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Dual functions of alcohol dehydrogenase 3: implications with focus on formaldehyde dehydrogenase and S-nitrosoglutathione reductase activities

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Abstract. Alcohol dehydrogenase 3 (ADH3) is highly conserved, ubiquitously expressed in mammals and involved in essential cellular pathways. A large active site pocket entails special substrate specificities: short-chain alcohols are poor substrates, while medium-chain alcohols and particularly the glutathione adducts S-hydroxymethylglutathione (HMGSH) and S-nitrosoglutathione (GSNO) are efficiently converted under concomitant use of NAD^+/NADH. By oxidation of HMGSH, the spontaneous glutathione adduct of formaldehyde, ADH3 is implicated in the detoxification of formaldehyde. Through the GSNO reductase activity, ADH3 can affect the transnitrosation equilibrium between GSNO and S-nitrosated proteins, arguing for an important role in NO homeostasis. Recent findings suggest that ADH3-mediated GSNO reduction and subsequent product formation responds to redox states in terms of NADH availability and glutathione levels. Finally, a dual function of ADH3 is discussed in view of its potential implications for asthma.

Keywords. Alcohol dehydrogenase, formaldehyde, metabolic interaction, S-nitrosoglutathione, protein S-nitrosation.

Introduction

Alcohol dehydrogenase 3 (ADH3) is of widespread occurrence in both prokaryotic and eukaryotic organisms [1–6]. Species variability assessed by comprehensive genome screens and sequence comparisons among members of the alcohol dehydrogenase (ADH) family shows that ADH3 is the ancestral form [4, 5, 7]. The universal presence and structural conservation imply that ADH3 performs essential functions in living organisms.

Accordingly, ADH3 is implicated in essential signaling and metabolic pathways [8–17]. By oxidizing S-hydroxymethylglutathione (HMGSH), the spontaneous glutathione (GSH) adduct of formaldehyde, ADH3 constitutes a key enzyme in the detoxification of endogenous and exogenous formaldehyde [18–20]. Other functions attributed to ADH3 include first-pass ethanol metabolism, contribution to retinoic acid formation and oxidation of ω-hydroxy fatty acids [8, 17, 21, 22]. Furthermore, since ADH3 has been discovered to efficiently reduce the S-nitrosothiol S-nitrosoglutathione (GSNO), an important role of this enzyme also in NO homeostasis has been suggested [12, 13, 15, 23]. Within this review, we describe the unique features and the complexity of the ADH3 enzyme.

Throughout time, different ADH nomenclatures have been used which may result in confusion, particularly considering early reports where ADH3 was referred to as glutathione-dependent formaldehyde dehydrogenase, χχ alcohol dehydrogenase or class III alcohol dehydrogenase. Moreover, since ADH3 has been discovered to exhibit GSNO-reducing activity, several reports have referred to ADH3 as GSNO reductase

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(GSNOR) [24, 25]. Further complexity is added by the fact that the gene nomenclature was not updated in parallel with the protein nomenclature [1, 26], and the gene coding for human ADH3 has been named *ADH5*. Here, we follow the most recently published recommendation [26].

Tissue distribution and associated functions

Extensive studies on ADH distribution in human and rodent tissues including fetal tissues have established that ADH3 is expressed ubiquitously and with relatively little inter-tissue variation in mammals, in contrast to other ADHs [27–34]. Notably, the tissues examined include major targets for damage from the inhalation of formaldehyde such as the nasal and buccal mucosa and the lower respiratory tract, suggesting that ADH3 is the main scavenger of exogenous formaldehyde [19, 28, 32]. However, formaldehyde is also released during intracellular metabolism of endogenous compounds or xenobiotics [35–40]. Expression of ADH3 might thus fulfill a protective role against DNA damage resulting from endogenous as well as exogenous sources of formaldehyde [38]. Supportive of this conclusion, ADH3 has been demonstrated to be localized not only to the cytoplasm but also to the nucleus [41, 42]. Correspondingly, formaldehyde toxicity in *Adh3* null mutant mice was shown to be significantly increased relative to that in wild-type mice [43].

Additionally, in its function as GSNO reductase, ADH3 appears to be involved in the regulation of protein S-nitrosation, due to the dynamic equilibrium between S-nitrosothiols and GSNO [12, 13, 15, 24]. Protein S-nitrosation, the post-translational modification of a cysteine side chain by the attachment of a nitrosyl group, is emerging as an omnipresent mechanism in cellular functions and pathways and underlies as such a large part of NO signaling [44]. Interestingly, ADH3 is the only ADH present in rodent and human brain tissue, where NO acts as a neuroprotectant or neurotoxicant in a concentration-dependent manner, both effects involving protein S-nitrosation reactions [28–30, 33, 45, 46]. Several reports have demonstrated the presence of GSNO in brain tissue [47, 48], and ADH3 activity has been shown to influence levels of cellular S-nitrosothiols [23, 24].

