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A mathematical approach to the carbohydrate catabolism in *Ascaris lumbricoides*

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Abstract

Metabolic pathways within a biological cell serve as a key for the various functions of the living organism. In this article, the authors have considered a mathematical study of the carbohydrate catabolism of the nematode, *Ascaris lumbricoides*. The technique involves setting up of a Stoichiometric matrix (S) for the biochemical reactions involved in the process and calculating biochemically meaningful basis vectors for the null space of S. In the present study, the stoichiometric matrix S is of order 26x40 for which fourteen column vectors are obtained as forming the "basis". It is found that twelve column vectors represent the reversible reactions involved in the metabolic pathway and two-column vectors represents stoichiometry of the network.

Keywords: *Ascaris lumbricoides,* Carbohydrate metabolism, Nematode, Mathematical approach.

Introduction

Metabolic pathway acts as an engine for the function of the living organism. A metabolic pathway is described mathematically using the concept of 'basis' in linear algebra, a set of constraints are imposed so that the basis obtained is not only mathematically correct but also biochemically feasible. The approach adopted was used to study the metabolic reaction network of the human erythrocyte (Christophe & Bernard, 1998).

The process of converting a "substrate" to a "product" with the help of a biocatalyst (enzyme) is called a biochemical reaction. The sum of all physical and chemical processes of anabolism and catabolism taking place within an organism is a metabolic pathway. Anabolism is the constructive metabolism by which the food materials are transformed into the body tissues, and energy for growth, repair and general functions of the body. Catabolism is the destructive metabolism by which the substances are broken down into the simpler substances which are usually excreted. The collection of metabolic pathways is called a metabolic network. Flux in biology relates to movement of a substrate between compartments. Metabolic flux refers to the rate of flow of metabolites along a metabolic pathway or even through a single enzyme. The principle of law of conservation of mass is used in mass balances to evaluate energy and the conversion of substrate to products. A region where the process takes place is a system, while the surroundings are everything outside the system. An imaginary barrier around the system is referred to as system boundary. An open system is one where mass is transferred from the system to its surroundings or vice versa. A closed system is one where mass does not enter or leave the system boundary. Mass balances are based on the law of conservation of mass, defined under general balance equations, which is represented by

Input + Generation - (Output + Consumption) = Accumulation. (1)

This equation can be used for any material that enters or leaves the system being evaluated, including compounds, molecules and atoms. When there is a continuous flow of constituents into and out of the system, the process is a continuous process. In reacting systems the mass balance equations take the form of equation (1) (Buckingham *et al*., 1994). For steady state flow processes (accumulation = 0) the mass balance reduces to

$$\sum_{kii} input + \sum_{kig} generation = \sum_{kio} output + \sum_{kic} consumption$$

Where kig (kic) is the number of reactions where component 'i' is generated (or consumed). Reactants are present in the stoichiometric proportions if they are present in the same proportions shown in the balanced reaction equation. For any given biochemical reaction network a system boundary can be drawn around all of the chemical reactions occurring within a system. A cell is considered as an open system where there is exchange of metabolites within and outside the cell.

Materials and methods

Carbohydrate catabolism in Ascaris lumbricoides

Ascaris lumbricoides is an endo-parasite present in the digestive tract of the human, which absorbs carbohydrate to cause digestive disorder in the host. The parasite has lost metabolic functions in the course of their evolution and has a rather different glycolytic design well suited for a parasitic life (Fairlamb,1989). The parasitic nematodes have their evolutionary origin in environments where oxygen is present in low concentrations or completely absent (Bryant ,1994). The first observed metabolic differences between the parasitic helminths and their hosts was the identification of novel anaerobic mitochondrial energy generating pathways wherein the initial end products are succinate and pyruvate (Saz & Bueding,1966). The ratio of the activity of the enzyme that act on phosphoenolpyruvate, Pyruvate kinase : Phosphoenolpyruvate kinase (Pk:PEPCK) decides whether the metabolism of a helminth relies on glycolysis and produce lactate or fix CO_2 and obtain anaerobic energy generating pathways (Bueding & Saz, 1968). Helminth parasites absolutely depend on carbohydrate

