathionine γ-lyase promoter is critical for LPS-induced cystathionine γ-lyase expression

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Abstract. Hydrogen sulfide (H_2S) is regarded as the third endogenous gaseous signaling molecule. Cystathioine γ-lyase (CSE), one of the three enzymes in the transsulfuration pathway, is responsible for the production of endogenous H_2S. The H_2S/CSE signaling pathway is involved in the inflammation induced by lipopolysaccharides (LPS). Therefore, in this study, we investigated the effects of the binding site (on the CSE promoter) for the transcription factor, nuclear factor (NF)-κB, on the transcriptional regulation of the CSE gene in mammalian cells treated with LPS. For this purpose, HEK-293 and COS-7 cells were transfected with 5 μg pGL4.12-KM1478 or 5 μg pGL4.12-KM1478m (mutant) together with the pRL-CMV control vector (0.032 μg for the HEK-293 cells, 0.0032 μg for the COS-7 cells). Subsequently, the cells were treated with LPS for 6 h. The expression of CSE was measured by RT-qPCR. cDNA pooled from J774.1A and RAW264.7 cells treated with LPS for 6 h was used to estimate the quantity of the transcripts. Our results revealed that LPS markedly increased the mRNA and protein expression levels of the CSE gene in the J774.1A and RAW264.7 cells following treatment with LPS for 6 h. In addition, we found that the GGGACATTCC DNA sequence on the promoter of the CSE gene was closely associated with the transcriptional regulation of the CSE gene in the HEK-293 and COS-7 cells treated with LPS. Taken together, our data suggest that the NF-κB binding site on CSE promoter is critical for LPS-induced CSE expression in mammalian cells.

Introduction

Hydrogen sulfide (H_2S), a well-known toxic gas, is regarded as the third endogenous gaseous signaling molecule (1,2). Cystathioine γ-lyase (CSE), one of the three enzymes in the transsulfuration pathway, is responsible for the production of endogenous H_2S using L-cysteine or L-homocysteine as a substrate (2-5). The generation of CSE mRNA occurs along with the generation of H_2S in the rat aorta (6,7) that is provided with exogenous L-cysteine. Generally, cystathionine β-synthase (CBS) is considered to play a crucial role in the development and maintenance of the central nervous system, and radial glia/astrocyte dysfunction may be involved in the complex neuropathological features (8). Moreover, 3-mercaptopyruvate sulfurtransferase (3MST), another H_2S-producing enzyme, is localized to neurons in the brain and to the vascular endothelium (9).

Previous studies have shown that the H_2S/CSE signaling pathway is involved in the inflammation induced by endotoxins, such as lipopolysaccharides (LPS). H_2S may represent a novel endogenous mechanism of cytoprotection in the inflamed joint, suggesting a potential opportunity for therapeutic intervention (10). The inhibitory effects of LPS on endothelium-dependent relaxation resulting in pulmonary hypertension may also be mediated by H_2S (11). NaHS (an H_2S donor) has been shown to dose-dependently inhibit LPS-induced chemokine receptor CX3CR1 expression in macrophages (12). Inhaling H_2S has also been shown to prevent inflammation and improve survival after LPS challenge by altering sulfide metabolism in mice (13). The decrease in pulmonary surfactant (PS) levels is the most important physiopathological process of acute lung injury (ALI) induced by LPS. Exogenously applied H_2S can attenuate the process of ALI, possibly since H_2S can adjust the composition and secretion of PS (14). The downregulation in CSE/H_2S expression is involved in the pathogenesis of LPS-induced ALI, and LPS can stimulate CSE expression and H_2S production (15,16).

Nuclear factor (NF)-κB is a heterodimer involving a variety of signaling pathways (17). The transcription factor, NF-κB, regulates inflammatory responses by inducing the expression of a variety of genes (18). H_2S can inhibit NF-κB activation in LPS-stimulated macrophages (19). In a previous study, S-propargyl-cysteine (SPRC), a novel H_2S-modulated agent, exerted beneficial effects by inhibiting IκB-α degradation and the phosphorylation of transcription factors associated with nuclear factor-κB p65 activation induced by LPS (20).

