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Induction of nano pore in *Agrobacterial* hemoglobin

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Abstract

Introduction: A variety of oxygen-transport and -binding proteins exist in organisms including bacteria, protozoans, and fungi all have hemoglobin-like proteins. In addition to dealing with transport and sensing of oxygen, they may also deal with NO_2, CO_2, sulfide compounds, and even O_2 scavenging in environments. Also they detoxified chlorinated materials like P450 enzymes and peroxidases and use as a detector of nitrate and hydrogen peroxide. Pore-forming bacterial globins are interested for filtration.

Materials and methods: Although there are data for bacterial toxin as a filter, here we used *Agrobacterial* hem to induce nano pore in the heme structure using point mutation.

Results: Investigations showed that three amino acids leucine 76, alanine 83 and histidine 80 are important for pore formation in *Agrobacterium* hemoglobin. A point mutation on leucine 76 to glycine, histidine 80 to asparagine and alanine 83 to lysine step by step led to create the nano pore 0.7- 0.8 nm in the globin.

Discussion and conclusion: These mutations in bacterial hemoglobin increase the stability when mutation is with it's at pH7. This mutation decreases the aliphatic index however increase the stability index.

Key words: Hemoglobin, *Agrobacterium*, Molegro Virtual Docker

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Introduction

Hemoglobins have seen in some bacteria, the flavohemoglobins from *Escherichia coli* (1), *Bacillus subtilis* (2) and *Alcaligenes eutrophus* (3) have been studied more. The three-dimensional structure has been determined for the *Alcaligenes* flavohemoglobin (4). The homology between the bacterial and eukaryotic hemoglobins has been reported according to structure and folding. The *Alcaligenes* flavohemoglobin has been implicated in catalyzing a reduction reaction. The hemoglobin in Vitreoscilla is not fused with a flavoprotein domain (5).

A hemoglobin encoded within a nif operon in the cyanobacterium Nostoc commune (6) is similar to the hemoglobins found in the unicellular eukaryotes. No hemoglobins have been reported in the archeobacteria to date, but it would be surprising if they were truly absent. Even though the hemoglobin genes have been diverging for an extremely long time, relationships among them can be analyzed by multiple alignment of amino acid sequences of the encoded proteins followed by construction of phylogenetic trees. When the respiratory electron transport system is not in a divided intracellular compartment, as in bacteria, some species still use hemoglobin under hypoxic conditions, perhaps to provide oxygen as electron acceptor. Certainly, the flavohemoglobins appear to catalyze redox reactions, with the heme playing a straight role in electron transfers as it does with the cytochromes. Maybe these proteins offer clues about the purpose of the ancestral globins.

Sequence analyses illustrate that dissimilar residue have evolved into distinct group of bacteria, possibly reflecting adaptation to exact environmental conditions. The presence of His (42) CD1 is distinctive of group II 2/2Hbs from Proteobacteria, while in the vast majority of 2/2Hbs and Hbs, the CD1 site is taken by a severely preserved Phe residue that shields the heme from solvent. The existence of His at site CD1 is thus very odd and likely indicates useful adaptation. Another exception to the "PheCD1 rule" is found in group II 2/2Hbs from *Actinobacteria* where Tyr occupies the CD1 position (7). In this work we had a point mutation at leu 76, His 80 and Ala 83 to get smaller pore in the molecules.

Materials and Methods

The 3D structure of proteins was obtained from the PDB (Protein Data Bank) server[1]. The PDB format accordingly provides for the description and annotation of protein and nucleic acid structures including atomic coordinates, observed side chain rotamera, secondary structure assignments, as well as atomic connectivity. Structures are often deposited with other molecules such as water, ions, nucleic acids, ligands and so on, which can be described in the PDB format as well (8).

Multi alignment of different bacterial hemoglobins was operated by MVD(Molegro Virtual Docker) software (9) and used to verify accommodation of the structure of this proteins in various bacteria and identification the conserved and semi-conserved areas.

CASTp server and MVD software were used to finding hydrophobic cavities in this protein and survey and calculate the volume and surface of cavities.

