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ORIGINAL ARTICLE

An in silico model of enterocytic glutamine to citrulline conversion pathway

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Abstract Enterocyte is one of the main sites of amino acids metabolism and particularly of the citrulline biosynthesis. Working at the cellular scale and applying ordinary differential equations (ODEs) formalism, we have built a mathematical model of the enterocytic glutamine to citrulline conversion in the fasting state. This model enables us to test different physiopathological scenarios of enzyme activity loss. Results from two different approaches were compared: a standard approach (KA) based on the Michaelis–Menten assumptions and an association–dissociation approach (VH) based on the kinetic mass action law. For both approaches, ODEs system was numerically solved using Mathematica™. In both cases, the model correctly predicts the physiological plasma citrulline steady-state, but the two approaches present clear differences for metabolites of enzymes having a complex mechanism, challenging the validity of the KA approach in such cases. When physiopathological scenarios of enzyme activity loss are simulated, both approaches predict a very sharp transition from the physiological citrulline plasma level to the lack of its production: the concentration profiles of these simulations show a clear threshold of which characteristics vary with the involved enzyme. Moreover, amongst all enzymes included in the model, the ornithine aminotransferase (OAT) shows the highest sensitivity in the system whatever the approach used. This model points out the limits of the Michaelis–Menten approach to model complex enzyme mechanisms. It highlights the key role of OAT in the studied citrulline synthesis pathway and also suggests an order of magnitude about the optimal ratio of enzyme concentrations in this pathway.

Keywords Citrulline · Glutamine · Enterocyte · Mathematical modelling · Ordinary differential equations

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Introduction

L-Arginine (Arg) and L-glutamine (Gln) are respectively considered as semi or conditionally essential amino acids. Indeed, while the body synthesises enough quantities of these amino acids in most physiological conditions, their production becomes insufficient in catabolic states induced by injuries such as surgery or sepsis (Castillo et al. 1993, 1994; Marc Rhoads and Wu 2009). These amino acids, connected through several metabolic pathways distributed over different organs and cell types, play a key role in the regulation of several physiological functions such as the immune response or nitrogen homeostasis (Alican and Kubes 1996; Appleton 2002; van de Poll et al. 2007; Souba et al. 1985).

One of the main metabolic intermediaries between Arg and Gln is L-citrulline (Cit), a non-essential amino acid produced mainly from Gln in the intestine (Wu 1998). Contrary to Arg, which is actively taken up by the liver and degraded by arginase, Cit escapes liver uptake and is converted in the kidney (Windmueller and Spaeth 1981) into Arg which is then available for other organs and cells.

Therefore, Cit can be considered as the true precursor of body Arg. The enterocyte can use Gln from both its luminal and basolateral sides, basolateral Gln being mainly synthesised by muscles. Taking into account the important roles of these amino acids in physiological and pathological situations, a better understanding of these amino acid fluxes would enable to improve both the prevention of chronic disease such as the metabolic syndrome and the treatment of catabolic situations. This has been the focus of clinical (Burlina et al. 1999; McCudden and Kraus 2006), experimental and theoretical (Caldara et al. 2008; Curis et al. 2007; Qiao et al. 2005; dos Santos et al. 2010) studies. Among various methods, which can be used for theoretical studies, mathematical modelling based on ordinary differential equations (ODE) is the most common one allowing direct simulations of each species concentrations and fluxes as a function of time.

In this paper, we present an ODE-based mathematical model of Cit synthesis pathway in the enterocyte and apply two modelling approaches at the cellular scale, that differ in the mathematical description of the involved metabolic reactions: a classical one, based on Michaelis–Menten like approximations (designed as King–Altman approach or KA approach), and a more seldom used, based on a microscopic description of the enzyme mechanism requiring less assumptions (designed as Van't Hoff approach or VH approach). The essential aim of this paper is to compare advantages and weaknesses of these two formalisms. In order to check the predictive capacity of the model in both approaches, several simulations related to modified enzyme activities, as could be encountered in some inherited diseases on the studied pathway, were carried out.

After the modelling basis presentation where we describe the metabolic pathways constituting the system and its associated mathematical formalism, we give the main features of the two KA and VH approaches.

Modelling basis

The system

The model represents the Cit production pathway in an enterocyte. This cell is oriented with an apical pole in contact with gut lumen and a basolateral side in contact with the plasma via the extracellular matrix. We do not take into account the diffusion within the extracellular milieu and consider a direct exchange from basolateral membrane to plasma. Moreover, all the studied reactions taking place in the mitochondria, the transport from plasma to mitochondria (hereafter resumed by the term basolateral exchanges) is modelled by one transport equation. Therefore, the system presents three distinct compartments: (i) the intestinal luminal side (ii) the intracellular space (iii) the plasma side. Each compartment is considered homogeneous.

In this work, we only consider the fasting state, so exchanges between the gut lumen and the cell have not been simulated. Thus, the model includes the intake of Gln from plasma at the basolateral side, the conversion of Gln into Cit in the enterocyte and the release of Cit into the plasma, also through the basolateral membrane. This conversion goes through the following enzymatic reactions (Fig. 1): Gln is converted into glutamate (Glu) through glutaminase; Glu is then transformed by Δ1-pyrroline-5-carboxylate (P5C) synthase into P5C; ornithine aminotransferase (OAT) uses P5C to produce ornithine (Orn); finally, ornithine is converted into Cit by ornithine carbamoyl transferase (OCT). Since P5C synthase presents two different active sites, it was considered as two enzymes in this model: a glutamyl kinase (GKin) and a glutamyl phosphate reductase (GRed).

