J. Biochem. 2011;149(4):357–359 doi:10.1093/jb/mvr018

JB THE JOURNAL OF BIOCHEMISTRY

JB Commentary

Production of H_2S by 3-mercaptopyruvate sulphurtransferase

Received December 25, 2010; accepted January 24, 2011; published online February 15, 2011

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Hydrogen sulfide (H_2S) has been established as the third gaseous signaling molecule following nitric oxide and carbon monoxide and participates in a variety of cellular functions such as modulation of neuronal transmission, endothelium-dependent vasorelaxation, stimulation of angiogenesis and regulation of insulin release. Although cystathionine β-synthase and cystathionine γ-lyase have been regarded as the main producers of H_2S in many tissues including brain, liver and kidney, Kimura and his colleagues have recently communicated that 3-mercaptopyruvate sulphurtransferase coupled with cysteine (aspartate) aminotransferase is responsible for the production of H_2S in the vascular endothelium of the thoracic aorta [Shibuya *et al.* (2009) *J. Biochem.* 146, 623–626]. This finding provides a new insight into the production of the physiologically important signaling molecule.

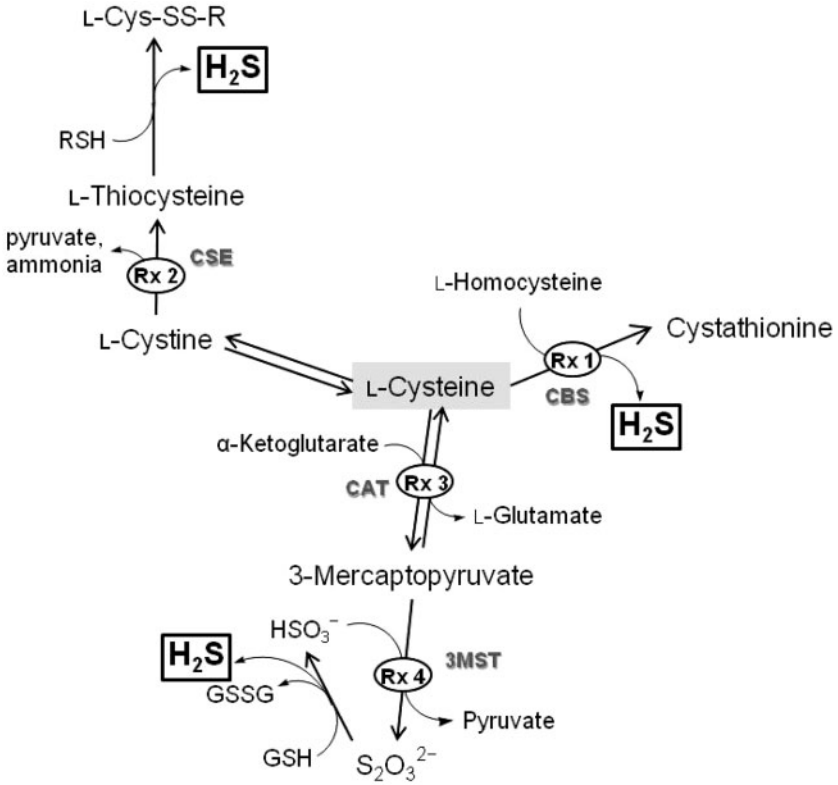
Keywords: endothelium/hydrogen sulfide/3-mercaptopyruvate sulphurtransferase/smooth muscle/vasorelaxation.

Abbreviations: CAT, cysteine (aspartate) aminotransferase; CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase; 3MST, 3-mercaptopyruvate sulphurtransferase.

Although hydrogen sulfide (H_2S), the so-called rotten egg gas, has been long known for its very high toxicity, studies over the past two decades have revealed that H_2S generated endogenously in mammalian tissues exhibits various physiological functions [for a recent review, see Ref. (*1*)]. Kimura and his colleague (*2*) first demonstrated that H_2S produced in the brain acts as a neuromodulator, which facilitates the induction of hippocampal long-term potentiation by enhancing the activity of NMDA receptors in neurons and increases the Ca^{2+} influx into astrocytes (*3*). They also showed that H_2S produced in the thoracic aorta, portal vein and ileum plays as a smooth muscle vasorelaxant in synergy with nitric oxide (NO) (*4*). These pioneer findings by Kimura's group facilitated numerous subsequent studies by other groups, which finally led to the establishment of H_2S as the third gaseous signaling molecule following NO and carbon monoxide (CO).