Due to the ubiquitous expression pattern in mammalian tissues, ADH3 has been ascribed a housekeeping role in living organisms. However, a defined expression pattern has been found in zebrafish and a distinctly tissue-specific pattern during embryonic development in amphioxus (*Branchiostoma*), the sea squirt (*Ciona intestinalis*) and the fruit fly (*Drosophila*) [3, 49]. These results suggest important ADH3 roles in embryonic development.

Spatial distribution of ADH3 has also been studied in plants, albeit to a lesser extent. Transcript assessment demonstrated similar levels of ADH3 mRNA in all plant organs of *Arabidopsis thaliana* [11]. However, more recently, these studies were complemented by assessment of protein and activity levels showing, in contrast to the ubiquitous transcript, differential distribution of the protein with the highest levels in roots and rosette leaves [50].

Structure-function relationships

Like other enzymes of the medium chain dehydrogenase/reductase (MDR)-ADH family, ADH3 exhibits a dimeric structure with two zinc ions per 40 kDa subunit [51]. While one of the zinc ions is considered to serve a structural function only, the other zinc ion functions as a Lewis acid and activates the substrate in the active site, which is located in a cleft between the catalytic and the coenzyme binding domain of the enzyme. ADH3 differs markedly from the classical ethanol-oxidizing ADHs (ADH1 enzymes) in substrate specificity and kinetic properties. These differences are consistent with structural changes in the immediate vicinity of and within the active site [51; Eklund, this series].

Relating to the substrate specificity, residues 53–59 and 113–120 are positioned away from the catalytic cleft, thus enlarging the active site substantially versus the classical class I type and generating a broader entrance to the substrate-binding pocket [51]. Hence an active site is created that cannot be saturated with ethanol, but accommodates larger substrates such as HMGSH, GSNO, medium-chain alcohols, aldehydes and ω-hydroxy fatty acids [2, 20, 52]. Several residues within the active site which are different from those in the ADH1 enzymes play critical roles. Among those, Arg114 provides a positive charge in the substrate-binding pocket and facilitates the binding and correct orientation of negatively charged substrates, activators and inhibitors [51–54]. As a result, Arg114 is essential for hydrophobic anion activation of ethanol dehydrogenase activity and for the binding of ω-hydroxy fatty acids [22, 53, 55]. Arg114 and the active-site zinc, as well as to a lesser extent residues Thr46, Asp55 and Glu57 are responsible for the efficient binding of HMGSH [51, 54, 55]. Most likely the same residues are responsible for the binding of GSNO (Fig. 1). Another ADH3-specific property is low sensitivity towards 4-methylpyrazol, a potent inhibitor of ADH1 enzymes, which is mainly attributed to the