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The NF-κB pathway can be rapidly activated by a large spectrum of chemically diverse agents and stress conditions, including bacterial LPS, microbial and viral pathogens, cytokines and growth factors (18). SPRC has been shown to exert anti-inflammatory effects in LPS-stimulated H9c2 cells partly through the CSE/H_2S pathway by impairing IκB-α/NF-κB signaling (21).

However, the transcriptional regulation of the CSE gene in mammalian cells treated with LPS remains largely unknown. Based on the fact that the H_2S/CSE signaling pathway is involved in the inflammation induced by endotoxins, such as LPS, in the present study, we investigated the effects of the binding site of the transcription factor, NF-κB, on the transcriptional regulation of the CSE gene in mammalian cells treated with LPS.

Materials and methods

Construction of luciferase reporter under the control of the mouse CSE promoter. A 1.5-kb DNA fragment upstream of the transcription start site (-1453 to +25) of the mouse CSE gene was amplified by PCR using pGL4.12-KM1716 [the construct with the mouse CSE gene promoter previously constructed in our laboratory (22)] as the template (forward primer, 5'-CGGGG TACCGTGGAAGGGACATTCCTGTGAATAG-3' and reverse primer, 5'-CCGCTCGAGAGGAGTGCGAGGTGTTGCTTT GGCT-3'). The thermal cycling conditions were as follows: initial denaturation at 94˚C for 3 min, followed by 30 cycles of 95˚C for 30 sec, 60˚C for 45 sec, 72˚C for 2 min and a final elongation step at 72˚C for 8 min. The PCR product was digested with the restriction enzymes, *Kpn*I and *Xho*I (both from Takara Biotechnology, Co., Ltd., Dalian, China), and cloned into the promoterless pGL4.12 vector (Promega, Madison, WI, USA) that contains a Firefly luciferase gene driven by the inserted promoter. The resultant construct was designated as pGL4.12-KM1478. The inserted DNA fragment was confirmed by DNA sequencing (Biosune Biotechnology Co., Ltd., Shanghai, China). The construction of the reporter with the mutant CSE promoter was the same as above, the only difference being that an alternative forward primer (5'-CGGGG TACCGTGGAAATCTCATTCCTGTGATAG-3') was used during PCR amplification.

Cell culture and treatments and luciferase assay. For transfection, HEK-293 and COS-7 cells were grown to 70-80% confluence Thereafter, 5 μg pGL4.12-KM1478 or 5 μg pGL4.12-KM1478m together with the pRL-CMV control vector (0.032 μg for the HEK-293 cells, 0.0032 μg for the COS-7 cells) were transfected into the cells using Xfect™ transfection reagent (Clontech Laboratories, Inc., Palo Alto, CA, USA) according to the manufacturer's instructions. Twelve hours later, the transfected cells were trypsinized and seeded into 35-mm dishes. Following treatment with LPS for 6 h, both Firefly lucierase and *Renilla* luciferase activities were assayed using the Dual-Luciferase® reporter assay (Promega) according to the manufacturer's instructions with the SpectraMax® microplate reader (Molecular Devices Corp., Sunnyvale, CA, USA), according to the manufacturer's instructions. The Firefly luciferase activity was normalized against the *Renilla* luciferase activity.

