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System wide cofactor turnovers can propagate metabolic stability between pathways

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ABSTRACT

Metabolic homeostasis, or low-level metabolic steady state, has long been taken for granted in metabolic engineering, and research priority has always been given to understand metabolic flux control and regulation of the reaction network. In the past, this has not caused concerns because the metabolic networks studied were invariably associated with living cells. Nowadays, there are needs to reconstruct metabolic networks, and so metabolic homeostasis cannot be taken for granted. For metabolic steady state, enzyme feedback control has been known to explain why metabolites in metabolic pathways can avoid accumulation. However, we reasoned that there are further contributing mechanisms. As a new methodology developed, we separated cofactor intermediates (CIs) from non-cofactor intermediates, and identified an appropriate type of open systems for operating putative reaction topologies. Furthermore, we elaborated the criteria to tell if a multi-enzyme over-all reaction path is of in vivo nature or not at the metabolic level. As new findings, we discovered that there are interactions between the enzyme feedback inhibition and the CI turnover, and such interactions may well lead to metabolic homeostasis, an emergent property of the system. To conclude, this work offers a new perspective for understanding the role of CIs and the presence of metabolic homeostasis in the living cell. In perspective, this work might provide clues for constructing non-natural metabolic networks using multi-enzyme reactions or by degenerating metabolic reaction networks from the living cell.

1. Introduction

Metabolic reaction networks entail thousands of enzymes and connect to a myriad of metabolites, with intermediate number being of the order of 10^3 and average concentration 32 μM for a prokaryote (Atkinson, 1968; Palsson, 2011). A remarkable feature of such networked reaction system is that all the intracellular metabolic intermediates (or internal metabolites) are kept at metabolic homeostasis (in a physiological term) or low level steady state (in an engineering-orientated term). Such networks have been observed to be inherently robust (Stephanopoulos and Vallino, 1991; Ishii et al., 2007; De la Fuente et al., 2014), predominantly operated at thermodynamic non-equilibrium (Prigogine, 1955; Fell, 1997), and are able to accommodate a widely varied metabolic fluxes (Stephanopoulos, 1998; Villadsen, 2016).

Metabolic control and regulation take place notably at metabolic and gene-expression levels (Reich and Sel'kov, 1981; Palsson, 2015). The time constant (or time scale) can be used to differentiate study regimes (Palsson, 2011; Reich and Sel'kov, 1981). Control and regulation at metabolic level is a spontaneous, dynamic reaction process. Representative time constants at this level are in the range of 0.1–10 s (Reich and Sel'kov, 1981; Wiechert, 2002). In contrast, gene-expression and transcriptional control and regulation regimes for remapping metabolic fluxes involve biosynthesis/biodegradation of large molecules (e.g. enzymes), and their typical time constants are >1 h (Wiechert, 2002; Palsson, 2011). The present work concerns with the control and regulation at the metabolic level with small time constants.

An important milestone in understanding metabolic regulation at metabolic level came from the discovery of enzyme negative feedback control by inhibition (Umbarger, 1956; Yates and Pardee, 1956; Gerhart and Pardee, 1962). Supported by mechanism explorations, such feedback control mechanism largely avoids metabolite build-up (Monod et al., 1963; Savageau, 1969, 1974; Chandra et al., 2011; He et al., 2013). The most efficient feedback

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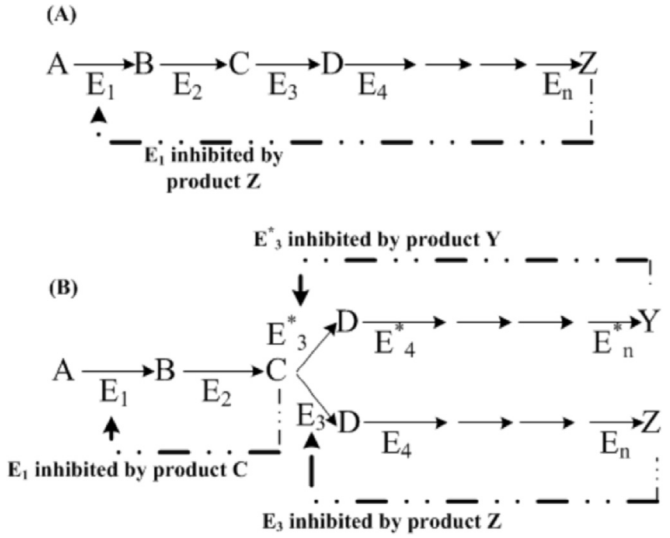

Fig. 1. End-product feedback control by the minimal inhibition loops for (A) linear pathway with only one inhibition loop, and (B) bifurcated pathway with 3 inhibition loops.

control for a linear pathway at metabolic level is end-product inhibition, which may be termed as the optimal design of feedback inhibition control (Savageau, 1974). It is optimal because such feedback control requires only one inhibition for a linear carbon-backbone pathway (Fig. 1(A)), and at least 3 feedback inhibition loops for a bifurcated pathway system (Fig. 1(B)) (Savageau, 1974, 1976). Even in the case that this mechanism is not functioning, the cell can still use a contingency mechanism to enzymatically hydrolyse the end-product to avoid its build-up (Reaves et al., 2013).

