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Slow Generation of Hydrogen Sulfide from Sulfane Sulfurs and NADH Models

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Abstract

Here we report the model studies of the reactions between NADH models (using HEH and BNAH) and sulfane sulfurs (using polysulfides). Such reactions could lead to the oxidation of NADH models and the production of hydrogen sulfide (H₂S). Kinetics of the reaction between BNAH and elemental sulfur S₈ were determined in ethanol and the second-order rate constant was found to be 0.074 M⁻¹min⁻¹ (at 37 °C) suggesting this is a slow process.

Graphical Abstract

Keywords

Ammonium Tetrathiomolybdate; Hydrogen Sulfide; Donor; Oxidative damage

Hydrogen sulfide (H₂S) is known as a gasotransmitter which has shown nitric oxide-like biological activities.^{1–4} Endogenous generation of H₂S is attributed to at least three enzymes including cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfur-transferase (3-MST).^{5–7} These enzymes use cysteine or cysteine derivatives as substrates to produce H₂S. These enzymatic pathways are believed to be the major sources for H₂S formation in cells. On the other hand, non-enzymatic pathways of

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H₂S production are also known.^{8–11} For example, biologically existing sulfane sulfurs (such as protein-bound polysulfides or S₈) are considered as H₂S precursors. Cellular redox or pH changes may be able to mobilize H₂S from these species for physiological actions.¹² However, the detailed mechanisms behind these processes have not been carefully studied. NADH/NAD⁺ is a dominant redox regulation system in biology.^{13–15} As a potent electron donor, NADH participates in many electron-transfer processes and functions as a powerful reductant. As NADH is ubiquitous in cells, we wondered if NADH could directly react with intracellular sulfane sulfurs to produce H₂S. Herein we report the results using a model system to understand the reaction between NADH and sulfane sulfurs.

In NADH mediated reactions, it usually delivers two electrons or hydrides (H[−]) to the receptor molecules. Sulfane sulfur refers to sulfur atom with six valence electrons but no charge (represented as S⁰). Biologically important sulfane sulfur compounds include persulfides (R-S-SH), hydrogen persulfide (H₂S₂), polysulfides (R-S-S_n-S-R), and protein-bound elemental sulfur (S₈). These molecules can serve as electrophiles to receive electrons or hydride from NADH (Scheme 1). As such, hydrosulfide (HS[−]) can be released. It should be noted that HS[−] is the equivalent of H₂S in aqueous systems. Meanwhile, NAD⁺ and the reduced sulfane sulfur should be the other products from the reaction. We note that the reduction of the S⁰ species by NADH to generate H₂S could be spontaneous. This can be inferred from the two-electron standard reduction potentials for NAD⁺ + 2e[−] + H⁺ → NADH and S + 2e[−] + H⁺ → H₂S in aqueous solution, which are −0.320 V and 0.144 V, respectively.^{16,17}

In mechanistic studies of NADH mediated reactions, Hantzsch ester (HEH) and 1-benzyl-1,4-dihydronicotinamide (BNAH) are often used as the model compounds.^{18,19} They could be stronger two-electron (equivalent to hydride) reducing agents than the NADH. The hydride releasing abilities of HEH and BNAH are 22.2 and 43.5 kJ/mol stronger than that of a very close NADH model in which the 1-substituent in the 1,4-dihydronicotinamide contains a ribose skeleton.²⁰ We first tested the reaction between HEH and a cysteine-derived polysulfide **1** to see if any meaningful reaction could be observed. The reaction was monitored by TLC and GC. The formation of oxidized HEH, e.g. **2**, was used to indicate the reaction progress. To avoid the effects of oxygen the reactions were carried out under argon atmosphere. As shown in Table 1, HEH was quite stable in the absence of polysulfide as the control experiments gave only trace amounts of the oxidized product **2**. However, in the presence of polysulfide, a clean transformation to **2** was observed, albeit the process was found to be slow. As expected, solvents and reaction temperature changes could change the outcomes. For example, we identified the most efficient condition to promote this transformation was in EtOH under 37 °C, which gave almost quantitative yield of **2** after 20 hours.

BNAH was also tested in this reaction and clean conversion of BNAH to **3** was again observed. As shown in Table 2, BNAH appeared to be a stronger reductant than HEH as the reactions were found to be a lot faster. Since the solubility of polysulfide in ethanol was found to be much better than in acetonitrile, ethanol was selected as the solvent for the following experiments.

After confirming the formation of oxidized products from BNAH and HEH, we then studied the products originated from polysulfide **1**. When excess of reductants were used, the isolated product from **1** was found to be the corresponding disulfide. Next we wondered if H₂S was formed in the reactions and trapping experiments were carried out to address this question. As illustrated in Figure 1, the reaction solution A (HEH+**1**) was placed in a sealed 20-mL glass vial under argon. In the same vial a 1.5 mL Eppendorf vial containing the trapping solution B was also placed to absorb H₂S generated from solution A. Both the standard methylene blue (MB) assay²¹ and fluorescence assay (using a fluorescent probe SeP2)²² were used to analyze the trapping solution after the reaction was carried out at 37 °C for 20 hours. Control experiments (with only polysulfide **1**) were also performed for comparison. As shown in Figure 2 and Figure 3, the obvious increases of UV and fluorescence signals demonstrated the formation of H₂S in these systems. Moreover, GC-chemiluminescence assay was also performed and obvious production of free H₂S from the reaction between the NADH model compound and polysulfide was observed (Figures S1 and S2). It should be noted that, these assays cannot determine the exact amounts of H₂S due to the volatilization of H₂S in solutions.