Isolation of RNA and quantitative reverse-transcription PCR (RT-qPCR). Total RNA was isolated using TransZol Up reagent (TransGen Biotech Co., Ltd., Beijing, China), according to the manufacturer's instructions, after the treated cells were rinsed twice with 1X dPBS buffer (Thermo Scientific, Rockford, IL, USA). The cells were directly lysed in a culture dish by the addition of 1 ml of TransZol Up to a 3.5-cm dish, and passing the cell lysate several times through a pipette. The homogenized samples were then incubated for 5 min at 15-30˚C to permit the complete dissociation of the nucleoprotein complexes. In total, 0.2 ml of chloroform per 1 ml of TransZol were added. The tubes were vigorously shaken by hand for 15 sec and were then incubated at room temperature for 3 min. The samples were cetrifuged at 10,000 rpm for 15 min at 4˚C. Following centrifugation, the mixture was separated into a lower red, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. The aqueous phase was transferred to a fresh tube. The RNA from the aqueous phase was precipitated by mixing with isopropyl alcohol. A total of 0.5 ml of isopropyl alcohol per 1 ml of TransZol Up was used for the initial homogenization. The samples were incubated at room temperature for 10 min and centrifuged at 10,000 rpm for 10 min at 4˚C. The RNA precipitate, often invisible before centrifugation, forms a gel-like pellet on the side and bottom of the tube. The supernatant was carefully removed. The RNA pellet was washed once with 75% ethanol, adding at least 1 ml of 75% ethanol per 1 ml of TransZol Up used for the initial homogenization. The sample was mixed by vortexing and centrifugation at no more than 7,000 rpm for 5 min at 4˚C. At the end of the procedure, the RNA pellet was air-dried for 5-10 min. The RNA was dissolved in RNase-free water by passing the solution a few times through a pipette tip, incubating for 10 min at 55-60˚C and cooling to room temperature. The RNA samples were quantified by absorbance at 260 nm (Biophoto, Eppendorf, Germany) and the integrity, purity and amount of RNA were verified by the visualization of rRNA following agarose gel electrophoresis. The fluorescence intensity was determined for the RNA concentrations using a Qubit™ RNA assay kit (Invitrogen Life Technologies, Shanghai, China) according to the manufacturer's instructions. First-strand cDNA was synthesized by incubation at 42˚C for 30 min using an anchored oligo$(dT)_{18}$ primer. The total volume of the the reaction mixture was 20 μl containing 2 μg of RNA, 1 μl of anchored oligo$(dT)_{18}$, 10 μl of 2X TS Reaction mix and TransScript™ RT/RI Enzyme mix as well as RNase-free water (TransGen Biotech Co., Ltd.). The reaction was terminated by incubation at 85˚C for 5 min and the reaction mixture was stored at -20˚C and used for RT-qPCR.

RT-qPCR was performed in a final volume of 25 μl containing 11 μl cDNA diluted with double-distilled H_2O (1:20), 0.5 μl of 0.2 μM each primer, 0.5 μl Passive Reference Dye II and 12.5 μl of 2X TransStart™ Green qPCR SuperMix (TransGen Biotech Co., Ltd.). All reactions were run in triplicate on an Agilent Mx3000P QPCR System (Agilent Technologies, Santa Clara, CA, USA) and MxPro QPCR software (Agilent Technologies) using a SYBR-Green fluorescence quantification system with the following conditions: an initial denaturation step at 95˚C for 10 min, and 45 cycles of 30 sec at 95˚C, 30 sec at 60˚C and 10 sec at 72˚C. The primer pair Q CSE Forward Primer/Q CSE Reverse Primer (Table I) was designed to

Table I: Primers used for RT-qPCR.

Gene	GenBank accesion no.	Forward/reverse primer	Exon	Amplicon size
CSE	NM_145953.2	5'-TGGGCTGCCCTCTCATCCACA-3'	2	112 bp
		5'-TCCTCCCAAGCTCTCGGCCA-3'	2	
ACTB	NM_007393.3	5'-GCGAGCACAGAGCCTCGCCTTT-3'	2	128 bp
		5'-CCTTGCACATGCCGGAGCCGT-3'	2	

CSE, cystathioine γ-lyase (CSE); ACTB, β-actin gene.

determine the relative expression of CSE. The primers specific to our PCR template were created by Blast biological software (http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome). The fluorescence was measured at the end of the extension step at 72˚C. Controls for genomic DNA and primer contamination were routinely performed with non-RT or no template PCR reactions, respectively. Dissociation curves were performed for each set of oligonucleotides to test primer specificity and to confirm the presence of a unique PCR product. To estimate PCR efficiencies, standard curves were performed based on 5 serial dilutions of a cDNA stock. PCR efficiencies of all primer sets were between 95 and 100%. After verifying that the β-actin gene (*ACTB*) and CSE mRNA primers had similar amplification efficiencies, the comparative Ct method $2^{-\Delta\Delta Ct}$ was used to for performing relative quantification analysis of the mRNA levels, as previously described (23). The relative amount of each mRNA was normalized to the housekeeping gene, *ACTB*. Each sample was run and analyzed in triplicate. The average of the relative amount of each mRNA in the control group is defined as 1.0. The quantity of the transcripts was estimated from a standard line derived from 20-fold serial dilutions of cDNA pooled from J774.1A or RAW264.7 cells treated with LPS.

