OPEN ACCESS Freely available online

Journal of Proteomics & Bioinformatics

Research Article

An Investigation of the Effect of T15458C and T15663C Mutations in the *CYB* Gene on Respiration via an *In silico* Method

Mulyani R, Destiarani W, Rahmawati UM, Yusuf M, Subroto T, Iman P. Maksum*

Universitas Padjadjaran, Jl. Raya Bandung Sumedang KM.21, Hegarmanah, Jatinangor, Kabupaten Sumedang, Jawa Barat 45363 Raya Jatinangor, Indonesia

ABSTRACT

Diabetic mellitus and cataracts are common mitochondrial diseases in Indonesia, occurring as a result of a lack of ATP, which is produced in mitochondria via oxidative phosphorylation (OXPHOS). The OXPHOS chain is composed of five complex proteins encoded by genes from nuclear DNA and mitochondrial DNA. One such gene is the *CYB* gene, with its locus in mitochondrial DNA. The *CYB* gene encodes part of the complex III subunit, cytochrome b, an electron transfer agent. A novel mutation has been identified at T15458C in type 2 diabetes mellitus patients and T15663C in cataract patients, both of which are in the *CYB* gene, leading to amino acid alterations S238P and I306T. This study investigated the relationship between T15458C and T15663C mutations in the *CYB* gene of mitochondrial DNA to the OXPHOS process via an *in silico* method. Wild type and mutant *CYB* were modelled via homology modelling using Modeller 9.19 with a 5XTE template, then evaluated using the Ramachandran plot, packaging quality score, and the DOPE profile. The results of the S238P mutant structure analysis showed that proline acts as a helix-breaker due to the loss of a hydrogen bond, while I306T causes a hydrophobic interaction as the helix stabiliser is lost in the mutant. It is assumed the threonine was phosphorylation mutations also destabilises the complex, as proven by the $\Delta\Delta G < 0$. In conclusion, S238P and I306T mutations affect the function of complex III as an electron transfer agent in the respiratory process.

Keywords: Cataract; Cytochrome b; Diabetes mellitus; Mitochondrial disease

INTRODUCTION

Mitochondrial disease was first discovered in 1988 and mitochondrial DNA has since been the focus for the identification of pathogenetic mutations [1]. As of July 2018, the MITOMAP database website listed 349 pathogenetic point mutations. Some diseases caused by mutations in mitochondrial DNA are LHON (Leber heredity optic neuropathy), MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes), MERRF (myoclonus epilepsy associated with ragged red fibres), MIDD (maternally inherited diabetes and deafness), breast cancer, thyroid cancer and prostate cancer [2,3]. In Indonesia, the most common mitochondrial diseases are diabetes and cataracts [4,5]. Diabetic mellitus is a maternal disease caused by ATP deficiency in the pancreas which disrupts insulin secretion, whereas cataracts occur by the inhibition of the protein renaturation process in the eyes [6,7]. Based on statistical analysis, the 3243(A>G) was associated with type 2 diabetes and cataracts ($p<0.001$) [8]. An indicator-based electrochemical DNA biosensor for the detection of mitochondrial DNA A3243G tRNALeu point mutation related to maternally inherited diabetes (MID), MIDD and type 2 diabetes mellitus (DM) was evaluated with both synthetic and real samples [9]. The A3243G mutant template by Site-Directed Mutator (SDM) was used as a positive control in PASA-mismatch three bases to determine the optimisation condition of SDM. Normal mtDNA was isolated from lymphocytes, then the adenine base in position 3243 of tRNA Leu(UUR) gene was mutated to guanine and amplified using a mutant forward primer Mt3243 F and reverse primer Mt3243 R. A mutant template with 100× optimum dilution was used as a PASA-mismatch three base positive control in A3243G mtDNA mutation analysis [10]. Regarding the MITOMAP database 2013, there were 446 mitochondrial mutations related to mitochondrial diseases, with 211 mutations in the coding region. The largest number of mutations located on phosphorylation with [11]. Oxygen is used by aerobic organisms to synthesise energy in the form of ATP (adenosine triphosphate) via oxidative phosphorylation (OXPHOS) [12]. In eukaryotes, OXPHOS occurs in the mitochondrial membrane via five complex

Correspondence to: Iman P. Maksum, Universitas Padjadjaran, Jl. Raya Bandung Sumedang KM.21, Hegarmanah, Jatinangor, Kabupaten Sumedang, Jawa Barat 45363 Raya Jatinangor, Indonesia, Tel: +6282240839482, E-mail: iman.permana@unpad.ac.id

Received: September 22, 2020, **Accepted:** December 08, 2020, **Published:** December 15, 2020

Citation: Mulyani R, Destiarani W, Rahmawati MU, Yusuf M, Subroto T, Maksum IP, et al. (2020). An Investigation of the Effect of T15458C and T15663C Mutations in the *CYB* Gene on Respiration via an *In silico* Method.. J Proteomics Bioinform. 13:519.