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either in the form of glycogen or exogenous glucose as their main energy source. The pathways of carbohydrate catabolism are essentially anaerobic. Parasites are divided into two major groups based on the end products of the carbohydrate catabolism. The type-I rely on glycolysis and produce lactate or some other derivative of pyruvate as end products of carbohydrate breakdown. The type-II fixes carbon-dioxide and can be described as the *Ascaris* type metabolism. The regular glycolytic conversion of glucose to lactate is present in the larvae stage of the parasite which require oxygen for their development, the energy metabolism is aerobic (Takamiya *et al*., 1993). In the adult stage of the parasite the energy metabolism is predominantly anaerobic and the initial end products, succinate and pyruvate are converted by a series of steps into the branched chain fatty acids 2-methylvalerate and 2-methylbutyrate respectively, the major fermentation products of *Ascaris* metabolism (Suarez de Mata *et al*., 1983; Zadila *et al*., 1997).

In adult parasite, the anaerobic glycolytic pathway, PEPCK-succinate pathway can adapt to low oxygen tension and ATP can be produced under the low oxygen conditions. The glycolytic pathway in *Ascaris lumbricoides* has the normal glycolytic sequence till PEP. The activity of the enzyme pyruvate kinase is low, so instead of forming pyruvate, CO_2 fixation takes place and PEP is converted to oxaloacetate by the action of the enzyme phosphoenolpyruvate carboxykinase. The oxaloacetate thus formed is reduced in the cytoplasm to malate by malate dehydrogenase. Malate is then transported into the mitochondria via a phosphate dependent translocase. Inside the mitochondria malate undergoes a dismutation reaction, a part is oxidatively decarboxylated to pyruvate via an NAD^+ linked malic enzyme and a part to fumarate which is in equilibrium with malate via the enzyme fumarase. In *Ascaris,* fumarase occurs both in the mitochondria and cytoplasm. Fumarate is reduced to succinate by fumarate reductase. The pathway from oxaloacetate to succinate is the same as the second span of the TCA cycle, only that in this case it is working in the reverse direction. *Ascaris* is often observed to possess a partial reverse TCA cycle. The initial end products of carbohydrate breakdown in helminths which fix CO_2 are thus pyruvate and succinate, both produced in the mitochondria. The study of energy metabolism of *Ascaris lumbricoides* is of immense interest since it differs from that of the host, human. The PEP branch point is very crucial in helminth metabolism and at this juncture there is a difference in the metabolism of the host and the parasite. Comparison of host and parasite metabolism may find potential target for drugs such that the drug derange the metabolism of the parasite and not of the host. There is a divergence in the pathway of the host and parasite at PEP branch point. In mammals PEP is converted to pyruvate by the activity of pyruvate kinase which is then reduced to lactate or ethanol in the cytoplasm or translocated into the mitochondria for further oxidation by the tricarboxylic acid cycle. The enzyme PEPCK is present in the parasite *ascaris* and human (host) but its function is different. In the host this enzyme catalyses the conversion of oxaloacetate to phosphoenol pyruvate and CO_2, while in the parasite it catalyses the conversion of phosphoenol pyruvate and CO_2 to oxaloacetate in the mitochondrion anaerobically (Mansour, 2002). This functional difference is related to significant differences in the molecular properties of PEPCK enzyme. This is the first divergent step in the metabolic pathway of the host and parasite. The reaction network analyzed is shown in Fig.1 (Barrett, 1984; Komuniecki & Tielens, 2003) and it is translated into a stoichiometric matrix of order 26x40. The Stoichiometric matrix S is a matrix representation of a biochemical reaction. It is an m x n matrix with m number of metabolites and n number of reactions taking place within the network. Each row in S corresponds to a metabolite and each column in S corresponds to a reaction. It is constructed by the following rule:

$$S = \begin{cases} +c \text{ if the reaction produces metabolite X} \\ -c \text{ if the reaction consumes metabolite X} \\ 0 \text{ if the reaction neither produces nor consumes metabolite X,} \end{cases}$$

c is the stoichiometric constant.