Western blot analysis. For total protein extraction, $1x10^6$ cells were incubated in 120 μl of RIPA lysis buffer (mild) (Biomiga, Inc., San Diego, CA, USA), supplemented with 1 mM PMSF proteinase, 0.25 U/μl Benzonase, inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA). The cells were incubated on ice for 30 min, and the lysate was cleared by centrifugation at 12,000 x g at 4˚C for 15 min. Following centrifugation, glycerol, 2-mercaptoethanol, 10% sodium dodecylsulfate (SDS), 1 M Tris-HCl (pH 6.7) and bromophenol blue were added to the supernatant, and then denatured at 95-100˚C for 10 min. The proteins were separated by electrophoresis on a 10% [for the detection of cystathionine-γ-lyase (CSE), and α-tubulin (B-7)(TUBA)] SDS-polyacrylamide gel (Sigma-Aldrich) and transferred onto an polyvinylidene difluoride (PVDF) membrane (0.45 μm, Immobilon-P; Millipore, Billerica, MA, USA). The membrane was blocked with a blocking solution containing 5% skim milk, 137 mM NaCl, 0.1% Tween-20 and 20 mM Tris-HCl (pH 7.6). After a wash, the membrane was incubated overnight at 4˚C with anti-CSE (30.7; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) monoclonal antibodies (1:1,000 dilutions) or anti-α-tubulin (B-7; Santa Cruz Biotechnology, Inc.) monoclonal antibodies (1:15,000 dilutions). After another wash, the membrane was incubated with a horseradish peroxidase (HRP)-conjugated goat anti-mouse antibody (1:10,000) (Santa Cruz Biotechnology, Inc.), for the detection of CSE and TUBA. Positive bands for CSE or TUBA were identified around 43-47 or 52-58 kDa, respectively, by SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific). The resulting films (Kodak Co., Ltd., Xiamen, China) were scanned and quantified using densitmetric analytical software (the Bio-Rad Quantity One software; Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Statistical analysis. All data are expressed as the means ± SEM of at least 4 experiments. Statistical significance was assessed by either one-way or two-way ANOVA for repeated measures followed by Turkey's test. A value of $P<0.05$ was considered to indicate a statistically significant difference.

Results

Effects of LPS on CSE gene expression at the mRNA level. To determine the effects of LPS on the transcription of the CSE gene, we examined the mRNA expression level of CSE in the J774.1A and RAW264.7 cells. As shown in Fig. 1A, following treatment with LPS (0.1, 0.5 and 1 μg/ml) for 6 h, the CSE mRNA levels in the J774.1A cells increased, at the 0.5 μg/ml dose in particular. However, high concentrations of LPS (1.0 μg/ml) had less significant effects, possibly caused by the toxicity of LPS to the cells. As shown in Fig. 1B, following treatment with LPS (0.1, 0.5 and 1 μg/ml) for 6 h, the CSE mRNA levels in the RAW264.7 cells gradually increased in a dose-dependent manner; this suggests that the RAW264.7 cells are possibly more tolerant to the toxicity induced by LPS. As LPS has both immune-stimulating effects and a toxic activity, it may exert varying effects on different cell types.

Effects of LPS on CSE gene expression at the protein level. We examined the effects of LPS on the protein expression of CSE in the J774.1A and RAW264.7 cells. As shown in Fig. 2A, following treatment with LPS (0.1, 0.5 and 1 μg/ml) for 6 h, the CSE protein levels in the J774.1A cells increased, particularly at the dose of 1 μg/ml. As shown in Fig. 2B, following treatment with LPS (0.1, 0.5 and 1 μg/ml) for 6 h, the CSE protein levels in the RAW264.7 cells also increased, although not as significantly as the CSE protein levels in the J774.1A cells.