Nevertheless, spontaneous establishment of metabolic homeostasis does not rely only on allosteric enzyme regulation mechanisms. Morowitz et al. were probably the first in reporting the very nature of such autonomous homeostasis – in their wording "passive stability in a metabolic network" (Morowitz et al., 1964). These authors attributed a number of factors to the occurrence of metabolic homeostasis. Among their claims, the role of carbon backbone topology has been well substantiated (Morowitz et al., 2000; Smith and Morowitz, 2004), but the role of the cofactor topology, superimposed onto the carbon backbone topology, has never been evidenced.

As a broad objective, this work is aimed to shed light for understanding why a metabolic network is stable and why all metabolite concentrations are kept at low levels. With low level steady state being taken for granted, up to now the metabolic control and regulation community has given priorities to understanding how metabolic fluxes are amenable to changes in magnitude and distribution (Hofmeyr and Cornish-Bowden, 1991). Such convention had been directed to curate genome-based metabolic reaction networks without involving metabolite concentrations (Palsson, 2015). Understanding of metabolic homeostasis observed for living cells (Savageau, 1976) has long been separated from research on metabolic flux control and regulation (Kacser and Burns, 1973; Heinrich and Rapoport, 1974). In the past, this arbitrary separation had not caused concerns because the metabolic networks investigated were invariably associated with living cells where metabolic homeostasis is out of question. Nowadays, there are challenging needs for reconstructing metabolic networks in synthetic biology or metabolic engineering. In contrast, metabolic homeostasis for multi-enzyme reaction systems under investigation can no longer be taken for granted (Rollin et al., 2015; Keller et al., 2016). In detail, the present work will describe an emergent property resulted from interactions between cofactor intermediate (CIs) turnovers and enzyme feedback inhibition. This property may play a pivotal role to the emergence of metabolic homeostasis.

2. Theoretical framework

In order to unfold this research, we first separated CIs from non-cofactor intermediates (NCIs) to reveal the important role of the CIs, and then conceptually connect the systems used to various multi-enzyme reaction systems. We quantitatively illustrated how the metabolic homeostasis of in vivo systems is robust against an environmental perturbation (i.e. substrate concentration variation). This outcome then guided us in validating a suitable type of working open systems.

2.1. CIs versus NCIs

The metabolic network entails 2 types of internal metabolites: (i) a limited number of paired or grouped CIs and (ii) a vast number of standalone NCIs. Enzymes utilise NCIs for stepwise chemical conversion from substrate(s) to desired end products. CIs are usually paired and each paired CI pool size is fixed for global uses at the metabolic level. Common CI pairs/groups include ATP/ADP/AMP (for energy transduction), NAD^+/NADH, $NADP^+$/NADPH, Q/QH_2 (for redox transfer), and CoA-SH/acetyl-CoA/malonyl-CoA/succinyl-CoA (for carbon chain translocation). The energy CI pool should be referred to as ATP+ADP+AMP+Pi at the metabolic reaction time scale (Wiechert, 2002). The catabolic and anabolic redox CIs pool sizes are NAD^++NADH and $NADP^+$+NADPH respectively. The CoA-related CI pool size is the sum of CoASH in free form and covalently bound to certain carbon backbone chains (e.g. acetyl-CoA). Hence, the CoA-bound metabolites are both CIs and NCIs. Besides, metabolism contains further unique reaction topologies comparable to CI turnovers. For instance, cyclic-like reactions for transferring nitrogen-containing groups are different in that there is a net consumption of e.g. NH_2 groups.

Each CI pool size is inherently small (e.g. ATP+ADP+AMP pool typically at 2 mM) (Atkinson, 1977; Palsson, 2011). To simultaneously sustain so many reactions in a homogeneous space, ATP (ADP) has no options but to turn around at globally balanced rates through numerous ATP (ADP)-consuming and generating reactions. Usually, an NCI conversion reaction is realised by utilising a CI as the second reactant. In such network topologies, each CI [e.g. B (Q) out of the B + Q pool, Fig. 2(A)–(C)] can only stay either in steady or oscillating status (Chassagnole, 2002; De la Fuente et al., 2014). At such metabolic homeostasis, the net B-to-Q or Q-to-B flux can be termed as CI turnover flux or CI flux. To make a distinction, the well-understood carbon backbone flux (Stephanopoulos et al., 1998; Villadsen, 2016) can thus be termed as NCI flux. Each reaction stoichiometry connects a CI flux to relevant NCI flux (es). Without metabolic homeostasis, any discussions on fluxes will become groundless and meaningless. This work underpins how metabolic homeostasis may be constructed from scratch, and metabolic flux regulation is not the focus.

2.2. The in-vivo-like system

In the synthetic biology era, the boundary separating an in vivo and an in vitro systems is becoming more and more blurred. In order to discuss this subject systematically, it is useful to define a new concept termed the in-vivo-like system. In the present context, it refers to a multi-enzyme reaction system which possesses CI turnover and metabolic homeostasis. Such a system can be either completely man-made, or derived from native cells. Indeed,