Reversible S-glutathionylation of human 6-pyruvoyl tetrahydropterin synthase protects its enzymatic activity

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6-Pyruvoyl tetrahydropterin synthase (PTS) catalyzes the second step of the de novo biosynthesis of tetrahydrobiopterin (BH4), an essential cofactor of aromatic amino acid hydroxylases, nitric oxide synthases, and nitric-oxide synthases. Neopterin derived from 7,8-dihydroneopterin is a critical enzyme for the de novo synthesis of tetrahydrobiopterin, an essential cofactor for aromatic amino acid hydroxylases and nitric-oxide synthases. Neopterin derived from 7,8-dihydroneopterin is secreted by monocytes/macrophages, and is a well-known biomarker for cellular immunity. Because PTS activity in the cell can be a determinant of neopterin production, here we used recombinant human PTS protein to investigate how its activity is regulated, especially depending on redox conditions. Human PTS has two cysteines: Cys-43 at the catalytic site and Cys-10 at the N terminus. PTS can be oxidized and consequently inactivated by H2O2 treatment, oxidized GSH, or S-nitrosoglutathione, and determining the oxidized modifications of PTS induced by each oxidant by MALDI–TOF MS, we show that PTS is S-glutathionylated in the presence of GSH and H2O2. S-Glutathionylation at Cys-43 protected PTS from H2O2-induced irreversible sulfenylation and sulfoxylation. We also found that PTS expressed in HeLa and THP-1 cells is reversibly modified under oxidative stress conditions. Our findings suggest that PTS activity and S-glutathionylation is regulated by the cellular redox environment and that reversible S-glutathionylation protects PTS against oxidative stress.

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2 The abbreviations used are: PTS, 6-pyruvoyl tetrahydropterin synthase; AsA, ascorbic acid; BH4, tetrahydrobiopterin; GCH, GTP cyclohydrolase I; Grx, glutaredoxin; GSNO, S-nitrosoglutathione; NH2TP, 7,8-dihydroneopterin triphosphate; NO, nitric oxide; PTSSG, S-glutathionylated PTS; ROS, reactive oxygen species; Trx, thioredoxin; DMEM, Dulbecco’s modified Eagle’s medium; MWCO, molecular mass cut-off.

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Redox regulation of PTS by S-glutathionylation

Here, we report that PTS is a target protein of \( S \)-glutathionylation at Cys-43, protecting this residue from irreversible oxidation. GSH can reduce the modification and activate PTS at physiological concentrations. Moreover, we show that the cysteine residue of PTS is modified in HeLa and THP-1 cells under oxidative stress conditions.

Results

Oxidative inactivation and reductive reactivation of PTS

First, we purified recombinant PTS protein using the buffer without DTT as a thiol-protective reagent and found that PTS enzymatic activity was increased by the treatment with DTT as previously reported (25), suggesting that the PTS protein could be subject to oxidative modification. To determine the modifications, we purified the reduced form of PTS in the presence of DTT, which was removed just before the analyses.

We first examined whether the enzymatic activity of PTS is regulated by the redox status. The reduced PTS protein was incubated with \( H_2O_2 \) and GSH plus \( H_2O_2 \) for 10 and 30 min, resulting in loss of PTS activity (Fig. 1A). PTS activity was decreased to about 50% even at 100 \( \mu M \) \( H_2O_2 \) with and without GSH. The inactivation was slightly enhanced at 0.32 and 1 \( \mu M \) \( H_2O_2 \), in the presence of GSH, implying \( S \)-glutathionylation. To reveal reversibility of the oxidative inactivation, excess DTT was added for the reduction of PTS after oxidation with 1 \( \mu M \) GSSG to promote \( S \)-glutathionylation, 1 \( \mu M \) \( H_2O_2 \) and GSH plus 1 \( \mu M \) \( H_2O_2 \) (Fig. 1B). GSSG- and GSH + \( H_2O_2 \)-oxidized PTS restored PTS activity, indicating reversible modifications of PTS. In contrast, PTS oxidized with \( H_2O_2 \) alone for 30 min showed no reversible activation, whereas PTS oxidized for 10 min demonstrated reductive restoration, suggesting that the oxidative states of the cysteine(s) proceeded from reversible sulfoxylation to irreversible sulfhydration and sulfonylation.

