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Computational regulatory model for detoxification of ammonia from urea cycle in liver

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Abstract: A nondeterministic finite automaton was designed to monitor enzymatic regulation and detoxification of excess ammonia in the urea cycle and its disorders. The designed machine is used for the diagnosis of deficiency and for regulating the expression of any of the enzymes involved with acceptance and rejection states in the urea cycle. The urea cycle is the metabolism of excess nitrogen produced by the breakdown of protein and other nitrogen-containing molecules in liver. Disorder in the urea cycle may lead to the accumulation of toxic ammonia in the blood, which leads to hyperammonemia. The elevation of plasma ammonia concentration may ultimately lead to cerebral edema in infants and severe brain damage due to the toxicity of ammonia. The diagnosis of urea cycle disorder is based on evaluation of clinical, biochemical, and molecular data. In this study, a new therapeutic approach for urea cycle disorders is developed based on a computational model. It is used to observe the normal process of the cycle through the state of acceptance. The state of rejection denotes a deficiency in the respective enzymatic activity. Subsequently, it assists in the creation of targeted treatment for brain damage and related enzymatic deficiency disorders.

Key words: Ammonia, enzymes, nondeterministic finite automata, urea cycle disorder

1. Introduction

Mathematical biology is an interdisciplinary area of research that intersects the fields of mathematics and biology. As the field of mathematics plays a vital role in all types of computing, those results can be used to solve biological problems. Kari (1997) explored computational models in biology. Simulation of a biomolecular process using finite automata with 2 states and 2 symbols was introduced by Benenson et al. (2001). This method was experimentally tested in vitro. Through this idea, Cavaliere et al. (2005) proposed a theoretical model; the implementation of a push-down automaton built on circular DNA using a class II restriction enzyme. In addition, they extended the idea to implement push-down automata with 2 stacks using 2 circular molecules using DX molecules and class II restriction enzymes. Nowak and Plucienniczak (2008) described the nondeterministic finite state automaton based on DNA strands. This automaton was used to analyze DNA molecules, noting whether they were described by specified regular expression. Later, the ideas of Cavaliere et al. (2005) were improved by Krasinski et al. (2012) using a push-down automaton. Selvakumar et al. (2013) constructed a computational model for the extraction of human erythropoietin from the collection of proteins using a finite automaton. Muhammad et al. (2013) designed a push-down automaton to monitor the glycolysis process in cancer cells.

Amino acids are catabolized in the liver to generate ammonia. Ammonia is used for the synthesis of biomolecules, and the excess ammonia is converted into urea for excretion through a series of biochemical reactions for the production of urea (Balistreri and Carey, 2011). Ammonia can also be converted into a nontoxic compound such as glutamine or alanine. These serve as an amino group carrier in the blood to transport ammonia from the tissues to the liver for the conversion of ammonia into urea.

The improper disposal of ammonia in the form of urea is indicated by urea cycle disorders (UCDs). There are several inherited conditions that can cause problems in the waste-removal process. These disorders can be due to the absence or mutation of the gene responsible for the respective enzymes needed to break down ammonia in the body. Six enzymes are involved in the urea cycle that detoxifies toxic ammonia into urea, which is excreted in the urine. Infants with a UCD often initially appear normal

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but rapidly develop cerebral edema and the related signs of swelling of the brain (Lanpher et al., 2003).

When using computational biology, practical modeling for the optimization process of cell metabolism is essential for its application in health care (Weber et al., 2011). The utilization of intracellular enzymes for the purpose of both analytical and medical researches is highly interesting (Aly et al., 2013). In the present study, a monitoring procedure based on a nondeterministic finite automaton (NFA) was designed. It is constructive in the case of diagnostic procedures such as genetic tests, liver biopsy, enzyme-targeted therapy, and liver transplantation. Additionally, this designed machine is used to enhance the existing treatment procedures for brain damage and other diseases related to urea cycle metabolic disorders.

