

Enzyme-malfunctioning in the light of pathway analysis

An example from nucleotide metabolism

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1 Introduction

Many diseases occur as a consequence of enzyme deficiencies. The missing or incompletely operating enzyme can imply the interruption of a synthesis pathway and, thus, the lack of a product, or it can lead to the accumulation of an intermediate that is toxic when present in high concentration, for example, by inhibiting another essential enzyme. A clinically important and well known example is the enzyme hypoxanthine-guanine ribosyltransferase (HPRT, EC. 2.4.2.8) – an enzyme of the nucleotide metabolism. When this enzyme does not operate properly, uric acid accumulation, kidney stones, gout, and even the so-called Lesch-Nyhan syndrome occur, depending on the degree of malfunctionality [1].

For understanding the metabolic effects resulting from enzyme deficiencies, flux balance analysis, in particular the concept of elementary flux mode (EFM), can be of great help. Its advantage consists in coping with systems having available only the stoichiometric properties. An EFM represents a minimal set of enzymes that could operate at steady state with all irreversible reactions used in the right direction [2]. A biochemical system can be decomposed into a unique set of EFMs, which stand for all significant biochemical interconversions. A necessary step is to split the set of metabolites into external and internal metabolites. The source and the sink metabolites have to be external. Sometimes it is useful to consider also those metabolites as external which interconnect many different branches of metabolism. The remaining metabolites are internal.

2 Results

In the reaction scheme depicted in Fig.1, AMPase, PNPase and HPRT are low specificity enzymes, being able to catalyse more than one reaction. In order to calculate the EFMs, each function was numbered. In terms of enzyme functions, the corresponding modes, which also coincide with the vectors of the convex basis of the nullspace of the stoichiometric matrix, read:

1: PNPase2 PNPase3 AMPase1 AMPase3 ADA Xd XOR PRPPs HPRT2

2: PNPase2 PNPase4 AMPase1 AMPase4 ADA Xd XOR PRPPs HPRT3

3: (2 PNPase2) AMPase1 AMPase2 ADA Xd XOR PRPPs HPRT1

4: PNPase2 PNPase1 (2 AMPase1) ADA Xd XOR PRPPs APRT.

In terms of overall reactions these four modes perform the same biochemical interconversion:



If the definition of an EFM is correctly applied [3], there are actually two EFMs, containing enzymes {PNPase, AMPase, ADA, Xd, XOR, PRPPs, HPRT} and {PNPase, AMPase, ADA, Xd, XOR, PRPPs, APRT} respectively. If HPRT does not operate, PRPP accumulates and at the first sight, it seems that the yield of urate decreases. But this is not the case. Accumulation of PRPP forces the cell to start the *de novo* pathway (Fig. 2) that involves enzyme PPAT, which is able to consume PRPP (glutamine + PRPP = Ribosylamine5P + PP_i + glutamate). This pathway pumps new nucleotides (IMP) into the system, which, if not used, have to be removed by degradation to urate.

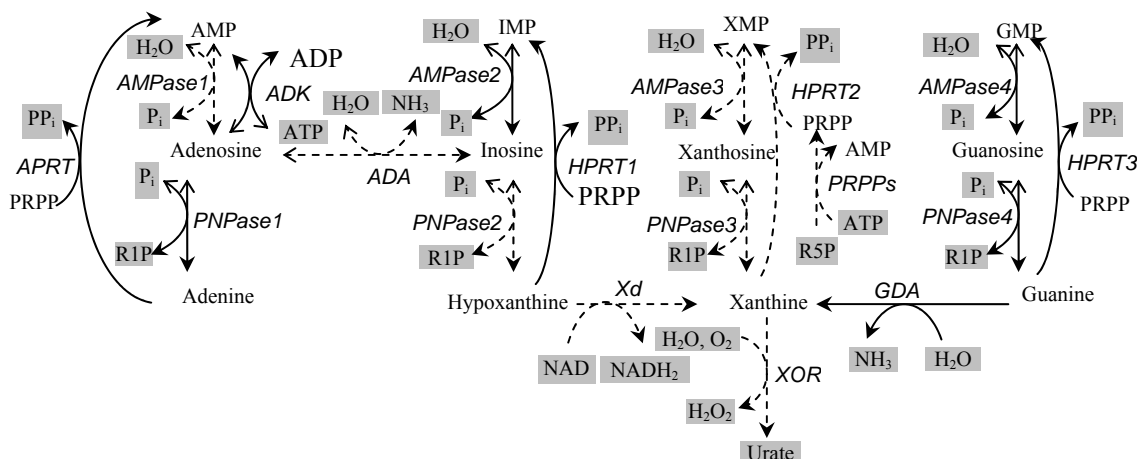


Figure 1: Part of the purine metabolism. (Ir)reversible reactions are depicted through (one) double-headed arrows. Their full ends indicate the main direction of the corresponding reaction. Enzyme names are given in italics. The metabolites written in grey boxes are assumed to be external, while the others are assumed to be internal. The enzymes taking part in the first EFM are depicted with interrupted arrows.

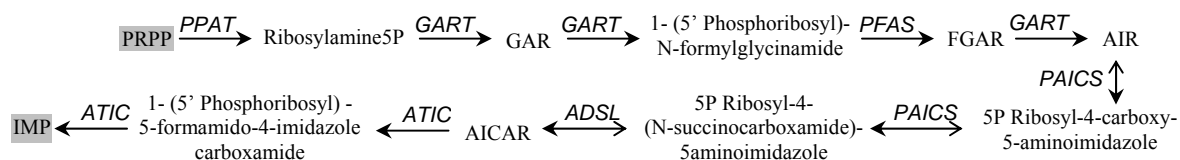


Figure 2: *De novo* EFM. *GART*, *PAICS* and *ATIC* are enzymatic complexes. The enzyme names conform with KEGG database [4]. Cofactors were omitted for the sake of simplicity.

A proper way to avoid urate accumulation is to inhibit the enzyme XOR having urate as its direct product: $\text{Xanthine} = \text{urate} + \text{H}_2\text{O}_2$. A potent inhibitor of that enzyme is allopurinol. However, after inhibition of XOR all flux modes providing urate before will then produce xanthine, through enzymes as: *Xd*, *PNPase2*, *GDA*. Unfortunately, xanthine accumulation is still dangerous, leading to xanthinuria and stones formation [5], which are, nevertheless, milder than Lesch-Nyhan syndrome and gout.

3 Discussions

The EFM concept is a reliable instrument, that offers the possibility to detect risk enzymes and to develop eventually approaches to cure the corresponding diseases. Considering the relation between the nucleotide metabolism and the DNA replication, the analysis of purine and pyrimidine metabolism can be extended to obtain some points for control of cancer and parasite infections.

References

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