Redox states of the cysteine residue

To investigate whether the inactivation and activation that were dependent on the redox conditions were due to the cysteinylation modification, we employed the maleimide labeling method for the separation of the reduced and oxidized forms of the proteins. The thiol-modifying maleimide-PEG\(_{11}\)-biotin specifically binds to thiol and lowers protein mobility on SDS-PAGE, allowing titration of the number of oxidized cysteine residues. As shown in Fig. 1C, labeled and separated PTS, which has 2 cysteine residues, was found with three distinct mobilities, indicating that the number of oxidized cysteines can be determined by this method (the red, ox1, and ox2 bands correspond to 0, 1, and 2 oxidized cysteines, respectively). Approximately one-half of PTS was found in the ox1 band, corresponding to a single cysteine oxidized form, even in the presence of 10 \( \mu M \) DTT. The fully reduced form (red band) was decreased and shifted to the ox1 band by oxidizing treatments. Reduction by 5 \( \mu M \) DTT recovered the proportion of reduced and oxidized forms in GSSG- and GSH + \( H_2O_2 \)-oxidized PTS. In contrast, DTT had little effect on \( H_2O_2 \)-oxidized PTS. The proportions of fully reduced PTS and its reversibility corresponded to the redox-dependent changes in activity, indicating that modification of a cysteine regulates the enzymatic activity of PTS.

S-Nitrosylation of PTS

We next assessed the possibility of \( S \)-nitrosylation, the other reversible modification of cysteine residues. The incubation of PTS with 1 \( \mu M \) \( S \)-nitrosothiol (GSNO), which is a major physiological donor of NO, resulted in the inactivation of PTS. Because \( S \)-nitrosothiol is specifically reduced by ascorbic acid (AsA), GSNO-oxidized PTS was further incubated with AsA. Although DTT could fully restore PTS activity, AsA reduction showed partial recovery (Fig. 2A). In contrast to GSH- and \( H_2O_2 \)-induced oxidation (Fig. 1), two cysteine residues were oxidized by GSNO as shown in Fig. 2B (ox2 band). Consistent with the activity, DTT could recover the redox state in the reduced form. However, the ox2 band was reduced partially by reduction with AsA, whereas the fully reduced form (red band) was not observed. The difference between the reduction patterns with DTT and AsA suggested that GSNO treatment induced different oxidative modifications; one was AsA reducible, and the other was not. To further clarify the \( S \)-nitrosylation of PTS, the GSNO-oxidized PTS was analyzed by biotin switch assay (27). The ox1 bands were found in both apply and elution.
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Cys-43 is the main target of oxidative modification

PTS protein contains 2 cysteine residues at positions 10 and 43. Cys-43 is a catalytic residue and is therefore conserved in almost all PTSs (1, 28). To identify the critical cysteine(s) for redox regulation, we prepared Cys-to-Ala substitution mutants (C10A and C43A). Because the C43A mutant showed no activity due to the loss of a catalytic residue, we compared the changes in the activity of the C10A mutant protein with the WT protein. The C10A mutant showed the same inactivation and reactivation as the WT protein when oxidized by GSH/H$_2$O$_2$ and GSNO (Fig. 3). The reversible modification by GSH/H$_2$O$_2$ on the C10A mutant was shown by maleimide labeling. The GSNO-treated C10A mutant was not reduced by AsA, suggesting that the modification of Cys-43 was not S-nitrosylation. The results indicate that Cys-43 is a major target of the oxidative modifications and is therefore a regulatory cysteine. In the case of the C43A mutant, Cys-10 was modified by GSNO and partly reduced by AsA, indicating S-nitrosylation of Cys-10.

GSH is a potential intracellular reducer of PTS

GSH is the most abundant (~10 mM) low-molecular-weight thiol in the cell and has an important role for the maintenance of cellular redox states. The cellular redox state is also coupled with the Trx and Grx systems. We investigated the intracellular reducing system for S-glutathionylated PTS (PTS-SG). DTT
and GSH were used as sources of reducing equivalents for the Trx and Grx systems, respectively. If PTS is a target of Trx or Grx, the reduction of PTS must occur at lower concentrations of reducing equivalents in the presence of Trx or Grx. However, no enhancement of the restored activity was observed with any concentration of DTT in the presence of Trx, suggesting that Trx is not a reducer of PTS-SG (Fig. 5, left panel). Although Grx1 and Grx2c slightly enhanced the reactivation of PTS-SG, GSH alone could restore sufficient PTS activity (Fig. 5, right panel), suggesting that the redox state of PTS depends on the intracellular GSH redox potential.