For example consider the system of reactions,

$S_1 \rightarrow S_2$,

$S_2 \leftrightarrow S_3$

This system has three reactions and three metabolites. The stoichiometric matrix of the system of reactions S =

$$\begin{bmatrix} -1 & 0 & 0 \\ 1 & -1 & 1 \\ 0 & 1 & -1 \end{bmatrix}$$

S connects all the metabolites in a defined metabolic system. S contains both internal and exchange reactions. A system boundary can be drawn around all of the chemical reactions occurring within a system. The internal fluxes are referred to as $v_1 - v_i$ which are the reactions and their relative activity in the system.'i' is the number of internal fluxes. Only those fluxes corresponding to the transport of a metabolite are permitted to cross the system boundary. Those fluxes constitute sources and sinks in the system and will be referred to as exchange fluxes denoted as b_1-b_j where *'j'* is the total number of exchange fluxes. All of the exchange fluxes are defined as positive if the activity is draining a metabolite or leaving the system. To obtain theoretically and biochemically feasible basis two constraints are imposed on both the internal and exchange fluxes (Christophe & Bernard, 1998). i) We constrain all internal fluxes to be positive; $v_i \geq 0$ (all internal fluxes), ii) we relatively impose further restriction on the values of the exchange fluxes.

$b_j \geq 0$ (exit only) and $b_j < 0$ (enter only).

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Table 1. Names and abbreviations of the twenty six metabolites included in the reaction network

Internal	Exchange	Abbreviation	Metabolite
	x	GLU	Glucose
x		G6P	Glucose-6-phosphate
x		F6P	Fructose-6-phosphate
x		F1,6 P	Fructose-1-6-diphosphate
x		GA3P	Glyceraldehyde-3-phosphate
x		DHAP	Dihydroxyacetone phosphate
x		1,3DPG	1-3-diphospho glycerate
x		3PG	3-Phosphoglycerate
x		2PG	2-Phosphoglycerate
x		PEP	Phosphoenol pyruvate
x		OAA	Oxaloacetate
x		MAL	Malate
x		FUM	Fumarate
	x	SUC	Succinate
	x	PYR	Pyruvate
	x	ATP	Adenosine triphosphate
	x	ADP	Adenosine diphosphate
	x	NAD^+	Nicotinamide dinucleotide
	x	$NADH^+$	Reduced Nicotinamide dinucleotide
	x	ITP	Inosine triphosphate
	x	IDP	Inosine diphosphate
	x	CO_2	Carbon dioxide
	x	H_2O	Water
	x	PI	Inorganic phosphate
	x	H+	Hydrogen ion
	x	CoA	Co factor A

Following the above definitions the pathway leading to the formation of succinate and pyruvate in the parasite consist of twenty six metabolites and forty fluxes (both internal and external). The 26 metabolites involved in the reaction network are classified as internal or exchange metabolite corresponding to the presence of an exchange flux for a particular metabolite and their names and abbreviations with the classification type are given in Table 1.

The name of enzymes involved in the network and the corresponding flux vectors are listed in Table 2. External metabolites are often located at the boundaries of the mode. Currency metabolites such as ATP, ADP, NAD^+, NADH, PI, metabolites buffered by connection to reservoirs such as H_2O and CO_2, metabolites such as glucose, glycogen that serve as entry and exit metabolites are termed as external. Other than external ones, all remaining intermediates are termed as internal metabolites, they are either consumed or produced, and their concentrations change accordingly. The involvement of the exchange fluxes namely ATP, ADP, NAD, $NADH_2$, ITP, IDP, CO_2 , H_2O, Pi, CoA, H^+ are shown in Fig.1. The reactions involved in the stoichiometric matrix are shown in Fig.1.

The Stoichiometric matrix S is a tool for deriving conservation relations of a chemical system. In following the law of conservation of mass and with the stoichiometric matrix S, a flux vector v, the biochemical network can be written in the form of a homogenous linear equation in the matrix form as

$$S.v = 0 \quad (2)$$

v is an nx1 matrix. The Stoichiometric matrix S contains important information about the structure of the metabolic network. The Stoichiometric matrix S remains constant at all times and the flux vector v is a variable indicating different flux distribution patterns. Furthermore the matrix S is defined from the metabolic genotype of an organism. It is aimed at using the Stoichiometric matrix S for identifying the inherent structure and connectivity of the metabolites within a metabolic network. In terms of mathematics, this is the vector space containing all possible solutions of the system of equations (2). The set of all possible solutions of system (2) is a subspace of the vector space and it is the null space of S. The dimension (Dim) of the null space of system (2) is n-r where n is the number of unknowns (number of reactions) and r is the rank of the stoichiometric matrix. The number of free variables in the original set of linear equations is referred to as the rank of S, which is given by the rank theorem (Gilbert Strang, 2006):

