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Research Article

For which Extend could *Arthrospira platensis* Bilins affect the α-globin Macromolecules?

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Abstract

Arthrospira platensis is an edible cyanobacterium that grows naturally, resists harsh conditions and has many vital natural products including bilins. It could support both humans and animals against different diseases at once. It has been proven to have antiviral, antioxidant and antisickling effects. It contains vital nutrients, and is edible for its bilins, which are evaluated for their antisickling effect using molecular modeling against of one normal and three mutants of the α-globin macromolecules.

The five used bilins [(1) Red bilin, (2) 21H-Bilin-1(22H)-one, (3) 21H-Bilin-1(24H)-one, (4) 1H-Bilin 1 one, and (5) 22H-Biline (21-bilin)] were investigated using protein modeling and docking software. MODELLER v 9.8, Hex ver 8.0.0 and Discovery Studio 4.1 Client 4.1.0.14169 (Accelrys software Inc.) software were used for modeling and docking. The E-Total of the various docking processes were determined. Red-Bilin shows the best E-Total results. One can decide which bilins should be used for treating a particular disease case based on the differences in the E-Total results of the bilins and α-globin (increase or decrease). The study proves that in general the E-Total of the five used bilins were close to that of the porphyrin ring, which proposes different applications concerning the hemoglobin. The study suggests using strategy for comparing the normal and mutants α-globin macromolecules in presence and absence of the natural ligand (the porphyrin ring), as in order to be used as a model for evaluating any drugs under investigation to by evaluating their its interaction with a particular protein. The natural ligand will represent the control for both, of the docking processes and the E-Total value(s). Even if it will cause a change in the used macromolecules from the quaternary to the tertiary structure, it still it is the most perfect structure, that could be used as a control. We recommended inducting more investigation concerning the *A. platensis* bilins as well as using our tactic as a general model for investigating various interactions between alternative ligands and those existing naturally.

Keywords: Bilin; α-globin; Protein modeling; Structure/Function/Specificity

Introduction

A. platensis is a bioactive nontoxic cyanobacterium, which is eco-tolerant to various ecological conditions, with wide range of biological activities. Cyanobacteria like plants depend on chromatophores in their vital biological processes including light capturing and energy transportation. The traditional medicines, folk activities and memory show that, *A. platensis* (Spirulina) was used widely in the sub-Sahara region in Africa, particularly around Lake Chad. The native population observed the Flamingo birds' tribes after their long emigration, which was interested to feed themselves on the Spirulina. The birds recovered energy and gained strength. In our experiences with *A. platensis* for years, we found that this edible cyanobacterium has an extra property, which enable it to survive in a closed flask for twelve years. In Egypt, farmers use the floating algae to feed their animals and birds [1]. Seeing the migrating birds feeding safely on Arthrospira, as well as the flamingos encourages its use by local individuals as food.

Recently, Amara and Steinbüchel (2013) proved that not only the alkalinity but also the salinity are responsible for the dominance of the *A. platensis* in the alkaline lakes [1,2].

Kebeda (1997) reported that in Ethiopia, farmers and herdsmen living in areas close to the soda lakes make their cattle drink Arthrospira water about once a month and believe that it has therapeutic effects and compensates for some lack in dietary food [2,3].

Arthrospira contains high levels of proteins (50-70%), lipids (7-16%), vitamins, and omega-3 fatty acid [3-8]. *A. platensis* was proved to be efficient in controlling the herpes simplex virus infection. In previous studies conducted on the β-globin macromolecules, the bilins of the *A. platensis* were proven to have docking properties with it. Hemoglobin molecules are highly sensitive to the O_2/CO_2 exchange. The sensitivity level of hemoglobin molecules can find differences between O_2 and CO_2 molecules. The folded hemoglobin macromolecules are able to differentiate between O_2 and CO_2. The four subunit composition of normal adult hemoglobin (HbA) is $\alpha_2\beta_2$. The other variants collectively can be found in the fetal hemoglobin (HbF: $\alpha_2\gamma_2$), sickle cell hemoglobin (HbS: α_2S_2), and a minor adult hemoglobin (HbA2: $\alpha_2\delta_2$). Amara (2015) suggested that prototype mutants in β-globin could be a hidden source or sleeping mutants

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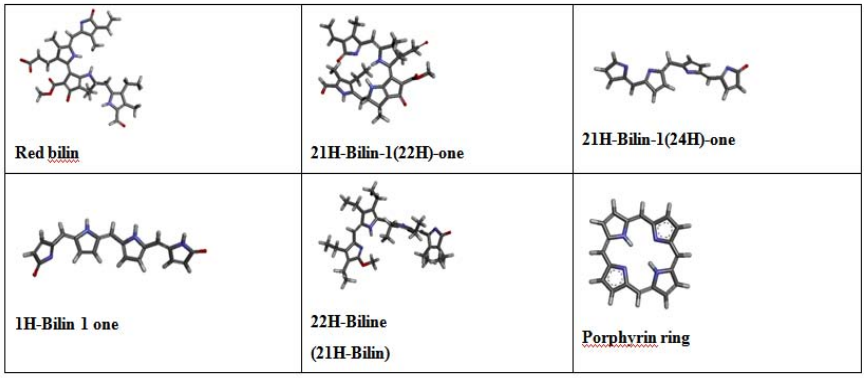