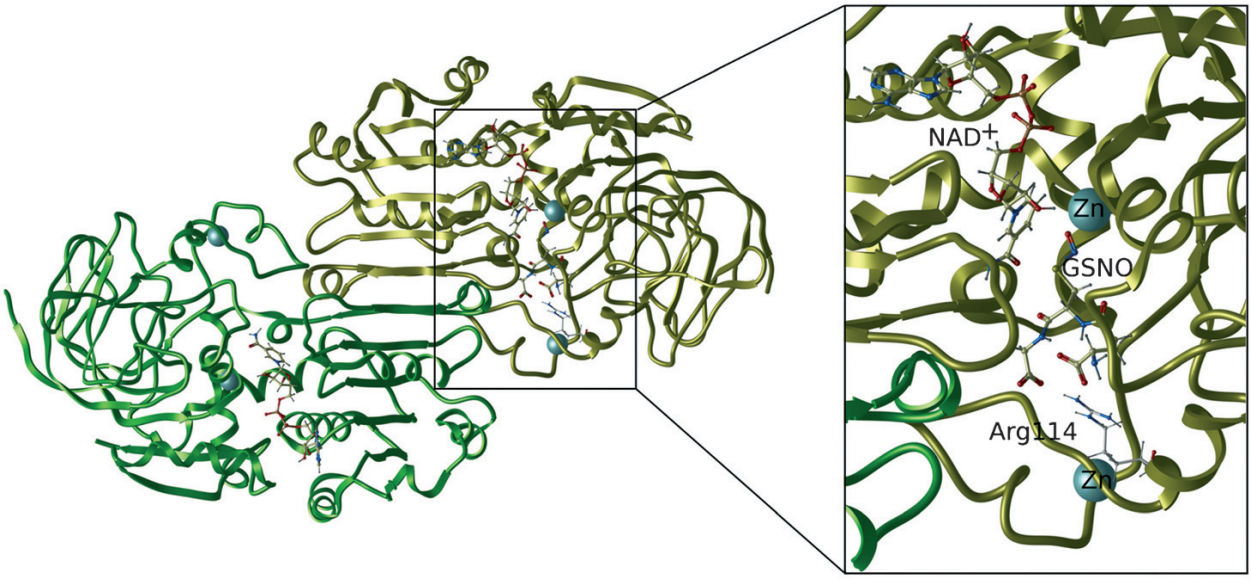

Figure 1. Dimeric structure and active site of ADH3. (Left:) The dimeric structure of ADH3, showing different monomers (green and yellow) and the position of zinc atoms (cyan), coenzymes and GSNO (only yellow monomer). (Right:) Close-up view of the active site with the coenzyme and the substrate GSNO, demonstrating orientation of the substrate between the active-site zinc and Arg114. GSNO was docked into ADH3 (pdb-file 1MC5) using ICM software (Molsoft) [104].

substitution of Phe93 in ADH1 with Tyr in ADH3 (Tyr92) [56].

Oxidation of HMGSH and 12-hydroxydodecanoic acid under concomitant conversion of NAD^+ appears to follow a random bi-bi kinetic mechanism in agreement with the noticed tertiary structure differences between the apoenzyme and binary and ternary complexes [52–54, 57]. Thus, binding of the substrate or NAD(H) does not entail significant domain movements as observed upon coenzyme binding in ADH1 enzymes, where an ordered bi-bi kinetic mechanism is predominant. In contrast, domain closure in ADH3 is executed upon ternary complex formation [57].

ADH3 in oxidative pathways

Formaldehyde

Because of the polarized carbonyl group formaldehyde is a highly reactive compound that can be attacked either by a nucleophile or an electrophile and can participate in substitution and addition reactions. In a cellular milieu, there are a variety of potential reactants for formaldehyde, including thiols which will form hemithioacetals as e.g. HMGSH, spontaneously formed from GSH and formaldehyde, or amines which will form Schiff bases. Importantly, the latter reaction represents the first step in the formation of DNA-DNA, DNA-protein and protein-protein cross-links, where amino groups present in DNA and proteins are covalently linked via a methylene bridge derived from formaldehyde [58]. Ultimately, the high DNA reactivity leads to mutagenic effects and chromosomal changes which combined with formaldehyde-associated activation of proliferation are considered to form the basis for formaldehyde carcinogenicity [59, 60].

Formaldehyde is part of the one carbon pool, which includes the metabolism of serine and glycine, and is also a product of oxidative demethylation of xenobiotics by cytochrome P450 enzymes [35–40]. In the light of the genotoxic and cytotoxic effects it is not surprising that several formaldehyde detoxification systems have evolved. In humans, two members of the divergent aldehyde dehydrogenase (ALDH) superfamily, namely a cytosolic (ALDH1A1) and a mitochondrial (ALDH2) enzyme, can metabolize formaldehyde directly, but exhibit K_m values for free formaldehyde that are several magnitudes higher than the one ADH3 exhibits for HMGSH [61–64]. Clearly, ADH3 is the most efficient formaldehyde-metabolizing enzyme *in vitro* among all elucidated enzymes so far, and most likely equally efficient under cellular redox conditions, i.e. with glutathione present in millimolar concentrations and a high NAD^+/NADH ratio. In contrast to the vast number of studies carried out *in vitro*, only a few functional studies have been performed. Nonetheless, these studies uniformly argue for the importance of ADH3 in formaldehyde resistance of prokaryotes exemplified by an *Escherichia coli* strain, and eukaryotes, exemplified by *Arabidopsis thaliana* and mice [43, 65, 66].

Ethanol

ADH3 shows very poor activity towards ethanol, and the human enzyme exhibits non-hyperbolic kinetics with ethanol concentrations up to 3.5 M [21, 22, 67, 68]. ADH3 is thus considered to play only a minor role in hepatic alcohol metabolism because ethanol concentrations rarely exceed 50 mM (equivalent to 2.3‰ blood concentration). The main pathway for ethanol detoxification in the liver involves the ADH1 enzymes, which may be assisted by an ethanol-inducible pathway, mediated by cytochrome P450 2E1 [69]. However, during ethanol intake a significant amount is metabolized already in the gastric lumen, where ethanol concentrations can be as high as 1.5 M [70]. At high ethanol concentration ADH3 displays positive cooperativity with ethanol (0.5–3.5 M) compatible with a contribution to first-pass metabolism *in vivo,* despite negligible activity with ethanol at lower concentrations [21].