MVD software was used to implement the point mutations and subsequent optimization and minimization of energy in the new structure (11). Selected positions were submitted to I-Mutant 2.0 server and in the output data energy level of protein for changing a specific position to any

other 19 possible amino acids was received (12). This was done in all positions that had potentiality to perform mutation.

I-Mutant 2.0 was used to predict protein stability changes upon single point mutations in protein sequence and structure. This tool based on information taken from ProTherm that currently is the most comprehensive source of information about protein mutations (12).

ProtParam[2] is a tool which allows the computation of various physical and chemical parameters for a given protein stored in Swiss-Prot or for a user entered sequence. The computed parameters include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (13).

Results

The structure of hemoglobin

Three-dimensional structure studies showed that this protein is composed of 6 alpha Helix and 6 turn and heme group acts as a cofactor in the center of protein (Fig. 1).

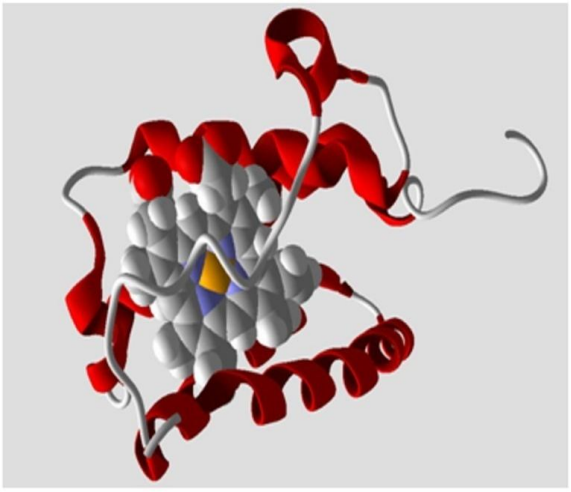

Fig. 1- 3D structure of bacterial Hemoglobin

Heme group is located in a cavity with volume 226.81 angstrom and the amino acids that form the cavity structure are including F25, Y26, M29, C38, I41, H42, P43, G48, S49, E50, K52, F53, Y54, D55, Y56, L57, Y60, Y67, H71, P74, L76, R79, H80, F81, V82, A83, P84, I85, G86, E89, R90, D91, W93, L94, F97, P117, V118, E119, R120, L121, A122, F123, M125 & Q126 (Fig. 2).

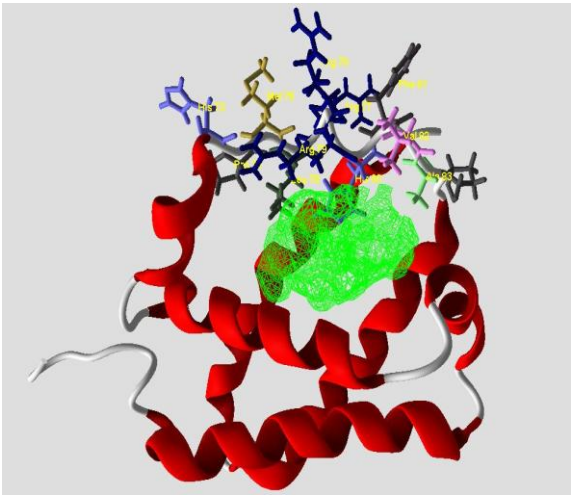

Fig. 2- Hole forming amino acids of hemoglobin

Multi alignment

Five hemoglobin producing bacteria that their crystallographic structure (X-Ray) was available on PDB server including: Agrobacterrium (PDB ID:2XYK), Geobacillus (PDB ID:2BKM), Mycobacterium tuberculosis (PDB ID: 1S61), Vitroescilla (PDB ID: 2VHB), Synechocystis (PDB ID: 1RTX)) were selected in order to investigate multi alignment (multi sequence) operation by MVD software (Fig. 3). Then desired point mutations were studied in non-conserved areas. In the next step, through the bacteria mentioned above, Agrobacterium was chosen for the next studies.