Figure 1: Different used bilins and the porphyrin ring.

could be converted to complete mutants (in future). Accumulatively, different types of prototype mutants might be avoided if detected early by avoiding marriage among individuals harboring the responsible genes [4]. Even if the primary structures of the β, γ, and δ chains of human hemoglobin are highly conserved, humans still have stages of modifications concerning the functional hemoglobin. In general, a fetus initially synthesizes a $\zeta_2\varepsilon_2$ tetramer. After the first trimester, ζ and γ subunits will be replaced by α and ε subunits. While synthesis of β subunits begins in the third trimester, β subunits do not completely replace γ subunits to yield adult HbA ($\alpha_2\beta_2$) until some weeks postpartum [5].

Over 800 known mutants of the human hemoglobin's are both extremely rare and benign, presenting no clinical abnormalities. Hemoglobinopathy is a term given for the mutants that compromise biological functions [5,6]. It is still not fully proved whether a subunit is completely absent (α^0 or β^0) or its synthesis is reduced (reduced α^+ or β^+) [5,7,9]. Avoiding the marriage among patients harboring heterozygote chromosomes will reduce the disease or even eliminate it completely by the time as proposed by Amara (2013) [10]. The aim of this study was to evaluating natural products expected to have antisicklig activity, which are represented in the *A. platensis* bilins to help in reducing the side effect of this degenerative disease.

Material and Methods

Bilins

The three-dimensional structure for the five *A. platensis* bilins were used in this study to investigate their abilities to be docked with the different hemoglobin and α- globin protein models. Each bilin was adjusted to pdb format using the software Discovery Studio 4.1. Client 4.1.0.14169 (Accelrys software Inc.). The chemical formula and name of the used bilins are as follows:

1. Red bilin [Also known as CPD-7063, (7S,8S,101R)-8-(2-carboxyethyl)-17-ethyl-19-formyl-101-(methoxycarbonyl)-3,7,13,18-tetramethyl-2-vinyl-8,23-dihydro-7H-10,12-ethanobiladiene-ab-1,102(21H)-dione]. It has the molecular formula $C35H38N4O7^{-2}$; molecular weight of 626.69882 g/mol; InChI Key HMDDKKOMBDRDIA-DSJLEYPNSA-L. Its IUPAC name is [3-[(2Z,3S,4S,5Z)-5-[(4-ethenyl-3-methyl-5-oxopyrrol-2-yl)methylidene]-2-[2-[(3-ethyl-5-formyl-4-methyl-1H-pyrrol-2-yl)methyl]-5-methoxycarbonyl-3-methyl-4-oxido-2,3-dihydro-1H-cyclopenta[b]pyrrol-6-ylidene]-4-methylpyrrolidin-3-yl] propanoate].

2. 21H-Bilin-1(22H)-one, 2,3,7,8,12,13,17,18-octaethyl-19-methoxy-, 113435-10-2, with the molecular formula C36H48N4O2, molecular weight 568.79192 g/mol, and InChI Key QCYVKTHGTVWFSV-UHFFFAOYSA-N. Its IUPAC name is [5-[[5-[[5-[(3,4-diethyl-5-methoxypyrrol-2-ylidene)methyl]-3,4-diethyl-1H-pyrrol-2-yl]methylidene]-3,4-diethylpyrrol-2-ylidene] methyl]-3,4-diethylpyrrol-2-one].

3. AGN-PC-0O2GJ1, **21H-Bilin-1(24H)-one,** 19-hydroxy-, 21H-Biline-1,19-dione, 22,24-dihydro-, 142550-15-0, 58828-89-0, with the molecular formula C19H14N4O2, molecular weight 330.34006 g/mol, InChI Key MQHWQQCOXHUNCS-UHFFFAOYSA-N, and its IUPAC name is 5-[[5-[[5-[(5-oxopyrrol-2-ylidene)methyl]-1H-pyrrol-2-yl]methylidene]pyrrol-2-ylidene] methyl]pyrrol-2-one].

4. 1H-Bilin-1-one [Also known as AGN-PC-0OFTAO, 66560-67-6] which has the molecular formula C19H12N4O; molecular weight of 312.32478 g/mol; InChI Key VGJBOZZPXZVBBI-UHFFFAOYSA-N. Its IUPAC name is 5-[[5-[[5-(pyrrol-2-ylidenemethyl)pyrrol-2-ylidene]methyl]pyrrol-2-ylidene]methyl] pyrrol-2-one.

5. 22H-Biline, 21H-Bilin, 22H-Bilin, AC1OAGP5, SureCN139406, AGN-PC-02LS4D.

Molecular Formula: C19H14N4, Molecular Weight: 298.34126 g/mol, InChI Key: PPRBOEHFGAHFGC-UHFFFAOYSA-N. Its IUPAC name is: 2-(pyrrol-2-ylidenemethyl)-5-[[5-(pyrrol-2-ylidenemethyl)-1H-pyrrol-2-yl]methylidene]pyrrole.

Another bilins exist but were not included in this study.

The five bilin molecules were downloaded from PubChem (www.ncbi.nlm.nih.gov/pccompound) and saved as SDF format files [13]. The chemical structure of the molecules is given in Figure 1.