proteins encoded by nuclear DNA and mitochondrial DNA [13]. Complex III, known as the cytochrome bc1 complex of cytochrome bc1 oxidoreductase, is an electron transfer agent and a homodimer consisting of 11 subunits, the biggest of which are ISP subunits (iron-sulphur binding protein), cytochrome b, and cytochrome c1 [14]. Cytochrome b is the only subunit coded by mitochondrial DNA, consisting of 380 amino acids, mostly hydrophobic [15]. Maksum et al. [16] found two mutation points that have not been reported before in patients with type 2 DM, namely 15458T>C and 15663T>C in cataract patients [16] located in the *CYB* gene that encodes cytochrome b protein, complex subunit III. These mutations caused changes in amino acids S238P and I306T, and theoretically, could interfere with protein stability, directly affecting electron transfer. Both unreported point mutations could provide a novel understanding of mitochondrial DNA alteration in ATP deficiency. In other studies, the mutation m.9053G>A in the ATP6 gene was identified in patients with type 2 DM and cataracts. The TP6 gene encodes the protein subunit of ATPase6, a part of ATP synthase, which is important in electron transfer and proton translocation in the intracellular respiratory system [17]. This study aimed to investigate the effect of 15458T>C and 15663T>C in the cytochrome protein b complex subunit III, as an electron transporter in human respiration via an *in silico* method.

EXPERIMENTAL PROCEDURE

Methods

Cytochrome b templates were obtained using MITOMAP. Cytochrome b amino acid sequences were taken from NCBI, access number NC_012920 AC_000021 version NC_012920.1. Cytochrome b amino acid sequences were aligned using BLAST (Basic Local Alignment Search Tool) with the PDB (Protein Data Bank) database to obtain templates. The criterion for the selected 3D protein templates was the highest homology to 5XTE.

Model development

Cytochrome modelling, mutant S238P, and I306T mutants were conducted using the Modeller 9.19 programme with a protein template 5XTE PDB code. Fifty models were created and one model with the lowest DOPE (Discrete Optimised Protein Energy) value was selected to model the cytochrome b native protein, mutant S238P, and the I306T mutant.

Evaluation of the model

The model with the lowest DOPE value was evaluated using the PROCHECK programme, which determines the torsion angle values (*phi*) and (*psi*) on the Ramachandran plot. Residues in the permitted area must be more than 90% to obtain good structural quality. The second evaluation was performed by the WHAT-IF programme to obtain the packaging quality value to determine if the interaction between atoms on each residue was stable, the number of permitted packaging quality must be greater than -5.0. The last evaluation was to construct a DOPE profile plot to determine whether the DOPE profile model matched the template.

Mutation analysis

Molecular dynamic simulations on helices were performed for natives and mutants for S238P and I306T. It also analyses the effect on the complex by calculating the RMSD value (root mean square deviation) as the value of the mutant model deviation of the native model and ΔΔG.

RESULTS AND DISCUSSION

Sequence analysis

Cytochrome b native protein, S238P, and I306T mutants were modelled via a homology modelling method using the sequence of cytochrome b proteins in the NCBI database as target proteins. To obtain a protein with a homologous sequence, the cytochrome b sequence was aligned with the database using BLAST to determine the template with 100% identity homology with the target protein. In this study, 5XTE (crystalline structure of human respiratory complex III) (cytochrome bc1 complex) was used as the modelling template, with a resolution of 3.4 Å to allow visualisation of each atom. Previously, Maksum et al. [16] used 1L0L (structure of bovine mitochondrial cytochrome bc1 complex with a bound fungoxadone), [16] Anugolu et al. [18] used 1BGY (cytochrome bc1 complex from bovine) [18] and Husen et al. [19] used 1ZRT (Rhodobacter capsulatus cytochrome bc1 complex with stigmatellin bound) [19]. The 5XTE template was released in PDB on 30th August 2017, as yet, no research publications have reported the use of this template despite the high validation as a reference for the structure of the cytochrome bc1 III complex. 5XTE is a three-dimensional structure of cytochrome bc1 complex III with an amino acid number of 4224. S238P and I306T mutations occur in the cytochrome b subunit, which is one of the cytochrome bc1 complex III subunits (Figure 1).

Model development

Using the Modeller 9.19 programme, the cytochrome b protein was modelled and the model with the lowest DOPE value was selected, as this indicates that the structure has the highest stability. The DOPE value is the potential energy to assess the quality of a molecular model, the smaller energy, the more stable the molecule. The native model had a DOPE value of -39920.27, mutant model S238P -39778.17, and the mutant model I306T -39879.07. The best model was evaluated using the model evaluator programme.

Evaluation of Cytochrome Model b

The native model and the two mutants were evaluated with several programmes. The PROCHECK programme provides an evaluation