Redox states of PTS in HeLa and THP-1 cells

Finally, we investigated whether PTS altered the redox states depending on the cellular environment. Because the level of endogenous PTS was too low to be detected in HeLa and THP-1 cells, we transfected a FLAG-PTS-expressing plasmid into cells. THP-1 cells were differentiated into macrophage-like cells with phorbol 12-myristate 13-acetate. The oxidative stress condition was generated by the addition of 1 mM H$_2$O$_2$ to the culture medium. The redox states were then detected by Western blotting following the maleimide labeling. Approximately 20 and 40% of FLAG-PTS was found in the ox1 band in HeLa and THP-1 cells without oxidative treatment, respectively (Fig. 6, B and C), suggesting that one of the cysteine residues (probably Cys-43) is highly reactive and subject to the oxidative modification. The addition of H$_2$O$_2$ led PTS to become more oxidized within 10 min. After the addition of 1 mM DTT to the medium or the removal of H$_2$O$_2$ after the oxidative stress, the redox states of PTS tended to be reduced slightly (Fig. 6), indicating that the reversible modification of the PTS protein occurred in the cells.

Discussion

The redox state of a protein strongly impacts its physiological role by changing the stability, function, and enzymatic activity of the protein, depending on the cellular conditions. Although the requirement of thiol reductants such as dithioerythritol, 2-mercaptoethanol, and DTT for in vitro PTS activity and inhibition by alkylation reagents are known, the redox regulation of
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PTS remains unclear (25, 26). Here, we revealed that S-glutathionylation of PTS reversibly regulates its enzymatic activity.

All of the oxidants that we employed inactivated PTS through modifications of cysteine residues (Figs. 1 and 2). Oxidation with H₂O₂ showed a time-dependent loss of the reversible activation via the irreversible sulfenylation and sulfonylation (Fig. 4). In the presence of GSH during H₂O₂ oxidation (GSH + H₂O₂), PTS was protected from this irreversible modification by S-glutathionylation (Figs. 1 and 4). Therefore, S-glutathionylation of PTS is suggested to be a protective mechanism against irreversible oxidation. This finding further implies that PTS protein is maintained as a recoverable form to be able to respond more rapidly to environmental changes than protein expression.

The regulatory and protective S-glutathionylation occurred at Cys-43 (Figs. 3 and 4). Because the Cys-43 of PTS is considered to be ionized and attract a proton from the substrate during the catalytic reaction (28, 30), Cys-43 must be acidic and have a low pKᵈ compared with free cysteine. The ionized (deprotonated) Cys is both a good catalyst for enzymatic reaction and a good target for oxidative modifications. In agreement with this, approximately one-half of WT and C10A PTS protein, expressed in Escherichia coli and purified with DTT, was found in the irreversibly oxidized form.

In addition to catalytic, and thus conserved Cys-43, human and ape PTS have one additional cysteine residue at position 10. This cysteine was specifically modified with GSNO and confirmed as an S-nitrosylation (Figs. 2–4). It is difficult to distinguish how the S-nitrosylation at Cys-10 contributes to the regulation of PTS activity, because GSNO treatment simultaneously induced S-glutathionylation of Cys-43. Protein–protein interactions, stability, and sensitivity to oxidative modification at Cys-43 of PTS might be regulated by the modification at Cys-10 in the cell. Considering the requirement of BH₄ for NO synthesis, S-nitrosylation could be involved in a feedback mechanism to prevent the excess production of BH₄ and/or NO. Further investigations will elucidate the significance of the S-nitrosylation of human and ape Cys-10.

GSH, Trx, and Grx are major antioxidant systems in the cells and maintain the protein thiol as a reduced form. Although cytosolic Grxl and Grx2c slightly enhanced the reduction of PTS-SG, the activation of PTS-SG was mainly dependent on GSH (Fig. 5). Most GSH is found in the cytosol at 1–10 mM, and the ratio of GSH to GSSG is 30–100:1 in unstressed cells (31, 32). When cells were exposed to oxidative stress, GSH was immediately oxidized (32, 33). Therefore, the observed H₂O₂-induced oxidation of FLAG-PTS must be caused by the decrease in the GSH/GSSG ratio and subsequent binding of GSH, as shown in Fig. 6. The slight recovery of the redox states of FLAG-PTS by the removal of H₂O₂ from the culture medium might be due to enhanced production of NADPH by glucose-6-phosphate dehydrogenase, and subsequent recovery of intracellular GSH potential by degradation of H₂O₂ and reduction of GSSG into GSH by thioredoxin and GSH reductase systems (34). Furthermore, FLAG-PTS in THP-1 cells were found as more oxidized states than in HeLa cells after each treatment. This might reflect the more oxidizing conditions such as protein carbonylation (35), methionine sulfoxidation (36), and GSH potential (33) by stimulation with phorbol 12-myristate 13-acetate in THP-1 cells (Fig. 6).

