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Tailoring the Subunits of Ubiquinol Oxidase of *Acetobacter aceti* with Molecular Engineering Approach towards Microbial Fuel Cell Application

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Abstract : Acetobacter aceti is the most promising electrigen for microbial fuel cell applications. Ubiquinol oxidase, the terminal oxidase plays a key role in cell respiration. Cytochrome bo3 ubiquinol oxidase is one of the key players in the electrocatalysis of *Acetobacter aceti*. This work is aimed at constructing the three dimensional structure of the ubiquinol oxidase subunits isolated from *Acetobacter aceti.* Subunits I, II, III and IV of ubiquinol oxidase were constructed with the Modeller 9.11 software to construct the three dimensional structure. The stereochemical quality of the modeled subunits of ubiquinol oxidase was validated by the PROCHECK server. The newly built structures were also validated by z-score analysis with the ProSA server. The Q site finder is used for finding the ligand binding site on three-dimensional structure of the modeled structures. The experimental results provide a framework for understanding the structure and the possible binding sites of the four different subunits of ubiquionol oxidase of *Acetobacter aceti* for electrochemical investigations and catalytic applications in Microbial Fuel Cells.
Keywords: ubiquinol oxidase, electrogenic activity, molecular modeling, microbial fuel cells.

Introduction

Acetic acid bacteria which predominantly include the Acetobacter are believed to be the model organisms for electrochemical investigations. Acetic acid bacteria are more prevalent in nature and it includes seven genera and 33 species. *A. aceti* and *A. pasteurianus* prevail in spoiled grapes.[1] Acetobacter has the remarkable ability to oxidize wide range of substrates including ethanol and lactate and seem to be the most suitable organisms for power generation.[2] Acetobacter also finds its applications in electrochemical sensors for the detection of hydrogen peroxide.[3,4]

They are the most promising electrigens for Microbial Fuel Cell applications. Recently several reports have been made on the bioelectricity generation from *A. aceti*.[5-7] Ubiquinol oxidase which constitutes the heme-copper oxidase superfamily of enzymes along with cytochrome c plays a key role in driving the cell respiration process.[8] It conserves energy from the reduction of dioxygen to water by translocation of protons across the bacterial membrane. Subunits I, II, III and IV of ubiquinone oxidase was isolated form *A. aceti* and the molecular masses of the subunits were found to be 72, 34, 21, and 13 kDa, respectively.[9] Ubiquinol oxidase, the terminal oxidase plays a most crucial role in several bioelectrochemical reactions in biological systems. Analysing the structure of the ubiquinol oxidase subunits of *A. aceti* might offer several information leading to developments in Microbial fuel cells. Modeling of these subunits will offer several details about their

catalytic activity. NMR analysis and XRD provides information about structure the structure of protein. If the size of the proteins is large and if they are too difficult to crystallize, then the structure prediction with NMR and XRD becomes cumbersome. Molecular modeling is the process by which the three dimensional structure of the native protein is built from the target amino acid sequence with related homologous protein with maximum similarity as the template. The quality of the constructed homology model is directly proportional to the percentage of similarity with the chosen template. Homology modeling is the best and ideal option for predicting the three-dimensional (3D) structure of proteins.[10]

In the present study, the modeled structure of the subunits of the ubiquinone oxidase protein and its validation is reported. The modeled structures will aid to explore their catalytic activities and to identify inhibitors.

Materials and Methods

The Swiss-Prot database contains several sequences of proteins.[11] The homology modeling is used to predict the structure for the subunits of ubiquinol oxidase from *A. aceti*. The amino acid sequence of the four different subunits namely subunits I, II, III and IV of ubiquinol oxidase from A. aceti was retrieved from the accession numbers P98009, P50653 Q43890 and Q43891 respectively of Swiss-Prot database. It consists of 664, 307, 201and 109 amino acids respectively. PSI-BLAST is a useful program to search the protein from the database.[12] Then the template sequences for subunits I, II, III and IV with maximum similarity was identified using PSI-BLAST search.