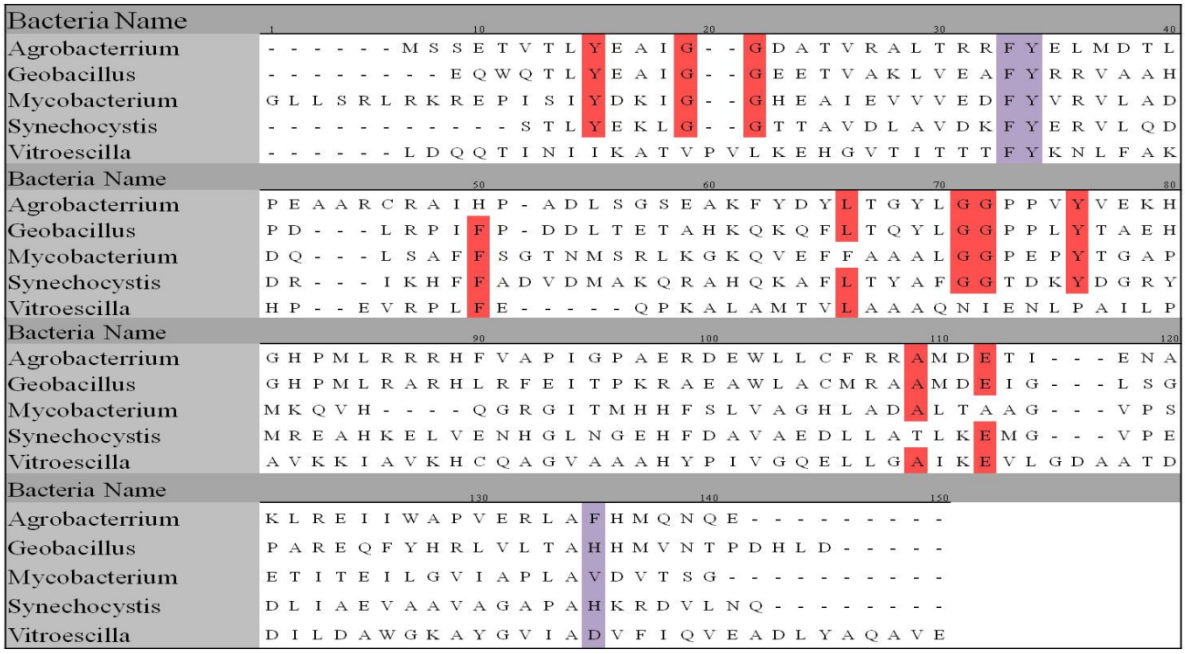

Fig. 3- Alignment of deduced amino acid residues of bacterial Hemoglobin. Conserved and semi conserved residues are placed in blue & red boxes respectively.

Information obtained by the multi alignment of five different bacterial hemoglobin (Fig. 3), showed that amino acids, F25, Y26 and A122 are conserved and amino acids, Y9, G13, G14, L57, G62, G63 Y67, A100 and E103 are semi-conserved so manipulation and mutation in these areas lead to disrupt the overall structure of the protein.

Finding hydrophobic cavities

Studies showed that the cavity that heme cofactor takes place in it, has the volume 232.448 and surface 535.04, and in other hand this cavity only has one gate that its aperture diameter is 1.5286 nm in maximum measure (Fig. 4A) and 0.8478 nm in minimum measure (Fig. 4B). Appropriate mutations in non-conserve points were carried out to avoid disarrangement of the natural structure in order to change in size and more equalizing of cavity diameter and create an exit aperture on the other side of the protein.