Fatty acids and ω-hydroxy fatty acids

Dependent on carbon chain length and preferably at alkaline pH, fatty acids can act as activators of short-chain alcohol oxidation. Ethanol oxidation, for instance, is considerably activated by octanoic acid [22, 55]. The optimal size of activator/substrate pair lies around 10 carbon atoms: For instance, activation of ethanol oxidation is highest with octanoic acid, while activation of methylcrotyl alcohol oxidation is highest with pentanoic acid [22]. In contrast, dodecanoic acid inhibits ADH3 irrespective of substrate, and of all ω-hydroxy fatty acids, 12-hydroxydodecanoic acid is the best substrate for ADH3 [8, 53, 71]. Clearly, these chain length-dependent activities reflect the dimensions of the large active site. As described above, Arg114 is essential for activation by fatty acids, as well as for oxidation of 12-hydroxydodecanoic acid and HMGSH.

It is unclear whether activation or inhibition by fatty acids is of physiological importance. Activation of ethanol oxidation by fatty acids is probably not powerful enough at physiological pH to allow ADH3 to contribute significantly to ethanol detoxification. It is conceivable that inhibition of ADH3 by medium-chain fatty acids as e.g. dodecanoic acid plays a role *in vivo*, because fatty acids are ubiquitous and readily transported into the cell [72]. Furthermore, cytochrome P450 enzymes and other enzymes that are capable of catalyzing ω-hydroxylation of fatty acids exist in bacteria, animals and plants, resulting in endogenous generation of 12-hydroxydodecanoic acid and other ω-hydroxy fatty acids [73, 74].

Retinoid metabolism

Participation of ADH3 in retinol oxidation has been studied with the mouse enzyme, where oxidation of *all-trans*-retinol *in vitro* is very low in comparison to the more efficient enzymes ADH1 and ADH4 [17]. Nonetheless, *Adh3*-deficient mice demonstrate significantly decreased levels of *all-trans*-retinoic acid in serum, providing evidence for the involvement of ADH3 in retinoic acid formation *in vivo.* It has been proposed that the ubiquitous expression pattern, and the co-expression with retinaldehyde dehydrogenases, could compensate for poor catalytic activity [17]. In contrast, expression of ADH4, an efficient retinol oxidation catalyst *in vitro*, is limited to the gastric epithelium, does not overlap with expression of potential enzymes for the second step in retinoic acid biosynthesis, and *all-trans*-retinoic acid levels in serum of *Adh4*-deficient mice were not significantly altered [31, 43].

ADH3 in NO homeostasis: importance of cellular redox state

Protein S-nitrosation and GSNO

NO synthase activity has been discovered in bacteria, yeast, protozoa and metazoa. As a biological messenger it thus seems similarly widespread as the ADH3 function [2, 75]. Intracellular NO signaling is in part mediated through S-nitrosation, the attachment of an NO moiety to sulfhydryl groups of cysteine residues [44]. S-nitrosated proteins appear to be in transnitrosation equilibrium with GSNO, the most common low molecular weight S-nitrosothiol [23, 24, 76]. In the presence of NADH, GSNO is efficiently and irreversibly reduced by ADH3, an activity which appears to be conserved from prokaryotic ADH3 [13, 77, 78] to eukaryotic ADH3, including plant [14], yeast [42], non-vertebrate [16] and mammalian ADH3 enzymes [12, 13, 15, 64]. Currently, more than 100 potential targets for S-nitrosation have been identified, including proteins involved in cellular processes as diverse as apoptosis, membrane trafficking and iron homeostasis [44]. It is thus not surprising that deregulated levels of cellular S-nitrosothiols are often associated with disease [24, 79–81].

Metabolism of GSNO

At present, metabolism of S-nitrosothiols is not well understood. Several enzyme systems have been proposed to be involved in human GSNO metabolism (Table 1) [64, 82–85]. Among those, ADH3 stands out by virtue of three properties: First, it is the only GSNO-converting activity so far detected that does not result in NO release (see note added in proof).

Table 1. Human GSNO converting enzymatic activities (see also note added in proof).

