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Metabolite damage and repair in metabolic engineering design

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ABSTRACT

The necessarily sharp focus of metabolic engineering and metabolic synthetic biology on pathways and their fluxes has tended to divert attention from the damaging enzymatic and chemical side-reactions that pathway metabolites can undergo. Although historically overlooked and underappreciated, such metabolite damage reactions are now known to occur throughout metabolism and to generate (formerly enigmatic) peaks detected in metabolomics datasets. It is also now known that metabolite damage is often countered by dedicated repair enzymes that undo or prevent it. Metabolite damage and repair are highly relevant to engineered pathway design: metabolite damage reactions can reduce flux rates and product yields, and repair enzymes can provide robust, host-independent solutions. Herein, after introducing the core principles of metabolite damage and repair, we use case histories to document how damage and repair processes affect efficient operation of engineered pathways – particularly those that are heterologous, non-natural, or cell-free. We then review how metabolite damage reactions can be predicted, how repair reactions can be prospected, and how metabolite damage and repair can be built into genome-scale metabolic models. Lastly, we propose a versatile 'plug and play' set of well-characterized metabolite repair enzymes to solve metabolite damage problems known or likely to occur in metabolic engineering and synthetic biology projects.

1. Introduction

Metabolic engineering and metabolic synthetic biology (SynBio) focus on pathways and the fluxes through them, as does metabolic biochemistry in general. While essential, this focus tends to lead to an idealized, 'pathway-centric' mindset that sees enzymes as perfectly specific and their intermediates and products as the only relevant compounds in chemical space (Danchin, 2017; Lerma-Ortiz et al., 2016; de Lorenzo et al., 2015). Excessively pathway-centric thinking is problematic for metabolic engineering and SynBio as well as for basic understanding of metabolic networks because it downplays metabolism's dark underside (Golubev, 1996). In this neglected underside, metabolites continuously undergo damaging chemical and enzymatic side-reactions in vivo, and cells ceaselessly fight this damage with a suite of repair enzymes (Van Schaftingen et al., 2009). The products of metabolite damage are basically wasteful and often toxic. If allowed to accumulate, they can disrupt the function of both native and engineered pathways (Collard et al., 2016; Schwander et al., 2016).

After decades on the margins, metabolite damage reactions and the matching repair enzymes are now entering mainstream metabolic biochemistry, and examples of damage and repair are being uncovered at an increasing rate (reviewed by Hanson et al., 2016; Keller et al., 2015; Linster et al., 2013; Van Schaftingen et al., 2013). There is also a growing case list of metabolic engineering and metabolic SynBio projects whose design has taken metabolite damage and repair into account – with positive outcomes. To help consolidate the place of metabolite damage and repair in engineering project design, in this review we first summarize the core principles involved using engineering-relevant examples. We then illustrate these principles in action using a series of engineering case-histories, and describe how metabolite damage and repair reactions can be predicted and modeled. Lastly, we propose a set of repair enzymes that are likely to prove useful in diverse engineering projects. Fuller treatments of metabolite damage and repair – including the iconic oxygenase and other side-reactions of the photosynthetic CO_2-fixing enzyme RubisCO – are available in recent reviews (Erb and Zarzycki, 2016; Hanson et al., 2016; Linster et al., 2013).

2. Chemical and enzymatic damage to metabolites

As just noted, metabolite damage has two sources: spontaneous chemical reactions that take place under in vivo conditions, and

Abbreviations: SynBio, synthetic biology; NAD(P)HX, NAD(P)H hydrates

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enzyme errors. These errors involve an enzyme acting, at a low rate, on a substrate other than the physiological one (enzyme promiscuity or sloppiness), or catalysis of an abnormal ('misfire') reaction on the physiological substrate. Spontaneous reactions may involve only metabolites, i.e. small molecules, or attack by a metabolite on large molecules, i.e. proteins or nucleic acids. Whether of spontaneous or enzymatic origin, damage products are essentially a wasteful diversion of pathway flux, and are often inhibitory to enzymes or otherwise actively detrimental or functionally compromised (Linster et al., 2013). It is important to note at the outset that metabolite damage reactions are favored by high metabolic flux rates and large pool sizes (Ito et al., 2016; Rzem et al., 2007; Sullivan et al., 2013) because metabolic engineering approaches generally aim to achieve high fluxes and involve expanded pools. Engineering efforts thus often aggravate metabolite damage and must find ways to cope with it. While it is in principle possible to reduce damage from enzyme promiscuity or misfires by enzyme engineering, in practice this is not easy because of the trade-off between improved specificity and catalytic activity (Tawfik, 2014). Another potential way to reduce damage is by sequestering chemically reactive metabolites or problematic substrates for promiscuous enzymes within multienzyme complexes (metabolons) or special compartments, but as such approaches are in their infancy they are again not easy to implement (Singleton et al., 2014; Young et al., 2017). Fig. 1 illustrates the various types of metabolite damage using examples from three core sectors of primary metabolism: cofactors, sugar phosphates and amino acids. Damage reactions like those in Fig. 1 – chemical or enzymatic – occur in almost all cells almost all the time, and in at least some cases are accelerated by suboptimal conditions (Intlekofer et al., 2017; Piedrafita et al., 2015).