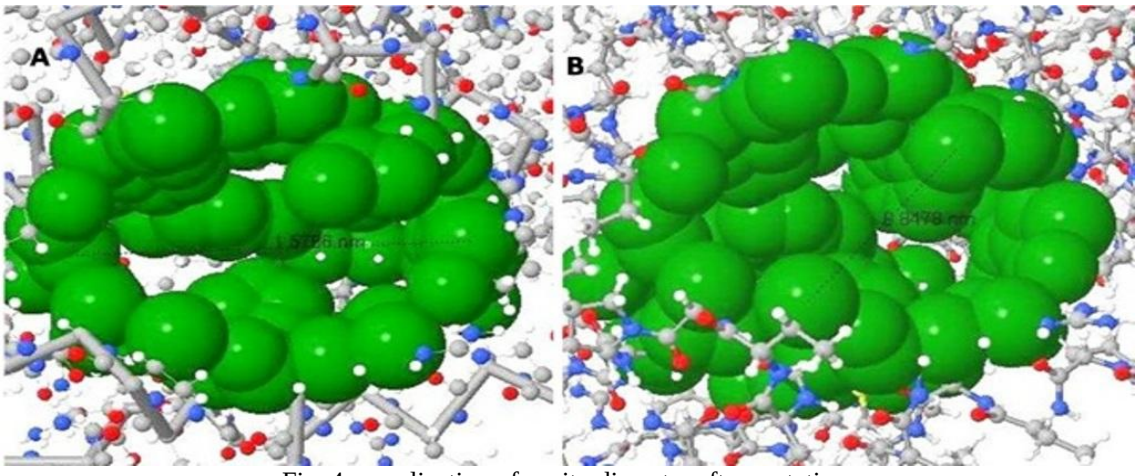

Fig. 4- equalization of cavity diameter after mutation

Point mutation

Point mutation by MVD software was performed in positions that had not any significant effect in 3D configuration of the hemoglobin protein. Based on the output of the I-Mutant 2.0 server, best spots for performing point mutation in a manner that the protein cavity becomes smaller and more equalize were in positions including: Leu 76, His 80 and Ala 83.

In this way a structure is more stable when DDG score is larger and positive and the structure is more unstable when DDG score is smaller and negative (Table 1).

Table 1- Properties of hemoglobin after mutation

Position	WT	NEW	DDG	PH	T	RSA
76	Leu	Gly	-2.74	7.0	25	12.0
80	His	Asn	-0.41	7.0	25	12.0
83	Ala	Lys	-1.07	7.0	25	12.0

WT: Amino acid in Wild-Type Protein,
NEW: New Amino acid after Mutation,
DDG: DG (New Protein) -DG (Wild Type) in Kcal/mol, DDG<0: Decrease Stability, DDG>0: Increase Stability,
T: Temperature in Celsius degrees,
PH: -log [H+], **RSA:** Relative Solvent Accessible Area

So with this awareness more negative amino acid was used to structural changes in the target area. First, point mutation converted leucine 76 to glycine and led to create the exit aperture (with diagonal: 0.788 nm) (Fig. 5C). In the next mutation, histidine 80 was converted to asparagine and cavity was more equalize along the cavity track. In the final step, point mutation on alanine 83 converts this amino acid to lysine and led to entrance hole was smaller (1.2 in the maximum measure (Fig.5A) and 0.85 in minimum measure (Fig.5B)).