α-globin used models

The normal α-globin used sequence was obtained from a sequence published by Liebhaber et al. (1980), and represent the following amino acids as appeared in the published paper [11]:

"val leu ser pro ala asp lys thr asn val lys ala ala trp gly lys val gly

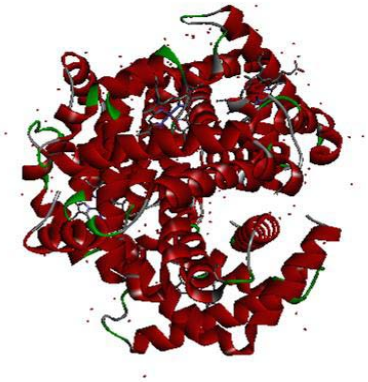


Figure 2: Pdb1gzx hemoglobin quaternary structure protein model [15].

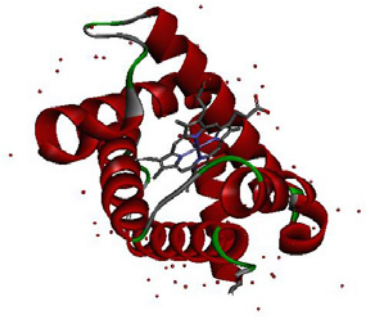


Figure 3: α-globin obtained from the pdb1gzx hemoglobin model, the surface energy and the porphyrin ring still exist [15].

ala his ala gly glu tyr gly ala glu ala leu glu arg met phe leu ser phe pro thr thr lys thr tyr phe pro his phe asp leu ser his gly ser ala gln val lys gly his gly lys lys val ala asp ala leu thr asn ala val ala his val asp asp met pro asn ala leu ser ala leu ser asp leu his ala his lys leu arg val asp pro val asn phe lys leu leu ser his cys leu leu val thr leu ala ala his leu pro ala glu phe thr pro ala val his ala ser leu asp lys phe leu ala ser val ser thr val leu thr ser lys tyr arg"

The three letter of the amino acids from the published sequence were converted to a single letter code using Zbio.net > Calculations > Sequence tools > Three- / one-letter code http://molbiol.ru/eng/scripts/01_17.html [12]

The sequence then was saved as FASTA format and searched in BlAST search and the structure similarity as well as three mutant pdb files were determined [13,14].

The hemoglobin used model was pdb1gzx as in Figure 2 [15]. This structure which contains four globin macromolecules was reduced to a single α-globin macromolecule by removing three out of four using the software Discovery Studio 4.1. Client 4.1.0.14169 (Accelrys software Inc.). Both of the original surface energy and the porphyrin ring were kept also as in Figure 3.

Normal α-globin amino acid sequence

VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSF
PTTKTYFPHFDLSHGSAQVKGHGKKVADALTNAV
AHVDDMPNALSALSDLHAHKLRVDPVNFK
LLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVLTSKYR

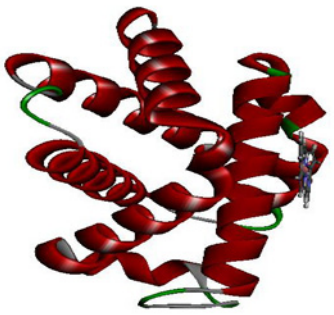


Figure 4: Normal α-globin model obtained from the Modeller software, the normal α-globin sequence and three hemoglobin models.
VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPH
FDLSHGSAQVKGHGKKVADALTNAVAHVDDMPNALSA
LSDLHAHKLRVDPVNFKLLSHCLLVTLAAHLPAEFTPAVHAS
LDKFLASVSTVLT SKYR

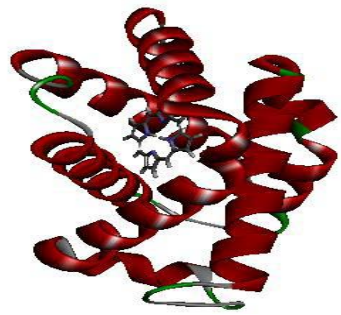


Figure 5: Mutant 1 α-globin model obtained from the Modeller software, the mutant 1 α-globin sequence and three hemoglobin models.
VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTHFPHFDLSH
GSAQVKGHGKKVADALTNAVAHVDDMPNALSALSDLH
AHKLRVDPVNFKLLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVS
TVLTSKYR

Two other published hemoglobin macromolecules were used to build the α-globin various models by the MODELLER v 9.8 software; 3OO5.pdb [16] and 5NI1.pdb [17].

Generated models

Software for Modeling: One published sickle hemoglobin model was used [15]. The α-globin protein model was generated using the software MODELLER v 9.8 [18].

Software for docking: "Hex" is a Molecular Graphic Program (Hex's Home Page: http://www.loria.fr/~ritchied/hex/) for calculating and displaying feasible docking modes of pairs of protein and DNA molecules. Hex software can also calculate Protein-Ligand Docking, assuming the ligand is rigid, and it can superpose pairs of molecules using only knowledge of their 3D shapes. It uses Spherical Polar Fourier (SPF) correlations to accelerate the calculations and it is one of the few docking programs which was built in graphics to view the result. Simply, the protein pdb is loaded from the "File>open>receptor" and the bilin or the porphyrin ring loaded from "File>open>ligand" and then from the control option docking is selected and the parameter in Figure 1 is used. The binding energy result is normally negative, stating that a better binding affinity is established from the highest negative result. Low (negative) energy indicates a stable system [19].