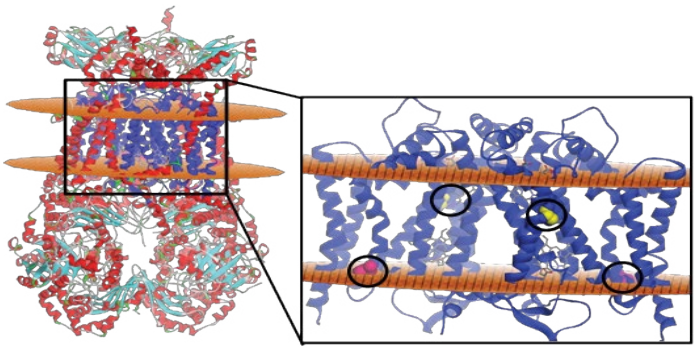
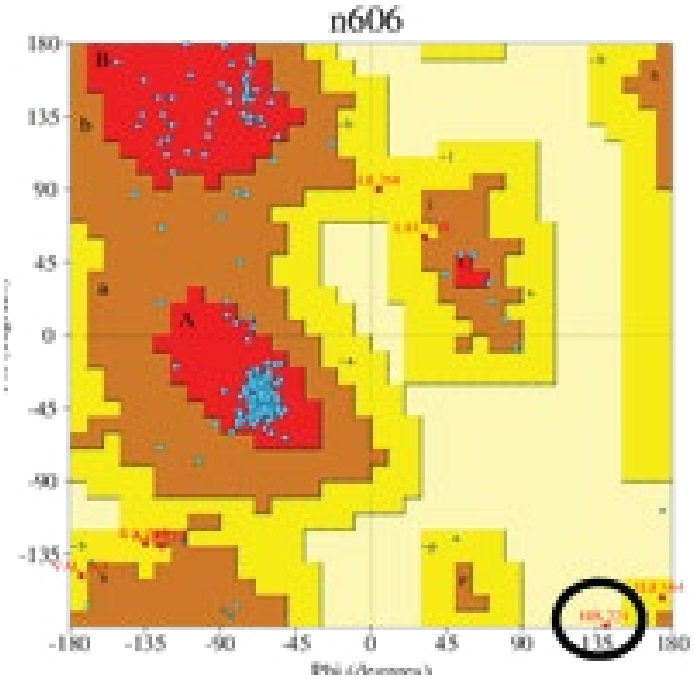


Figure 1: Structure of the cytochrome bc1 complex III in the mitochondrial membrane and enlargement of the cytochrome b subunit indicated in blue. The point mutations are marked by a black circle and yellow atomic colour for the mutation point S238P and pink for the mutation point I306T.

in the form of the Ramachandran plot, which illustrates the presence of amino acid residues to assess the stereochemical quality of protein structures. The model is acceptable if the percentage of residues in the permitted area is more than 90%. The negative model Ramachandran plot (Figure 2) shows that there is one amino acid in the area that is not permitted, namely H221, therefore loop optimisation was performed on the loops surrounding H221 amino acid, which are residual loops 206-223. In the optimisation of this loop, a low-energy loop conformation state is preferable, so the conditions are permitted. After optimisation, all residues were in the permitted area as shown in Figure 3, that is, in the absence of H221, the model was considered stereochemically good. There were no amino acid residues in the area not permitted in the Ramachandran plot for the S238P mutant model (Figure 4) and I306T mutant model (Figure 5), so loop optimisation was not needed. The models were also evaluated via the WHAT-IF programme to obtain the packing quality. The evaluation method for this programme is DACA (Directional Atomic Contact Analysis), which observes atoms from each amino acid and their interaction with other amino acids from different proteins. The packing quality is present in each amino acid residue in the model, if the residue has a value of -5.0 or less, then the residue makes contact symmetry, contact with the ligand or ion, or something goes wrong [20]. The average packaging quality model for 380 amino acids was -1.206, -1.249 for the mutant model S238P, and -1.221 for the mutant model I306T, indicating good packaging quality, hence,

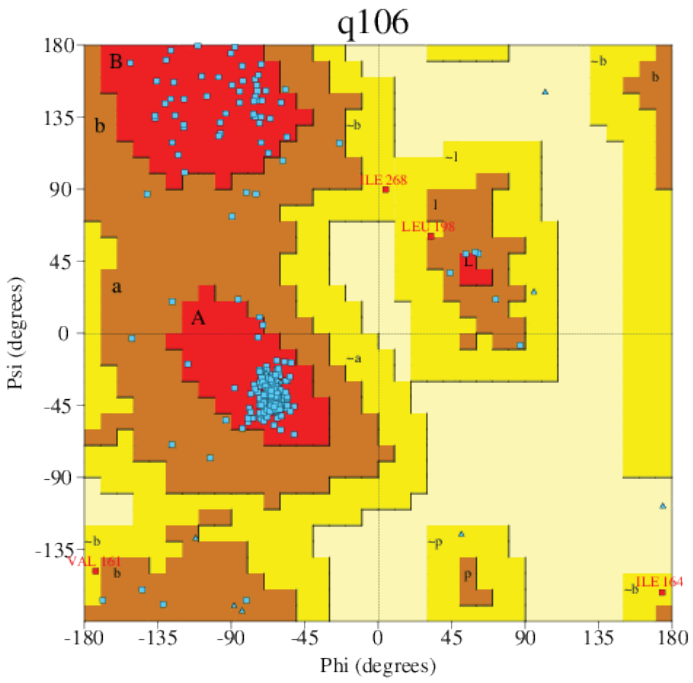


PROCHECK statistics

1. Ramachandran Plot statistics

		No. of residues	%-tage
Most favoured regions	[A,B,L]	303	91.5%
Additional allowed regions	[a,b,l,p]	21	6.3%
Generously allowed regions	[~a,~b,~l,~p]	6	1.8%
Disallowed regions	[XX]	1	0.3%*
Non-glycine and non-proline residues		331	100.0%
End-residues (excl. Gly and Pro)		2	
Glycine residues		24	
Proline residues		23	
Total number of residues		380	

Figure 2: Ramachandran plot for the negative model, showing one amino acid in the area that is not permitted, the H221 residue (dark circle).

