COMPUTER ANALYSIS OF THE EFFECTS OF MUTATIONS IN LDL RECEPTOR GENE ON THE REGULATION OF CHOLESTEROL BIOSYNTHESIS IN THE CELL

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Resume

Motivation: Study of the normal and pathological cholesterol transports from blood plasma into the cell mediated by LDL receptors has become a topical problem. Impairments of this system play a key role in development of hypocholesterolemia and atherosclerosis of humans and various animals.

Results: The mathematical model simulating cholesterol biosynthesis in the cell and its exchange with blood plasma cholesterol developed earlier with additionally verified values of its parameters was used for computer analysis of the effects of various mutations in LDL receptor gene on the system in question.

Availability: http://wwwmgs.bionet.nsc.ru/mgs/gnw/gn_model/

Introduction

The main fraction of cholesterol in blood plasma is contained in low-density lipoproteins, or LDL (Murray et al., 1988). Cholesterol is transported into the cell via a system mediated by LDL receptors. This system maintains a certain LDL level and, correspondingly, a certain cholesterol level, in blood plasma. The relevant experimental data demonstrate that impairments of this system play a key role in development of hypocholesterolemia and atherosclerosis of humans and various animals (Klimov, Nikul’cheva, 1999).

So far, over 300 mutations in LDL receptor gene have been detected and described. The major part of these mutations are large deletions or rearrangements, while the rest result from deletions of one or several base pairs or, rarer, insertions, or point nucleotide substitutions (Hobbs et al., 1990; Soutar, 1992).

In this work, computer analysis of the effects of various mutations in LDL receptor gene on the system studied was performed.

Methods and Algorithms

A computer model of gene network functional dynamics developed earlier (Ratushny et al., 2000) was used for the analysis. The model was developed in the context of chemical kinetic approach to simulation and is a system of ordinary differential equations describing the operation of this gene network supplemented with a set of discrete expressions used to imitate the external factors (Likhoshvai et al., 2001). Additional information is available from (Ratushny et al., 2000) and the papers used to describe this gene network in GeneNet (http://wwwmgs.bionet.nsc.ru/systems/mgl/genenet/).

Identification of the parameters of mathematical model. The values of reaction constants (the parameters in question) were determined differently. All the parameters used in this mathematical model fall into the following groups: (1) constants of enzymatic reactions; (2) constants of macroprocesses (transcription, translation, etc.); and (3) the constants characterizing interactions of the gene network regulating cholesterol biosynthesis with its cellular and organismal environment. Values of the majority of enzymatic constants are compiled in the electronic databases WIT (http://www-unix.mcs.anl.gov/compbio/), BRENDA (http://bRENDA.bc.uni-koeln.de/), and other.

Values of the constants of macroprocesses were selected taking into account the biological data on their typical rates. For example, the translation rate constant is taken equal to 0.1 sec\(^{-1}\), as (i) the distance between A-sites of the neighboring ribosomes in mRNA cannot be less than 20–60 nucleotides due to steric limitations and (ii) the mean elongation rate amounts to about 3–10 codons/sec (Spirin, 1986).

The parameters characterizing interactions of gene network with environment were estimated from general biological grounds, such as lifespans or half-lives of gene network components, their equilibrium concentrations, contents per cell, durations or rates of the processes considered, etc.

The values of parameters absent in the literature were verified using evolutionary method (Likhoshvai et al., 2002). Fig. 1 exemplifies the achieved compliance of the model with experimental data.
Implementation and Results

The model allows stationary characteristics and dynamics of the gene network, both in norm and in the presence of mutations, upon various effects to be studied.

Bold lines in Fig. 2 demonstrate the calculated response of the studied gene network in norm to a twofold increase in the inflow of LDL into blood plasma continuing over 8 h (hatched region). These conditions cause a monotonic increase in blood LDL, reaching an approximately fourfold level by 10 h of the experiment (a, n). In this process, the concentration of free receptors on the cell surface decreases (c, n), whereas the concentration of cholesterol in the cell changes insufficiently (b, n), which is explained by the negative feedback decreasing the rate of cholesterol biosynthesis in the cell upon its increased inflow from outside the cell. All the variables of this system take stationary values approximately 6 h after the internal effect is stopped.

