A stochastic modeling of isotope exchange reactions in glutamine synthetase

N V Kazmiruk¹, S E Boronovskiy¹ and Ya R Nartsissov¹

¹Institute of cytochemistry and molecular pharmacology, Moscow 115404, Russia

Abstract. The model presented in this work allows simulation of isotopic exchange reactions at chemical equilibrium catalyzed by a glutamine synthetase. To simulate the functioning of the enzyme the algorithm based on the stochastic approach was applied. The dependence of exchange rates for $^{14}$C and $^{32}$P on metabolite concentration was estimated. The simulation results confirmed the hypothesis of the ascertained validity for preferred order random binding mechanism. Corresponding values of $K_{0.5}$ were also obtained.

1. Introduction

Up to the present time, the isotope exchange kinetics at chemical equilibrium has been recognized as an important experimental approach in the study of chemical reactions mechanisms. In particular, it appears as a powerful tool for validation of their pathways and structure of transition states [1,2]. In biomedical research the method of equilibrium isotope exchange is often used for the investigation of the substrate binding in the active site of the enzyme. Variation of structurally related reactant-product pairs yields information about substrate binding order and the relative rates of association/dissociation, whereas the equilibrium exchange rates are used to make a distinction between the processes of fully random or preferred order random binding of substrates [3]. Glutamine synthetase (GS), which is known to be a key component of the metabolism of glutamic acid, is among the enzymes that are under active consideration with the help of this technique.

It catalyzes the synthesis of glutamine and provides the regulation of ammonium level in almost all organisms, in particular in astrocytes of human brain, where it is a critical enzyme for metabolism of glutamate, GABA and ammonia. Since GS is an essential part of a complex astrocyte–neuron signaling process, changes in enzyme expression and activity will lead to neurological dysfunction. A number of different brain pathologies are associated with alterations in GS expression/activity [4]. The mechanisms by which the expression and activity of this enzyme are regulated under normal and pathological conditions (e.g. under hyperammonemia) still remain unclear [5]. It is important to note that there is the major evidence that the active site of the enzyme is rather conservative suggesting an identical catalytic mechanism of the various forms of the enzyme [6]. The biosynthesis reaction of GS is quite complex, and as a first approximation it can be described in two processes Figure 1.

![Reaction Scheme]

L-glutamate + ATP + NH$_4^+$ → ADP + P$_i$ + L-glutamine

The first step consisting in the formation of the activated intermediate γ-glutamyl phosphate is followed by the second one, which is the ammonia attack on the intermediate and glutamine release [7-9].
Figure 1. Structure of active center of bacterial glutamine synthetase (PDB code 1F1H); n1 and n2 are the high- and low-affinity binding sites for divalent cations [10].

Nevertheless, stochastic simulation requires the detailed mechanism, taking into account all the possible variations of binding metabolites with a certain probability. Such a mechanism is «Random Ter-Ter», which has been proposed in the earlier studies [11]. Thus, the study of bacterial glutamine synthetase using the computational model of such a method allows to obtain the dependence of exchange rate between the substrates and the reaction products on the concentrations of various metabolites and makes the conclusions concerning the substrates binding mechanism. Furthermore, it yields a great perspective of Gln-Glu cycle studies in silico.

2. Model

2.1. Equilibrium Isotope Exchange in Enzyme Catalysis

Generally, isotope exchange reactions can be defined as the spontaneous processes of redistribution of isotopes between the molecules, which are not accompanied by chemical changes of the molecules (i.e., changing only their isotopic composition). However, the exchange may occur between the molecules located in the same or different phases and within the molecules. In the study of GS exchange reactions $[^{14}\text{C}]\text{Glu} \leftrightarrow[^{14}\text{C}]\text{Gln}$, $[^{14}\text{C}]\text{ADP} \leftrightarrow[^{14}\text{C}]\text{ATP}$, $[^{32}\text{P}]\text{P}_i \leftrightarrow[^{32}\text{P}]\text{ATP}$ were simulated, which were used for carrying out classical earlier experimental studies [12,13].

The equation for calculating speeds of the equilibrium exchange reaction $X \leftrightarrow Y$ is as follows:

$$R = -\frac{[X][Y]}{[X]+[Y]} \ln (1-f) \quad (1)$$

where $f$ is the fraction of isotopic equilibrium, defined as:

$$f = \frac{[X]+[Y]}{[Y]} \times \left(\frac{y}{x+y}\right) \quad (2)$$

where $x$ and $y$ are disintegrations per minute (dpm) of radiolabel in the $X$ and $Y$ pools. The dependence of the fraction of isotopic equilibrium from the half-time period of is determined by the formula:

$$-\ln (1-f) = \ln \frac{2}{t_{0.5}} \quad (3)$$
This equation allows to determine $f$ according to experimental data and to find the value of the rate of exchange $R$.