The alignment of two different protein sequences can be done with ClustalW.[13] The sequences of subunits I, II, III and IV and their corresponding chosen template sequences are then aligned using ClustalW. The homology modeling is carried out for the four subunits against the chosen template using Modeller 9.11. The outcomes of the modeled structures are ranked on the basis of an internal scoring function, and those with the least internal scores were identified and utilized for model validation.

The PROCHECK program helps to assess the overall stereochemical quality of the modeled protein.[14,15] After modeling the subunits I, II, III and IV, Ramachandran plot analysis is performed using the PROCHECK program. The quality of the consistency between the templates and the modeled ubiquinone oxidase subunits is evaluated using ProSA.[16] The z-scores of the modeled subunits of ubiqinone oxidase and their corresponding template structures are obtained with the ProSA server.

The binding pockets of the four subunits of ubiquinol oxidase protein from *A. aceti* are identified using the Q Site Finder. The Q site finder provides complete information about the active binding sites of the protein and functional residues located on proteins.

Results and Discussion

Structure of ubiquinol oxidase from *Escherichia coli* (PDB id 1FFT) is chosen as the template for ubiquinol oxidase subunit I, II and III whereas the crystal structure of the 5'->3' Exoribonuclease Xrn1, E178q Mutant (PDB id 3PIE) is the most similar template for ubiquinol oxidase subunit IV. The chosen templates had the maximum similarity of 67%, 55%, 60% and 33% with the four target subunits I, II, III and IV respectively. The alignment of the target and the template sequences are performed with ClustalW and the result of alignment is shown below.

Alignment between Target (Ubiquinol oxidase subunit I) and Template sequence (ubiquinol oxidase from *E. coli*):

```
Target      MLGRLSLSAIPLDVPILVGTFIGVVIVGVAVLGLITYYGKWGYLWKEWFTSVDHKRLAAM
Template    MFGKLSLDAVPFHEPIVMVTIAGIILGGLALVGLITYFGKWTYLWKEWLTSVDHKRLGIM
            *.*.***.*.*.. **.. *. *... *.*..******.*** *******.********. *

Target      YIILALVALFRGFADAIMMRTQLALAYAGNPGYLPPHHYDQIFSAHGTIMIFFLAMAFMT
Template    YIIVAIVMLLRGFADAIMMRSQQALASAGEAGFLPPHHYDQIFTAHGVIMIFFVAMPFVI
            ***.*.* *.***********.* *** **. *.***********.***.******.** *.
```

```
Target      GLFNFIVPLQIGARDVAFPFLNNLSFWMTAVAFILVNVSLFIGEFSQCGWLAYPPLSENQ
Template    GLMNLVVPLQIGARDVAFPFLNNLSFWFTVVGVILVNVSLGVGEFAQTGWLAYPPLSGIE
            **:*:.:**********************:*.*.:******* .:***:* ********** :

Target      FSPGVGVDYYIWAVQISGVGTLLTGVNFFVTIVKMRAPGMTWRKMPVFTWTALCASILIM
Template    YSPGVGVDYWIWSLQLSGIGTTLTGINFFVTILKMRAPGMTMFKMPVFTWASLCANVLII
            :*********:**.:*:**:** ***:*******:******** ********.:***.:**:

Target
VAFPVLTVAVGLLGMDRYFGMHFFTNDGGGNQMMYLNLIWAWGHPEVYILVIPAFGVFSE
Template    ASFPILTVTVALLTLDRYLGTHFFTNDMGGNMMMYINLIWAWGHPEVYILILPVFGVFSE
            .:**:***:*:** .:***:* ******* *** ***:****************:.*:******

Target      VVPAFSGKPLFGYSTMVYATCSIMVLSFLVWVHHFFTMGAGPDVNAFFGIATMIISIPTG
Template    IAATFSRKRLFGYTSLVWATVCITVLSFIVWLHHFFTMGAGANVNAFFGITTMIIAIPTG
            :. :** * ****:..*:** .* ****:**:********* :********:****:****

Target      IKLFNWLFTMYKGRIQFHACMYWAVGFMITFTIGGMTGVMLAIPGADFVLHNSLFLIAHF
Template    VKIFNWLFTMYQGRIVFHSAMLWTIGFIVTFSVGGMTGVLLAVPGADFVLHNSLFLIAHF
            :*:*********:*** **:.* *:.:**:.:**:.*******:**:*****************

Target      HNTIIGGVYFGYICGMNFWFPKVMGFKLDETWGKRAFWFWFVGFYCAFVPLYIVGFEGMT
Template
HNVIIGGVVFGCFAGMTYWWPKAFGFKLNETWGKRAFWFWIIGFFVAFMPLYALGFMGMT
            **:***** ** :.**.:*:**.:*****:**********:.:**:. **:*** :** ***

Target
RRLNHYDNPAWHPWLLVAEVGAVLVMLGIACQLTQLYVSIRDRNLPQNRDVTGDPWNGRT
Template    RRLSQQIDPQFHTMLMIAASGAVLIALGILCLVIQMYVSIRDRD--QNRDLTGDPWGGRT
            ***:. :* :*  *:.*  *****: *** * : *:********:  ****:***** ***

Target      LEWSTSSPPPVYNFAIVPHVHELDTFMLDKENGIDTRQAGAQYEAIHMPKNTSFGSGLCK
Template    LEWATSSPPPFYNFAVVPHVHERDAFWEMKEKGEAY-KKPDHYEEIHMPKNSGAGIVIAA
            ***:*******:*****:****** *:*  **:*  : :** *******:: * :.

Target      CSALIFGFAAVWYIWWLAAVGLVGVIGTVIARSADKDIDYYIPAEEVARIENEHTRKLMA
Template    -FSTIFGFAMIWHIWWLAIVGFAGMIITWIVKSFDEDVDYYVPVAEIEKLENQHFDEITK
            : ***** :*:***** **:.*:* * *.:* *:*:****:*. *:.::**:* ::

Target      QAAE---
Template    AGLKNGN
            . :
```