Activated phagocytic cells generate ROS mainly by NADPH oxidase, xanthine oxidase, and myeloperoxidase as a part of an inflammatory response. The generated ROS not only changes phagocytic cells to the oxidative condition but are also released into the extracellular environment, causing damage to the cells around inflammation sites (37). At the same time, macrophages synthesize and secrete neopterin and 7,8-dihydroneopterin at the expense of BH₄ synthesis, although the physiological role of pteridines is not fully understood. The significant increase in GCH expression up to 50-fold in macrophages (13, 38) is thought to be a cause of neopterin secretion. Along with this, the inactive S-glutathionylation of PTS must be involved in the BH₄ and neopterin synthetic pathways. A recent study revealed that lipopolysaccharide injection into the abdominal cavity induced a rapid increase in neopterin in the serum and hippocampus within 15 min (8), suggesting the post-translational regulation of neopterin production and secretion prior to the increased expression of GCH. Considering the immediate response of ROS production from phagocytic cells (39–41), the redox states of PTS must be affected by S-glutathionylation and thus regulate the amount and/or ratio of BH₄ and neopterin for anti- or pro-inflammatory effects to macrophages and surrounding cells at the time of inflammation.

In conclusion, PTS was a target of reversible S-glutathionylation at Cys-43 to prevent irreversible modification with H₂O₂. S-Glutathionylation and deglutathionylation are dependent on the oxidized and reduced forms of GSH, suggesting that the intracellular GSH redox condition is the determinant of the extent of modification and thus the activity of PTS. Further investigation of the association of the intracellular redox states with the synthesis of BH₄ and neopterin should elucidate the role of redox regulation of PTS.

Experimental procedures

Proteins

A gene encoding E. coli GCH (EcGCH) was amplified with a Hisᵩ tag at the N terminus and cloned into pET23a expression vector (Novagen, Madison, WI). E. coli strain BL21 (DE3) harboring the obtained plasmid was cultured at 37 °C in LB medium supplemented with 50 µg/ml of ampicillin. When A₆₀₀ reached 0.4, protein expression was induced by the addition of 0.4 mM isopropyl β-D-1-thiogalactopyranoside followed by further culture at 25 °C overnight. EcGCH protein was purified using a HisTrap HP column (GE Healthcare, UK) in a solution containing 25 mM Tris-HCl (pH 7.5), 100 mM NaCl. The column was washed with 100 mM imidazole, and the protein was eluted with 200 mM imidazole. The obtained protein was dialyzed against 25 mM Tris-HCl (pH 7.5), 100 mM NaCl, concentrated with Amicon Ultra-15 (molecular weight cut-off (MWCO) 30 kDa, Millipore, Bedford, MA), and stored at −80 °C in the presence of 15% (v/v) glycerol.

Human PTS gene was fused with Hisᵩ₆ tag at the N terminus and cloned into pET16b (Novagen). Site-directed mutagenesis for cysteine substitution was performed using the PrimeSTAR Mutagenesis Basal Kit (TAKARA, Kyoto, Japan) according to...
the manufacturer’s instructions. Based on the numbering of the amino acids according to UniProt, His10 tag and linker upstream of the first methionine of PTS are excluded from the numbering. WT and mutated PTS proteins were expressed in the same way as EcGCH. The protein was purified using HisTrap HP column in a solution containing 25 mM Tris-HCl (pH 7.5), 100 mM NaCl, and 1 mM DTT twice, concentrated with Amicon Ultra-15 (MWCO 30 kDa), and stored at −80 °C in the presence of 15% (v/v) glycerol. DTT was removed with NAP-5 desalting column (GE Healthcare) before analyses. To prepare PTS-SG, after the elution in the absence of DTT from the HisTrap column, PTS was incubated with 1 mM GSSG for 30 min at 37 °C. The PTS-SG was separated from GSSG and concentrated with Amicon Ultra-15 (MWCO 30 kDa).