Table 1: Various E-Total energy of different docked models (receptor) with the different bilins (ligands).

Ligand name	Normal wild structure α globin with structure porphyrin*	Model wild structure α globin with docked porphyrin§	Model Normal wild structure α globin without structure porphyrin	Model Mutant no 1 with docked porphyrin	Model Mutant no 2 with docked porphyrin	Model Mutant no 3 with docked porphyrin	Model Mutant no 1 without docked porphyrin	Model Mutant no 2 without docked porphyrin	Model Mutant no 3 without docked porphyrin
	E-Total								
1 H Bilin 1 one	-280	-280.8	-284.2	-276	-269.3	-287.5	-263.4	-265.2	-273.5
21 H Bilin-1 (22 H) - one	-311.3	-324.5	-334.53	-321.1	-306.4	-389.1	-316.1	-318.8	-370.7
21 H Bilin-1 (24 H) - one	-270.2	-272.4	-267	-263.9	-265.6	-272	-274	-268.1	-267.1
22 H Bilin-1 (21 H) - one	-320.8	-303.5	-309.1	-292.1	-251.4	-378.4	-311.7	-333.8	-374.6
Red Bilin	-373.8	-390.1	-209.8	-366.8	-349.7	-381.4	-338.2	-354	-378.8

*Outer-shell energy was not removed
§ Outer-shell energy was removed

To determine the behavior of both of the protein molecules under study and whether we need high negative energy or lower ones; porphyrin ring has been docked firstly against both of the normal and the sickle α-globin molecules which were obtained from the MODELLER software and the published sickle hemoglobin model as mentioned above.

The five used bilins and one porphyrin ring, which were used in this study, are summarized in Table 1. The docked molecules' 3D structures have been saved as pdb files and put in Table 2 and 3 to show the different interactions.

Software for study of the molecules: The software Discovery Studio 4.1. Client 4.1.0.14169 (Accelrys software Inc.) was used to visualize and analyze the docking of the bilins with the protein models and to show the ligands binding sites [6].

For better 3D structure, the background of the images has been converted to the white color and the 3D image has been adjusted and saved. All of the docking images have been put in Table 2 and 3 to enable better comparisons between the α-globin and the bilins.

After finishing the hemoglobin quaternary structure reduction as well as the modeling of the various models, the following models were obtained and saved as pdb files:

- Model 1: Normal α-globin with both of its surface energy and the porphyrin ring which are obtained from the published model pdb1gzx of the hemoglobin (Figure 2).
- Model 2: Normal α-globin without the surface energy and the porphyrin ring which are obtained from the published model pdb1gzx, 5NI1.pdb, 3OO5.pdb by building the α-globin model using the sequence obtained from Liebhaber et al. (1980). The software MODELLER v 9.8 was used to build the model (Figure 4).
- Models 3, 4 and 5 which represent the mutants 1, 2 and 3 and were built using the published model pdb1gzx, 5NI1.pdb, 3OO5. pdb. The software MODELLER v 9.8 was used to build the models (Figure 5, 6 and 7).
- Models 6, 7, 8 and 9 were in contrast to Model 2, 3, 4 and 5 built without the porphyrin ring. The software Hex has rebuilt all of them without the porphyrin ring. The software Hex ver 8.0.0 was used to dock the porphyrin ring with each model (Figure 8, 9, 10 and 11).

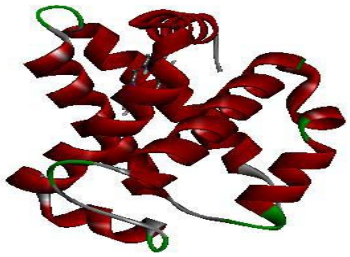


Figure 6: Mutant 2 α-globin model obtained from the Modeller software, the mutant 2 α-globin sequence and three hemoglobin models.
V L S P A D K T N V K A A W G K V G A H A G E Y G A E A L ERMFLSFPTTKTYFPHFDLSHGSAQVKGWGKKVADALTNAVAHVDDMPN ALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHLPAEFTPAVHASL DKFLASVSTVLTSKYR

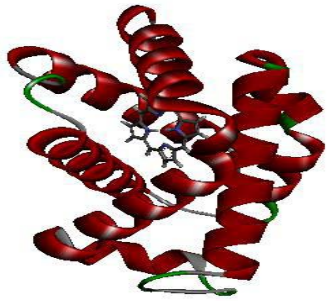


Figure 7: Mutant 3 α-globin model obtained from the Modeller software, the mutant 3 α-globin sequence and three hemoglobin models.
V L S P A D K T N V K A A W G K V G A H A G E Y G A E A L ERMFLSFPTTKTYFPHFDLSHGSAQVKGLGKKVADALTNAVAHVDDM PNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHLPAEFTP AVHASLDKFLASVSTVLTSKYR

Cluster analysis

The different values of the E-Total of the various docking processes were clustered using the classic cluster option in the Past Software. The algorithm is adjusted to Paired group (UPGMA). The

Table 2: Mutant models (with porphyrin) and the various docked bilins.

