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Review

Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes ☆

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ABSTRACT

Background: Glutathione-dependent catalysis is a metabolic adaptation to chemical challenges encountered by all life forms. In the course of evolution, nature optimized numerous mechanisms to use glutathione as the most versatile nucleophile for the conversion of a plethora of sulfur-, oxygen- or carbon-containing electrophilic substances.

Scope of review: This comprehensive review summarizes fundamental principles of glutathione catalysis and compares the structures and mechanisms of glutathione-dependent enzymes, including glutathione reductase, glutaredoxins, glutathione peroxidases, peroxiredoxins, glyoxalases 1 and 2, glutathione transferases and MAPEG. Moreover, open mechanistic questions, evolutionary aspects and the physiological relevance of glutathione catalysis are discussed for each enzyme family.

Major conclusions: It is surprising how little is known about many glutathione-dependent enzymes, how often reaction geometries and acid–base catalysts are neglected, and how many mechanistic puzzles remain unsolved despite almost a century of research. On the one hand, several enzyme families with non-related protein folds recognize the glutathione moiety of their substrates. On the other hand, the thioredoxin fold is often used for glutathione catalysis. Ancient as well as recent structural changes of this fold did not only significantly alter the reaction mechanism, but also resulted in completely different protein functions.

General significance: Glutathione-dependent enzymes are excellent study objects for structure–function relationships and molecular evolution. Notably, in times of systems biology, the outcome of models on glutathione metabolism and redox regulation is more than questionable as long as fundamental enzyme properties are neither studied nor understood. Furthermore, several of the presented mechanisms could have implications for drug development. This article is part of a Special Issue entitled Cellular functions of glutathione.

1. Introduction

Glutathione is the central redox agent of most aerobic organisms. Its reduced form (GSH≡γ-L-glutamyl-L-cysteinylglycine) serves as a ubiquitous nucleophile in order to convert a variety of electrophilic substances under physiological conditions. Glutathione-dependent enzymes significantly accelerate most of these chemical reactions in numerous metabolic pathways. Accordingly, tens of thousands of articles on glutathione-dependent enzymes and pathways have been published since the disputed discovery of glutathione by Hopkins as well as Hunter and Eagles in the 1920s [1]. It is therefore rather surprising that many fundamental mechanistic questions still remain to be solved in order to precisely understand the role of glutathione metabolism at the cellular and organismic level. This review is a (doomed) attempt to summarize the knowledge on glutathione-dependent catalysis and to outline the relevance of the current mechanistic models. I will approach the topic from two perspectives: In Section 2, I will start with a focus on the substrates. I will present theories on the origin and benefits of glutathione-dependent processes, summarize the properties of this extraordinary molecule and provide an overview of the glutathione-dependent enzymes and pathways. The mechanisms of glutathione-dependent enzymes and their physiological relevance will be subsequently discussed and compared in Sections 3–8.

2. Theories on the benefits, functions and evolution of glutathione catalysis

2.1. Two chemical challenges for life

Why do we need a glutathione system? Life as we know it has encountered several chemical challenges in the course of evolution. In fact, countless "natural" chemicals—including electrophilic substances—are carcinogens, mutagens, teratogens and clastogens [2,3]. In addition to xenobiotics, two of the presumably most important chemical challenges are (i) the formation of reactive oxygen species (ROS) due to an

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aerobic atmosphere, and (ii) the formation of 2-oxoaldehydes (2-OA) due to glycolysis and other fundamental metabolic pathways.

2.1.1. The formation of reactive oxygen species

Oxygenic photosynthesis most likely caused the first global "environmental pollution crisis". As a consequence of anoxygenic and oxygenic photosynthesis, the presumably reducing, hydrogen sulfide-enriched oceans and atmosphere changed to oxidizing, oxygen-enriched habitats with two significant oxygenation boosts occurring approx. 2.5–2.2 and 0.8-0.5 billion years ago (Fig. 1) [4,5]. Under the present conditions, electrophilic ROS are expected to be easily formed in all aerobic organisms with the help of light, flavins, semiquinones as well as iron, copper and other metal ions (Fig. 2A) [6–9]. H_2O_2 and $O_2^{\bullet-}$ can both react with selected proteins containing Fe/S-clusters, liberating their iron ions. Free or complexed Fe^{2+} reduces H_2O_2, yielding $OH^{\bullet}$ which unspecifically modifies all kinds of biomolecules at a diffusion-limited rate. Hence, radicals, sulfenic acids, disulfides and (hydro)peroxides are directly or indirectly formed by ROS (Fig. 2B). These ROS-dependent modifications result in inactivated proteins, damaged membranes and mutations [8–10].

However, thiyl radicals, disulfides, sulfenic acids and ROS can also fulfill vital functions: (i) Some ROS are not only involved in the defense against pathogens, but can also serve as signal mediators in the redox regulation of metabolism and transcription. Accordingly, there are several proteins and enzymes that either sense or even generate ROS [7,11,12]. Excellent examples for the latter enzymes are myeloperoxidases, producing HOCl, and NADPH-oxidases, generating $O_2^{\bullet-}$ [13]. (ii) Some cysteine-derived thiyl radicals, sulfenic acids and disulfides are pivotal intermediates during catalysis or could serve as signal mediators [7,11,14,15]. Of note, the reduction of ribonucleotides is a peculiar example for a fundamental thiyl radical-dependent as well as disulfide-dependent physiological process in all domains of life [16,17]. (iii) The importance of protein disulfide bonds is furthermore underlined by the fact that bacteria and eukaryotes established non-related analogous machineries to stabilize secreted and intracellular proteins in the periplasmic space, the endoplasmic reticulum and the mitochondrial intermembrane space [18–20].