Effects of LPS on the wild-type or mutant promoter activity of the CSE gene. We also determined the effects of LPS on the transactivation of the promoter activity of the CSE gene using the transient transfection experiment. As mentioned

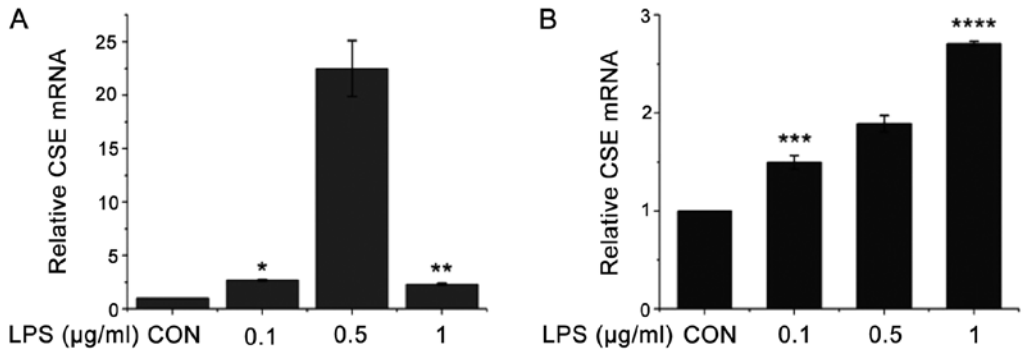

Figure 1. Effects of lipopolysaccharides (LPS) on cystathioine γ-lyase (CSE) expression in (A) J774.1A cells and (B) RAW264.7 cells at the mRNA level. Following treatment with LPS for 6 h, total RNA was isolated from these cells and used as a template for reverse transcription. The CSE mRNA levels were then quantified by RT-qPCR and normalized against the mRNA level of the β-actin gene (*ACTB*). *P<0.01 vs. control (CON) and 0.5, **P<0.01 vs. CON, ***P<0.05 vs. CON and 0.5, ****P<0.01 vs. 0.5 and CON.

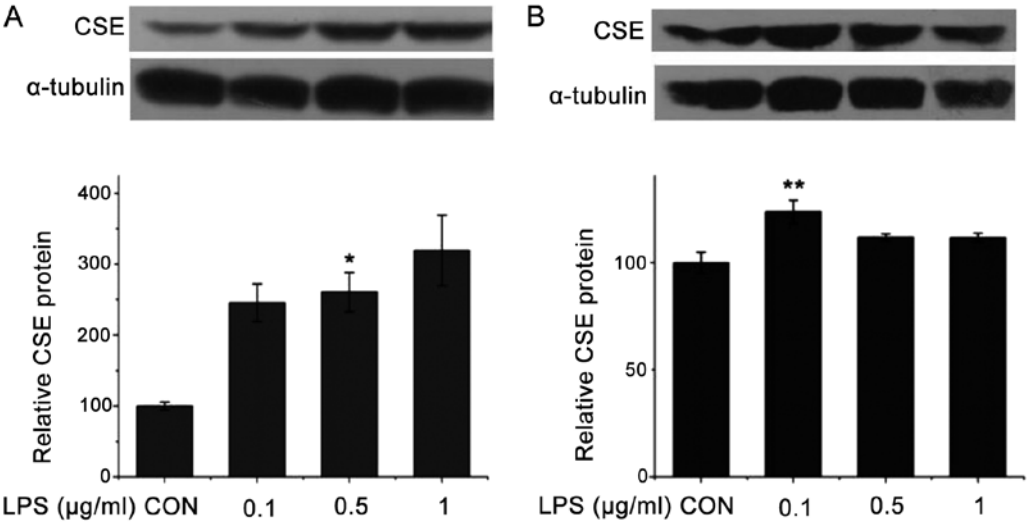

Figure 2. Effects of lipopolysaccharides (LPS) on cystathioine γ-lyase (CSE) expression in (A) J774.1A cells and (B) RAW264.7 cells at the protein level. Follwoing treatment with LPS for 6 h, the cells were lysed and the extracted proteins were subjected to sodium dodecylsulfate (SDS)-PAGE. After being electro-transferred onto a polyvinylidene difluoride (PVDF) membrane, the bands of CSE and α-tubulin were visualized by western blot analysis using anti-CSE and anti-tubulin antibodies, respectively. The bands were quantified using Bio-Rad Quantity One software and the CSE protein levels were normalized against the band of α-tubulin. *P<0.05 vs. control (CON), **P<0.01 vs. CON.

