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Review

Gas biology: Tiny molecules controlling metabolic systems☆

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ABSTRACT

It has been recognized that gaseous molecules and their signaling cascades play a vital role in alterations of metabolic systems in physiologic and pathologic conditions. Contrary to this awareness, detailed mechanisms whereby gases exert their actions, in particular *in vivo*, have been unclear because of several reasons. Gaseous signaling involves diverse reactions with metal centers of metalloproteins and thiol modification of cysteine residues of proteins. Both the multiplicity of gas targets and the technical limitations in accessing local gas concentrations make dissection of exact actions of any gas mediator a challenge. However, a series of advanced technologies now offer ways to explore gas-responsive regulatory processes *in vivo*. Imaging mass spectrometry combined with quantitative metabolomics by capillary-electrophoresis/mass spectrometry reveals spatio-temporal profiles of many metabolites. Comparing the metabolic footprinting of murine samples with a targeted deletion of a specific gas-producing enzyme makes it possible to determine sites of actions of the gas. In this review, we intend to elaborate on the ideas how small gaseous molecules interact with metabolic systems to control organ functions such as cerebral vascular tone and energy metabolism *in vivo*.

1. Gas signaling *in vivo*: why difficult to study?

Gas-transducing signaling involves many regulatory roles including neurotransduction, transcription, vascular resistance, and metabolism, and has attracted much attention. However, investigation of gas-transducing signaling is a challenge. Criteria that must be fulfilled for a standard signaling such as hormonal signaling include: (i) specific receptor triggering the change of functions of target molecules; (ii) transducing the initial change to downstream effectors; and (iii) reversibility allowing the cascade to be controlled. Unlike hormonal signaling where specific targets are identified, mechanisms that mediate gas signaling are relatively unsolved. There are reasons why it is difficult to characterize the molecular nature involving each of the three steps above. First, gas has an ability to coordinate with metal centers of prosthetic groups of proteins (*e.g.* heme, and transition-metal center other than iron), but also some gases (*e.g.* NO and H_2S) exert their actions by covalently modifying the sulfhydryl group of cysteines in target proteins, processes designated as *S*-nitrosylation by NO and *S*-sulfhydration by H_2S (Mustafa et al., 2009). Thus, gas actions are pleiotropic in nature (Fig. 1). Second, their small-size and neutral-charge provide gases with the ability to permeate through cell membrane and inside the macromolecular structure, allowing gases to contact rapidly with various functional groups of different molecules. Third, the redox state of a metal center modulates the affinity of the binding of a gas ligand to a metal atom. Since the alteration of redox states is a hallmark of disease conditions such as ischemia and metabolic disorders, it needs to be taken into account. However, it adds a further challenge to elucidation of gas-signaling mechanisms *in vivo*. See review (Hishiki et al., 2012; Kajimura et al., 2010) for more comprehensive account on this subject.

Recent biochemical investigations of purified enzymes to correlate molecular structure of a heme binding pocket with functional relation (*e.g.* catalytic reaction) have found many answers for gas-sensing and gas-transduction mechanisms on the specific protein *in vitro*. How can we make a bridge between findings *in vitro* and solving problems *in vivo*? One approach could be to examine not only expressions of enzymes but also the abundance of substrates and cofactors of a gas-producing enzyme that is more likely to determine the rate of gas formation in the tissues with

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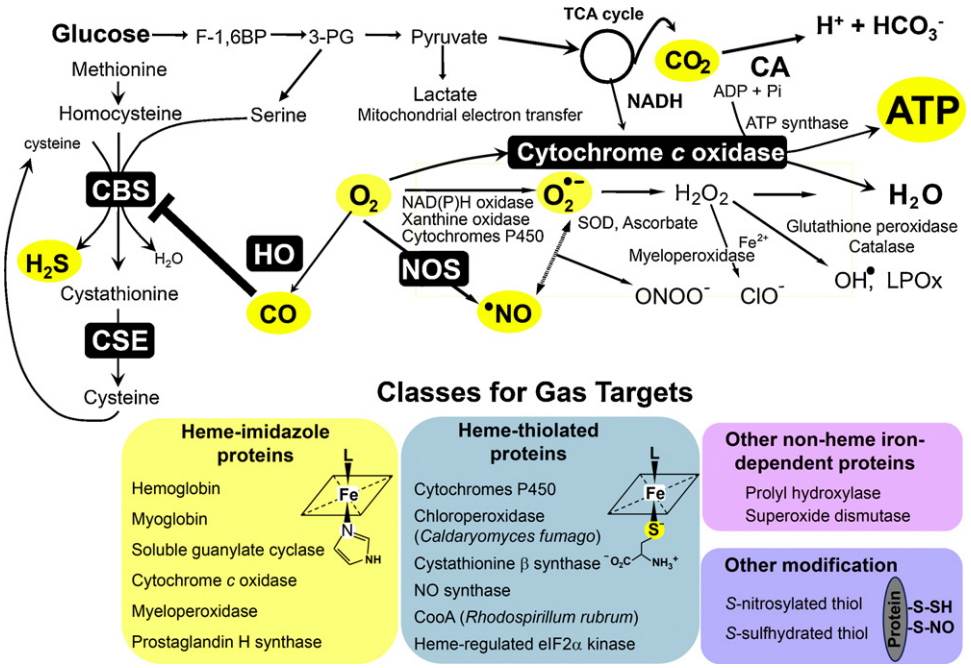