Enzyme	kinetic parameters	Reaction products
ADH3 [64]	$K_m = 11\ \mu M$ $k_{cat} = 1200\ min^{-1}$ $k_{cat}/K_m = 110\ min^{-1}\ \mu M^{-1}$	GSNHOH[a] as intermediate which can be: a) rearranged to form $GSONH_2$ [12, 15, 64] or b) intercepted by GSH to form GSSG and NH_2OH [12, 64]
Cu/Zn superoxide dismutase [82]	$K_m = 5.6\ \mu M$ $k_{cat} = 0.3\ min^{-1}$ $k_{cat}/K_m = 0.05\ min^{-1}\ \mu M^{-1}$	GSSG and NO
Glutathione peroxidase [83]	ND[b]	GSSG and NO
Thioredoxin system [84]	$K_m = 60\ \mu M$ $k_{cat} = 36\ min^{-1}$ $k_{cat}/K_m = 0.6\ min^{-1}\ \mu M^{-1}$	GSH, NO and O_2^{-}[c]
Xanthine oxidase [85]	ND[b]	GSH, NO and O_2[d]

[a] Reaction is NADH-dependent.
[b] ND, not determined.
[c] Reaction is oxygen-dependent.
[d] Reaction is dependent on xanthine oxidase-mediated formation of O_2.

Instead, the NO moiety is reduced and irreversibly removed from the NO pool, compatible with an NO terminase function [12, 15, 64]. Second, with a k_{cat}/K_m value of $110\ min^{-1}\ \mu M^{-1}$, it is the most efficient GSNO-converting activity among all suggested activities, provided the appropriate cofactor NADH is present [64]. Third, product formation appears to respond to GSH levels, which will be discussed in more detail below. There is evidence for the functional involvement of ADH3 in the regulation of intracellular S-nitrosothiols in living cells [23, 24]. For instance, one study showed that the degree of down-regulation of ADH3 by RNA interference correlated inversely with nitrosothiol levels in cultured cells [23]. Thus, in its function as GSNO reductase, ADH3 appears indirectly to govern levels of protein S-nitrosothiols and is therefore likely to serve an important regulatory function in NO signaling [44].

Influence of the cellular redox potential: glutathione

The current body of data suggests that the occurrence of ADH3-mediated GSNO reduction as well as product formation is under control by the cellular redox potential [64]. S-nitrosoglutathione is reduced by ADH3 to form an intermediate which can either rearrange to the corresponding glutathione sulfinamide ($GSONH_2$) or be intercepted by GSH to form glutathione disulfide (GSSG, Fig. 2). Typical cellular GSH concentrations in the millimolar range decelerate the ADH3-catalyzed reaction and preclude the rearrangement to the sulfinamide by quantitative interception of the intermediate semimercaptale [12, 64]. However, cellular GSH levels are subject to intracellular fluctuations, and glutathione depletion occurs under various disease conditions [86–89]. *In vitro*, low GSH concentrations favor the rearrangement to $GSONH_2$ [12, 64]. $GSONH_2$ in turn is spontaneously hydrolyzed to the corresponding sulfinic acid, GSO_2H, and it is conceivable that, under sustained or extreme oxidative stress, the sulfinic acid could be oxidized further to form the sulfonic acid, GSO_3H. Interestingly, the three products have increasing potential to inhibit glutathione transferases, important enzymes in the cellular defense towards xenobiotics [64]. This could represent a novel toxicity mechanism, where severe nitrosative/oxidative stress leads to the formation of GSH-dependent detoxification enzymes. On the other hand, this might also be a mechanism to prevent further GSH loss in order to conserve residual GSH for scavenging of reactive oxygen species (ROS). Notably, the products involved show several parallels to redox state-dependent modification of protein cysteine thiols and might constitute a low molecular weight sensor for the redox state of the cell [44]. Compatible with that hypothesis, *Arabidopsis thaliana* mutants with modified ADH3 expression seem incapable of detecting intracellular changes in the GSH pool [50]. However, future work has to clarify the physiological relevance of $GSONH_2$ and its derived products relative to GSSG.

Influence of the cellular redox potential: NADH

Under normal redox conditions, the cellular free NAD^+/NADH ratio can essentially be considered unfavorable for reductive pathways [90, 91]. In contrast, the $NADP^+$/NADPH ratio is usually kept low, which allows for the utilization of NADPH in biosynthetic reductive pathways [92]. ADH3, however, cannot efficiently use the alternative cofactor NADPH for GSNO reduction [15]. Hence, ADH3-mediated GSNO reduction appears to be strongly governed by NADH accessibility, and increasing intracellular NADH levels are likely to trigger GSNO reduction. Various factors could induce such