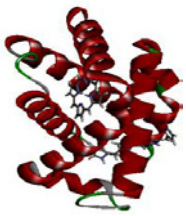
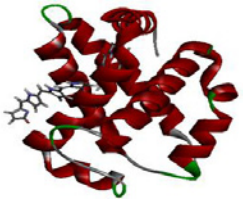
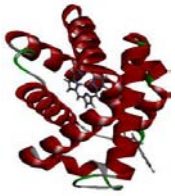
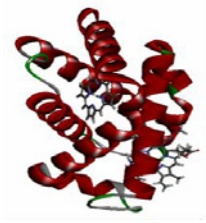
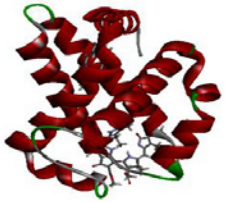
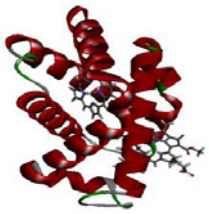
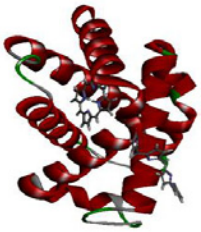
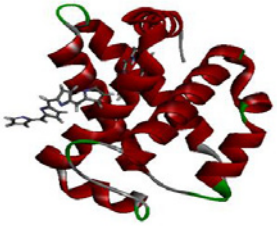
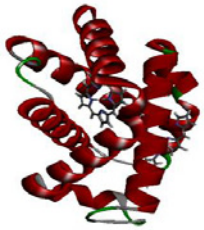
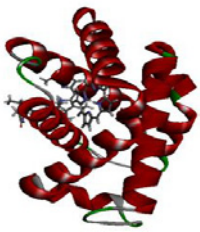
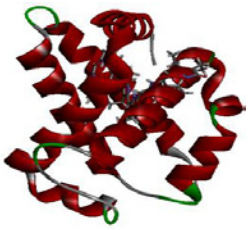
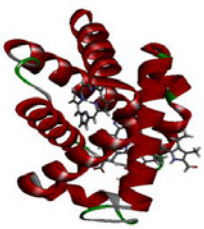
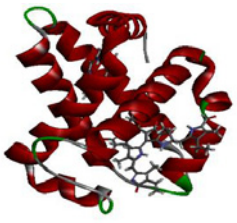
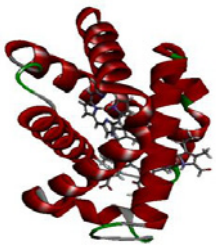
Ligand name	Model Mutant no 1 with porphyrin	Model Mutant no 2 with porphyrin	Model Mutant no 3 with porphyrin
1 H Bilin 1 one	- 276 	- 269.3 	- 287.5 
21 H Bilin-1 (22 H) - one	- 321.1 	- 306.4 	- 389.1 
21 H Bilin-1 (24 H) - one	- 263.9 	- 265.6 	- 272 
22 H Bilin-1 (21 H) - one	- 292.1 	- 251.4 	- 378.4 
Red Bilin	- 366.8 	- 349.7 	- 381.4 

Table 2: Mutant models (with porphyrin) and the various docked bilins.

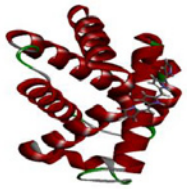
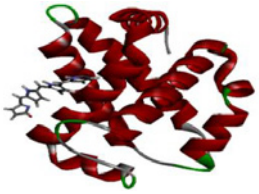
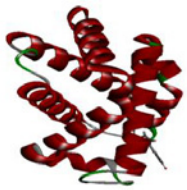
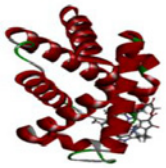
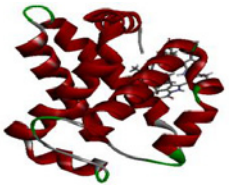
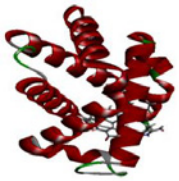
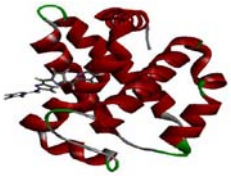
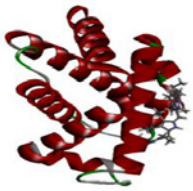
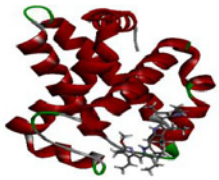
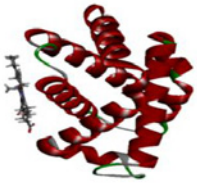
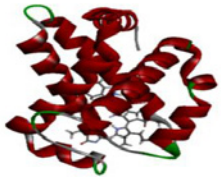
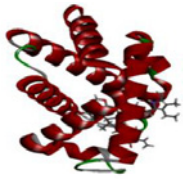
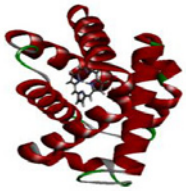
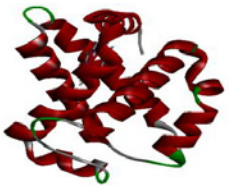
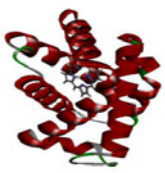
Ligand name	Model Mutant no 1 without porphyrin	Model Mutant no 2 docked porphyrin	Model Mutant no 3 without porphyrin
1 H Bilin 1 one	- 263.4 	- 265.2 	- 273.5 
21 H Bilin-1 (22 H) - one	- 316.1 	- 318.8 	- 370.7 
21 H Bilin-1 (24 H) - one	- 274 	- 268.1 	- 267.1 
22 H Bilin-1 (21 H) - one	- 311.7 	- 333.8 	- 374.6 
Red Bilin	- 338.2 	- 354 	- 378.8 
Porphyrin	- 232.8 	- 215.2 	- 221.5 