Mathematical formalism

As stated above, since the compartments are considered to be homogeneous, no spatial information is taken into account, and so the model is represented by simple mathematical formalisms of ODE. Basolateral exchanges transports were modelled using Michaelis–Menten kinetics.

The rate of production or consumption of each species is determined by the reaction rates of each individual reaction: the variation of the concentration as a function of time for a

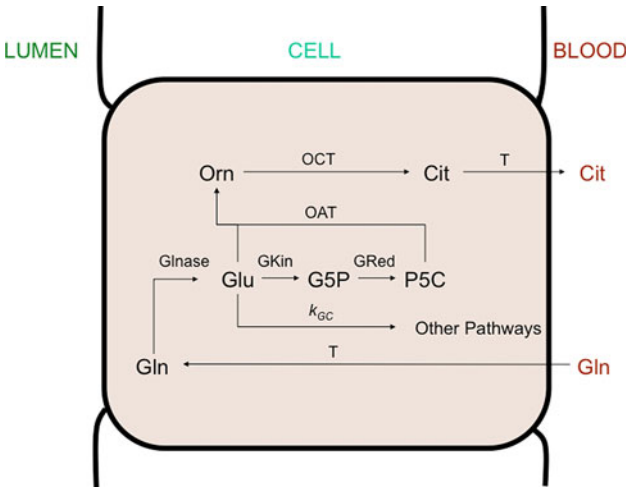

Fig. 1 Metabolic pathways of Cit production from Blood Gln in the enterocyte. Amino acids: Gln (glutamine), Glu (glutamate), G5P (Glutamyl-5-Phosphate), P5C (1-pyrroline-5-carboxylate), Orn (ornithine), Cit (citrulline); Enzymes: Glnase (glutaminase), P5C synthase: GKin (glutamyl kinase) and GRed (glutamyl phosphate reductase), OAT (ornithine aminotranferase), OCT (ornithine carbamoyltranferase). T: represents the transporters from/to plasma to/from mitochondria. In this figure, carbamoyl phosphate (co-substrate of OCT) is not mentioned as supposed as a non-limiting element

given species is defined as the linear combination of all reaction rates weighted with the algebraic stoichiometric coefficient of the species in the reaction. This coefficient is equal to: 0 if the species is not used in the simulation, +1 if the species is produced and −1 if it is consumed.

The two modelling approaches presented hereafter differ by the choice of equations used to describe each enzymatic reaction and the level of details for its description.

Description of the two approaches

Standard enzymatic kinetic approach (KA)

This approach is based on the traditional King–Altman method (King and Altman 1956). Herein, each enzyme reaction is described by a Michaelis–Menten like equation, using K_m and V_{max} as parameters. Reaction rate expressions are selected according to the class of enzyme mechanism, which describes the corresponding enzyme reaction. The data extracted from the literature (Leskovac 2003) are summarised in Table 1. For instance OAT is described as a ping-pong mechanism, with a change in the enzyme conformation during the reaction.

This KA approach relies on two assumptions: (i) the system is at quasi steady-state and (ii) enzyme concentrations are much smaller than substrate concentrations and enzyme active sites are then saturated. These two hypotheses are not always fulfilled in physiological situations and can weaken models using this approach. Nevertheless, this method reduces the number of equations and allows using available experimental values of the kinetic parameters from the literature.

For example the rate of the reaction catalysed by OAT, an enzyme known to follow a Michaelis–Menten mechanism, is described by the following equation:

$$v_{OAT} = \frac{V_{maxOAT}[Glu][P5C]}{K_{mGluOAT}[P5C] + K_{mP5C}[Glu] + [Glu][P5C]}$$

where V_{maxOAT} is the maximum rate of the reaction, $K_{mGluOAT}$ and K_{mP5C} are the Michaelis constants, respectively, for Glu and P5C in the OAT reaction.

Association–dissociation approach (VH)

With this approach, each enzyme reaction is described by a succession of association–dissociation elementary reactions between the enzyme and its substrates. This assumes that the reaction rate is limited by substrate diffusion and this is the only hypothesis used to model each elementary step. Such elementary reactions follow the Van't Hoff's law, often also referred to as the mass action law. As we must take into account all the enzyme–substrate complexes, the number of species in the system increases, and therefore, the number of ODEs and kinetic parameters also increases. The set of association–dissociation reactions was determined from the class of the enzyme mechanism. As an example, taking into account the ping-pong mechanism displayed by OAT, the