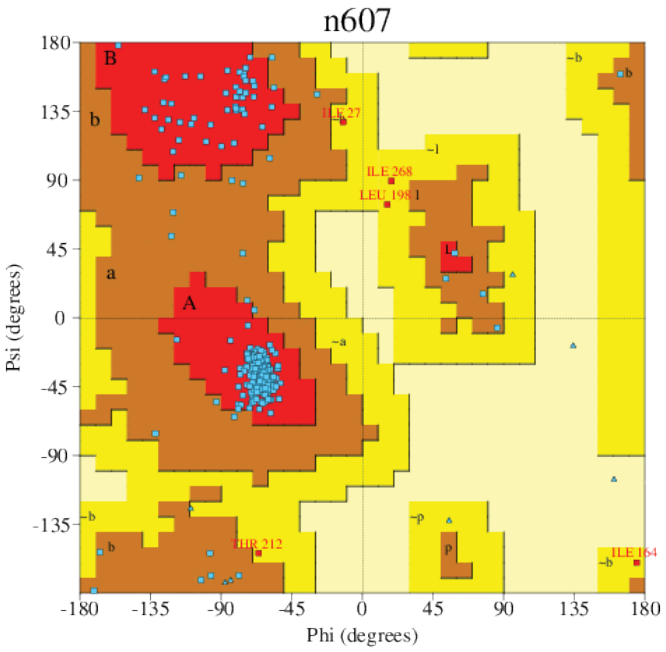


PROCHECK statistics

1. Ramachandran Plot statistics

		No. of residues	%-tage
Most favoured regions	[A,B,L]	306	92.4%
Additional allowed regions	[a,b,l,p]	21	6.3%
Generously allowed regions	[~a,~b,~l,~p]	4	1.2%
Disallowed regions	[XX]	0	0.0%
Non-glycine and non-proline residues		331	100.0%
End-residues (excl. Gly and Pro)		2	
Glycine residues		24	
Proline residues		23	
Total number of residues		380	

Figure 3: Ramachandran plot for the negative model after loop optimisation, with all residues in the permitted area.

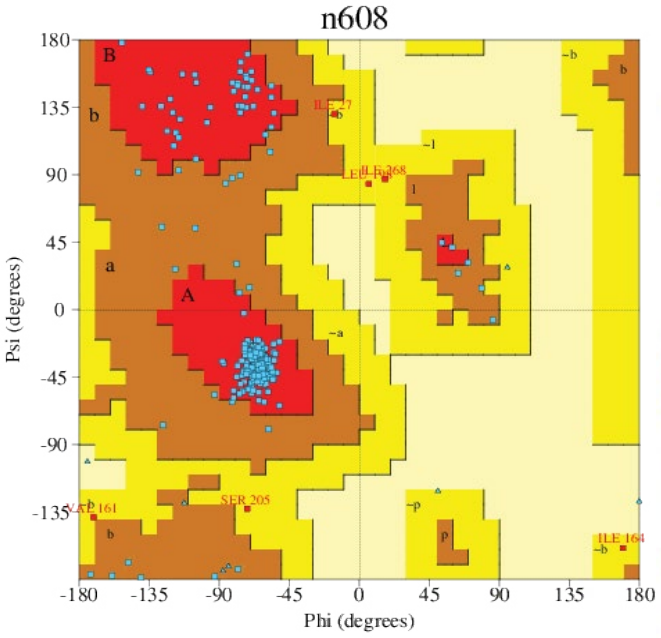


PROCHECK statistics

1. Ramachandran Plot statistics

		No. of residues	%-tage
Most favoured regions	[A,B,L]	301	91.2%
Additional allowed regions	[a,b,l,p]	24	7.3%
Generously allowed regions	[~a,~b,~l,~p]	5	1.5%
Disallowed regions	[XX]	0	0.0%
Non-glycine and non-proline residues		330	100.0%
End-residues (excl. Gly and Pro)		2	
Glycine residues		24	
Proline residues		24	
Total number of residues		380	

Figure 4: Ramachandran plot for the S238P mutant model, with all residues in the permitted area.



PROCHECK statistics

1. Ramachandran Plot statistics

		No. of residues	%-tage
Most favoured regions	[A,B,L]	303	91.5%
Additional allowed regions	[a,b,l,p]	22	6.6%
Generously allowed regions	[~a,~b,~l,~p]	6	1.8%
Disallowed regions	[XX]	0	0.0%
Non-glycine and non-proline residues		331	100.0%
End-residues (excl. Gly and Pro)		2	
Glycine residues		24	
Proline residues		23	
Total number of residues		380	

Figure 5: Ramachandran plot for the I306T mutant model, with all residues in the permitted packaging area.

each amino acid fragment has stable atomic interaction. The third validation involved a comparison of the DOPE profile between the 5XTE template and the native model, the mutant S238P, and I306T, indicating that the model has good quality (Figure 6).

Analysis of S238P mutation

The mutation S238P was found in homoplasmic type 2 DM patients and occurs in cytochrome b, which could interfere with electron transfer in the process of oxidative phosphorylation, thereby disrupting ATP production. Proteins have certain functions when forming tertiary structures with distinctive characteristics formed by folding. To study its function, proteins must have structural stability according to their physiological conditions, when the structural stability is interrupted, the protein cannot function properly or even not function.