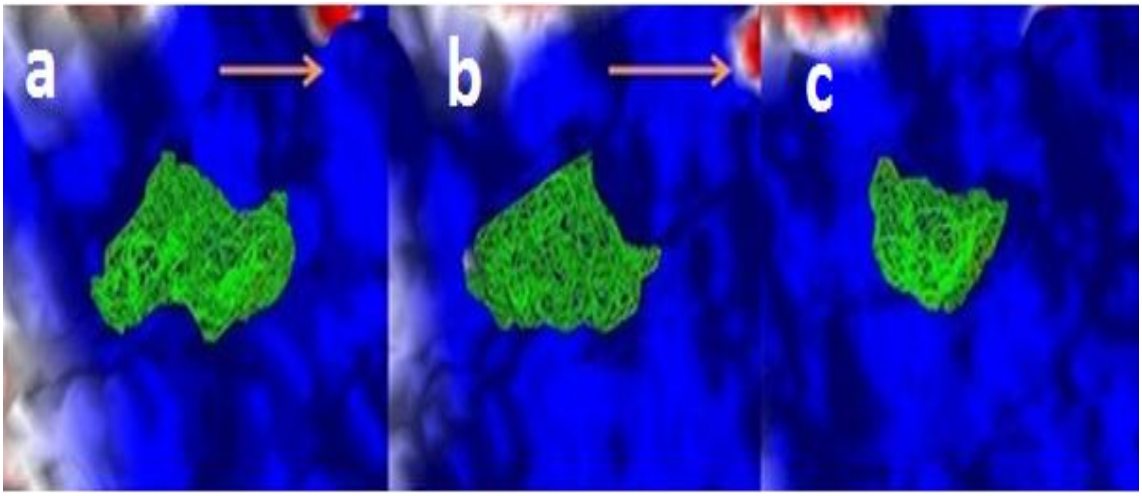

Fig. 5- Reduction in hole diameter after first (a), second (b) and third (c) point mutation.

Discussion and conclusion

CASTp server uses the weighted Delaunay triangulation and the alpha complex for shape measurements. It provides identification and measurements of surface accessible pockets as well as interior inaccessible cavities, for proteins and other molecules. It measures analytically the area and volume of each pocket and cavity, both in solvent accessible surface (SA, Richards' surface) and molecular surface (MS, Connolly's surface). It also measures the number of mouth openings, area of the openings, and circumference of mouth lips, in both SA and MS surfaces for each packet (10).

Our aim of this study was reducing in diameter of the entrance hole and creating an outlet opening beyond the protein with maintaining protein structure and minimization of structural changes. In addition studies showed that amino acids 73 to 84 are located in turn in the edge of protein and form the gate (Fig. 3), so three amino acids Leucine 76, Histidine 80 and Alanine 83 were selected to change and

reduction of pore diameter and appropriate amino acids were selected to replace using I-mutation server based on DDG score.

Pore-forming bacterial proteins are interested for filtration. Agrobacterial hem is one of these proteins that we can induce and improve nano pore in it by point mutation in three amino acids leucine 76, alanine 83 and histidine 80**.** These mutations also increase the stability of the protein. This procedure may be used for other pore-forming bacterial proteins.

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القا نانو حفره در هموگلوبین باکتریایی

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چکیده

مقدمه: تعداد زیادی از پروتئین‌های متصل شونده و انتقال دهنده اکسیژن در ارگانیسم‌های مختلف از جمله باکتری‌ها، پروتوزوآ و قارچ‌ها وجود دارد که همگی پروتئین‌های شبه هموگلوبین هستند. این پروتئین‌ها می‌توانند اکسیژن را با انواع مختلفی از مولکول‌ها از جمله NO_2، CO_2 و ترکیبات سولفیدی تبادل کنند. به علاوه آن‌ها می‌توانند موجب سمیت زدایی از مواد کلرینه مانند آنزیم‌های P450 و پراکسیدازها شوند و همچنین، به عنوان شناساگر نیترات و هیدروژن پراکسید به کار می‌روند.

مواد و روش‌ها: حفره هموگلوبین‌های باکتریایی می‌تواند برای تولید فیلترهای با اندازه نانو به کار روند. در این مطالعه، هموگلوبین آگروباکتر برای تولید نانو فیلتر از طریق جهش‌زایی نقطه‌ای استفاده شد.

نتایج: بررسی‌ها نشان داد که سه آمینو اسید لوسین ۷۶، آلانین ۸۳ و هیستیدین ۸۰ در تشکیل حفره در هموگلوبین باکتریایی بسیار مهم هستند.

بحث و نتیجه‌گیری: جهش نقطه ای و تبدیل لوسین ۷۶ به گلیسین، هیستیدین ۸۰ به آسپارژین و آلانین ۸۳ به لیزین، مرحله به مرحله موجب تشکیل نانو حفره ۰/۷ تا ۰/۸ نانومتری در هموگلوبین شد. این جهش‌ها همچنین موجب افزایش پایداری پروتئین در اسیدیته ۷ شدند.

واژه‌های کلیدی: هموگلوبین، آگروباکتریوم، مولگرو ویرچوال داکر

* نویسنده مسؤول مکاتبات

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