Table 1 Role and mechanism of the citrulline pathway enzymes

Enzyme	Catalysed reaction	Mathematical expression by the King–Altman method
Glutaminase	This enzyme catalyses the deamination of glutamine for the biosynthesis of Glu. It is a Uni-Bi reaction	$v_{Glnase} = \frac{V_{maxGlnase}[Gln]}{K_{mGln}+[Gln]}$
Pyrroline-5-Carboxylate (P5C) synthetase	Firstly, production of glutamate-5-phosphate (G5P), is catalysed by the first domain of the enzyme, the glutamyl kinase (GKin); secondly, glutamate semi-aldehyde is synthesised from the glutamate-5-phosphate under the action of the second domain of the enzyme, the glutamyl phosphate reductase (GRed). The glutamate semi-aldehyde produced is the hydrated form of the P5C. This enzyme is represented by two equations in the models, each of them corresponding to a domain	$v_{GKin} = \frac{V_{maxGKin}[Glu]}{K_{mGluGKin}+[Glu]}$ $v_{GRed} = \frac{V_{maxGRed}[G5P]}{K_{mG5P}+[G5P]}$
Ornithine Aminotransferase (OAT)	It allows the biosynthesis of ornithine and 2-oxoglutarate from Glu and P5C. This enzyme has a ping-pong mechanism characterised by an important conformational change of the enzyme during the reaction	$v_{OAT} = \frac{V_{maxOAT}[Glu][P5C]}{K_{mGluOAT}[P5C]+K_{mP5C}[Glu]+[Glu][P5C]}$
Ornithine carbamoyl tranferase (OCT)	It catalyses the synthesis of citrulline from ornithine. It follows an ordered Bi–Bi mechanism where the carbamoyl phosphate binds first, followed by the ornithine after what the products are released: citrulline first and phosphate last	$v_{OCT} = \frac{V_{maxOCT}[Orn][CP]}{K_{mCP}[Orn]+K_{mOrn}[CP]+[Orn][CP]}$
Incoming and outgoing fluxes (transporters)	The model includes the glutamine uptake from the blood side and the release of citrulline in blood. The potential release of glutamine into the blood in case of an excessive concentration in the cell was also included. All the transporters (T) are represented by a Michaelis–Menten equation in both approaches	$v_T = \frac{V_{max}[S]}{K_m+[S]}$

v Initial rate of the reaction; V_{max} maximum velocity of the reaction and K_m Michaelis–Menten constant for the substrate of the reaction

reaction catalysed by this enzyme can be recapitulated by the following association–dissociation equations:

$$\mathrm{Glu} + \mathrm{E}_4 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{GluE}_4 \xrightarrow{k_2} \mathrm{E}'_4 + \mathrm{Oxo}$$

$$\mathrm{P5C} + \mathrm{E}'_4 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \mathrm{P5CE}'_4 \xrightarrow{k_4} \mathrm{E}_4 + \mathrm{Orn}$$

where E_4 is the initial form of the enzyme and E'_4 its transitory form, Oxo stands for oxoglutarate, $GluE_4$ is the complex of Glu with the initial enzyme form, and $P5CE'_4$ is the complex of P5C with the transitory enzyme form. The microscopic constants are k_1, k_{-1}, k_2, k_3, k_{-3}, and k_4.

Therefore OAT reaction is described by the following equations:

$$\frac{\mathrm{d}[\mathrm{Glu}]}{\mathrm{d}t} = -k_1[\mathrm{Glu}][\mathrm{E}_4] + k_{-1}[\mathrm{GluE}_4]$$
$$\frac{\mathrm{d}[\mathrm{Oxo}]}{\mathrm{d}t} = k_2[\mathrm{GluE}_4]$$
$$\frac{\mathrm{d}[\mathrm{GluE}_4]}{\mathrm{d}t} = k_1[\mathrm{Glu}][\mathrm{E}_4] - k_{-1}[\mathrm{GluE}_4] - k_2[\mathrm{GluE}_4]$$
$$\frac{\mathrm{d}[\mathrm{E}_4]}{\mathrm{d}t} = k_{-1}[\mathrm{GluE}_4] + k_4\left[\mathrm{P5CE}'_4\right] - k_1[\mathrm{Glu}][\mathrm{E}_4]$$
$$\frac{\mathrm{d}[\mathrm{P5C}]}{\mathrm{d}t} = -k_3[\mathrm{P5C}]\left[\mathrm{E}'_4\right] + k_{-3}\left[\mathrm{P5CE}'_4\right]$$
$$\frac{\mathrm{d}[\mathrm{Orn}]}{\mathrm{d}t} = k_4\left[\mathrm{P5CE}'_4\right]$$
$$\frac{\mathrm{d}\left[\mathrm{P5CE}'_4\right]}{\mathrm{d}t} = k_3[\mathrm{P5C}]\left[\mathrm{E}'_4\right] - k_{-3}\left[\mathrm{P5CE}'_4\right] - k_4\left[\mathrm{P5CE}'_4\right]$$
$$\frac{\mathrm{d}\left[\mathrm{E}'_4\right]}{\mathrm{d}t} = k_2[\mathrm{GluE}_4] + k_{-3}\left[\mathrm{P5CE}'_4\right] - k_3[\mathrm{P5C}]\left[\mathrm{E}'_4\right]$$

Link between macroscopic and microscopic constants

The exact expression of the reaction rate is obtained by the King–Altman method, starting from the detailed mechanism of each reaction, decomposed in elementary steps. This classical approach will not be described in this article [for a review see (Leskovac 2003)].