Dim (null space S) + Rank(S) = number of columns

The dimension n-r is called the nullity of the matrix S. The null space of S spans the steady state pathway space of a biochemical network (Bernhard O Palsson, 2006). A set of vectors called spanning set can be generated to define the entire null space. The most efficient way to span a null space is by using its basis. The minimum number of linearly independent vectors spanning a vector space is called the basis of that vector space. The number of vectors in a basis of a vector space is the dimension of the vector space (dimension of the null space of S). Our aim is to find a theoretically and

Table 2. Name of the enzymes included in the reaction network

Flux	Name of the enzyme	EC No.
V_1	Hexokinase	2.7.1.1
V_2,V_3	Glucose-6-Phosphate isomerase	5.3.1.9
V_4,V_5	6-Phophofructokinase	2.7.1.11
V_6	Fructose biphosphate aldolase	4.1.2.13
V_7,V_8	Triose-phosphate isomerase	5.3.1.1
V_9,V_{10}	Glyceraldehyde-3-phosphodehydrogenase [phosphorylating]	1.2.1.12
V_{11},V_{12}	Phosphoglycerate kinase	2.7.2.3
V_{13},V_{14}	Phosphoglycerate mutase	5.4.2.1
V_{15},V_{16}	Enolase	4.2.1.11
V_{17},V_{18}	Phosphoenolpyruvate carboxykinase [GTP]	4.1.1.32
V_{19},V_{20}	Malate dehydrogenase	1.1.1.37
V_{23},V_{24}	Fumarate hydratase	4.2.1.2
V_{25},V_{26}	Fumarate reductase	1.3.1.6
V_{21},V_{22}	Malic enzyme	1.3.1.38

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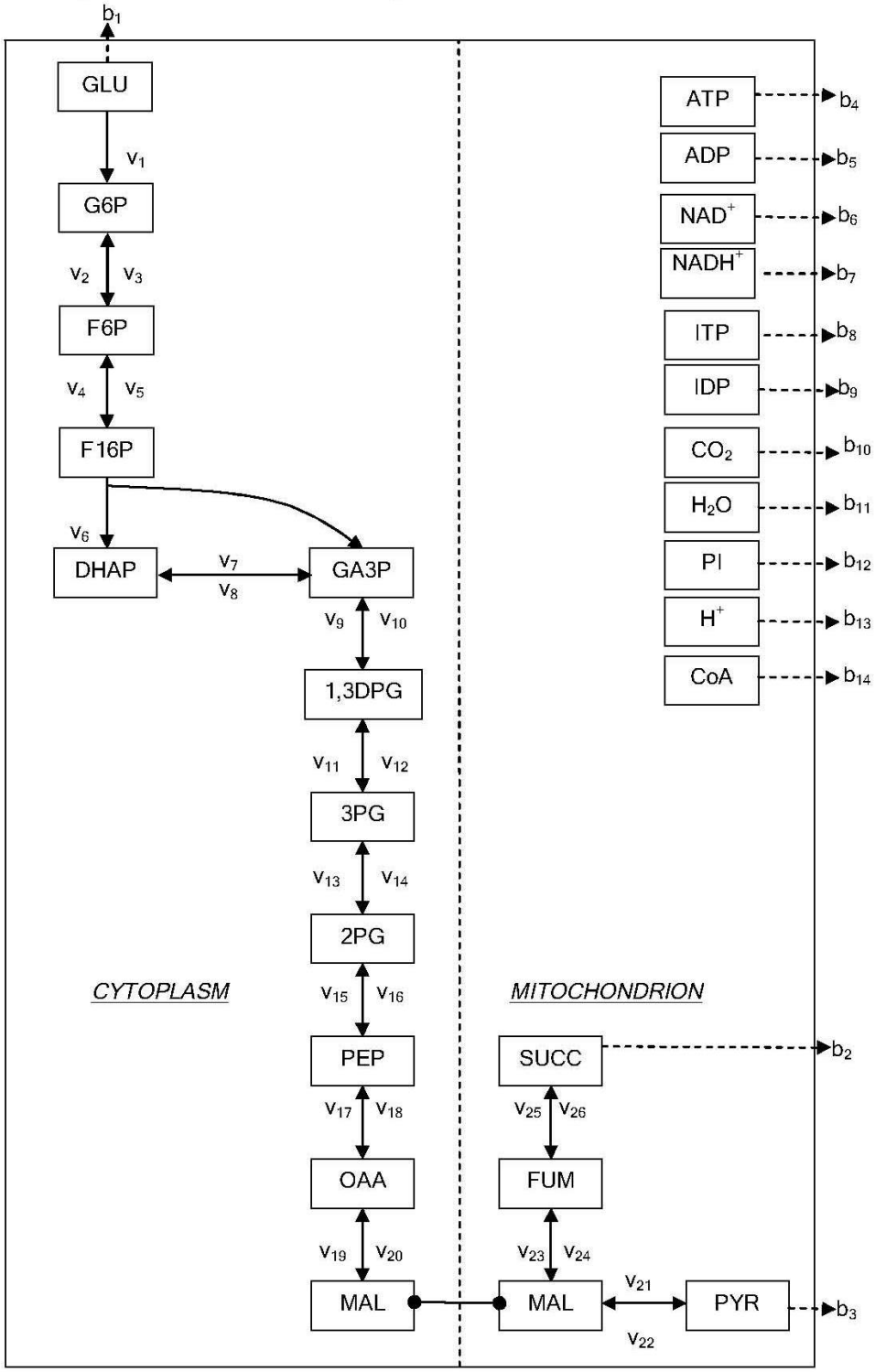
Fig.1. Reaction network of the carbohydrate catabolism in Ascaris lumbricoides