Table 3: Mutant models (with porphyrin) and the various docked bilins.

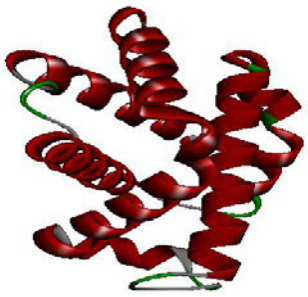


Figure 8: Normal α-globin model obtained from the Modeller software, the normal α-globin sequence and three hemoglobin models (without porphyrin ring).
VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLS HGSAQVKGHGKKVADALTN AVAHVDDMPN ALSALSDLHAHKLRVDPVNF KLLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVLTSKYR

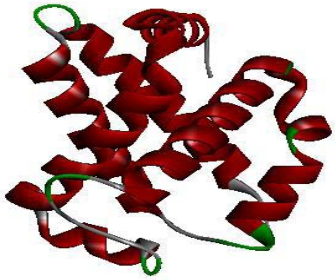


Figure 10: Mutant 2 α-globin model obtained from the Modeller software, the mutant 2 α-globin sequence and three hemoglobin models (without porphyrin ring).
VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFD LSHGSAQVKGWGKKVADA LTNAVAHVDDMPNA LSALSDLHAHKLRVDPV NFKLLSHCLLVTLAAHLPA EFTPAVHASLDKFLASVSTVLTSKYR

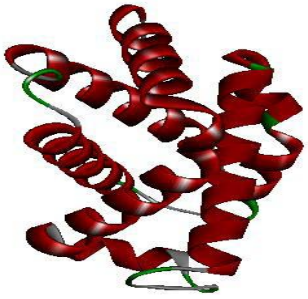


Figure 9: Mutant 1 α-globin model obtained from the Modeller software, the mutant 1 α-globin sequence and three hemoglobin models (without porphyrin ring).
V L S P A D K T N V K A A W G K V G A H A G E Y G A E ALERMFLSFPTTKTHFPHFDLSHGSAQVKGHGKKVADALTNAV AHVDDMPNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHLP AEFTPAVHASLDKFLASVSTVLTSKYR

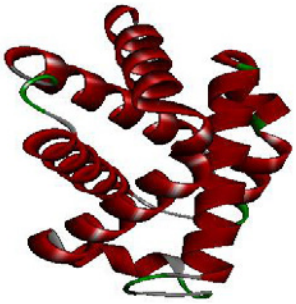


Figure 11: Mutant 3 α-globin model obtained from the Modeller software, the mutant 3 α-globin sequence and three hemoglobin models (without porphyrin ring).
VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLS HGSAQVKGLGKKVADALTNAVAHVDDMPNALSALSDLHAHK LRVDPVNFKLLSHCLLVTLAAH LPAEFTPAVHASLDKFLAS VSTVLTSKYR

similarity index was Rho with two-way. The result was obtained as image file.

Protein sequences alignment

The used models of the normal and three mutants of the α-globin were obtained from either the reduced hemoglobin model or the generated (α-globin model(s)) ones of the normal and mutants using the software Discovery Studio 4.1. Client 4.1.0.14169 (Accelrys software Inc.). The alignment was generated using ClustalW option in the BioEdit ver 7.2.3 [20]. GeneDoc 2.7. was used to visualize the aligned sequences [21].

Result and Discussion

This study is concerned with investigating the proposed activity of *A. platensis* bilins as antisickling agents. α-globin was used to investigate the docking activity of the bilins. The used normal α-globin macromolecule was obtained from a published pdb file. The molecule was a part of the hemoglobin quaternary structure. Three out of four globin macromolecules with the attached porphyrin rings were removed using the Studio and Discovery software. The existing surface energy was kept. Another but with similar amino acids constituents α-globin model was built using the software MODELLER. The amino acid sequences of three mutants were obtained from the BLAST protein database and then were used to build three protein models by the MODELLER software. All the built models without porphyrin ring were docked against it. Five bilins were adjusted as pdb format for investigating their proposed docking activities against each of the normal structure obtained from the published model solved α-globin macromolecule using x-ray analysis, the normal built α-globin macromolecule model, and the models of the three mutants under investigation.