Having demonstrated HEH and BNAH can indeed react with polysulfide **1** to form H₂S, we turned to study the generality of this reaction. Four additional polysulfides (**4–7**, Scheme 2) were tested. Under the optimized conditions (37 °C, EtOH), all of the polysulfides could effectively react with HEH and BNAH to form the corresponding oxidized products and produce H₂S (Table 3).

To further understand the reactions between NADH models and polysulfides, we studied the kinetics of reaction of BNAH with S₈ in ethanol containing dichloromethane (3/1, v/v). S₈ was selected in this study because of its high purity. BNAH has a characteristic UV absorption peak at 350 nm while S₈ has a very weak absorbance at this wavelength. Monitoring the decay of BNAH's UV absorption at 350 nm with time under *pseudo*-first-order conditions allowed us to determine the *pseudo*-first-order rate constants (k_{obs}). In these experiments, BNAH (1 mM) was treated with large excess of S₈ (20, 30, 40 and 50 mM) at 37 °C. The *pseudo*-first-order reaction rate constants were determined by linear fits of $-\ln(A_t - A_\infty)$ plotted versus reaction time (t) (see the supporting information). The effect of S₈ concentration on the *pseudo*-first-order rate constants gave rise to a linear plot with an intercept close to zero, showing that the reaction is also first-order in S₈ (Figure 4). The second-order rate constant of this reaction was calculated to be 0.074 M⁻¹min⁻¹.

In conclusion, we report here the model studies of the reactions between NADH models and sulfane sulfurs. HEH and BNAH were used as NADH models and polysulfides were used as sulfane sulfur models. We confirmed that such reactions could lead to the oxidation of NADH models and the production of H₂S. This might be another source of H₂S generation in cells. As can be expected, the non-enzymatic H₂S production process was found to be slow, indicating its limited contribution to overall H₂S biosynthesis in cells. However, it provides a new way to achieve controllable H₂S delivery which could have other interesting applications. Such studies are undergoing in our laboratory.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Procedure for detecting H₂S generated from the reaction between HEH and polysulfide **1**.

Figure 2.

UV absorption spectra of methylene blue assay. 0.5 mL trapping solution was mixed with 0.5 mL water, 0.5 mL FeCl_3 , and 0.5 mL DMPD and then incubated for 30 min before UV measurement. Solid line: the reaction between HEH and **1**; dot line: control.

Figure 3. Fluorescence spectra of the trapping solution treated with SeP2 ($\lambda_{\text{ex}}=498$ nm). Solid line: the reaction between HEH and **1**; dot line: control.

Figure 4. Linear plot of the pseudo-first-order rate constant $k_{obs}(\text{min}^{-1})$ versus the concentrations of S_8 . The slope is the second-order rate constant $k(\text{M}^{-1}\text{min}^{-1})$ for this reaction.

Scheme 1.

Proposed reaction between NADH and sulfane sulfurs

Scheme 2.
Structures of the additional polysulfides tested

Table 1

The reactions between HEH and polysulfide **1** (the reactions were conducted under dark. The concentration of HEH was 50 mM and the concentration of **1** was 100 mM).

Entry	Equiv of 1	Solvent	Temp.	Yield of 2
1	0	EtOH	r.t.	<5%
2	2	EtOH	r.t.	26%
3	0	EtOH	37 °C	<5%
4	2	EtOH	37 °C	96%
5	0	ACN	r.t.	<5%
6	2	ACN	r.t.	12%
7	0	ACN	37 °C	<5%
8	2	ACN	37 °C	18%

Table 2

The reactions between BNAH and polysulfide **1** (the reactions were conducted under dark. The concentration of BNAH was 50 mM and the concentration of **1** was 100 mM)

Entry	Equiv of 1	Solvent	Temp.	Yield of 3
1	0	EtOH	r.t.	<5%
2	2	EtOH	r.t.	60%
3	0	EtOH	37 °C	<5%
4	2	EtOH	37 °C	98%
5	0	ACN	r.t.	<5%
6	2	ACN	r.t.	96%
7	0	ACN	37 °C	<5%
8	2	ACN	37 °C	98%

Table 3

Reactions between HEH/BNAH and compounds **4–7** (reactions were conducted in ethanol under dark. The concentrations of HEH or BNAH were 50 mM and the concentrations of **4–7** were 100 mM).

Entry	Polysulfide	Yield of 2	Yield of 3
1	4	94%	95%
2	5	90%	98%
3	6	94%	99%
4	7	90%	75%