Alignment between Target (Ubiquinol oxidase subunit II) and Template sequence (chain B, ubiquinol oxidase from Escherichia coli):

```
Target      MKNKLLAR-VARLGGLSSALLLAGCELDVLDPKGPVGEGVKTLIATSTVAMLIVVIPTIL 59
Template    MRLRKYNKSLGWLSLFAGTVLLSGCNSALLDPKGQIGLEQRSLILTAFGLMLIVVIPAIL
60
            *:.:  : :. *.:.::**:**:. :***** :*  ::** *:  ********:**

Target      ETLLFAWQYRQSNTSAEYLPKWCHSNKIEVTIWGVPSLIILFLAVITYQTCHSLDPYKPL
119
Template
MAVGFAWKYRASNKDAKYSPNWSHSNKVEAVVWTVPILIIIFLAVLTWKTTHALEPSKPL 120
            :: ***:** **.:*:* *:*:*****:*.::* ** ***:*****:*::* *:*:* ***

Target      EAEANTKPLHVEVVALDWKWLFIYPEQGIATVNQLAIPVNTPIDFNITSDSVMNSFFIPR
```

```
179
Template      AHDE--KPITIEVVSMDWKWFFIYPEQGIATVNEIAFPANTPVYFKVTSNSVMNSFFIPR
178
              :  **: :***:.*****:***********:.:*:* ***: *:.**:**********

Target        LGSMIYAMAGMQTQLHLLASEPGDYLGESANYSGRGFSDMKFHTLAVSG-
DEFNAWVEKV 238
Template
LGSQIYAMAGMQTRLHLIANEPGTYDGISASYSGPGFSGMKFKAIATPDRAAFDQWVAKA 238
              *** **********:***:*:*** * * **:*** ***:***:::*...  *: ** *.

Target        K-SSSEQLDSQTYPKLAAPSE-NPVEYFAHVEPGMFNTIVAKYN-NGMVMDKSTGKMIQV
295
Template
KQSPNTMSDMAAFEKLAAPSEYNQVEYFSNVKPDLFADVINKFMAHGKSMDMTQPEGEHS 298
              * *..  * :: ******* * *****.:*:*.:* :: *: :*  ** : : :

Target        QQSAMSDMNMKE----- 307
Template      AHEGMEGMDMSHAESAH 315
              :.:*.:*:*..
```