The already-mentioned microscopic constants are scarcely available in the literature and have to be determined from the available ones (K_m and V_{max}). Let us note that even in the simplest case of an irreversible michaelian enzyme, the number of microscopic constants (k_1, k_{-1}, k_2) is superior to the number of the michaelian parameters (K_m and V_{max}). For instance in the case of a basic Michaelis–Menten mechanism we get:

$$K_m = \frac{k_{-1} + k_2}{k_1}$$
$$V_{max} = k_2[\mathrm{E}]_0$$

Microscopic constant determination is done for every enzyme by finding the mathematical link between the ODE system describing the enzymatic mechanism studied in this approach and the Michaelis–Menten like kinetic equation, given by the King–Altman method. Therefore, some of the microscopic constants have to be arbitrarily chosen. Nevertheless, Bulik et al. (2009) have clearly demonstrated that as long as microscopic parameters remain consistent with the macroscopic constants, simulated concentration profiles do not depend on the arbitrarily set values. Thus, these so-called "arbitrarily set constants" are derived in order to satisfy the relationships between microscopic and macroscopic parameters.

Hereafter, we first introduce the hypotheses founding the model construction, underlining the shared part between the two approaches, then the specific tools applied to each approach. In a second step, we describe the model settings.

Model construction

Shared part between the two approaches

Hereafter, we successively present the input and output of the model, the modelling of Glu consumption in the enterocyte and the modelling transport systems.

In the fasting state, enterocytes use plasma Gln to synthesise Cit, which is released in the plasma. In such a state and under physiological conditions, plasma Gln and Cit concentrations are steady as the result of their balanced production and utilisation. Indeed the production of Gln, mainly from the muscle, compensates its consumption; therefore, plasma Gln concentration is constrained to be constant in the model.

Concerning Cit produced by the intestine, its plasma level is regulated by a consumption pathway. We modelled this regulation step by a velocity parameter v_{cc} to be determined. It has been shown in rat models (Dhanakoti et al. 1990) that renal Cit uptake is proportional to circulating Cit concentration. Since the kidney is the main organ consuming this amino acid, we propose to represent its consumption rate v_{cc} by a first order equation:

$$v_{cc} = k_{cc} \times [\mathrm{Cit}]_{\mathrm{Pl}}$$

where $[Cit]_{Pl}$ is Cit plasma concentration and k_{cc} plasma Cit consumption rate constant.

It is worthy of note that Glu produced from Gln can be consumed in numerous other pathways with several kinetic mechanisms and parameters. Since these pathways do not further interfere with Cit synthesis, they were summarised in the models through a generic first order process, with a consumption rate v_{gc} as:

$$v_{gc} = k_{gc} \times [\mathrm{Glu}]$$

where [Glu] is the cell concentration of Glu and k_{gc} the Glu consumption rate constant.

Concerning the transporters no data are found in the literature to express a link between the macroscopic and microscopic approaches and so we chose to conserve a unique kinetic relation for the transporters. As a consequence, in both approaches, transport was assumed to follow a Michaelis–Menten kinetic:

$$v_T = \frac{V_{max}[S]}{K_m + [S]}$$

V_{max} is the maximum speed of the reaction, K_m is the constant of Michaelis expressing the enzyme affinity for its substrate (S), [S] is the substrate concentration.

Specific modelling tools for each of the two approaches

For both approaches the modelling calculations are performed with Mathematica™. The KA approach equations are introduced directly into the solver. The VH approach equations are generated using kMech, a Cellerator language extension for Mathematica™, that generates the mathematical equations relative to the association–dissociation reactions of a given enzyme mechanism (Yang et al. 2005b). The values of the microscopic constants, even arbitrarily set ones, are estimated by kMech Lambda method from the kinetic constants k_{cat} and K_m available in literature (Yang et al. 2005b). This method, which takes into account the rapid equilibrium approximation, reduces the number of unknown parameters.

Once the system of ODEs is written, the NDSolve function in Mathematica™ is used to numerically solve it with the default algorithm.

Model settings

At this step, the model requires some parameters values that should, as much as possible, be deduced from the literature; the shared parameters between the two approaches are set at the same value to ensure comparability.

These parameters include the concentrations of various substrates and products, the enzyme concentrations, the rate constants for plasma Cit and enterocyte Glu consumption, the kinetic characteristics of transporters.

If correctly parameterised the model should reach a steady-state for all the metabolites. We focused on plasma Cit concentrations in agreement with experimental data from the literature. Our model is required enabling to reach a normal plasma Cit steady-state concentration of 40 μM starting from plasma Gln concentration constrained to be constant. Normal plasma citrulline values are defined experimentally between 20 and 50 μM (Pappas et al. 2002).

Plasma Gln concentration has been set at 600 μM, which is the mean value for a fasted human adult (Parry-Billings et al. 1990). All the concentrations for the main metabolites—intracellular Gln, Glu, Δ1-pyrroline-5-carboxylate (P5C), ornithine, intracellular and plasma Cit—have been initially set at 0 μM and authorised to vary during simulations. The secondary metabolite carbamoyl phosphate (CP) must be taken into account since it is the co-substrate of OCT. We know that NH_3 is the CP precursor and that NH_3 is continuously provided by Glu metabolism for energy production. Consequently, CP is not considered as a limiting factor and thus its concentration is set constant at 100 μM to perform the simulations.