above, LPS increased the expression of CSE in 2 lymphocyte cell lines. Bioinformatics analysis using the search algorithm TFSEARCH: Searching Transcription Factor Binding Sites (version 1.3; http://mbs.cbrc.jp/research/db/TFSEARCH.html) as previously described (24) revealed the potential NF-κB binding site on the promoter of the mouse CSE gene with the DNA sequences of 5'-GGGACATTCC-3'. To determine whether NF-κB is involved in regulating the expression of the CSE gene, we constructed a reporter assay in which the reporter luciferase expression was either controlled by the wild-type (with NF-κB binding site) or the mutant (without NF-κB site) control region of the *CSE* gene (Fig. 3).

Following treatment with LPS (0.1, 0.5 and 1 μg/ml) for 6 h, the wild-type promoter activity of the CSE gene in the transfected COS-7 cells increased with the increment in the LPS concentration (Fig. 4A). On the contrary, the mutant promoter activity of the CSE gene gradually decreased with the increment in the LPS concentration (Fig. 4B). Following treatment with LPS (0.1, 0.5 and 1 μg/ml) for 6 h, the wild-type promoter activity of the CSE gene increased in the transfected HEK-293 cells treated with LPS at 0.1 μg/ml (Fig. 5A). The CSE promoter activity did not increase significantly in the cells treated with LPS at 0.5 and 1 μg/ml. However, following treatment with LPS (0.1, 0.5 and 1 μg/mL) for 6 h, the mutant promoter activity of the CSE gene gradually decreased with the increment in LPS concentration (Fig. 5B).

Discussion

H_2S is regarded as the third endogenous gaseous signaling molecule (1,2). CSE, which produces H_2S from cysteine or homocysteine (25), is mainly expressed in the liver, kidneys, lungs, thoracic aorta, ileum, portal vein, uterus and the brain, as well as in pancreatic islets and the placenta in mammals (7,26-33). A number of studies have shown that the H_2S/CSE signaling pathway is involved in the inflammation induced by endotoxins,