Fig. 1. Simplified metabolic links among cellular energetics, gas synthesis and gas targets. Molecular oxygen functions primarily as a terminal acceptor of electrons of the electron transport chain in the mitochondria. Most of the oxygen is reduced to generate water through the reaction of cytochrome *c* oxidase. However, a small fraction of oxygen is used to generate compounds including CO, NO, and reactive oxygen species. Gases exert biologic activity through interactions with macromolecules. These interactions involve; (i) the coordinate bonding of a gas ligand to prosthetic metal complexes in their receptor proteins leading to a wide range of interactions, depending on the strength of the bond formed, and (ii) their binding to the critical region for the protein function such as the cysteine thiol group. The interactions are further modulated by the redox state of the metal before ligand binding which is not shown in this scheme. Here gas targets are classified by four groups depending on the nature of the bond. L, ligand; CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase; HO, heme oxygenase; NOS, nitric oxide synthase; CA, carbonic anhydrase.
Adapted by permission from Suematsu et al. (2003).

spatial and temporal resolution. Imaging mass spectrometry combined with quantitative metabolomics can satisfy these criteria as it provides spatio-temporal profiles of many metabolites simultaneously. Comparing the metabolic footprinting from an animal model with a targeted deletion of a specific gas-producing enzyme induces logic to identify the sites of actions of the gas. This article aims to outline how these technical advances can help solve critical issues laid out above, with focus on physiological significance of coordinate actions of CO and H_2S and their relation to O_2 metabolism *in vivo* proposed in the recent literature.

2. Interaction of CO and H_2S: cystathionine β-synthase as a specific CO sensor

Recent literature indicates that heme oxygenase (HO)/CO and cystathionine β-synthase (CBS)/H_2S systems interface (Morikawa et al., 2012). What is a molecular mechanism of this interaction? CO derived from HO can regulate the activity of CBS, an H_2S-producing enzyme, which has been known as a CO-specific sensor *in vitro* (Taoka et al., 2002, 1999). However, it is only within several years that CO was found to control the function of CBS *in vivo* (Shintani et al., 2009). We start this section by providing a brief summary of structural characteristics of purified CBS *in vitro*. Then we describe how metabolomic approaches can be used to examine altered functions of this enzyme by CO.

2.1. Fundamental mechanisms of CO sensing by CBS in vitro

Many heme enzymes including cytochromes P450 were once considered putative CO-sensitive signal transducers. However, the ferrous heme of these enzymes has been found sensitive to both CO and NO, ruling them out as CO-specific sensors. By contrast, CBS remained a strong candidate for a CO-specific sensor. CBS was discovered as an interesting soluble heme protein that showed an absorption peak at 448-nm on its reduction without addition of CO (Kim and Deal, 1976). Since the 450-nm absorption peak of the CO-ligated P450 in the reduced state is the hallmark of cytochrome P450, it was named H450 as a 'pseudo-cytochrome P450′ (Omura, 2005). Subsequently, Omura et al. (1984) identified that the axial ligand at the 5th coordinate position is a thiolated anion, and the 6th position is occupied by histidine, confirming the heme-thiolated nature of this protein (Fig. 2A and B). Authors showed that adding CO causes the spectral shift of the absorption peak from 448 to ~420 nm, indicating that the thiolate-anion ligand of the heme is replaced with CO to produce a spectrum similar to the CO-ligated heme–imidazole protein (Omura et al., 1984). This is the first study suggesting the gas-sensing function of this enzyme.

Why is the heme-thiolated form useful to function as a sensor? This effect might derive from a weak, reversible binding of CO to the heme. Coordination of thiolate anion to heme is weaker than that of the imidazol group, particularly when the iron atom of the heme is in the ferrous state. This labile nature of the thiolate-anion ligand in the heme–thiolate proteins explains the functions of the protein as a sensor for detecting CO. In such a case, binding of CO to the heme results in the displacement of the thiolate-anion ligand and induces a conformational change of the protein moiety, which is transduced to a change in its enzyme activity (Fig. 2B). See review by Omura (2005) for more comprehensive account on gas-sensing mechanisms by heme-thiolated proteins.

2.2. CBS inhibition by CO in vitro

CBS is unique in that it is the only known pyridoxal phosphate (PLP)-dependent enzyme that possesses prosthetic heme