Effect of mutations on the helix

In a previous study, Maksum et al. [16] assumed that the S238P mutation had an effect on heme [16]. Histidine, which interacts with heme, along with the helical mutation point, was observed for histidine fluctuations that interact with heme. Histidine fluctuations are obtained by conducting molecular dynamics simulations in a vacuum, which do not follow the presence of cytochrome b in the membrane and the presence of water. Nonetheless, there is a lack of information regarding the effect of mutations on the helical structure. The most probable and closest possible effects are likely that the mutations have a direct effect on the helix because the mutation is at the helical-forming residue. The affect of mutations on the interactions of surrounding amino acids was analysed to determine their structural effects. Mutations that occur from serine to proline in the cytochrome b affect the

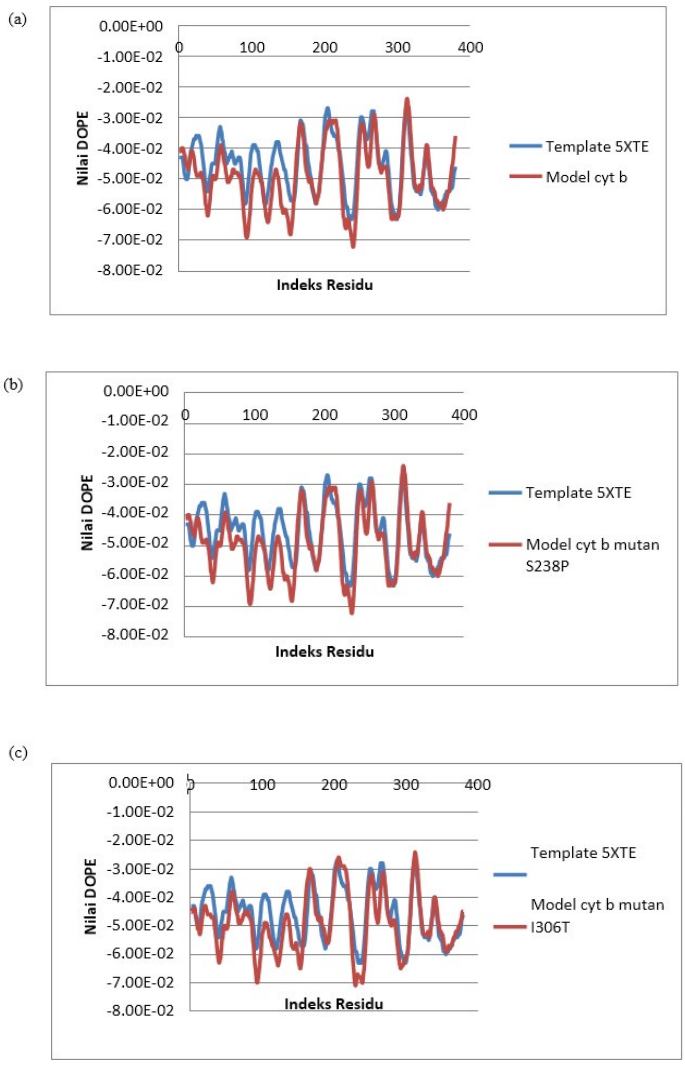


Figure 6: The comparison between the template and the model (a) cytochrome native, (b) mutant S238P and (c) mutant I306T, indicating good quality.

formation of the helical structure shown in Figure 7. In the native S238 model, hydrogen bonds present between the NH backbone serine238 with the CO backbone phenylalanine235, the OH side-chain serine238 with the CO backbone phenylalanine235, and the NH backbone is serine with leucine CO2 backbone, whereas in the P238 mutant, the three hydrogen bonds are lost. Hydrogen bonds are the main interactions that build a helical structure. Hydrogen bonds present in the main chain of amino acid residues n and n + 4 as evidenced by the hydrogen bonds in the main residue 238 with 234. When the hydrogen bonds were lost the helical structure was disrupted, hence, proline acts as a helical disruptor [15,21,22].

Effect of mutations on heme

The distance between the point mutations S238P and heme bL is about + 3 Å with two helices acting as an active site of heme b_L, namely helices a and b (written statement in Table 1). Heme b_L with a helix forms hydrophobic interactions with residues 41, 45, and 49, and with a helix b with residue 87 shown in Figure 8. Helical e disrupts due to the S238P mutation, affecting helix a and b by causing an obstacle forbidding interaction between heme and its active side. Therefore, heme activity as an electron transporter was disrupted.

Effect of mutations on complex proteins

To determine the structural differences between the cytochrome wild type and the S238P mutants, the native model was suppressed with the S238P mutant. The results of the model overlay (Figure 9) show that wild type (blue) and mutant (yellow) models do not differ in the overall structure of the A monomer, whereas in the B monomer, there is a slight shift in the helical structure. Quantitatively, RMSD was used to calculate how far the mutant model shifts from the negative model. The RMSD of the S238P mutant model on the wild type model is 2.014, which showed a distance gap in protein structure, but is still within the tolerance range of RMSD. To determine the effect of the mutations on the structure of complex III, the ΔΔG mutants was calculated against the values in complex III, ΔΔG is a prediction of the effect of mutations taking into account changes in the value of ΔG between mutants and the wild type. If the value of the mutant is greater, then ΔΔG is negative, which means that the mutation destabilises the complex, whereas, if the value of ΔG mutant is smaller, then

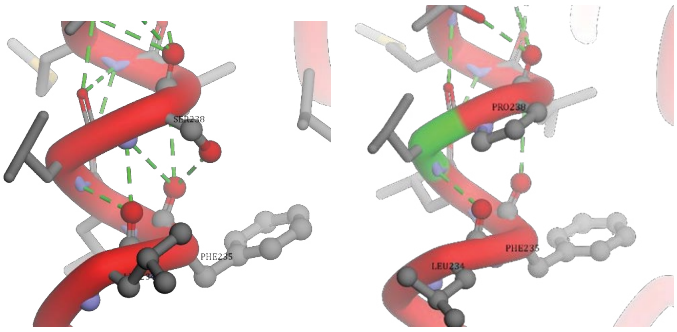


Figure 7: Differences in native (left) and mutant S238P (right) structures in forming a helix. In the native model, serine forms hydrogen bonds (green dotted lines) with L234 and F235, whereas no hydrogen bonds are formed in the proline mutant model, thereby disrupting the helical structure.