Alignment between Target (Ubiquinol oxidase subunit III) and Template sequence (ubiquinol oxidase from *Escherichia coli*):

```
Target        MAQNTTVQTAG--HDEHHHES--PVVFGFWVYLMTDCIIFGTLFAAFAVLHNQFNGGPTG
56
Template
MATDTLTHATAHAHEHGHHDAGGTKIFGFWIYLMSDCILFSILFATYAVLVNGTAGGPTG 60
              ** :* .:::  *:. **:. . :****:***:***:*. ***:.*** *  ******

Target        HELFEFGGLGLETALLLVSSITYGFGMIAAHKSQVSKVILWLGLTFLLGLGFVGLELREF
116
Template      KDIFELPFVLVETFLLLFSSITYGMAAIAMYKNNKSQVISWLALTWLFGAGFIGMEIYEF
120
              :::**: : :** ***.*******.. ** :*.: *:*** **.**:*:* **:*:*: **

Target        AHMIAEGAGPDRSAFLSAFFTLVSTHGLHVTCGLIWIVTLIVQLMGTTEIPERMMNKLTC
176
Template      HHLIVNGMGPDRSGFLSAFFALVGTHGLHVTSGLIWMAVLMVQIARR-
GLTSTNRTRIMC 179
              *:*.:* ******.*******:**.*******.****:..*:**:    :..  .:: *

Target        LSLFWHFLDIVWICVFTYVYLASMI 201
Template      LSLFWHFLDVVWICVFTVVYLMGAM 204
              **********.******** *** . :
```

Alignment between Target (Ubiquinol oxidase subunit IV) and Template sequence(Chain A, Crystal Structure Of The 5'->3' Exoribonuclease Xrn1, E178q Mutant):

```
Target        ----------------------------------MSNPHTSSSGE--------SHGSV 16
Template      MGIPKFFHFISERWPQISQLIDGSQIPEFDNLYLDMNSILHNCTHGDGSEVNSRLSEEEV 60
                                  * *.:.*:      *. .*

Target        SSYIIGFVLAVVLTVLS----------------------FGVVMTPQPSPAGTLAAIS 52
Template
YSKIFSYIDHLFHTIKPKQTFYMAIDGVAPRAKMNQQRARRFRTAMDAEKALQKAIENGD 120
              * *.:: :.*:.                 * .* .: :  ::  .

Target        ALA----------LVQVLVHLHYFLHMGGSSEQRWNNMCFVFT---------- 85
Template      ELPKGEPFDSNAITPGTEFMAKLTENLKYFIHDKITNDTRWQNVKVIFSGHEVPGEGQHK
```

```
180
            *.            :.:: :*:**:*  :.: **:*:.:*:

Target      ----------------------------------------VAF---------- 88
Template      IMDYIRAIRAQEDYNPNTRHCIYGLDADLIILGLSTHDHHFCLLREEVTFGKRSSSVKTL
240
                                        *:*

Target      ---------------------------------------VAILIVGTVFIMNNTEHM 106
Template      ETQNFFLLHLSILREYLALEFEEITDSVQFEYDFERVLDDFIFVLFTIGNDFLPNLPDLH 300
                                    .::: :*. *: * .:

Target      MSR----------------------------------------------------- 109
Template
LKKGAFPVLLQTFKEALQHMDGYINEQGKINLARFSIWLKYLSDFEYLNFEKKDIDVEWF 360
            ::.

Target      ------------------------------------------------------------
Template
NQQLENISLEGERKRTRMGKKLLMKQQKKLIGAVKPWLLKTVQRKVTSELQDADFEIFPL 420

Target      ------------------------------------------------------------
Template
EDKELVRANLDFLKEFAFDLGLILAHSKSKDLYYFKLDLDSINXXXXXXXXXXXXXXXXX 480


Target      ------------------------------------------------------------
Template
XXIYSERFVEWKDQYYKDKLDFSINDTDSLKEMTENYVGGLQWVLYYYYRGCPSWSWYYR 540


Target      ------------------------------------------------------------
Template      YHYAPRISDVIKGIDQNIEFHKGQPFKPFQQLMAVLPERSKNLIPVVYRPLMYDEHSPIL
600

Target      ------------------------------------------------------------
Template
DFYPNEVELDLNGKTADWEAVVKISFVDQKRLVEAMAPYDAKLSPDEKKRNSFGTDLIFI 660

Target      ------------------------------------------------------------
Template      FNPQVDTVYKTPLAGLFNDIEHNHCIEREFIPESMENVKFLFGLPKGAKLGASSLAGFPS
720

Target      ------------------------------------------------------------
Template      LKTLPLTAELAYNSSVVFNFPSKQQSMVLHIQDLYKENGISLSDLAKRHMGKIVYSRWPF
780

Target      ------------------------------------------------------------
Template      LRESKLLSLITEETVYEGVKSGKLTKVIERKPQDFERKEFRELKMTLKSNYQRTKAILLD
840

Target      ------------------------------------------------------------
Template      DISALAKVVPVNGLVRNSDGSYSKSFNETIEYYPLQLIVEDVKNKDERYIEKEPLPINKE
900

Target      ------------------------------------------------------------
Template
FPKGSKVVFLGDYAYGGEATVDGYNSETRLKLTVKKGSLRAEPNIGKVRAKLDSQALRFY 960
```