2.2. Stochastic description of the enzyme functioning

The stochastic algorithm based on probabilistic approach was applied for modeling of an isotopic exchange reaction. In contrast to the kinetic approach, which is employed to describe the behavior of the protein ensemble and has been previously used for exchange reactions modeling, a stochastic one allows us to use thermodynamic parameters (e.g. Gibbs energy) and to work directly with a single protein [14]. Therefore, in this work the equilibrium constants for the substrate binding and conformational changes acted as the basic parameters. The binding process can be represented using a simple scheme:

$$E + A \xrightleftharpoons[k_d]{k_a} EA \Rightarrow K_d = \frac{[A][E]}{[EA]} \quad (4)$$

where $A$ is a substrate, $E$ is a protein (GS), and the value of $K_d$ can be determined according to changes in the thermodynamic functions or measured in the experiments. The algorithm is based on a step-by-step calculation of state probabilities with the subsequent estimation of both rate constant and exchange rates for each «substrate-product» pair. If it is assumed that there are a number of independent states of the protein, it is necessary to calculate the probability of finding the protein in the free state or in complex with the substrate:

$$
\begin{align*}
P_E &= \frac{N_E}{N_{EA} + N_E} \\
P_{EA} &= \frac{N_{EA}}{N_{EA} + N_E} \\
N_E + N_{EA} &= N
\end{align*}

(5)$$

where $N_E$ is a number of protein molecules in the free state, and $N_{EA}$ is a number of protein molecules in a complex with the substrate. Then an average probability vector can be written as the following:

$$
\bar{P} = \frac{1}{1 + \sum_{j=1}^{N} (K_j^{d_j} \cdot [A_j]^{-j_j^{d_j}})} \cdot \frac{K_k^{d_k} \cdot [A_k]^{-j_k^{d_k}}}{1 + \sum_{j=1}^{N} (K_j^{d_j} \cdot [A_j]^{-j_j^{d_j}})} \cdot \frac{K_N^{d_N} \cdot [A_N]^{-j_N^{d_N}}}{1 + \sum_{j=1}^{N} (K_j^{d_j} \cdot [A_j]^{-j_j^{d_j}})} \quad (6)
$$

where $d$ and $s$ are the direction of a single transition and metabolite consumption respectively.

3. Results and discussion

Generally, to obtain saturation curves for an isotopic exchange reaction while maintaining chemical equilibrium, it is necessary to vary substrate and product concentration in a constant ratio. The rates for isotope exchanges $[^{14}C]Glu \leftrightarrow [^{14}C]Gln$, $[^{14}C]ADP \leftrightarrow [^{14}C]ATP$ and $[^{32}P]P \leftrightarrow [^{32}P]ATP$ were obtained using the single protein stochastic model.
Figure 2. Equilibrium exchange rates. The effect of varying all metabolite concentrations. The points indicate the mean±SD of 20 independent computer simulations of GS activity.

Figure 3. Equilibrium exchange rates. The effect of varying ATP concentrations. The points indicate the mean±SD of 20 independent computer simulations of GS activity. The value of equilibrium ratio is $\frac{[ATP]}{[P_i]} = 1/10$, which is similar to original experiments.

Figure 4. Equilibrium exchange rates. The effect of varying Glu concentrations. The points indicate the mean±SD of 20 independent computer simulations of GS activity. The values of equilibrium ratio are $\frac{[Glu]}{[P_i]} = 1/10$ and $\frac{[Glu]}{[ADP]} = 1/4$ respectively, which are similar to original experiments.
An information about the equilibrium level achieved in the experiments allowed to select the following values for the parameter $f$: 0.7 for the exchanges $[^{14}\text{C}]\text{Glu} \leftrightarrow [^{14}\text{C}]\text{Gln}$ and $[^{14}\text{C}]\text{ADP} \leftrightarrow [^{14}\text{C}]\text{ATP}$, and 0.4 for the exchange $[^{32}\text{P}]\text{P}_{i} \leftrightarrow [^{32}\text{P}]\text{ATP}$. It is important to note that there is no decrease in the exchange rate in the case of relative concentration modeling Figure 2. This result is the major evidence of the hypothesis which excludes strictly ordered sequential binding. Thus, there is a tendency for preferred ordered binding of substrates (i.e. presence of dominant pathways) as a part of completely random mechanism. The fact that saturation curves increase in a sigmoidal manner is in consistence with the «Random Ter-Ter» mechanism of the reaction. Obtained values of $K_{0.5}$ are in a good agreement with the in vitro experimental data.

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