Human Trx and human cytosolic Grxs (Grx1 and Grx2c) were fused with His6 tag at the C terminus and cloned into pET23a. The sequence of Grx2c is in accordance with a previous article (42). The expressions and purifications were performed in the same way as EcGCH.

All purification procedures were performed at 4 °C. Protein concentration was determined by a Bradford assay using bovine γ-globulin as a standard.

**Oxidation and reduction treatments**

PTS protein was incubated with indicated concentrations of H$_2$O$_2$, GSSG, and H$_2$O$_2$ in the presence of 0.5 mM GSH (GSH + H$_2$O$_2$), and GSNO at 37 °C for 10 and 30 min. After the incubation, 5 mM DTT or 5 mM AsA were added to the oxidized protein solutions and incubated for 10 min at 37 °C. The activity and redox state of the protein were assessed.

**Measurements of PTS activity**

PTS was incubated in a reaction mixture containing 25 mM Tris-HCl (pH 7.5), 100 mM NaCl, 8 mM MgCl$_2$, 58 μM NH$_2$TP, and 0.5–0.7 μM PTS at 37 °C for 30 min, and the reaction was quenched by the addition of 1 M perchloric acid and 75 mM EDTA. The resulting 6-pyruvoyl tetrahydropterin was converted into a stable pterin by the oxidation with 1% (w/v) I$_2$, 2% (w/v) KI in 1 M HCl for 30 min at room temperature. Excess iodine was removed by the addition of 2% (w/v) AsA for 10 min at 4 °C. Insoluble protein was removed by centrifugation at 20,000 × g for 10 min, and the pterin contents were analyzed by HPLC with fluorescence detection (43). All procedures were performed in the dark until AsA was added.

NH$_2$TP was prepared by incubating EcGCH with 1 mM GTP, 25 mM Tris-HCl (pH 7.5), and 100 mM NaCl for 1 h in the dark at 37 °C. EcGCH protein was removed with Amicon Ultra-15 (MWCO 10 kDa), and the flow-through was stored at −80 °C until use. The NH$_2$TP concentration was determined by conversion to neopterin by alkaline phosphatase (Sigma) and iodine oxidation, and quantified by HPLC with fluorescence detection.

**Visualization of the redox states of PTS**

The redox states of cysteines of PTS protein were determined by the shift of electrophoretic mobility by labeling with maleimide compound (44). After the incubation with oxidants and reductants, PTS was precipitated with 5% TCA, labeled with maleimide-PEG$_{11}$-biotin (Thermo Fisher Scientific, Waltham, MA), and resolved by nonreducing SDS-PAGE.

**Biotin switch assay**

S-Nitrosylation of PTS was determined based on a biotin switch assay with slight modifications (27). Reduced thiols of GSNO-oxidized PTS were blocked with 10 mM N-ethylmaleimide in the presence of 1% SDS for 30 min at room temperature.

**Cell culture and transfection**

HeLa cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (BioWest, Nuillé, France) at 37 °C in a 5% CO$_2$ incubator. FLAG-PTS protein was expressed in the cells using pFLAG-CMV2 vector (Sigma) and CalPhos mammalian transfection kit (Clontech, Mountain View, CA) as a transfection reagent. THP-1 cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1× antibiotic-antimycotic (Thermo Fisher Scientific) at 37 °C in a 5% CO$_2$ incubator. Flag-PTS protein was expressed in the cells using pFLAG-CMV2 vector (Sigma) and CalPhos mammalian transfection kit (Clontech, Mountain View, CA) as a transfection reagent. THP-1 cells were transfected with Flag-PTS plasmid and X-tremeGENE HP DNA (Roche, Basel, Switzerland).

**Western blot analysis**

The HeLa and THP-1 cells were exposed to 1 mM H$_2$O$_2$ containing medium for 10 min. The medium was replaced with fresh medium in the presence or absence of 1 mM DTT, or further incubated for 10 min (Fig. 6A). The cells were washed with ice-cold PBS three times, and ice-cold 10% TCA was added directly for an extraction and fixation of the redox states of proteins in the cells. The denatured proteins were collected by centrifugation and washed with ice-cold acetone. The obtained proteins were dissolved and labeled in the nonreducing sample buffer containing 3 mM maleimide-PEG$_{11}$-biotin, and sub-
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...jected to Western blot analysis according to a conventional procedure. FLAG-PTS protein was detected using a primary anti-FLAG antibody (Clontech) and an anti-mouse secondary antibody (GE Healthcare). Band intensities from at least three individual experiments were determined using ImageJ.