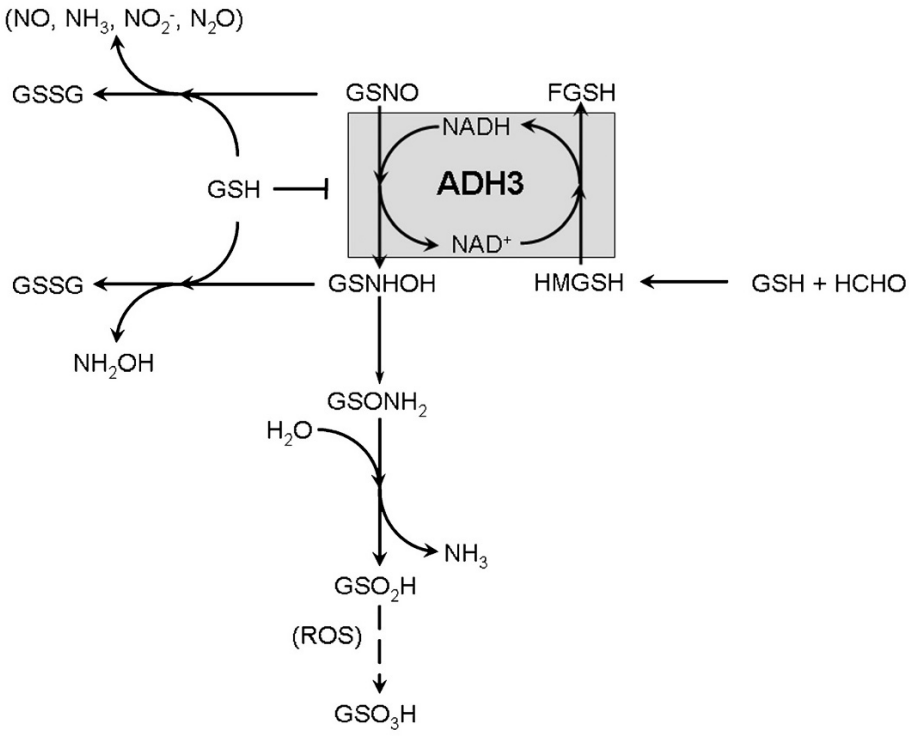

Figure 2. Model for product formation of ADH3-mediated GSNO reduction responding to local GSH concentration. GSH at millimolar concentrations decreases the rate of ADH3-mediated GSNO reduction. GSNO reduced by ADH3 to the intermediate S-(N-hydroxyamino)-glutathione is intercepted by GSH to yield GSSG and NH_2OH. Some GSNO is converted in a slow non-enzymatic reaction to yield GSSG and a variety of nitric species, dependent on local oxygen concentrations [105, 106]. Under conditions of GSH depletion, ADH3-mediated GSNO reduction is fast, and the intermediate S-hydroxylamino-glutathione can be spontaneously rearranged to the $GSONH_2$. $GSONH_2$ is partly hydrolyzed to GSO_2H, which is likely to be oxidized to GSO_2H under oxidative stress (ROS, reactive oxygen species). Invariably, NADH for GSNO reduction can be provided by the oxidative ADH3 pathways as e.g. by oxidation of the glutathione adduct of formaldehyde, HMGSH.

a change: For instance, ADH-mediated metabolism of ethanol in hepatocytes leads to a substantial redox shift [93]. Glutathione depletion in pheochromocytoma cell lines (commonly used as a neuron model) results in inhibition of mitochondrial complex I, NADH dehydrogenase and could presumably entail accumulation of NADH in the cell [94]. In addition, ADH3 itself catalyzes oxidative reactions which produce NADH, most importantly the oxidation of formaldehyde. The question whether formaldehyde and other ADH3 substrates trigger GSNO reduction under conditions reflecting the normal cellular NAD^+/NADH ratio was addressed in a recent study [64].

Potential implications of a dual ADH3 function as formaldehyde dehydrogenase and GSNO reductase

ADH3-mediated oxidation of HMGSH leads to rapid depletion of GSNO *in vitro*

Oxidation of HMGSH and other alcohols by ADH3 yields NADH, a factor which should limit incidence of GSNO reduction under normal cellular redox conditions. However, it was unclear whether simultaneous presence of HMGSH, GSNO and both oxidized and reduced cofactor would lead to the formation of ternary dead-end inhibitory complexes which would hamper both formaldehyde detoxification and GSNO reduction. This question was addressed in *in vitro* experiments where ADH3-mediated alcohol oxidation was monitored in the absence and presence of GSNO using purified ADH3 as well as crude cell lysates [64] (Fig. 3). Reaction rates were determined under conditions where no net NADH production occurred as verified by fluorescence spectroscopy, i.e. where all NADH produced was immediately consumed for GSNO reduction. In the absence of GSNO, initial reaction rates were determined using the extinction coefficient of NADH, 6220 M^{-1} cm^{-1}. In the presence of GSNO, no fluorescence emission was observed, hence no net NADH production, and thus, reaction rates could be determined from the initial concurrent negative slope in absorbance at 340 nm – corresponding to the decrease in GSNO only – using the extinction coefficient of GSNO, 840 M^{-1} cm^{-1}