Table 1: Helical names for Figures 4, 8 and 12.

Helical name	Amino acid residue	Color
a	29-54	Red
b	77-104	Yellow
c	110-131	Navy
d	173-203	Light Blue
e	220-245	Pink
f	287-307	Green
g	319-338	Orange
Heliks Name	345-377	Brown

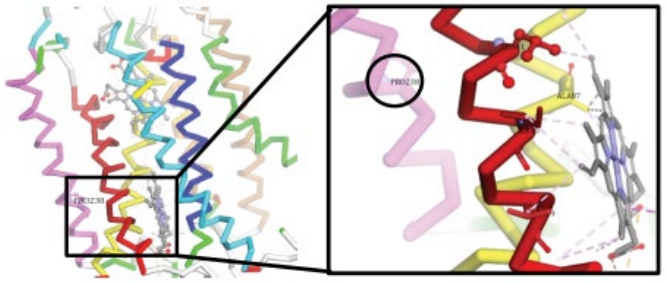


Figure 8: Interaction between helix a, b, and heme bL. The S238P mutation (circled) destabilises the helix e, thereby could affect helix a and b as the active side of heme which interferes with electron transfer.

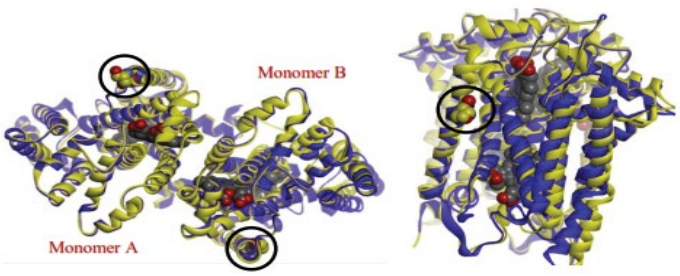


Figure 9: Wild type (blue) overlay and S238P (yellow) mutants. The left picture shows the appearance of the cytochrome b dimer model with a point mutation indicated by a black circle, the left image shows a side view on the B monomer.

ΔΔG is positive which means the mutation stabilises the complex [23]. The model is described in the SDM programme. Based on these calculations, the S238P mutation reduces the complex stability with ΔΔG -1.62 kJ/mol in the A monomer (ID chain J) and B monomer (ID V chain), shown in Figure 9. The reduced stability affects the function of complex protein III, so the S238P mutation in the cytochrome b subunit may impact the electron transfer process in the respiratory system (Table 2).

I306T mutation analysis in complex protein III.

The I306T mutation was identified in homoplasmic cataract patients. Previously, Maksum et al. [16], observed the effect of mutations on heme, reporting that histidine fluctuations interact with heme during the simulation under a vacuum. Unfortunately, the effect of mutations on the structure was not possible to observe [16] therefore, this study attempted to observe the effect of mutations on the helical structure (Figure 10). Structurally, an alteration in the amino acids in these mutations causes changes in polarity and size, the isoleucine lost hydrophobic interactions in mutants. As shown in Figure 11, the hydrophobic interaction shown by the dashed line occurs at I306 with M303 and L307, while in the T306 mutant, the hydrophobic interaction with M303 is lost. According to Ahn et al. [24] and Pace et al. [25], hydrophobic interactions can stabilise the helix, so the loss of hydrophobic interactions caused by mutations can affect the stability of the helical chain [24,25].

Effect of mutations on heme

The I306T mutation is on helix f (description in Table 1), which has a residue that directly interacts with heme bH, namely the L301 residue (Figure 12). Also, the I306T mutation is adjacent to helix c, in which the active site is located, the W113 residue. Reducing helical stability due to the mutations predicted could affect the active site of heme, hence disrupting the function of heme as an electron transporter.

Effect of mutations on complex proteins

The differentiation of wild type cytochrome and mutant models, wild type, and mutant models were suppressed with the results shown in Figure 12. The light blue mutant model shifted slightly to the edge of B monomer, while the A monomer remains as was. To determine structural deviation in the I306T mutant, RMSD was calculated using the Biovia Discovery Studio programme. The RMSD of the negative is 2.053, indicating that all mutations are within the tolerance limit. The effect of mutations on the subunits on the complex was calculated by the HR programme, ΔΔG -1.06 kJ/mol for A monomers and -1.5 kJ/mol for B monomers. These values indicate that point mutations in the subunit of cytochrome b destabilise the complex, resulting in electron transfer disturbances in complex protein III (Table 3).

Table 2: Stability change (ΔΔG) prediction of the HR programme stating that the S238P mutation can reduce the stability of complex III with ΔΔG -1.62 kJ/mol. Chain ID J is a cytochrome b subunit A, and V is a B monomer.