In summary, on the one hand, the ancestors of modern organisms had to develop numerous mechanisms to maintain reducing intracellular conditions, to avoid the formation of ROS, to detoxify ROS, and to reverse or repair ROS-derived damage [8–10]. On the other hand, partially oxidizing conditions as well as appropriate redox steady states in different cellular compartments became essential for life. So-called oxidative stress occurs only when the balance between the formation and the removal of ROS is disturbed, thereby resulting in the accumulation of oxidized and damaged biomolecules [10]. Please note that precise mechanistic definitions of oxidative stress at the molecular level are just beginning to emerge and seem to highly depend on the cell type or organism.

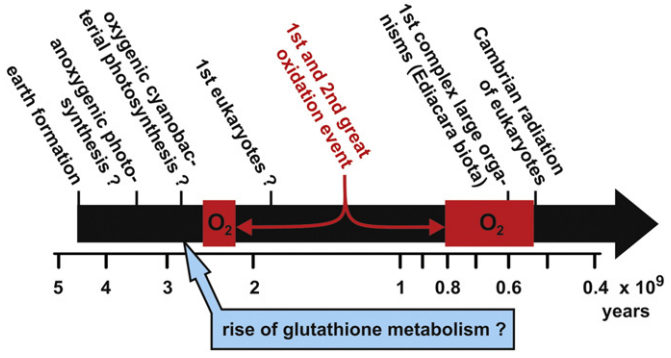

Fig. 1. The evolution of aerobic life and glutathione metabolism. Oxygenic photosynthesis resulted in an oxidation of the environment followed by a delayed increase of free oxygen in the atmosphere (during the so-called 1st and 2nd great oxidation event highlighted in red). Several glutathione-dependent enzymatic activities are found in contemporary eukaryotes as well as purple bacteria and cyanobacteria but seem to be absent in many other bacteria and archaea. Ondarza as well as Fahey and colleagues therefore suggested that glutathione metabolism evolved together with oxygenic photosynthesis [86,549–551]. More recent in silico analyses revealed that the domains of some glutathione-dependent enzymes such as Grx and GST are found in all kingdoms of life, including some archaea and all kinds of bacteria [203,479] (Deponte, unpublished). Thus, a putative earlier evolution of glutathione-dependent enzymes and a subsequent loss or replacement in bacteria and archaea cannot be fully excluded. Nevertheless, based on the current data, it seems more likely that the few genes encoding glutathione-dependent enzymes in archaea and bacteria originate from horizontal gene transfers.

2.1.2. The formation of 2-oxoaldehydes

Glycolysis-dependent ATP-formation is an imperfect process. During an "unwanted" side reaction of the Emden–Meyerhof–Parnas pathway, phosphate is eliminated from the triosephosphates glyceraldehyde-3-phosphate (GAP) and dihydroxyacetone-phosphate (DHAP) (Fig. 2C) [21–23]. The molecular architecture of the glycolytic enzyme triosephosphate isomerase (TIM) stabilizes the enediolate intermediate of the isomerization reaction and therefore significantly reduces this ubiquitous side reaction [24]. Nevertheless, the elimination product methylglyoxal (MG) is continuously generated at a low level. For example, in human red blood cells about 0.1% of GAP and DHAP were estimated to end up as MG [25]. Even archaea—using the Entner–Doudoroff instead of the Emden–Meyerhof–Parnas pathway—have a functional TIM for gluconeogenesis [26] and were shown to produce MG [27].

MG and other structural analogs of glyoxal (OCHCHO ≡ ethanedial) are 2-oxoaldehydes (2-OA). In addition to gylcolysis these compounds are also formed during lipid peroxidation as well as acetone, glycerol and threonine metabolism [21,23,28,29]. Owing to the adjacent carbonyl groups, 2-OA are strong electrophiles that spontaneously react with nucleophiles from proteins, lipids and nucleic acids, thereby yielding so-called advanced glycation endproducts (AGEs) (Fig. 2D). As a consequence, 2-OA are potentially cytotoxic and mutagenic, and their removal by a detoxification system is beneficial [30–32]. However, *Escherichia coli* and other bacteria sometimes even generate MG with the help of methylglyoxal synthase to metabolize DHAP under conditions of limited phosphate [21,28,33]. As outlined in Section 7.4, 2-OA can be also involved in signal transduction and cellular differentiation. Hence, the structures, cellular concentrations and effects of 2-OA highly depend on the often neglected biological context. In summary, 2-OA are ubiquitous electrophilic metabolites that are usually detoxified but that might also exert regulatory functions in analogy to the janus-faced hydroperoxides [31].

2.2. One single solution: glutathione

2.2.1. Overview of glutathione metabolism and catalysis

How are the chemical challenges outlined in Section 2.1 mastered? The glutathione system—together with the thioredoxin system—probably evolved very early in aerobic organisms (Fig. 1). Owing to the cysteine moiety of GSH, the whole system is based on common sulfur biochemistry (Fig. 3A). It therefore requires, (i) an electron relay, linking the universal reducing agent NADPH to thiol/disulfide-metabolism, and (ii) a thiol-containing adapter molecule to transfer electrons to a set of different acceptors. Flavoproteins are widely used as electron relays [18]. Hence, it is not surprising that the reducing equivalents from NADPH enter the glutathione system either with the help of the FAD-dependent enzyme glutathione reductase (GR) [34–36] or the thioredoxin reductase/thioredoxin couple (TrxR/Trx) [37–43]. The electrons are subsequently transferred to glutathione disulfide (GSSG), yielding two molecules of GSH (Fig. 3B). GSH either serves as a reducing agent for disulfides (Fig. 3C) and hydroperoxides (Fig. 3D), or is conjugated with 2-OA (Fig. 3E) and other electrophilic substances (Fig. 3F). Alternatively, GSSG can also oxidize thiols under