α-globin macromolecule as well as β-globin are sensitive macromolecules in which a single change in amino acids may lead to a degenerative disease. This might be strangely against the Darwain hypothesis about the evolution, in which a single nucleotide change in β-globin causes glutamic acid change to valine causing sickle cell anemia; a degenerative disease. Previously, Amara (2015) [4] proposed avoiding marriage among relatives to evade the existence of homologous alleles and to dilute the heterologous ones or even to let it to disappear against the time factors (after several generations).

Targeting the globin diseases must not stop only at the genetic level; even if it is the only one that provided endpoint solutions. Searching for natural products might also help in protecting against the sickling effect of the globin diseases. There are increasing reports about the antisickling effect of A. platensis. This cyanobacterium was used from ancient times by local tribes in Africa to improve the health

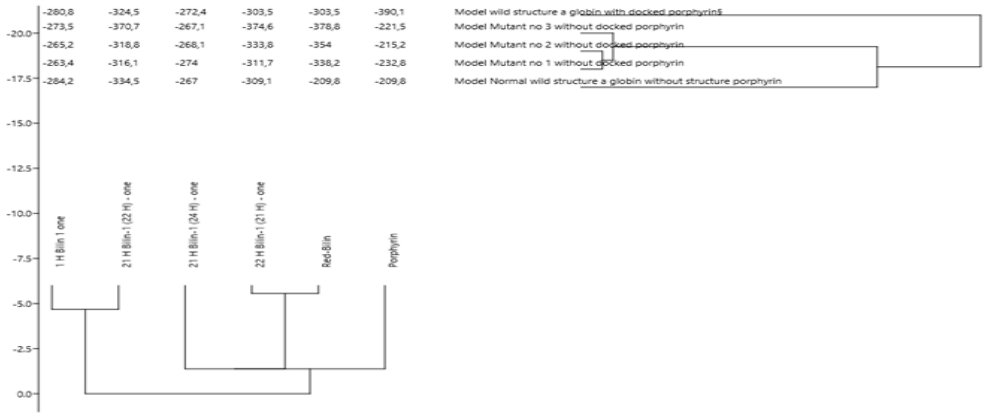


Figure 12: Cluster analysis for the various E-Total determined by docking the various α models against the used bilins.

of both animals and humans. The structure of the *A. platensis* bilins as well as their functions is similar for some extend to the porphyrin ring, which encourage us to conduct this study.

Five structures of bilins were adjusted as pdb format to enable the Hex software to dock them against the above mentioned five different α-globin macromolecules. The E-Total (total energy) of the docking was used to evaluate the different interactions between the molecules. The different protein models were used against the bilins in the presence or absence of the porphyrin ring. This enables the following investigations:

1- The similarity level between the porphyrin ring and the different bilins either in the docking total energy or in the attaching point with the protein model.

2- The differences between the various docking steps in case of presence or absence of the porphyrin ring.

3- Using different mutants will prove or disprove the sensitivity of the docking process as well as the 3D model to the variation in the protein structure, and whether the docking process is useful or not, particularly in the similar studies (to this one's). This study confirms that single amino acid change could dramatically affect a protein structure/function/specificity, which is against the Darwin hypothesis

4- Close E-Total energy might propose using bilins in treating the different globin diseases.

5- The sensitivity of the results obtained from this study might be used to evaluate the efficacy of different investigations. As an example, the repetition of the docking processes using the materials and the software included in this study even if different computers and windows versions are used.

From table 1, the E-Total of the Red Bilin with the generated α-globin model that has docked porphyrin showed the most stable molecule (E-Total equal to –390.1). In all cases where porphyrin existed, Red bilin showed the highest stability with the least E-Total. Considering the E-Total of the porphyrin as a control for guiding the other E-Total of other docked bilin, porphyrin showed -209.8 E-Total with α-globin model, which is higher than those of mutants 1, 2, and 3. However, the existence of differences proves the sensitivity of the macromolecules to any amino acid change as well as the sensitivity of the docking processes. However, knowing such differences might enable treatment, which might establish a science combine between different parameters to adjust the E-Total to adjust a particular function of a certain molecules or macromolecules. Such concept might be helpful particularly in the genetic diseases. Increasing the stability of the macromolecules in case of the mutants when docked against the porphyrin ring, if compared with the wild α-globin also proves that the case is not 'which one is more negative or more positive than the native form'. The E-Total increase either could positively or negatively affect the macromolecules-porphyrin stability, and consequently the Red blood cells stability and the respiration process in general.

In this case + and – changes from the native structure is effective either negatively or positively, but in generally will cause the imbalance of the process. In case of using native model structure or even the model generated in this study, docking porphyrin ring with the α-globin contain porphyrin ring is sense less and for that it is not included. Presence of two porphyrin molecules will interfere with the docking process. Repeating the docking processes did not give variation, so replica were not considered, hence no statistical analysis is included except the cluster analysis of the data (Figure 12).

The bilins/ α-globin interaction based on their stability could be ranked as:

1. Red bilin
2. 22 H Bilin 1 (21 H) one
3. 21 H Bilin 1 (22H) one
4. 1 H Bilin 1 one
5. 21 H Bilin 1 (24 H) one

Mutant 3 with docked porphyrin showed increase stability than the normal in case of 1 H Bilin, 21 H Bilin 1 (22H) one and 22 H Bilin 1 (21 H) one. In case of mutants without porphyrin, bilin 21 H bilin (22H) one, 22 H Bilin 1 (21 H) one and Red Billin showing increased stability, also porphyrin showed increase stability with mutant 3.Only 1 H Bilin 1 one and 21 H Bilin 1 (22H) one showed less stability with mutants 1 and 2.