Concerning enzyme characteristics, it must be noted that, from a general point of view, determining V_{max} requires that enzyme concentrations are known. However, these values are rarely available in literature for the enterocyte, probably because of their high sensitivity to physiological and environmental variations that render a "normal" value difficult to define. Only the glutaminase concentration (0.52 μM) could be roughly estimated from the V_{max} value (Robinson et al. 2007) and the k_{cat} value from the Brenda database (Hartman 1971). For all other enzymes, a value of 1 μM has been retained, as it is consistent with enzyme concentrations previously used in literature (Yang et al. 2005a); (Kuchel et al. 1977). Moreover, for this value, V_{max} equals k_{cat}, which allows straightforward comparison of the KA and VH approaches.

About the Cit plasma consumption, k_{cc} has been determined from Cit turnover rate measured in humans: 12 μmol/kg/h (Curis et al. 2005). Its value for a man of 70 kg, 5.5 l of blood and a mean plasma Cit concentration at 40 μM has been estimated as:

$$k_{cc} = \frac{12 \times 70}{5.5} \times \frac{1}{40} \times \frac{1}{3{,}600} = 0.00106\ s^{-1}$$

The Glu consumption constant k_{gc} has been adjusted so that the simulated plasma Cit steady-state level fits the above-cited 40 μM plasma concentration. Figure 2 presents the dependence of steady-state plasma Cit level as a function of the Glu consumption constant (k_{gc}) for KA and VH approaches. The k_{gc} adjusted value was the same in both VH and KA simulations (0.02).

Literature kinetic data of enzymes and transporters

While kinetic parameters may be found in the literature, activity values frequently cannot be readily used. In fact, the concentration of enzymes in the enterocyte being only rarely known, literature data presented as V_{max} values are often specific activities, expressed in terms of quantity (moles) of substrate transformed per time unit and per protein mass unit (milligrams). In order to use the

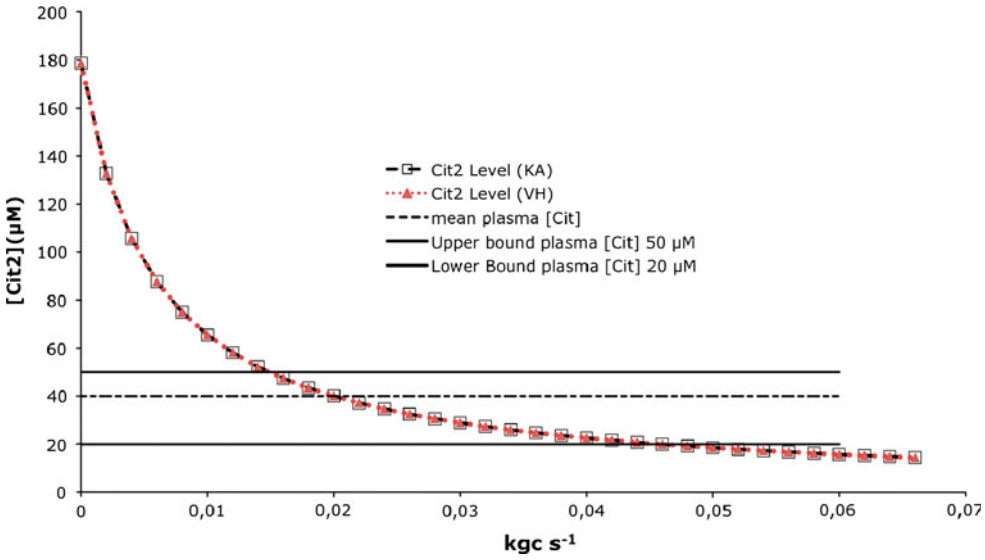

Fig. 2 Evolution of plasma Cit concentrations (µM) as a function of Glu consumption constant (k_{gc}) values. The straight lines show the upper (50 µM) and lower (20 µM) limits of the normal plasma Cit concentration from literature. The *dashed line* represents the plasma Cit selected mean value that corresponds a 0.02 s^{-1} k_{gc} value. No difference is found between the two approaches along the whole range of values

Michaelis–Menten expressions in simulations, the real V_{max} expressed as a quantity (moles) of substrate transformed per time unit and per volume unit is needed. This V_{max} is expressed as $V_{max} = k_{cat} \times [E]_0$ where $k_{cat} = a \times M$ is the catalytic constant of the enzyme, $[E]_0$ the total concentration of the active enzyme, a the specific activity and M the enzyme molecular mass. Therefore, the concentration $[E]_0$ of the enzyme in the enterocyte should be known in order to determine the actual V_{max} from literature specific activities a.

Unlike enzyme activity, transporter activity is generally given as a ratio to total protein mass in the cell and not specifically to the transporter mass itself. Since the mass fraction of transporters is unknown, there is no simple way to obtain k_{cat} from apparent specific activities. The V_{max} missing values for Cit transporters have been determined from the human experimental profile concentrations of plasma Cit (Moinard et al. 2008).

The K_m is well defined and is not subject to these questions.

Model's V_{max}, k_{cat} and K_m values are given in Tables 2 and 3.

In the coming section, we discuss the results obtained through preliminary simulations and their consequences on the parameter values. Then, we comment the observed differences between the two approaches and finally we apply this model to simulate a possible loss of enzyme activity.