2. Automata

Generally devices that are used to transform information from one form to another based on a definite procedure are called automata (Muhammad et al., 2013). It is a mathematical model of a system with distinct inputs and outputs. It deals with the logic of computation with respect to simple machines (Hopcroft and Ullman, 1979). Automata are abstract models of machines that perform computations on an input by moving through a series of states. At each state of computation, a transition function determines the next state on the basis of a finite portion of the present state. As a result, once the computation reaches an accepting state, it accepts a sequence of symbols called strings. This is an approach used to implement the computing devices through the acceptance of regular expressions.

2.1. Nondeterministic finite automata

An alphabet is a finite, nonempty set of symbols. Symbols are denoted by a small letter or digit. A string is a finite sequence of symbols (e.g., $\Sigma = \{a,b\}$; here Σ is an alphabet; a and b are symbols; and $abbb$, $abab$, and $ababab$ are strings) (Hopcroft and Ullman, 1979). A set of strings over a given alphabet is called a formal language.

Definition: A NFA is a 5-tuple, $R = (\Sigma, Q, q_0, F, \delta)$, where Σ is the input alphabet, Q the set of states, $q_0 \in Q$ the start state, $F \subseteq Q$ the final states, and $\delta : Q \times \Sigma \rightarrow 2^Q$ the transition function.

Two major elements in finite automata are states and inputs. Here the change of a state is fully governed by the input and the current state. The state moves from one place to another place due to the input and then reaches the final state. The input mechanism can move only from left to right, and it can read exactly one symbol at each step (Selvakumar et al., 2013).

3. Urea cycle

Every amino acid from the protein is degraded to generate ammonia. Ammonia enters the urea cycle, which occurs in both the mitochondria and cytoplasm (Figure 1). Ammonia and bicarbonate are combined to generate carbamoyl phosphate within the mitochondria in the presence of carbamoyl phosphate synthetase (CPS). Carbamoyl phosphate is converted to citrulline catalyzed by ornithine transcarbamylase (OTC) in the mitochondria. Citrulline is exported to the cytoplasm for conversion to argininosuccinate in the presence of argininosuccinic acid synthetase. Argininosuccinate is catalyzed by the enzyme argininosuccinate lyase (ASL) to form arginine. The amino acid arginine is cleaved by the enzyme arginase, producing

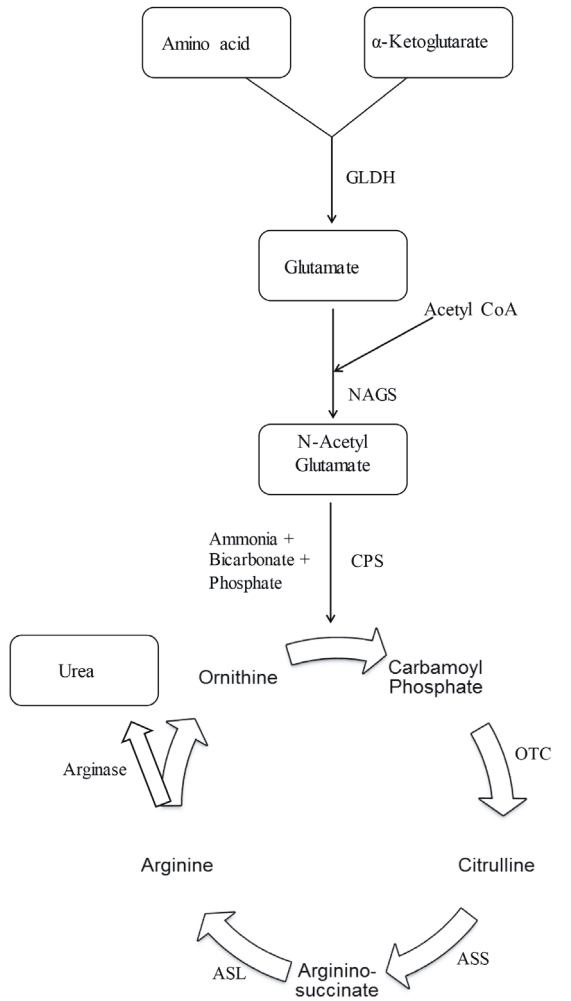

Figure 1. Overview of ammonia detoxification through the urea cycle.
Enzymes: GLDH - glutamate dehydrogenase, NAGS - N-acetylglutamate synthetase,
CPS - carbamoyl phosphate, OTC - ornithine transcarbamylase, ASS - argininosuccinate synthetase, ASL - argininosuccinate lyase.

urea and ornithine in the cytoplasm. The generated ornithine is recycled by entering the mitochondria to form citrulline. Each cycle consumes 3 ATPs and 4 high-energy nucleotides. These transport steps facilitate control but also are potential sites for genetic disease. The compound urea is the only end-product of this cycle, and the other by-products of the cycle are recycled. Overall urea formation can be explained as 2 ammonias, carbon dioxide, and 3 ATPs combined to produce urea.