The above logical analysis of the data is in agreement with the

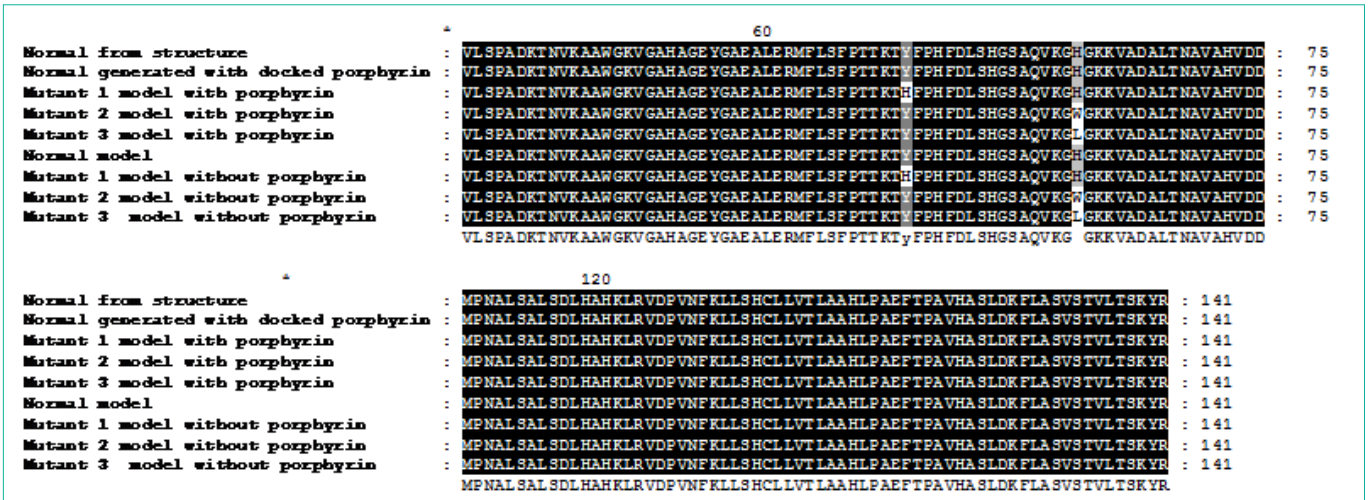


Figure 13: Alignment of the various used amino acids sequences, the different amino acids (mutants) were shaded with different color.

cluster analysis presented in Figure 12 which was conducted by the statistical software Past [22]. In the cluster analysis of the data, the porphyrin, red bilin and 22 H Bilin-1-(21H) – one was grouped together which prove the proposed similarity.

If one considers that +/- changes negatively affect the α-globin molecules in that case 21 H Bilin 1 (24 H) is the best molecule to be chosen as it gives E-Total similar to that of the porphyrin ring when docked with the α-globin and other forms. If one considers the increase in the stability is the best choice, Red Bilin will be the best billin which stably interacts with the α-globin macromolecules giving the lesser E-Total.

This study highlighted that:

1. The docking process is sensitive and stable and no error is detected even after using different computers and different windows versions.

2. The E-Total is a good candidate for evaluating the stability of the docked receptor and ligand, where the less E-Total energy needed the more stables the molecules.

3. +/- changes in the E-Total might not be the best choice, and molecules more close to the native form might give similar E-Total. However, the docking process could be differentiating between molecules if compared with the native or mutant macromolecules based on the E-Total results.

4. Single amino acid change will significantly change the E-Total of any docked molecules.

5. Arthrospira Bilins might be promising molecules as they give close E-Total to the porphyrin ring against the α-globin. Being edible might increase their attraction to be investigated as a proposed antisickling agent.

6. α-globin mutants side effect might be avoided or reduced by natural products which could interact with their macromolecules but this needs to be proved in an *in vivo* study.

This study recommended carrying out more research on the Arthrospira bilins to prove or disprove our hypothesis about their ability to support the α-globin macromolecules.

Simple alignment was generated just to show the different mutants (Figure 13).

Conclusion

This study tried to prove that structure of bilins which are naturally produced by *A. platensis* have similarity with the porphyrin ring and could support the α-globin molecules. For that aim, normal and three mutants models of the α-globin molecules were generated. They were represented either having the porphyrin ring or not. The various data prove that the electronically docking process is stable in various computers and under different windows. The E-Total of all used bilins are close to the porphyrin ring E-Total. The results show that porphyrin, red bilin and 22 H Bilin-1-(21H) – one, were so close and the cluster analysis of the data prove such conclusion. Even lot of efforts were conducted to maintain the accuracy of the finding including investigating the used sequence of each structure after its generation, using alignment to evaluate the sequences, repeating the processes in different computers and under different windows, however *in vivo* study should be conducted to prove/disprove our finding. Investigating structures obtained from edible foods or microbes will give extra-chances as they prove historically to be safe for humans hence they can bypass some of the long processes concerning their safety.

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