GGTACCGTGGAAGGGACATTCCTGTGAATAGAGAAATTTAAATCTTGGGCTGCCACCCCACAGCTTCCTCTAACTTTATCAGAAGCCTCTCATTTAGAA
KpnI
NF-κB normal point
ATCTCATTCC
NF-κB mutant point
AGTCTGTATAAGGGCCACTTTTCAAAGACCTCTTTGTGACAGTTACACTAAATAAAATGTCCAGTGGGAAGATGGCTCAGTGGGTAAATGCTTGCCGCA
CAGCCTGACACCCTGTGTTCTATCTCCAGCATCTACCTTTGTGCTGCTGTGTATCTGTAATAGCAGCTCTCCTACTGTAGAAGACAGAGAATCCACTGAA
GCTCCTGGGTGGTCTAGCCTGGGGTAAGCAGTGCAAAGACCCTGCGTCAACAGGGAGGTGGAAGACTGTCCTCTGACCTCTATGGGTGTGCCACACA
TTCCTCTGCACTCTCAAATACGATAAACAGACAAGTACACAAACAAATAGAAAACATCCATGTTTGTATAACCCAGGGCTTACCAACTGTACTTAGAAC
TGGACTCAGAACTGTTTCAGGCCCTTCTATTCCTCGGTGTTTTCTGTGATCTCATCAAAATGTACTTACGTCTTTAGGTTCCAGAACACCAGGGCTTGGC
TTCCTCTCCCACACGCAGATCACATCTGTTTCTAAACGCAGTTCCAGGGGGATCCAACAGCCTTTCTGACCTCTGTAGACATCAAGTATGCATGTGGTG
CAACACACACACACACACACACACACACACACACCGTTAGAATACACTCAGGCTGAGAACATACTTTAAAAGGAGAAATACTAATCAAGCAAGCATGGT
AACCCATTAGTGTTTCAGAGGCTACCCCCTTCCCCTGTGCCTCTGGCAGTGTACACTTGTTTCTGGAAGGTGATTGTGTCTGAAGCTAATTTGAAAAGC
ATACTGATTGTGTTTGCTCTTCGTAGCTTTTCCAAACCGTAGCTTTTAAAATCAGTTGTTTATTCCTTAACACCTTCCAAATTCGTTCAACAGTGTTTCAA
AGTTTTAGGTTCGACTTCCCAGGTACAGAAACTCCTTAAACTCCCCAGTTCTCGGACAAGGTGTAAAATTAGGTCAAAGCTACGTCTTTTTGGGAGCA
AAGCTTGGGCCTTAAGGCCTGATCTTGGCCAGAGTAGAGACGCGGGCGCTGGTGGCACAGCTGTGGCGGTCCTCAGATCCGAGGGAAGCACCTGCCT
GCGTGACCCTTGCCGGAACTCTGGGGCCACGCGAGGCTGCTGGAGGTTGCGTCCCTATCCTGCAGACAAAGGGGACCTCTGGGTTCAGTGCTGCAGC
CAGCTGCTGAGGTGTCCCACGTGGGCACTGCTCTGTGCCACTGGGAGGCGGGGCAGGAACGATCGGGGCAACACCTCCACTGCCGACCAATAAGCA
-1Transcriptional start
GGGCCGCTGCCTAGGGACCAGCGGTGATTGGTTGCGTCGGCCCCTCCCCACCCTGGATATAAGCGCCAAAGTCCAGAAGGTTTTAGCCAAAGCAACA
CCTCGCACTCCTCTCGAG
XhoI

Figure 3. The DNA sequences (5'-GGGACATTCC-3') of the wild-type promoter of the mouse cystathioine γ-lyase (CSE) gene, which was inserted into the pGL4.12 plasmid, and the DNA sequences (5'-ATCTCATTCC-3') of the mutant-type promoter of the mouse CSE gene.

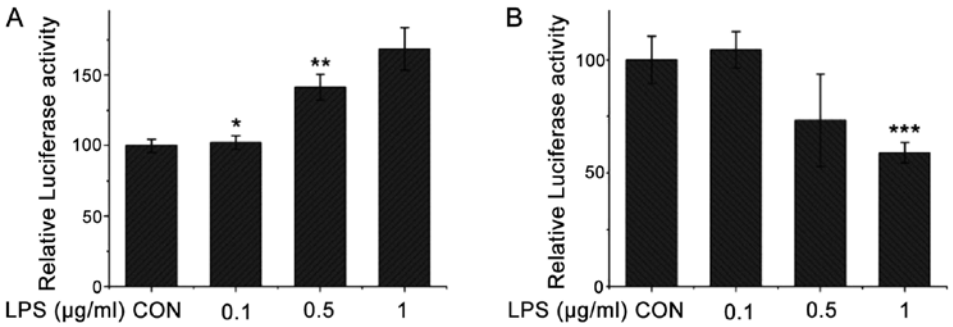

Figure 4. Effects of lipopolysaccharides (LPS) on the wild-type or mutant-type promoter activity of the cystathioine γ-lyase (CSE) gene in transfected COS-7 cells treated with LPS for 6 h. (A) The wild-type promoter of the CSE gene. (B) The mutant-type promoter of the CSE gene. After the transfected cells were treated with various concentrations of LPS for 6 h, the promoter activity was assayed. *P<0.01 vs. 0.5, **P<0.05 vs. control (CON) and 0.1, ***P<0.01 vs. CON and 0.5.