Parameters	Monomer A	Monomer B
PDB Code	5XTE	5XTE
Chain	J	V
Mutation	S238P	S238P
WT_SS	H	H
WT_RSA (%)	56.1	47.0
WT_Depth (Å)	3.3	3.4
WT_OSP	0.37	0.42
WT_SS	Incorrect	Incorrect
WT_SN	Incorrect	Incorrect
WT_SO	Correct	Correct
MT_SS	H	H
MT_RSA (%)	49.7	41.4
MT_Depth (Å)	3.7	3.5
MT_OSP	0.36	0.39
MT_SS	Incorrect	Incorrect
MT_SN	Incorrect	Incorrect
MT_SO	Incorrect	Incorrect
ΔΔG Prediction	-1.62 kJ/mol	-1.62 kJ/mol
Results	Decrease stability	Decrease stability

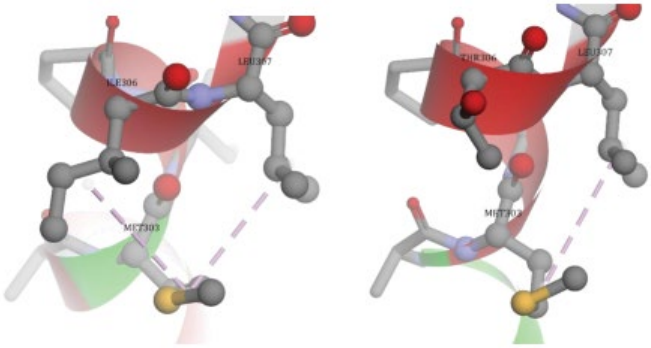


Figure 10: Negative model (left) and mutant I306T (right). Structural differences appear because of the loss of hydrophobic interactions shown by dashed lines. Wild type I306 forms a hydrophobic interaction with M303, while the interaction is lost in the T306 mutant.

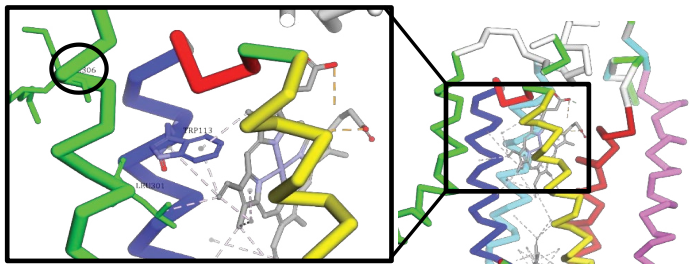


Figure 11: Interaction between helix f and c with heme bH. The I306T mutation (circled) is on the helix f and is adjacent to helix c, which is the active site of heme, residue 113.

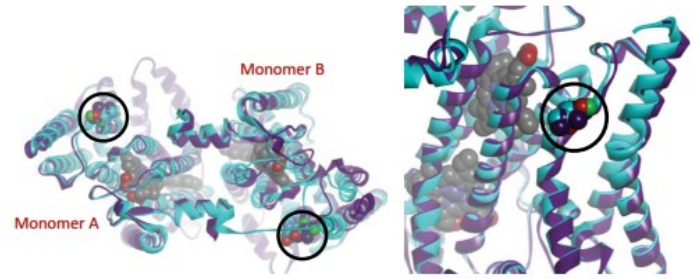


Figure 12: Wild type (purple) overlay and I306T mutants (light blue). The left picture shows the bottom view of the cytochrome b dimer model with the point of mutation shown by a black circle, the left image shows a side view on the B monomer.

Table 3: Predictions of stability changes (ΔΔG) showing that the I306T mutation can reduce the stability of complex III with ΔΔG -1.06 kJ/mol in A monomers and -1.5 kJ/mol in B monomers. Chain ID J is a cytochrome b subunit A, and V is a B monomer.

Parameters	Monomer A	Monomer B
PDB Code	5XTE	5XTE
Chain	J	V
Mutation	I306T	I306T
WT_SS	H	H
WT_RSA (%)	77.1	74.9
WT_Depth (Å)	3.4	3.5
WT_OSP	0.19	0.19
WT_SS	Incorrect	Incorrect
WT_SN	Incorrect	Incorrect
WT_SO	Incorrect	Incorrect
MT_SS	H	H
MT_RSA (%)	80.5	80.2
MT_Depth (Å)	3.4	3.3
MT_OSP	0.18	0.18
MT_SS	Incorrect	Incorrect
MT_SN	Incorrect	Incorrect
MT_SO	Incorrect	Correct
ΔΔG Prediction	-1.06 kJ/mol	-1.5 kJ/mol
Results	Decrease stability	Decrease stability

CONCLUSION

The S238P mutation showed that proline act as a helical disruptor by causing the loss of hydrogen interactions, whereas, the I306T mutation is a helical stabiliser. The ΔΔG of S238P was -1.62 kJ/mol in both monomers and I306T mutants, i.e., -1.06 kJ/mol in A monomers and 1.5 kJ/mol in B monomers, indicating that all mutations destabilise the complex. With the loss of structural stability in the complex, the S238P and I306T mutations may affect the function of complex III in electron transfer during respiration.