Target ----------
Template
PTQVXXXXXXXXXXXXXXXXXXXSSAEADSILKTVADWLSEARKPFVVVSLESDSLTKAS 1020

Target ----------
Template MAAVESEIIKYVSLPDSSEQKKLAKVPREAILNAESSYVLLRSQRFHLGDRVMYIQDSGK
1080

Target ----------
Template VPLHSKGTVVGYTSIGKNVSIQVLFDNEIIAGNNFGGRLQTRRGLGLDSSFLLNLSDRQL
1140

Target ----------
Template VYHSKASLEHHHHHH 1155

Homology modeling with the Modeler 9.11 successfully yielded 5 models for each subunit of the ubiquinol oxidase of *A. aceti*. The model with the minimum DOPE score is considered as the best model of protein. The DOPE scores of the best models for the four different subunits of ubiquinol oxidase are shown in table 1.

Table 1: DOPE Score of successfully produced models

S.No	Best model	DOPE score
1	TvLDH.B99990004.pdb	-76546.05469
2	TvLDH.B99990001.pdb	-25487.25391
3	TvLDH.B99990001.pdb	-18533.02539
4	TvLDH.B99990004.pdb	-8456.76465

The three dimensional structure of the ubiquinol oxidase subunits I, II, III and IV of the *A. aceti* is constructed and is shown in the Figure 1A, 1B, 1C and 1D respectively. It resulted in high quality structural models as the target sequences and the chosen template sequence had maximum similarity.

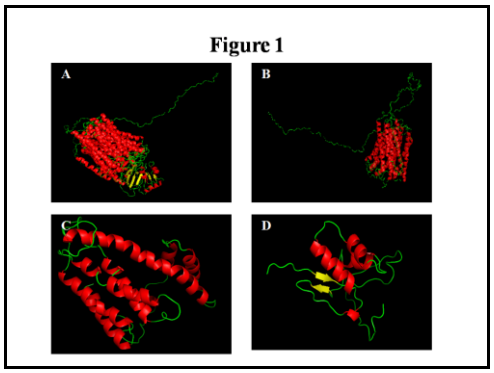

Figure 1: Modeled subunits of the ubiquinol oxidase of *A. aceti.*

The best model for each subunit which is identified based on the least DOPE score is then subjected to evaluation to check the quality of the generated model. The constructed three dimensional model of four protein subunits of the ubiquinol oxidase protein of *A. aceti* are validated further by calculating the ProSA Z-score, which gives the overall model quality based on the Cα positions. The z-plots of the template of subunit I is shown in the figure 2a and the z plots of subunits I, II and III are shown in figures 2b, 2c and 2d respectivity. Similarly the z-plots of the template and the target sequences of subunit IV are shown in figure 2e and 2f respectively.