In a state of intoxicated ammonia levels, the sensitive brain tissue enters a comatose state. The cells get rid of excess ammonia through reductive amination of α-ketoglutarate to form glutamate in the presence of glutamate dehydrogenase. The elevated ammonia concentration in the blood influences the conversion of glutamate to glutamine by utilizing ATP as the energy source. This results in depletion of ATP for brain function with increasing levels of ammonia in circulation. The normal catabolism in newborns combines with the immaturity of the neonatal liver to emphasize defects in these enzymes (Summar, 2001; Summar and Tuchman, 2001).

4. Computational model using finite automata

A programmable machine is designed to check the process of the urea cycle for the excretion of excess ammonia. It is a new approach to implement computing devices with an acceptance of regular expressions. A machine is constructed with the nondeterministic finite automaton to accept a string of enzymes. In a nondeterministic finite automaton, the state of acceptance is defined to accept the string of enzymes, and it attains the final state. If the machine cannot accept the string, this can be defined as the state of rejection (Muhammad et al., 2013).

The molecules are represented as states and are symbolized as follows:

States = Amino acid (*1*), α-Ketoglutarate (*2*), Glutamate (*3*), N-Acetylglutamate (*4*), Carbamoyl phosphate (*5*), Citrulline (*6*), Argininosuccinate (*7*), Arginine (*8*), Ornithine (*9*), Urea (*10*), Cytoplasmic ornithine (*11*).

The enzymes are represented as inputs and are symbolized as follows:

Inputs = Glutamate dehydrogenase (*A*), N-acetylglutamate synthetase (*B*), Carbamoyl phosphate synthetase (*C*), Ornithine transcarbamylase (*D*), Argininosuccinate synthetase (*E*), Argininosuccinate lyase (*F*), Arginase (*G*).

In the transition diagram (Figure 2), ε transition is a spontaneous transition that does not use any input. The automaton simply decides to change states without reading any symbol. There is no input from state *9* to state *11*; instead, a transporter is involved. The sequence of symbols, called a string, is accepted with ε transition. The transition function of a nondeterministic finite automaton for the state of acceptance is shown in Table 1.

4.1. State of rejection

In the case of a rejection state (Table 2), this machine could not read the input symbol; therefore, the process stopped. A rejection state may be due to the presence or absence of the active enzymes (inputs) needed to produce the respective molecules as a product. The metabolic potential of a cell can be investigated with the availability of the genome sequence for all known enzymes involved in the metabolic process (Gebert et al., 2006). A more refined approach to elusive drug development can be achieved by estimating the genome-wide network map accurately in order to specify targets of interested gene interactions (Altay et al., 2013). The current interdisciplinary methodology based on computational models supports investigation of metabolic products through inspecting the specific enzymatic molecule. Based on the literature, the expression levels of the following enzymes may play a vital role in the inhibition of the ammonia detoxification process. Under such conditions, the ammonia level can be regulated by any one of the following enzymes involved in the urea cycle. Such regulatory mechanisms can be used as therapy for UCD.