ACKNOWLEDGEMENT

The authors acknowledge the financial support from the second year doctoral funding grant Kemenristek DIKTI Indonesia, Nomor: 1827/UN6.3.1/LT/2020 and Academic Leadership Grant (ALG) DRPMI Universitas Padjadjaran, Nomor: 1427/UN6.3.1/LT/ 2020.

REFERENCES

1. Di-Mauro S. Mitochondrial diseases. Biochim Biophys Acta. 2004;1658(1–2):80-88.
2. Mahalaxmi I, Santhy KS. An overview about mitochondrial DNA mutations in ovarian cancer. Alexandria J Med. 2017;1(1):1-4.
3. Rossignol R, Faustin B, Rocher C, Malgat M, Mazat JP, Letellier T, et al. (2003) Mitochondrial threshold effects. Biochem J. 2003;370(3):751-762.
4. Dawkins RCH, Oliver GF, Sharma M, Pinto BM, Jeronimo B, Pereira B, et al. An estimation of the prevalence of diabetes mellitus and diabetic retinopathy in adults in Timor − Leste. Bio Med Central Research Notes. 2015;8(249):1-6.
5. Jakarta Eye Center. Cataract. 2014. https://jec.co.id/en/knowledge/33/cataract
6. Maksum IP. Patogenetika, Investigasi & Terapi Penyakit Mitokondria. Bandung: BIREAD Publishing. 2019.
7. Maksum IP. Varian Genom Mitokondria pada Pasien Diabetes Melitus Tipe 2 dan Katarak serta Kajian Pengaruh Mutasi pada Varian Genom Secara *In silico*. Bandung. Unpad Press. 2020.
8. Maksum IP, Natradisastra G, Nuswantara S, Ngili Y. The effect of A3243G mutation of mitochondrial DNA to the clinical features of type-2 diabetes mellitus and cataract. Eur J Sci Res. 2013;96:591-599.
9. Hartati YW, Topkaya SN, Maksum IP, Ozsoz M. Sensitive detection of mitochondrial DNA A3243G tRNALeu mutation via an electrochemical biosensor using Meldola's blue as a hybridization indicator. Adv Phys Org Chem. 2013;3(3A):20-27.
10. Maksum IP, Farhani A, Rachman SD, Ngili Y. Making of the A3243g Mutant template through site directed mutagenesis as positive control in pasa-mismatch three bases. Int J Pharmtech Res. 2013;5:974-4304.
11. Maksum IP, Alchumaira SF, Kamara DS, Rachman SD, Komalaningsih S. The relation of mitochondrial DNA mutation with mitochondrial diseases in coding region. Procedia Chemistry. 2015;17:84-92.
12. Kadenbach B, Hüttemann M. The subunit composition and function of mammalian cytochrome c oxidase. Mitochondrion. 2015;24(1567):64-76.
13. Nelson DL, Cox MM. Lehninger Principle of Biochemistry. (7th edn). New York: W. H. Freeman and Company, USA. 2017.
14. Lloyd RE, McGeehan JE. Structural analysis of mitochondrial mutations reveals a role for bigenomic protein interactions in human disease. PLoS One. 2013;8(7):1-14.
15. Zhuang X, Qu M, Zhang X, Ding S. A comprehensive description and evolutionary analysis of 22 grouper (perciformes, epinephelidae) mitochondrial genomes with emphasis on two novel genome organizations. PLoS One. 2013;8(8):1.
16. Maksum IP, Safitri S, Yusuf M, Natradisastra G, Nuswantara S, Suprijana O, et al. Structure and function of CYB in type-2 diabetes mellitus and cataract patients which associated with mitochondrial DNA mutation. In D. H. Tjahyono & V. S. Lee, (Eds). The 1st International Conference on Computational for Science and Technology. Chiang Mai: ITB-CMU-IAU, 2010;pp:103-110.
17. Maksum IP, Saputra SR, Indrayati N, Yusuf M, Subroto T. Bioinformatics study of m.9053G>A mutation at the *ATP6* gene in relation to type 2 diabetes mellitus and cataract diseases. Bioinform Biol Insights. 2017;11.
18. Anugolu RK, Gunda SK, Kakarla RV. Homology modeling and ligand interaction of cytochrome b protein. J Med Allied Sci. 2011;1(2):79-83.
19. Husen P, Solov'Yov IA. Mutations at the Qo-site of the cytochrome

bc1 complex strongly affect oxygen binding. J Phys Chem B. 2017;121(15):3308-3317.

20. Vriend G, Sander C. Quality control of protein models: Directional atomic contact analysis. J. Appl. Crystallogr. 1993;26(1):47-60.
21. Kim MEEK, Kang YKEE. Positional preference of proline in a-helices. Protein Sci. 1999;8(8):1492-1499.
22. Morgan AA, Rubenstein E. Proline : The distribution, frequency, positioning, and common functional roles of proline and polyproline sequences in the human proteome. PLoS One. 2013;8(1):1-9.
23. Pandurangan AP, Ochoa-monta B, Ascher DB, Blundell L. SDM : a server for predicting effects of mutations on protein stability. Nucleic Acids Research. 2017;1(1):1-7.
24. Ahn KH, Nishiyama A, Mierke DF, Kendall DA. Hydrophobic residues in helix 8 of cannabinoid receptor 1 are critical for structural and functional properties. Biochemistry. 2009;49(1):502-511.
25. Pace CN, Scholtz JM, Grimsley GR. Forces stabilizing proteins. FEBS Letters. 2014;588(14):2177-2184.