In an earlier study using the homogenates of rat liver and kidney (*5*), three enzymes present in mammalian tissues: (i) cystathionine β-synthase (CBS), (ii) cystathionine γ-lyase (CSE) and (iii) 3-mercaptopyruvate sulphurtransferase (3MST) in conjunction with cysteine (aspartate) aminotransferase (CAT), were reported to be possible H_2S-generators. CBS and CSE, which occur in many tissues including brain, liver, kidney, ileum, uterus and placenta, are both pyridoxal 5′-phosphate-dependent lyases (EC class 4) involved in the *trans*-sulphuration pathway and catalyse the synthesis of cystathionine from L-serine and L-homocysteine (β-replacement reaction) and the degradation of cystathionine into L-cysteine, α-ketobutyrate and ammonia (γ-elimination reaction), respectively. Beside these main reactions, CBS and CSE can also catalyse the synthesis of cystathionine from L-cysteine (instead of L-serine) and L-homocysteine, generating H_2S (Scheme 1, reaction 1) (*5*, *6*) and the β-elimination reaction with certain disulfides, including L-cystine, respectively (*5*). CSE is known to prefer L-cystine to L-cysteine for β-elimination and degrades it into pyruvate, ammonia and thiocysteine (reaction 2) (*7*), which may react non-enzymatically with L-cysteine or other thiols to form H_2S and L-cystine or the corresponding disulfide (Scheme 1) (*5*). However, the significance of L-cystine in the reducing intracellular environment remains to be clarified. Moreover, definitive evidence for the *in vivo* substrate directly used in the formation of H_2S is lacking for either of CBS and CSE. Nonetheless, it is widely assumed that desulfhydration of L-cysteine is the major source of H_2S in mammals and is catalysed by these *trans*-sulphuration pathway enzymes.

Recently, Kimura and his colleagues (*8*, *9*) have found that the third enzyme, 3MST in conjunction with CAT, contributes significantly in generating H_2S from L-cysteine in the presence of α-ketoglutarate in the brain and in the vascular endothelium of thoracic aorta. CAT, which is identical with aspartate aminotransferase, efficiently catalyses the transamination between L-cysteine and α-ketoglutarate to produce 3-mercaptopyruvate and L-glutamate (Scheme 1, reaction 3). 3MST then transfers sulphur from 3-mercaptopyruvate to sulphurous acid to produce pyruvate and thiosulfate (reaction 4), which is finally reduced to H_2S by another sulphurtransferase in the presence of reduced glutathione (GSH). Both CAT and 3MST were found to be localized not only in the brain but also in the vascular endothelium of thoracic aorta (*8*, *9*). Particularly, neither CSE nor CBS was found



Scheme 1 Possible pathways for production of H_2S in mammalian tissues. CBS, CSE, CAT and 3MST catalyse reactions (Rx) 1–4, respectively.

in rat endothelial cells (*9*), although they were detected in mouse endothelial cells (*10*), suggesting that there may be a species/tissues difference for production of H_2S. The H_2S generation was not observed in the absence of α-ketoglutarate and suppressed in the presence of L-aspartate, a preferred CAT substrate to L-cysteine (*9*). Thus, the enzyme system consisting of 3MST and CAT significantly contributes in generating H_2S as a smooth muscle relaxant in the vascular endothelium. This finding provides a new insight into the production of the physiologically important signaling molecule.

Aside from some uncertainties in the substrates for enzymatic generation of H_2S, there is accumulating evidence for various physiological roles of H_2S. Yang *et al*. have recently shown that the CSE-knockout mice have markedly reduced H_2S levels in the serum, heart, aorta and other tissues, and display pronounced hypertension and diminished endothelium-dependent vasorelaxation, providing further evidence that H_2S is an endothelium-derived relaxation factor possibly regulating the systemic blood pressure (*10*). Like NO and CO, the production of H_2S is enhanced by muscarinic cholinergic stimulation of the vascular endothelium, which activates the major H_2S-generating enzyme CSE through binding to calcium-calmodulin (*10*). However, the mechanism of action of H_2S is thought to be different from those of NO and CO, both of which activate soluble guanylate cyclase and increase the intracellular cGMP (*11*). Presumably, H_2S opens the K_{ATP} channel and thereby hyperpolarizes the membrane potentials of smooth muscle, leading to relaxation (*12*). Also, it should be noted that one gas regulates another to form intriguing feedback mechanisms among different gases (*13*, *14*).

H_2S also plays as a cytoprotectant rescuing neurons and cardiac smooth muscle from oxidative stress mainly by enhancing the cellular production of GSH and preserving mitochondrial function (*1*, *15*, *16*). Furthermore, H_2S is involved in the regulation of insulin release from the pancreatic β-cells (*17*, *18*) and in inflammatory reactions (*19*, *20*), stimulates the endothelium-related angiogenesis and wound healing through a K_{ATP} channel/MAPK pathway (*21*), and even brings small animals into a hibernation-like state by significantly down-regulating the energy metabolism (*22*). Due to such versatile activities, therapeutic exploitation of H_2S has been sought in clinical fields, expecting that administration of a low dose of H_2S or inhibitors for H_2S-generating enzymes would afford various clinical benefits (*23*, *24*).

In summary, the studies related to H_2S physiology are now expanding explosively and receiving wide attention.

Conflict of interest
None declared.

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