Results and discussion

Abnormal accumulation of P5C

In the KA simulation but not in the VH simulation, an abnormal accumulation of P5C was observed and no steady-state could be reached for this metabolite. This behaviour is either related to insufficient P5C consumption by OAT or to P5C overproduction by the P5C synthase (Fig. 3).

Table 2 Values of kinetic parameters for transporters

Zone	Transporter	Parameters		Source
Plasma	Gln absorption	K_m	1,000 µM	Estimated
		V_{max}	1 µmol/l/s	
	Cit	K_m	500 µM	
	Release	V_{max}	30 µmol/l/s	

Estimated after manual adjustment to obtain the appropriate steady-state (40 µM of plasma citrulline concentration)

The k_{gc} value could be a possible source of discrepancy between the two approaches. In order to check the mathematical influence of this parameter, we have set it to 0, unlikely situation where all Gln is metabolised into Cit. This k_{gc} 0 value did not remove the accumulation of P5C.

At this step of simulation, two choices are possible: either decreasing the P5C production by reducing the P5C synthase activity, or increasing its consumption by increasing the OAT activity. The first case would lead us to modify two metabolic steps (GKin and GRed) in our model, so, in view to alter the model as least as possible, we chose to vary OAT concentrations and we noticed that over 1.5 µM of OAT this accumulation is suppressed. Hence, OAT concentration is set to the round value of 2 µM in both approaches.

The results of these simulations are given in Table 4. Under these conditions, P5C concentration in the KA approach is more than fourfold its concentration in the VH approach. Interestingly, the same difference is found for ornithine concentration, which is almost fourfold higher in the KA approach than in the VH approach. The metabolite

Table 3 Values of the kinetic parameters for the enzymes in the two models

Enzyme	K_m (μM)	Molecular weight (g/mol)	kcat (s^{-1})	Concentration model VH (μM)	V_{max} for 1 μM of enzyme (μmol/l/s)
Glutaminase	Gln: 15,000 (Robinson et al. 2007)	73,461	1,270 (Hartman 1971)[a]	0.5249 (Hartman 1971; Robinson et al. 2007)[a]	666.67 (Robinson et al. 2007)[a]
P5C synthase (glutamyl kinase)	Glu: 82,000 (Pérez-Arellano et al. 2006)[a]	87,302	237.17 (Pérez-Arellano et al. 2006)[a]	1[c]	237.17 (Pérez-Arellano et al. 2006)[a]
P5C synthase (glutamyl phosphate reductase)	G5P: 10000[b]	87,302	145.50[b]	1[c]	145.50[b]
OAT	Glu: 38,790 (Matsuzawa 1974)[a] P5C: 2760 (Matsuzawa 1974)[a]	48,535	80.89[b]	2[b]	80.89[b]
OCT	Orn: 110 (Morizono et al. 1997)[a] CP: 50 (Morizono et al. 1997)[a]	39,935	41.60 (Morizono et al. 1997)[a]	1[c]	41.60 (Morizono et al. 1997)[a]

[a] References

[b] Estimated by manual adjustment to obtain the appropriate plasma Cit steady-state concentration (40 μM)

[c] Round values of the same order as average enzyme concentrations previously used in literature

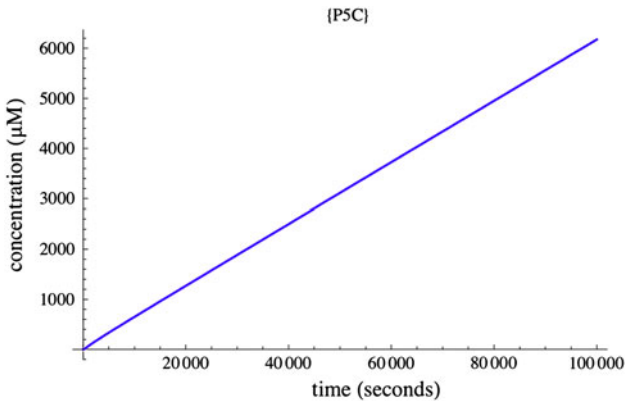

Fig. 3 Evolution of P5C concentration [μM] as a function of time (seconds) in the KA approach for OAT = 1 μM and $k_{gc} = 0$

ratios between the two approaches remain the same whatever is the chosen k_{gc} value (Table 4).

The steady-state

In both approaches, for a 2 μM OAT concentration, the model reaches an apparent steady-state, whatever the considered metabolite and its initial concentration. For instance Fig. 4, showing the evolution of cell Gln concentration as a function of time for each model, illustrates this feature with a plateau reached at 8.44 μM.

Why a different behaviour of the two approaches?

Obviously, the fundamental setting in both approaches being the same and the potential influence of k_{gc} having been cancelled, the discrepancies between the two approaches might come from the way the reaction mechanisms are expressed. Hence, the different description of the enzyme kinetic is the only possible origin of these differences.

Since steady-state concentrations are determined by the ratio between consumption and production rates, and since ornithine and P5C present the strongest discrepancies, OAT associated to ornithine production and P5C consumption, and OCT associated to ornithine consumption are the best candidates to originate such differences; we assumed that OAT, which produces ornithine and uses P5C, was a better candidate than OCT.