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References
1. Thöny, B., Auerbach, G., and Blau, N. (2000) Tetrahydrobiopterin biosynthesis, regeneration and functions. Biochem. J. 347, 1–16 CrossRef Medline
2. Blau, N., Schoend, G., and Curtius, H. C. (1989) Biosynthesis and significance of neopterin in the immune system. Eur. J. Cancer Clin. Oncol. 25, 603–605 CrossRef Medline
3. Werner, E. R., Blau, N., and Thöny, B. (2011) Tetrahydrobiopterin: biochemistry and pathophysiology. Biochem. J. 438, 397–414 CrossRef Medline
4. Weiss, G., Fuchs, D., Hansen, A., Reibnegger, G., Werner, E. R., Werner-Felmayer, G., Semenitz, E., Dierich, M. P., and Wacht, H. (1993) Neopterin modulates toxicity mediated by reactive oxygen and chloride species. FEBS Lett. 321, 89–92 CrossRef Medline
5. Cirillo, P., Pacileo, M., DE Rosa, S. Calabrò, P., Gargiulo, A., Angi, V., Granato-Corigliano, F., Fiorentino, I., Prevete, N., DE Palm, R., Mauro, C., Leonardi, A., and Chiaromel, M. (2006) Neopterin induces pro-atherothrombotic phenotype in human coronary endothelial cells. J. Thromb. Haemost. 4, 2248–2255 CrossRef Medline
6. Hoffmann, G., Schobersberger, W., Fred, S., Pelzer, L., Fandrey, J., Wacht, H., Fuchs, D., and Grote, J. (1996) Neopterin activates transcription factor nuclear factor-κB in vascular smooth muscle cells. FEBS Lett. 391, 181–184 CrossRef Medline
7. Schobersberger, W., Hoffmann, G., Hobisch-Hagen, P., Bock, G., Volk, H., Baier-Bitterlich, G., Wirleitner, B., Wacht, H., and Fuchs, D. (1996) Neopterin and 7,8-dihydropterin induce apoptosis in the rat alveolar epithelial cell line L2. FEBS Lett. 397, 263–268 CrossRef Medline
8. de Paula Martins, R., Ghisoni, K., Lim, C. K., Aguia, A. S., Jr, Guillem, G. J., and Latin, A. (2018) Neopterin pre conditioning prevents inflammasome activation in mammalian astrocytes. Free Radic. Biol. Med. 115, 371–382 CrossRef Medline
9. Ghisoni, K., Aguia, A. S., Jr, de Oliveira, P. A., Matheus, F. C., Gabach, L., Perez, M., Carlini, V. P., Barbeto, L., Mongeau, R., Lanfumey, L., Prediger, R. D., and Latin, A. (2016) Neopterin acts as an endogenous cognitive enhancer. Brain Behav. Immun. 56, 156–164 CrossRef
10. Duggan, S., Rait, C., Platt, A., and Giese, S. (2002) Protein and thiol oxidation in cells exposed to peroxyl radicals is inhibited by the macrophage synthesised pterin 7,8-dihydropterin. Biochim. Biophys. Acta 1591, 139–145 CrossRef Medline
11. Hoffmann, G., Wirleitner, B., and Fuchs, D. (2003) Potential role of immune system activation-associated production of neopterin derivatives in humans. Inflamm. Res. 52, 313–321 CrossRef
12. Fuchs, D., Hansen, A., Reibnegger, G., Werner, E. R., Dierich, M. P., and Wacht, H. (1988) Neopterin as a marker for activated cell-mediated immunity: application in HIV infection. Immunol. Today 9, 150–155 CrossRef Medline
13. Werner, E. R., Werner-Felmayer, G., Fuchs, D., Hansen, A., Reibnegger, G., Yim, J., Pfleiderer, W., and Wacht, H. (1990) Tetrahydrobiopterin biosynthetic activities in human macrophages, fibroblasts, THP-1, and T 24 cells: GTP-cyclohydrolase I is stimulated by interferon-γ and 6- pyruvyl tetrahydropterin synthase and siapteptin reductase are constitutively present. J. Biol. Chem. 265, 3189–3192 CrossRef Medline
14. Nakamura, H., Nakamura, K., and Yodoi, J. (1997) Redox regulation of cellular activation. Annu. Rev. Immunol. 15, 351–369 CrossRef Medline
15. Stamler, J. S., and Hadsland, A. (1998) Oxidative modifications in nitrosative stress. Nat. Struct. Biol. 5, 247–249 CrossRef Medline
16. Reddie, K. G., and Carroll, K. S. (2008) Expanding the functional diversity of proteins through cysteine oxidation. Curr. Opin. Chem. Biol. 12, 746–754 CrossRef Medline
17. Mannervik, B., and Axelsson, K. (1980) Role of cytoplasmic thioltrans- ferase in cellular regulation by thiol-disulphide interchange. Biochem. J. 190, 125–130 CrossRef Medline