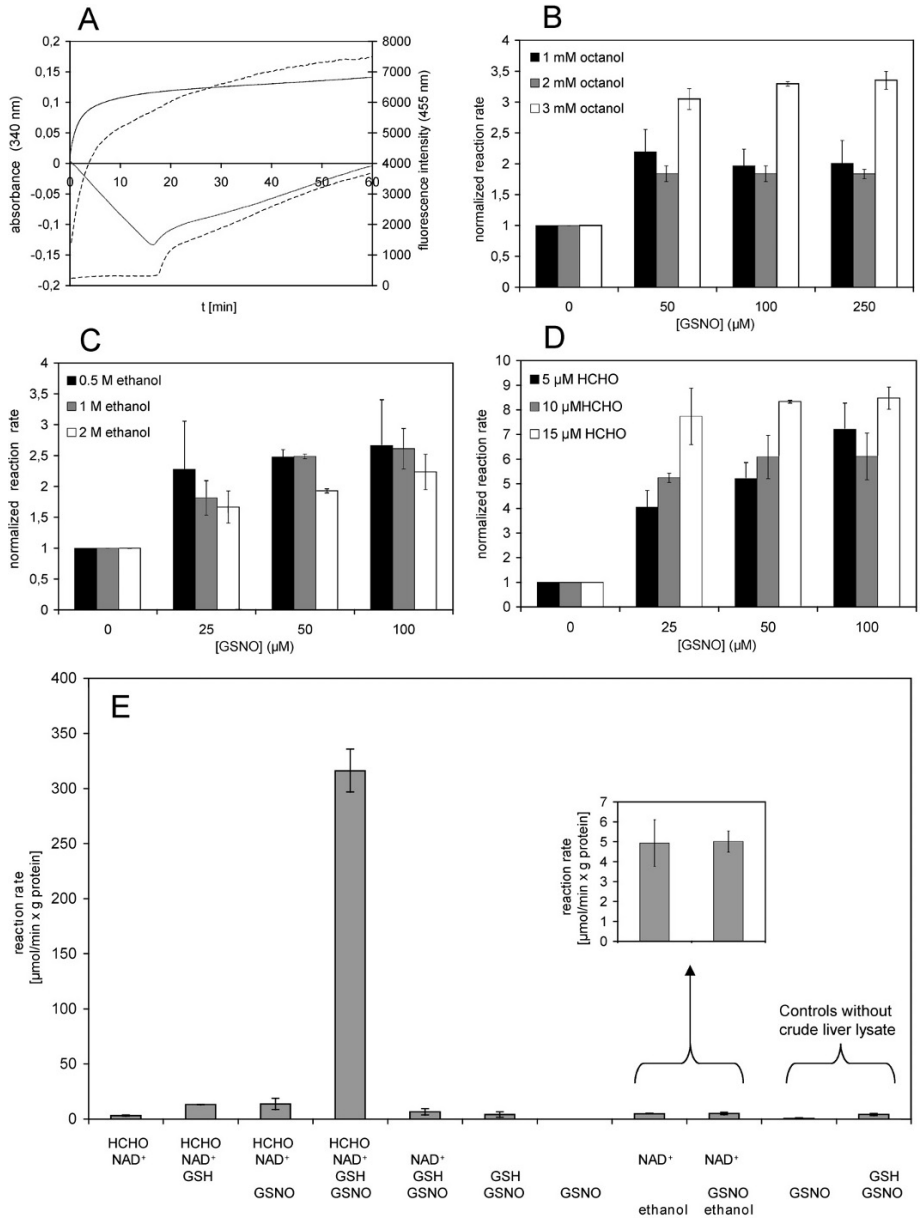

Figure 3. ADH3-mediated alcohol oxidation is promoted in the presence of GSNO. (*A*) ADH3-catalyzed octanol oxidation in the presence and absence of 250 μM GSNO. Reactions were carried out in 0.1 M potassium phosphate, pH 7.5, containing 2.4 mM NAD^+, 2.0 mM octanol and 62.5 μg/ml ADH3 in the presence (lower curves) and absence (upper curves) of 250 μM GSNO. Solid lines show reactions monitored by following the absorbance at 340 nm. Dashed lines show reactions monitored by the NADH-specific fluorescence emission at 455 nm with 340 nm as excitation wavelength. In the presence of GSNO, the initial negative slope coincides with absence of net NADH production. (*B–D*) Normalized rates of alcohol oxidation in the absence (rate = 1) and presence of GSNO for the substrates (*B*) octanol, (*C*) ethanol and (*D*) formaldehyde (in the form of HMGSH). For reactions with formaldehyde, the ADH3 concentration was 2.5 μg/ml, and the reaction mixtures also contained 1 mM GSH. (*E*) Similar experiments were performed with crude liver lysate instead of purified ADH3 at 37 °C in 0.1 M potassium phosphate, pH 7.5 containing 2.4 mM NAD^+ and 0.5 mg/ml protein. Different combinations of substrates (1 mM each) were added as indicated along the abscissa. Reactions were monitored by the absorbance at 340 nm. Results are expressed as μmol NADH formed, or for all reactions including GSNO, μmol GSNO degraded per min and g total protein. Results are presented as mean ± standard deviation of at least two experiments and agree within 10% for an independently prepared lysate.

(Fig. 3A). This approach revealed that oxidation rates for different ADH3 substrates, including octanol, ethanol and HMGSH, were two- to eightfold increased in the presence of GSNO, and this effect was most pronounced with the substrate couple HMGSH/GSNO (Fig. 3B–D). In an effort to study GSNO metabolism in a complex environment, crude lung and liver lysates were used in similar experiments, and an even more drastic increase in reaction rates (>20-fold) was observed for the substrate couple HMGSH/

GSNO (Fig. 3E). The results are consistent with immediate enzyme-bound cofactor recycling, as cofactor release is a step that is partially rate-limiting in HMGSH oxidation. In contrast, no such reaction rate increase was observed when ethanol was used as substrate at millimolar concentrations (Fig. 3E). At such low concentrations the contribution of ADH3 to ethanol oxidation is negligible, and ADH1 is the main enzyme. Thus, in this case NADH could not be provided in ADH3-bound form, and instead the rate of GSNO reduction was most likely limited by the rate of free NADH production following ADH1-mediated ethanol oxidation. Apart from relatively lower ADH3-activity, results were similar for lung lysates [64]. Overall, it appears that any source of NADH can trigger GSNO reduction, but that GSNO depletion is much more rapid when the redox cycle is constrained to ADH3.