Fine lines in Fig. 2 show the behavior patterns of the system when three different mutations are introduced. The first mutation (m₁; Fig. 2) exemplifies the class of mutations in LDL receptor gene preventing formation of immunodetectable protein (the so-called null alleles). Specific of a considerable fraction of the null alleles is increased concentrations of LDL receptor mRNA in cells of patients (Klimov, Nikul'cheva, 1999). Our numerical calculations simulating a twofold decrease in the rate of LDL receptor synthesis in the gene network demonstrate that the stationary number of free receptors on the cell surface decreases approximately 2.5-fold (m₁, c; Fig. 2). The stationary LDL concentration in blood increases approximately 1.5-fold, while the free cholesterol content in the cell decreases by 15% (m₁; Fig. 2). We explain a relatively small decrease in the intracellular cholesterol content with the ability of cell to compensate for the decrease in the external cholesterol inflow with its increased intracellular synthesis in combination with the negative feedback regulation of the cholesterol biosynthesis rate.

The graphs m₂ in Fig. 2 exemplify the class of mutations retaining the normal synthesis of LDL receptor and its transport to the cell surface; however, it displays a decreased ability to bind LDL. Most frequently, these mutations result from the deletion removing repeats 1 and 2 from the ligand-binding domain of LDL receptor gene (Russel et al., 1989). Using the model, we studied the effect of a fivefold (relative to the norm) decrease in the ability of the receptors to bind LDL. The calculations demonstrate that the mutation fails to deviate the LDL content in blood plasma and cholesterol concentration in the cell considerably from the norm (a, m₂; b, m₂), despite a drastic decrease in the LDL transport into the cell from intercellular space through the cell membrane. A compensatory effect of the negative feedback controlling transcription levels of the genes encoding enzymes of the cholesterol biosynthesis and LDL receptor gene in the cell underlies this phenomenon.

A fivefold decrease in the total LDL flow from blood plasma to the cell results in a fivefold increase in the transcription intensities of the corresponding genes according to a negative feedback mechanism. Consequently, both the production of endogenous cholesterol is elevated and the concentration of LDL receptors on the cell surface increases approximately fivefold (c, m₃), thereby normalizing the total LDL transport into the cell.
Cleavage of LDL receptor from its ligand in the acid medium of endosomes and its return to the cell surface complete the receptor conversion cycle in the cell. The mutation variant exemplified by curves m3 (Fig. 2) brings about formation of a truncated LDL receptor protein. This truncated receptor loses the ability to release LDL in endosomes, resulting in receptor degradation. The degradation rates of the LDL receptors impaired by mutations of this class may grow 5–10-fold, decreasing considerably the number of receptors on the cell surface (Fourie et al., 1992). The model allowed us to analyze the response to a tenfold increase in the receptor degradation in the cell relative to the normal rate. Fig. 2 demonstrates that the response to this mutation is qualitatively similar to that caused by the first mutation variants, but is more pronounced.

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The stationary number of free receptors on the cell surface reduces approximately 4.5-fold (c, m2); the concentration of free cholesterol, by approximately 25% (b, m2).

**Conclusion**

The computer analysis of the effects of different mutations in LDL receptor gene on the cholesterol biosynthesis on the cell has demonstrated that the stationary concentration of cholesterol in the cell is sufficiently insensitive to the mutations in question, whereas certain mutations change considerably the LDL level in blood plasma and, correspondingly, the cholesterol level. Certain mutations, such as m1 and m3 (Fig. 2) are capable of increasing significantly the LDL concentration in blood plasma, extending their circulation in blood of mutant individuals to 4–6 days (versus 2.5 days in intact individuals). Additionally, these LDLs become more susceptible to chemical modifications (peroxidation, glycosylation, etc.), as it is not native, but these LDL variants that acquire atherogenic properties (Klimov, Nikul’cheva, 1999). Such changes play the key role in development of hypcholesterolemia and atherosclerosis of humans and different animals. Mathematical simulation and computer analysis of the system regulating cholesterol biosynthesis allows new
approaches to prediction of these disease courses to be developed and optimal therapeutic strategies and methods for their corrections to be planned.

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