UCD can be controlled through up- or downregulation of enzymes that are responsible for urea formation. There is a severe deficiency in any of the first 4 enzymes (CPS,

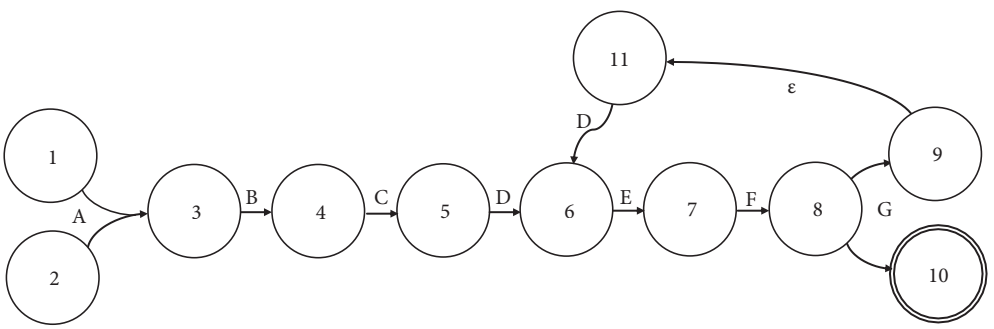

Figure 2. Transition diagram for urea excretion.

Table 1. The procedures and transition functions of NFA for acceptance.

Transition function	Explanation
δ (1,A) = 3	Start from the current state *1* with input *A*, and then it goes to state *3*
δ (2,A) = 3	Start from the current state *2* with input *A*, and then it goes to state *3*
δ (3,B) = 4	Start from the current state *3* with input *B*, and then it goes to state *4*
δ (4,C) = 5	Start from the current state *4* with input *C*, and then it goes to state *5*
δ (5,D) = 6	Start from the current state *5* with input *D*, and then it goes to state *6*
δ (6,E) = 7	Start from the current state *6* with input *E*, and then it goes to state *7*
δ (7,F) = 8	Start from the current state *7* with input *F*, and then it goes to state *8*
δ (8,G) = 9	Start from the current state *8* with input *G*, and then it goes to state *9*
δ (8,G) = 10	Start from the current state *8* with input *G*, and then it goes to state *10*
δ (9,*ε*) = *11*	Start from the current state *9* with ε transition, and then it goes to state *11*
δ (11,D) = 6	Start from the current state *11* with input *D*, and then it goes to state *6*

Table 2. The procedures and transition functions of NFA for rejection.

Transition function	Explanation
δ (1,A) = 3	Start from the current state *1* with input *A*, and then it goes to state *3*
δ (2,A) = 3	Start from the current state *2* with input *A*, and then it goes to state *3*
δ (3,B) = 4	Start from the current state *3* with input *B*, and then it goes to state *4*
δ (4,R) = 4, $\forall R \neq C$	In this transition the current state is *4*; if it could not get an input *C*, then it remains in state *4*

OTC, argininosuccinate synthetase, and ASL) in the urea cycle or the cofactor producer (NAGS) (Batshaw, 1984; Summar, 2001; Summar and Tuchman, 2001). Mutation in the gene encoding OTC is causative for deficiency of the respective enzyme (Tuchman et al., 1995). NAGS deficiency is due to mutation in the gene encoding the respective enzyme (Caldovic et al., 2003). Deficiency in the fifth enzyme, arginase, results in a chronic debilitating disease primarily affecting the nervous system (Batshaw, 1984). Deficiencies in any of the enzymes involved in urea synthesis have destructive effects on the production of urea due to the prominent level of ammonia concentration in the blood. These disorders include diseases such as argininosuccinic aciduria (argininosuccinate synthetase deficiency), CPS deficiency, and citrullinemia.

5. Conclusion

Biologists produce requisite gene-expression data that are used to construct an optimized network through mathematical applications. A NFA was designed to monitor the process of enzyme deficiency in UCD. The state of acceptance indicates the normal function of the enzymes. The state of rejection denotes an enzymatic disorder due to variations in the rates of transcription. Such a condition may show an altered effect in the expression level of enzymes involved in the urea cycle (Takiguchi and Mori, 1995). In the case of rejection, nitrogen is accumulated, resulting in improper excretion of ammonia in the form of urea. The elevated level of ammonia toxicity leads to brain damage and neurologic impairment in advanced stages of UCD. Deeper insights into the complex regulatory systems of the metabolic process can be analyzed and diagnosed by the development of new computational methods. Promising new therapies for UCD including clinical trials can result from the construction of such computational models for monitoring and diagnosing disorders in newborns and adults. Hence, the machine designed using NFA facilitates the development of biochips or biosensors for enzyme-targeted therapy in conjunction with newborn screening and genetic tests for enzymatic function.

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