To check this hypothesis, simulations following both KA and VH approaches of isolate enzyme reaction were performed separately, outside the system, for all enzymes involved in the model. As expected, no differences are found for glutaminase and P5C synthase kinetics, whereas we got significant differences for both OAT and OCT kinetics:

- Concerning OAT, the VH approach leads to a faster consumption of P5C compared to the KA approach, yielding about a fourfold decrease of P5C steady-state concentration (Table 4).
- For OCT, the VH approach also shows a faster consumption of its substrates compared to the KA approach, yielding about a fourfold decrease of ornithine steady-state concentration (Table 4). Interestingly, ornithine is the only metabolite in the model whose concentration is lower than the enzyme is (set to

Table 4 Steady-state concentrations (μM) of the pathway intermediates for $k_{gc} = 0$, 0.02 and OAT concentration at 2 μM

Metabolite	Concentration (μM) KA model	Concentration (μM) VH model	Concentration ratio KA/VH
$k_{gc} = 0$			
Gln (cell)	8.44	8.44	1
Glu	64.88	64.88	1
P5C	10.45	2.45	4.27
Orn	0.50	0.13	3.84
Cit (cell)	3.14	3.14	1
Cit (plasma)	178.57	178.57	1
$k_{gc} = 0.02$			
Gln (cell)	8.44	8.44	1
Glu	14.54	14.54	1
P5C	2.34	0.55	4.25
Orn	0.11	0.028	3.92
Cit1	0.70	0.70	1
Cit2	40.06	40.06	1

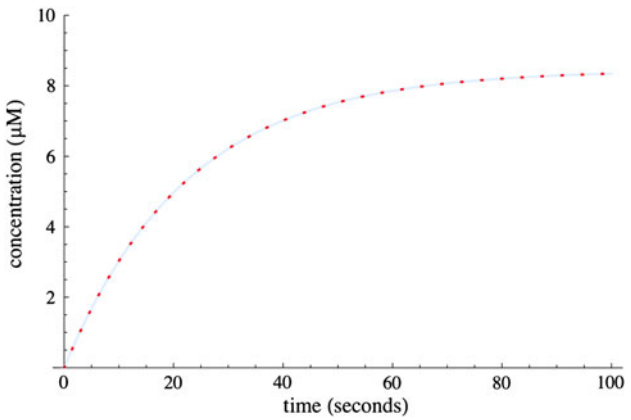

Fig. 4 Evolution of cell Gln concentration (μM) as a function of time (seconds) for the KA model (*thick line*) and the VH model (*dotted line*). No differences appear between the two approaches for simple michaelian kinetics

1 μM), challenging the validity of the KA standard method; such discrepancies between the approaches is then not so surprising and could be related to the Michaelis–Menten assumption violation about the substrate concentration saturation.

Simulation of enzyme mutation consequences on the modelled system

In this subsection, we simulate, following the two KA and VH approaches, a defect in one enzyme activity in the studied metabolic pathway. Such defects may be observed, for instance, in some inherited diseases of the metabolism with a mutation of the active site. In practice, in our model, manifestations of these mutations will be represented by a change in the K_m or, through a modification in the k_{cat} or in the active enzyme concentration, in V_{max}.

A wide variability of clinical mutation effects can be observed. The most obvious explanation for this variability is the enzymatic activity modification induced by the genetic mutation, and the homozygous/heterozygous status of the patient. A more subtle one might be a kinetic feature of the whole involved pathway leading to the existence of a threshold effect (Rossignol et al. 2003): as long as the activity of the enzyme stays above the threshold, the clinical effects remain unnoticed; once the activity falls below the threshold, the disease is declared. The delayed manifestation of these illnesses (many years sometimes) would be explained by the potential sensitivity of the threshold to the environmental and physiological conditions; the variability between patients, may be related to its sensitivity to small variations in the kinetic properties of all the enzymes involved in the pathway.

Assuming this hypothesis, the study of the steady-state of the system as a function of the enzyme activity level should give a sigmoid function reproducing this threshold as it is generally observed.

Simulations are carried out on the four enzymes of our model: glutaminase, P5C synthetase (as its glutamyl kinase and glutamyl phosphate reductase domains), OAT and OCT. The criterion is the evolution of plasma Cit as a function of the logarithm of the enzymatic activity. All the enzymes show this expected threshold. Table 5 presents the logarithm value of the deficiency coefficient (d) applied to the enzymatic activity in the two approaches to obtain, respectively, 10, 50 and 90% of the mean plasma Cit concentration. The results presented in that table, evidence no differences between the two approaches for glutaminase, Gkin and Gred, which are represented by a simple

michaelian mechanism. Differences are observed for OCT and OAT, which are complex mechanisms: slight variations for OCT and much more marked ones for OAT.

The threshold is quantified by the log d50 value where plasma Cit reaches 50% of its mean value. For many enzymes, on the sigmoid function graph, we observe a plateau characterising low threshold d50 values.

This plateau could be tentatively interpreted as a "natural safety system" developed by the cell allowing it to counterbalance the effects of genetic variations until a certain point. Similarly, in the case of sudden physiological modifications, the cell might have to quickly modulate the level of activity of a metabolic pathway. Such a low threshold in the activity might indicate the necessity for the cell to keep the enzyme concentrations slightly above the "sufficient" level: high enough to avoid a potential "accidental" loss of activity, but not too high to be able to promptly turn off (or on) the pathway when needed.