18. Klatt, P., and Lamas, S. (2000) Regulation of protein function by S-gluthathionylation in response to oxidative and nitrosative stress. Eur. J. Biochem. 267, 4928–4944 CrossRef Medline
19. Dalle-Donne, I., Rossi, R., Colombo, G., Giustarini, D., and Milzani, A. (2009) Protein S-glutathionylation: a regulatory device from bacteria to humans. Trends Biochem. Sci. 34, 85–96 CrossRef Medline
20. Hess, D. T., Matsumoto, A., Kim, S. O., Marshall, H. E., and Stamler, J. S. (2005) Protein S-nitrosylation: purview and parameters. Nat. Rev. Mol. Cell Biol. 6, 150–166 CrossRef Medline
21. Claiborne, A., Yeh, J. L., Mallet, T. C., Laub, J., Cran, E. J., 3rd, Charrier, V., and Parsonage, D. (1999) Protein-sulfenic acids: diverse roles for an unlikely player in enzyme catalysis and redox regulation. Biochemistry 38, 15407–15416 CrossRef Medline
22. Dickinson, D. A., and Forman, H. J. (2002) Cellular glutathione and thiols metabolism. Biochem. Pharmacol. 64, 1019–1026 CrossRef Medline
23. Kalinina, E. V., Chernov, N. N., and Saprin, A. N. (2008) Involvement of thioperoxo-, peroxi- and glutaredoxins in cellular redox-dependent processes. Biochem. (Moscow). 73, 1493–1510 CrossRef Medline
24. Subramani, J., Kundumann-Sridharan, V., Higler, R. H., Owens, C., and Das, K. C. (2016) Thioredoxin uses a GSH-independent route to deglutathionylate endothelial nitric-oxide synthase and protect against myocardial infarction. J. Biol. Chem. 291, 23374–23389 CrossRef Medline
25. Takikawa, S., Curtius, H. C., Redweik, U., Leimbacher, W., and Ghisla, S. (1986) Biosynthesis of tetrahydrobiopterin: purification and characterization of 6-pyruvoyl-tetrahydropterin synthase from human liver. Eur. J. Biochem. 161, 295–302 CrossRef Medline
26. Bürgisser, D. M., Thöny, B., Redweik, U., Hunziker, P., Heizmann, C. W., and Blau, N. (1994) Expression and characterization of recombinant human and rat liver 6-pyruvoyl tetrahydropterin synthase: modified cysteine residues inhibit the enzyme activity. Eur. J. Biochem. 219, 497–502 CrossRef Medline
27. Jaffrey, S. R., Erdjument-Bromage, H., Ferris, C. D., Tempst, P., and Snyder, S. H. (2001) Protein S-nitrosylation: a physiological signal for neuronal nitric oxide. Nat. Cell Biol. 3, 193–197 CrossRef Medline
28. Ploom, T., Thöny, B., Yin, J., Lee, S., Nar, H., Leimbacher, W., Richardson, J., Huber, R., and Auerbach, G. (1999) Crystallographic and kinetic investigations on the mechanism of 6-pyruvoyl tetrahydropterin synthase. J. Mol. Biol. 286, 851–860 CrossRef Medline
29. Kaneko, R., and Wada, Y. (2003) Decomposition of protein nitrosothiol matrix-assisted laser desorption/ionization and electrospray ionization mass spectrometry. J. Mass Spectrom. 38, 526–530 CrossRef Medline
30. Bürgisser, D. M., Thöny, B., Redweik, U., Hess, D., Heizmann, C. W., Huber, R., and Nar, H. (1995) 6-Pyruvoyl tetrahydropterin synthase, an enzyme with a novel type of active site involving both zinc binding and an intersubunit catalytic triad motif; site-directed mutagenesis of the proposed active center, characterization of the metal binding site and modeling of substrate binding. J. Mol. Biol. 253, 358–369 CrossRef Medline
31. Schafer, F. Q., and Buettner, G. R. (2001) Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic. Biol. Med. 30, 1191–1212 CrossRef Medline
32. Bhaskar, A., Munshi, M., Khan, S. Z., Fatima, S., Arya, R., Jameel, S., and Singh, A. (2015) Measuring glutathione redox potential of HIV-1-infected macrophages. J. Biol. Chem. 290, 1020–1038 CrossRef Medline
33. Dooley, C. T., Dore, T. M., Hanson, G. T., Jackson, W. C., Remington, S. I., and Tsien, R. Y. (2004) Imaging dynamic redox changes in mammalian cells with green fluorescent protein indicators. *J. Biol. Chem.* **279**, 22284–22293 CrossRef Medline

