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Cholera toxin A1 residues single alanine substitutional mutation and effect on activity with stimulatory G protein

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ABSTRACT

Cholera is a well-known gastrointestinal infection. The cholera toxin is an important pathological substance in pathogenesis of cholera diarrhea. Cholera toxin is composed of catalytic A1 subunit, an A2 linker, and a homopentameric cell-binding B subunit. In enterocyte, cholera toxin will attach to GM1 ganglioside receptors on the apical membrane and causes retrograde vesicular trafficking to endoplasmic reticulum. At endoplasmic reticulum, cholera toxin A1 is released from the rest of the toxin into cytoplasm. The cholera toxin A1 interacts will catalyze ADP ribosylation of subunits of stimulatory G protein resulting a persistent activation of adenylate cyclase and an elevation of intracellular cAMP which further result in diarrhea. The single alanine substitutional mutation can result in the reduction of the interaction activity between cholera toxin A1 and stimulatory G protein. In this study, the four well-known mutations, H55, R67, L71, S78, or D109, of cholera toxin A1 is focused. The author hereby calculates for the reaction energy for the reaction between cholera toxin A1 and stimulatory G protein in naïve case and mutated case. To calculate, the standard bonding energy calculation technique in mutation analysis was used. It can be seen that aberrant in reaction energy in each studied mutation is different and can imply the different effect on activity with stimulatory G protein.

1. Introduction

Diarrhea is a common problem in clinical practice. Infective diarrhea is still present public health problem in tropical countries. Of several diarrheal diseases, cholera is a well-known gastrointestinal infection. Severe watery stool passing is the clinical presentation and this can lead to fatality [1,2]. The pathogenesis of cholera is very interesting. The cholera toxin is an important pathological substance in pathogenesis of cholera diarrhea [2]. Cholera toxin is composed of catalytic A1 subunit, an A2 linker, and a homopentameric cell-binding B subunit [3]. In enterocyte, cholera toxin will attach to GM1 ganglioside receptors on the apical membrane and causes retrograde vesicular trafficking to endoplasmic reticulum [3]. At endoplasmic reticulum, cholera toxin A1 is released from the rest of the toxin into cytoplasm [3]. The cholera toxin A1 interacts will catalyze ADP ribosylation of α subunits of stimulatory G protein resulting in a persistent activation of adenylate cyclase and an elevation of intracellular cAMP which further result in diarrhea [3]. The effect of genetic aberration on the pathogenesis is very interesting. The single alanine substitutional mutation can result in the reduction of the interaction activity between cholera toxin A1 and stimulatory G protein [4]. In this study, the four well-known mutations, H55, R67, L71, S78, or D109, of cholera toxin A1 is focused.

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2. Materials and methods

This work is designed as a mathematical modeling study. The author hereby calculates for the reaction energy for the reaction between cholera toxin A1 and stimulatory G protein in naïve case and mutated case. To calculate, the standard bonding energy calculation technique in mutation analysis was used. The method is the standard method as used in previous publications [5,6]. Briefly, quantum chemical modeling analysis for energy reaction was done. As a primary assumption, the required reaction energy is firstly assigned to be "A" kCal/mol for one mole of cholera toxin A1 and stimulatory G protein product complex. Focusing on the amount of the substrates, it is assigned to be "B" g and "C" g for cholera toxin A1 and stimulatory G protein in general reaction with wild type of cholera toxin A1. The variability in this modeling study is the change in substrate, cholera toxin A1, due to mutation. The studied mutations are H55, R67, L71, S78, or D109. The required energy due to each mutation is assessed.

3. Results

The results from modeling are shown in Table 1. It can be seen that aberrant in reaction energy in each studied mutation is different and can imply the different effect on activity with stimulatory G protein. Based on the modeling, the required energy can be list from the least to the most as the followings: R67, S78, D109, wild, H55 and L71 types, respectively.

Table 1
Required energy for reaction in wild and mutated types of cholera toxin A1.

Types	Amount of cholera toxin A1 (g)	Require energy (kCal/mol)
Wild	B	A/B
H55	B – 174.20 + 155.16	A/B – 174.20 + 155.16
R67	B – 131.18 + 174.2	A/B – 131.18 + 174.2
L71	B – 155.16 + 131.18	A/B – 155.16 + 131.18
S78	B – 105.09 + 131.18	A/B – 105.09 + 131.18
D109	B – 115.13 + 133.10	A/B – 115.13 + 133.10

4. Discussion

In pathogenesis of diarrhea, cholera toxin (CT)-mediated adenosine diphosphate (ADP)-ribosylation of stimulatory G protein (Gsα) in enterocytes is the main step [3]. The increased intracellular cAMP in enterocyte is the main leading cause of profuse diarrhea and severe fluid loss in cholera [3]. To learn on molecular biological process is useful in understand the natural history of disease as well as finding of new effective drug.

Here, the authors use quantum chemical analysis for performing modeling study to assess the effect of five well-known mutations of cholera toxin A1. It can be seen that the mutations result in different outcome in the pathogenesis, different degrees of expression of disease can be expected. Based on the study, the mutations R67, S78 and D109 can result in increased susceptibility to disease expression and the mutations H55 and L71 can result in can result in decreased susceptibility to disease expression. Indeed, it is no doubt that some mutants of cholera toxin are less invasive than wild type. The application of reduced invasive type in influenza vaccine development is the good example [7].

Conflict of interest statement

We declare that we have no conflict of interest.

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