Implications for asthma

Several allelic variants have been detected for the gene of ADH3 in promoter and intron regions, and a recent report coupled two single nucleotide polymorphisms to asthma susceptibility [25, 95]. In this context, it is highly striking that two reverse substrates of ADH3 that trigger or even promote each others' conversion have opposite effects in asthma. It is very well known that formaldehyde acts as a bronchoconstrictor and exacerbates asthma symptoms, although the molecular causes are not well understood [96–102]. In contrast, GSNO acts as an endogenous bronchodilator in airway lining fluid, protects from hyperresponsivity and is depleted from airways of asthmatic patients [24, 76, 103]. The results described above might represent a mechanism by which formaldehyde exerts its asthma-exacerbating effects: Under asthmatic conditions, including lung epithelial cell damage, ADH3, GSH and NAD^+ are likely to be present in the airway lining fluid, where inhalation of formaldehyde could then lead to rapid depletion of GSNO, resulting in bronchoconstriction and enhanced airway hyperresponsivity [24, 64, 76].

Conclusions

ADH3 is involved in multiple cellular pathways, as diverse as formaldehyde detoxification, retinoid metabolism and NO homeostasis. A dual, coupled function as formaldehyde dehydrogenase and GSNO reductase would provide a molecular explanation for the turnover of NO. This may also be of interest in relation to asthma-exacerbating effects, where inhalation of formaldehyde may lead to depletion of the bronchodilator GSNO. Future studies should address interactions between NO signaling and additional pathways, such as retinoic acid formation, functions of ADH3 in the central nervous system, and the physiological significance of glutathione sulfinamide or other derivatives *in vivo*.

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Note added in proof. After acceptance of this review, a study was published that provides evidence for human carbonyl reductase acting as an additional GSNO reductase (Bateman, R.L. et al., Human carbonyl reductase 1 is an S-nitrosoglutathione reductase, J. Biol. Chem., in press). The kinetic constants for NADPH-dependent carbonyl reductase-mediated GSNO reduction were: $K_m = 30\mu M$, $k_{cat} = 450\ min^{-1}$, $k_{cat}/K_m = 15\ min^{-1}\ \mu M^{-1}$.

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