34. Tao, R., Zhao, Y., Chu, H., Wang, A., Zhu, J., Chen, X., Zou, Y., Shi, M., Liu, R., Su, N., Du, J., Zhou, H. M., Zhu, L., Qian, X., Liu, H., Loscalzo, J., and Yang, Y. (2017) Genetically encoded fluorescent sensors reveal dynamic regulation of NADPH metabolism. *Nat. Methods* **14**, 720–728 CrossRef Medline

35. Gieche, J., Mehlhase, J., Licht, A., Zacke, T., Sitte, N., and Grune, T. (2001) Protein oxidation and proteolysis in RAW264.7 macrophages: effects of PMA activation. *Biochim. Biophys. Acta* **1538**, 321–328 CrossRef Medline

36. Beck-Speier, I., Leuschel, L., Luippold, G., and Maier, K. L. (1988) Proteins released from stimulated neutrophils contain very high levels of oxidized methionine. *FEBS Lett.* **227**, 1–4 CrossRef Medline

37. Mittal, M., Siddiqui, M. R., Tran, K., Reddy, S. P., and Malik, A. B. (2014) Reactive oxygen species in inflammation and tissue injury. *Antioxid. Redox. Signal.* **20**, 1126–1167 CrossRef Medline

38. Liang, Y., Inagaki, H., Hao, Q., Sakamoto, M., Ohye, T., Suzuki, T., and Ichinose, H. (2013) Identification of an enhancer region for immune activation in the human GTP cyclohydrolase I gene. *Biochem. Biophys. Res. Commun.* **442**, 72–78 CrossRef Medline

39. Khan, M. A., Farahvash, A., Douda, D. N., Licht, J. C., Grasemann, H., Sweezey, N., and Palaniyar, N. (2017) JNK activation turns on LPS- and Gram-negative bacteria-induced NADPH oxidase-dependent suicidal NETosis. *Sci. Rep.* **7**, 3409 CrossRef Medline

40. Hazeldine, J., Harris, P., Chapple, I. L., Grant, M., Greenwood, H., Livesey, A., Sapey, E., and Lord, J. M. (2014) Impaired neutrophil extracellular trap formation: a novel defect in the innate immune system of aged individuals. *Aging Cell* **13**, 690–698 CrossRef Medline

41. Deng, J., Wang, X., Qian, F., Vogel, S., Xiao, L., Ranjan, R., Park, H., Karpurapu, M., Ye, R. D., Park, G. Y., and Christman, J. W. (2012) Protective role of reactive oxygen species in endotoxin-induced lung inflammation through modulation of IL-10 expression. *J. Immunol.* **188**, 5734–5740 CrossRef Medline

42. Lönn, M. E., Hudemann, C., Berndt, C., Cherkasov, V., Capani, F., Holmgren, A., and Lillig, C. H. (2008) Expression pattern of human glutaredoxin 2 isoforms: identification and characterization of two testis/cancer cell-specific isoforms. *Antioxid. Redox Signal.* **10**, 547–557 CrossRef Medline

43. Tani, Y., and Ohno, T. (1993) Analysis of 6R- and 6S-tetrahydriopterin and other pterins by reversed-phase ion-pair liquid-chromatography with fluorimetric detection by post-column sodium nitrite oxidation. *J. Chromatogr.* **617**, 249–255 CrossRef Medline

44. Kobayashi, T., Kishigami, S., Sone, M., Inokuchi, H., Mogi, T., and Ito, K. (1997) Respiratory chain is required to maintain oxidized states of the DsbA-DsbB disulfide bond formation system in aerobically growing *Escherichia coli* cells. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 11857–11862 CrossRef Medline

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