biochemically meaningful basis for the null space. The null space of S consists of all vectors that satisfy $S.v = 0$.

Results and discussion

Using MATLAB 7.0 a set of basis vectors, which spans the null space of S was determined. Fourteen column vectors were identified as constituting basis for the stoichiometric matrix S. They satisfy the condition (i) and (ii) without any basis transformation. A basis transformation is necessary if any of the internal fluxes v_i are less than zero and the exchange fluxes b_j is less than zero for exit and greater than zero for entry. They form a theoretically and biochemically feasible basis with negative value for exchange flux of glucose. Out of these fourteen column vectors, twelve column vectors represented the cycling of reversible reactions, which have no net effect on the input / output of the system as indicated by the absence in activity of any exchange fluxes. The remaining two vectors constitute true biochemical pathways of the system. The net balance equations of basis pathways are used to span the null space of the Stoichiometric matrix. The net reaction equations of the fourteen basis pathways are:

P_1: Glucose + 2IDP + 4 PI + 2 ADP + 2 $NADH^+$ + 2 H^+ + 2 CO_2 = 2 ITP + 4 H_2O + 2 ATP + 2 NAD^+ + 2 succinate

P_2: Glucose + 2 IDP + 2 PI + 2 NAD^+ = 2 ITP + 2 H_2O + 2 NADH + 2 H^+ + 2 Pyruvate

P_3–P_{14}: no net reaction.

The first pathway (**P_1**) refers to the catabolism of glucose through glycolytic pathway to the formation of Phosphoenol pyruvate and further metabolized to malate, which is reduced to succinate with the formation of ATP. This pathway is the energy producing one wherein there will be net gain of 2 moles of ATP and 2 moles of ITP.

The second pathway (**P_2**) also follows the glycolytic pathway and malate produced in the mitochondria is oxidized to pyruvate.

Balance in redox of this metabolism is maintained as the NADH formed in the cytoplasm is oxidized to NAD by the reduction reaction of oxaloacetate to malate, and

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NADH produced in the mitochondrion is oxidized by the reduction reaction of fumarate to succinate.

The net stoichiometry of the carbohydrate catabolism in *ascaris* is

Glucose + 2 IDP + ADP + 3 PI + CO_2 = Succinate + Pyruvate + 2 ITP + ATP + 3 H_2O .

The result obtained by the stoichiometric modeling using the concept of linear algebra is in agreement with *ascaris* type system of conversion of one mole of glucose to one mole each of succinate and pyruvate which gives a net yield of three ATP per mole of glucose catabolised (Barrett, 1994).

Conclusion

The carbohydrate catabolism of the parasite *Ascaris lumbricoides* is translated into a set of linear pathways by calculating biochemically meaningful basis vectors for the null space of the stoichiometric matrix. These linear pathways construct the transformation route from a given substrate to a given product which is biochemically feasible. In this work the anaerobic metabolism of the parasite is reviewed, modeled using stoichiometric analysis and found to be biochemically feasible.

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