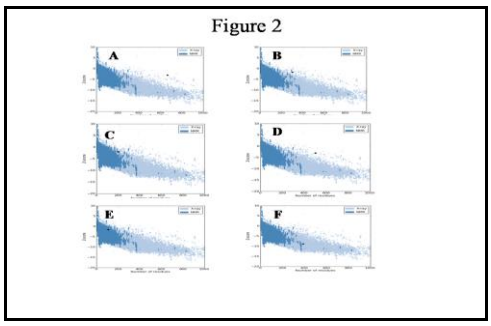

Figure 2: Z-scores of template and target subunits of ADH of *A. aceti*

The z-scores of the target and template structures for all the four subunits are shown in the table 2. The z-scores of the template and target are closer and they confirm the validity of the constructed model subunits.

Table 2: Z-score's of target and template structures

Subunits	Z score of target	Z- score of template
I	-3.03	−3.0
II	-1.74	−3.0
III	-1.74	−3.0
IV	-1.5	−8.87

Further evaluations of the modeled 3D structure of ubiquionol oxidase subunit I, II, III and IV protein of *A. aceti* are performed using the PROCHECK program by analyzing the Ramachandran plot. The percentages of phi and psi angles that occur in the allowed and disallowed regions were found for each of the predicted model. Ramachandran plots of subunit I, II, III and IV protein of *Acetobacter aceti* are shown in figure *3A*, 3B, 3C and 3D respectively.

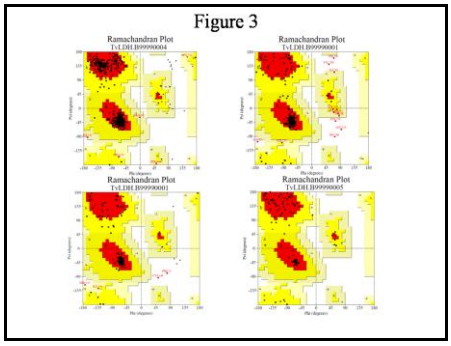

Figure 3: Ramachandran plot for Modeled subunits of the ubiquionol oxidase of *A. Aceti*

The good stereochemistry of the constructed protein subunits are evident from the Ramachandran plots and the Ramachandran plot statistics is shown in the table 3.

Table 3: Ramachandran plot statistics of Modeled structures of ubiquionol oxidase of *A. aceti*

Protein Subunits	Ramachandran Statistics			
	No of Residues in %			
	No. of Residues in (%) Most favored region	Additional allowed Region	Generously allowed Region	Disallowed Region
I	95.3	4.2	0.6	0.0
II	88.6	7.8	2.8	1.2
III	90.8	7.4	1.8	0.0
IV	82.1	17.9	0.0	0.0

It vividly indicates the quality of the model and confirms the excellent the stereo chemical quality of the model**.** The active sites for the modeled subunits I, II, III and IV of ubiquinone oxidase of *A. aceti* were found using the q site finder and is shown in the figures 4a-4d.

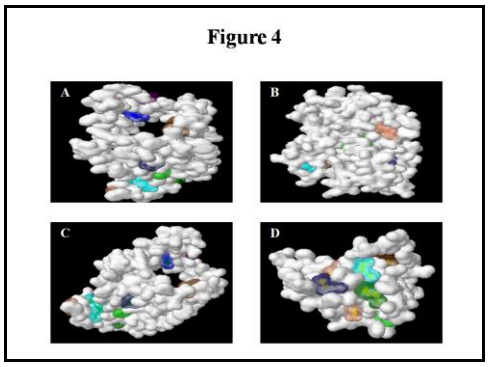

Figure 4: Active sites for Modeled subunits of the ubiquionol oxidase of *A. aceti*

Conclusion

In this work, the subunits I, II, III and IV of ubiquinol oxidase of *Acetobacter aceti* are modeled and validated. The validation experiments showed the good stereochemical properties of the modeled subunits. The active sites of the modeled structures are also identified. This work provides a platform to predict the structure and characterize the catalytic function of the modeled ubiquinol oxidase. This work provides a new path for understanding the mechanism of direct electron transfer from the cytochrome of the electrigen with different electrode materials. Further studies will be towards exploring the computational bioelectrochemical investigations of ubiquinol oxidase for Biological fuel cell applications.

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