2.1. Cofactor damage

NADH and NADPH hydrates (Fig. 1A) are formed by a side-reaction of the core glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase and also spontaneously, particularly at high temperatures (Acheson et al., 1988; Marbaix et al., 2011; Oppenheimer and Kaplan, 1974). The hydrates (abbreviated NAD(P)HX) exist as *R* and *S* epimers; they inhibit various dehydrogenases (Prabhakar et al., 1998; Yoshida and Dave, 1975) and consequently cannot be allowed to accumulate.

5-Formyltetrahydrofolate (Fig. 1B) is formed from 5,10-methylenetetrahydrofolate by a misfire reaction of the near-ubiquitous folate-dependent enzyme serine hydroxymethyltransferase (Stover and Schirch, 1990) and is also formed spontaneously from 5,10-methylenetetrahydrofolate at low pH (Baggott, 2000). 5-Formyltetrahydrofolate is potent inhibitor of folate-dependent enzymes, and therefore must be removed to prevent its build-up (Stover and Schirch, 1993).

2.2. Sugar phosphate damage

Methylglyoxal, also called pyruvaldehyde (Fig. 1C), forms spontaneously from the triose phosphate intermediates of glycolysis – glyceraldehyde 3-phosphate and dihydroxyacetone phosphate – via an elimination reaction that is further accelerated by a side-activity of triose phosphate isomerase (Richard, 1993). Methylglyoxal, a reactive dicarbonyl compound, is a potent glycating agent that reacts spontaneously with the amino or thiol groups of other metabolites, proteins, and DNA (Fig. 1C) (Richarme et al., 2016, 2017; Thornalley, 2008). The initial glycation products (aminocarbinols or hemithioacetals) can undergo a series of dehydrations, oxidations, and rearrangements (Maillard reactions) that produce Schiff bases, Amadori products, advanced glycation end products, and cross-links between macromolecules (Richarme et al., 2015). There is thus a cascade effect in which the initial damage product (methylglyoxal) goes on to create further damage.

4-Phosphoerythronate and 2-phospho-L-lactate (Fig. 1D) are generated by side-reactions of two glycolytic enzymes (Collard et al., 2016). 4-Phosphoerythronate is formed as a consequence of glyceraldehyde 3-phosphate dehydrogenase acting on the pentose phosphate pathway intermediate erythrose 4-phosphate, which is itself a reactive glycating agent (Van Schaftingen et al., 2012). 2-Phospho-L-lactate is formed by pyruvate kinase acting on L-lactate. 4-Phosphoerythronate inhibits the pentose phosphate pathway enzyme 6-phosphogluconate dehydrogenase and 2-phospho-L-lactate inhibits phosphofructokinase, which makes the glycolytic activator fructose-2,6-bisphosphate.

2.3. Amino acid damage

Deaminated glutathione (Fig. 1E), in which a keto group replaces the α-amino group of glutathione's glutamyl moiety, is produced by a side-activity of many transaminases (Peracchi et al., 2017) and at least one decarboxylase (Novogrodsky and Meister, 1964). The ketoacid moiety of deaminated glutathione cyclizes spontaneously, giving two anomeric forms; this cyclization makes the transamination reaction that produces deaminated glutathione essentially irreversible. Deaminated glutathione may interfere with the activity of glutathione-dependent enzymes, and its formation can represent a major drain on the glutathione pool (Peracchi et al., 2017).

5-Oxoproline (also called pyroglutamate) is the lactam form of glutamate (Fig. 1F). It is formed via spontaneous cyclization of glutamine (Tritsch and Moore, 1962), glutamate (Park et al., 2001), and the proline biosynthesis intermediate γ-glutamyl phosphate (Orlowski and Meister, 1971). 5-Oxoproline forms very readily from glutamine; the conversion rate is 10% per day in physiological conditions (pH 7.2, moderate ionic strength, 37 °C) (Tritsch and Moore, 1962). Although the mechanism is unclear, 5-oxoproline is widely toxic (Niehaus et al., 2017; Park et al., 2001). In addition to being a universal metabolite damage product, 5-oxoproline is a normal metabolite of glutathione in eukaryotes (Kumar and Bachhawat, 2012).

2.4. Damage products pervade metabolomics profiles

Chemically or enzymatically damaged metabolites are almost surely major contributors to the 'dark matter' of metabolomes, until now largely ignored (Fiehn et al., 2011; Showalter et al., 2017). All the damage product examples above are detectable by targeted or untargeted GC-MS or LC-MS metabolomics analyses (Collard et al., 2016; Niehaus et al., 2014; Peracchi et al., 2017; Ringling and Rychlik, 2013; Shaheen et al., 2014; Thornalley and Rabbani, 2014; van der Werf et al., 2007). It follows that the same should be true of the plethora of other damage products that side-reactions can generate from the enormous chemical diversity of metabolites (Fiehn et al., 2011; Peracchi et al., 2017). A major obstacle to identifying such damage products is that – like most of the examples above – they are not in metabolite libraries and they cannot be purchased. In a later Section 5.1 we show how computational chemistry can help overcome this obstacle by predicting the structures and properties of damage products for which no experimental data are yet available.

3. Damage repair enzymes

Damage repair enzymes, defined broadly, either undo damage by reconverting damaged molecules to normal ones, or dispose safely of harmful damage products by converting them to harmless ones. These activities are also called metabolite proofreading and damage preemption, respectively (Linster et al., 2013; Van Schaftingen et al., 2013). We illustrate repair principles using the enzymes that repair the cofactor, sugar phosphate, and amino acid damage products described above (Fig. 1).

3.1. Cofactor repair

NADHX and NADPHX are reconverted to NADH and NADPH by the