Let us notice that the levels of activity for which the threshold appears, are quite always the same in both approaches, except for the OAT. We find that the OAT and the glutamyl kinase in the KA approach, are the only enzymes not exhibiting a plateau before the threshold (log d high values). The Fig. 5 shows the results for the OAT in both approaches. This absence of plateau shows that OAT is one of the most sensitive in the system to the variations of its concentration. This difference might explain why in our preliminary simulations the P5C was insufficiently consumed in the KA approach but not in the VH approach: the concentration of 1 μM in the KA approach is too low relatively to the threshold. Noteworthily, OAT genetic mutations are extensively studied for their consequences. In the contrary, the OCT has a long plateau before reaching the threshold, even slightly longer in the VH model, showing an extensive capacity to maintain a sufficient level of activity keeping the pathway "turned on". Interestingly, clinical results (García-Pérez et al. 1995) show that some patients are able to live normally and reach the adult age despite a very important OCT activity deficiency, the determined residual activity varying from 1 to 3% of the

Table 5 The decimal logarithm values of the enzyme deficiency coefficient (d) applied to the two approaches, corresponding to 10, 50 and 90% of the normal plasma citrulline level

Enzyme	KA model and VH model (except for OAT and OCT)		
	Deficiency coefficient value		
	Log d value at 10%	Log d value at 50%	Log d value at 90%
Glutaminase	−4.09	−3.34	−2.94
P5C synthase (glutamyl kinase)	−1.10	−0.36	−0.06
P5C synthase (glutamyl reductase)	−4.02	−3.23	−2.75
OAT	−1.08 (KA)	−0.36 (KA)	−0.08 (KA)
	−1.50 (VH)	−0.79 (VH)	−0.51 (VH)
OCT	−3.81 (KA)	−3.11 (KA)	−2.82 (KA)
	−3.89 (VH)	−3.20 (VH)	−2.93 (VH)

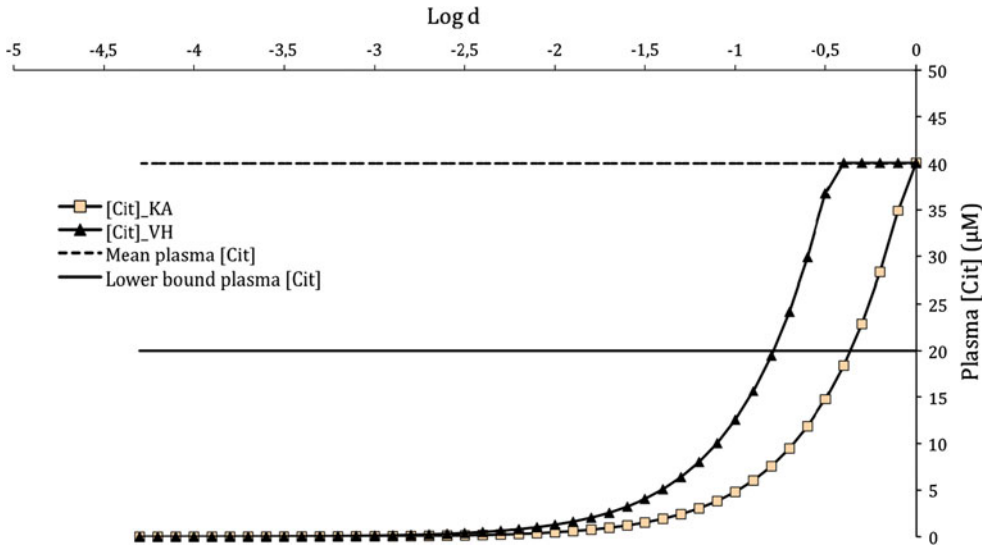

Fig. 5 Variations of plasma Cit concentration (μM) as a function of OAT deficiency constant Log values (Log d) applied in the two approaches. The *straight lines* show the upper (50 μM) and lower limits (20 μM) of the normal Cit plasma concentration, the upper bound coinciding with the *x*-axis. The *dashed line* represents the plasmatic Cit selected mean value (40 μM)

normal activity which is in agreement with the existence of such a long plateau before the threshold as determined by our model.

These results highlight the sensitivity of the OAT and its regulating role in the citrulline pathway. Moreover, they give a rough approximation about the relative OAT concentration value in this pathway expected to be the double of the other enzyme concentrations in the system.

Conclusion

A mathematical model of the intestinal conversion of Gln into Cit in the enterocyte has been built, in agreement with physiological data provided in literature, particularly the plasma Cit concentration.

Simulation results obtained by this model highlight the pivotal role played by the OAT in the system. Despite the lack of knowledge existing around the precise kinetic of this enzyme, we show that the discrepancies between the two approaches KA and VH, only affect metabolites closely related to OAT (P5C and ornithine). The observed differences for OCT only concern the ornithine level without affecting plasma Cit. These differences could be related to the Michaelis–Menten assumption violation about the substrate concentration saturation in the KA approach.

The model we describe is able to make predictions about the behaviour of the studied metabolic pathway when disrupted and more particularly about the threshold effect of the active enzyme concentrations. The simulations allow proposing some orders of magnitude about the "optimal" relative enzyme concentrations levels in the enterocyte.

Conflict of interest The authors declare that they have no conflict of interest.

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