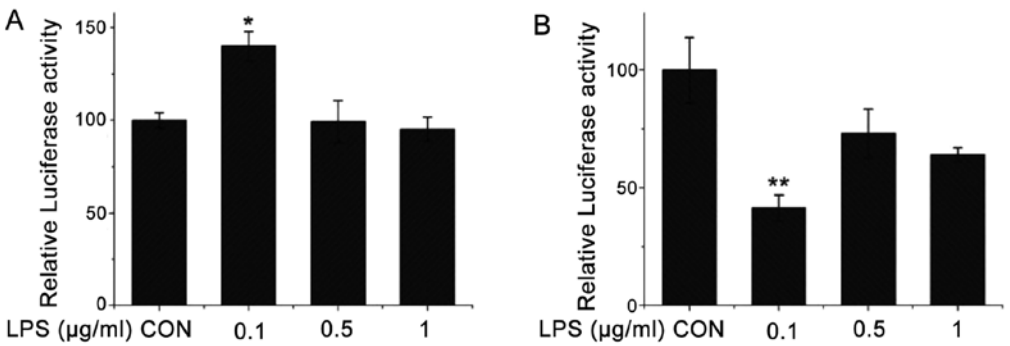

Figure 5. Effects of lipopolysaccharides (LPS) on the wild-type or mutant-type promoter activity of the cystathioine γ-lyase (CSE) gene in transfected HEK-293 cells treated with LPS for 6 h. (A) The wild-type promoter of the CSE gene. (B) The mutant-type promoter of the CSE gene. After the transfected cells were treated with various concentrations of LPS for 6 h, the promoter activity was assayed. *P<0.05 vs. control (CON), **P<0.05 vs. CON.

such as LPS (10,11,19-21,34). In the present study, our results revealed that the transcriptional and post-transcriptional regulation of the CSE gene is mediated by the binding site of NF-κB on the CSE promoter in mammalian cells treated with LPS.

Both COS-7 and HEK-293 cells (two mammalian kidney cell lines) have not only been successfully used in the study of the CSE/H_2S signaling pathway (35), but also in research on high DNA transfection efficiency in gene transfection experiment. Some typical mammalian cell lines, such as J774.1A and RAW264.7 cells, are frequently used in the study of the H_2S/CSE signaling pathway (12,36-39). Therefore, both the J774.1A and RAW264.7 cells are good models for testing the post-transcriptional regulation of the CSE gene by exogenous H_2S in mammalian cells, and the COS-7 and HEK-293 cells for testing the transcriptional activity.

Certain studies have indicated that H_2S plays an important role in inflammation, and LPS stimulates the expression of the CSE gene and the H_2S production rate. An increase in the plasma H_2S concentration, alongside augmented liver H_2S biosynthesis from exogenous cysteine, is also apparent in animals 4 h after an LPS injection (15,40). As previously demonstrated, the serine (Ser) 276 phosphorylation of p65 is increased by the LPS-mediated protein kinase A (PKA) activation in Raw264.7 murine macrophages (41). SPRC exerts beneficial effects by inhibiting Iκ B-α degradation and the phosphorylation of transcription factors associated with nuclear factor κ-B p65 activation induced by LPS (20). Our results demonstrated that LPS significantly increased the mRNA and protein expression levels of of the CSE gene in the J774.1A and RAW264.7 cells following treatment with LPS for 6 h. As LPS significantly affected the post-transcriptional regulation of the CSE gene, the binding site of the transcription factor, NF-κB, on the promoter of the CSE gene in mammalian cells may be involved in this regulation.

After the transfected HEK-293 and COS-7 cells were incubated with various concentrations of LPS for 6 h, the wild-type promoter activity of the CSE gene significantly increased. However, after the transfected HEK-293 and COS-7 cells were incubated with various concentrations of LPS for 6 h, the mutant-type promoter activity of the CSE gene significantly decreased. These results indicated that the GGGACATTCC DNA sequence on the promoter of the CSE gene was closely associated with the transcriptional regulation of the CSE gene in mammalian cells treated with LPS.

Taken together, our results suggest that the binding site of the transcription factor, NF-κB, on the CSE promoter is critical for LPS-induced CSE expression in mammalian cells. The data presnted in this study may significantly contribute to the further understanding of the regulatory mechanisms of the CSE gene in the inflammation process. Furthermore, these are important findings with potential clinical significance. However, the regulatory mechanisms involved in the effects of LPS on the transcriptional regulation of the CSE gene in mammalian cells via the NF-κB pathway require further investigation.

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