Structural analysis of antisickling agents in complex with carbonmonoxyhemoglobin in the context of sickle cell disease

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INTRODUCTION

Sickle cell disease is a group of red blood cells disorders of which Sickle cell anemia is the most common type. Sickle cell anemia is an autosomal recessive genetic disorder caused by a point mutation of beta-glu-6 in normal hemoglobin (Hb) to beta-val-6 in sickle Hb (Hb S)[1]. The mutated gene is generally inherited from the parent to the offspring. If one parent has the sickle cell disease and the another parent is normal then there is a 50% chance of child getting the sickle cell trait[2]. People with sickle cell trait are generally considered healthy and are known as carriers. If both the parents have sickle cell trait then there is a chance of the child getting infected with the sickle cell disease. The abnormalities in hemoglobin (Hb) leads to sickle shaped red blood cells which is unlike normal RBCs.These sickle shaped RBCs are not flexible and stick to the walls of the vessels which obstructs the blood flow(Fig.1). When the blood flow is blocked the oxygen is incapable to reach the nearby tissues which causes pain crises[3]. RBCs live upto 120 days whereas sickle cells last only 10 to 20 days which results in the decrease of RBCs than usual[3]. This condition is called anemia which deprives energy of a person. Usual symptoms are infections,pain and fatigue.Complications include chest pain, liver problems, kidney problems etc[4]. According to WHO, Africa is the most effected region with the highest prevalence in Uganda followed by republic of Congo, Gabon, Ghana and Nigeria (Source, WHO). In India, the most effected population are the tribals with more prevalence in the states of Gujarat, Maharashtra, Odisha and Chhattisgarh[5].

The agents that are used to alter the pathological conditions that lead to sickling of erythrocytes in sickle cell disease are known as antisickling agents. Hydroxyurea is the most commonly used antisickling agent[6]. Aromatic aldehydes are also in use because of their anti-polymerization property and their ability to increase the affinity of Hb for oxygen[7][8]. 5-HMF and Vanillin exhibited promising results as antisickling agents and this paved the way for the use of aromatic aldehydes in the treatment of sickle cell disease[9]. In this protein structure of carbonmonoxyhemoglobin, there is a mutation after Val1 where glutamic acid is absent (Fig.4). The drug molecule V1M is covalently linked to protein at Val1.

EXPERIMENTALS

The protein structure of carbonmonoxyhemoglobin (PDB ID - 6XDT) is downloaded from Protein Data Bank. The filters like refinement resolution: homosapiens, experimental method: X-ray diffraction and refinement resolution: 1.5 - 2.0 are used. The classification of the protein is oxygen transport and there is no expression system. The resolution value is 1.90 A^0. The R-Value free is 0.239 and R. Value work is 0.188. Two tests are done to determine the quality of the structure. 1) The difference between R-Free and R-Work values should be less than or equal to 0.05. 2) The R-Work value should be roughly equal to 1/10th of resolution. There are 2 macromolecules; Hemoglobin subunit alpha and Hemoglobin subunit beta. The small molecules include the ligands which are HEM, V1M and CMO. The secondary structure analysis and the hydrogen bonding analysis is done computationally using PyMOL software.

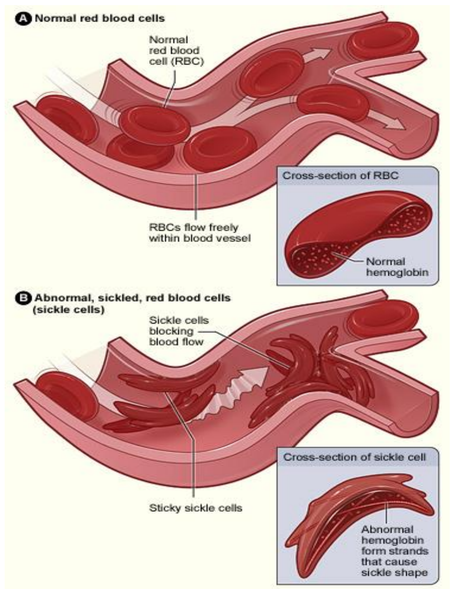

Fig.1:Difference between normal RBCs and sickle shaped RBCs. (Source: Wikipedia)

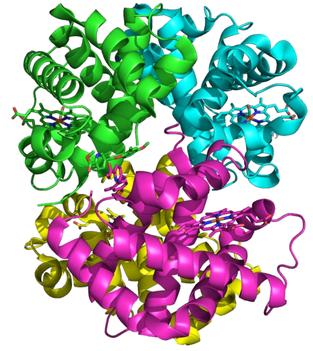

Fig. 2:Carbonmonoxyhemoglobin in complex with antisickling agent PDB ID: 6XDT. Pagare *et al.* 2020

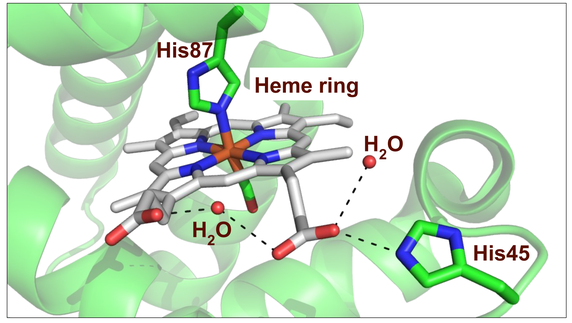

Fig.3:The ligand HEM forming four hydrogen bonds three with waters and one with His45.

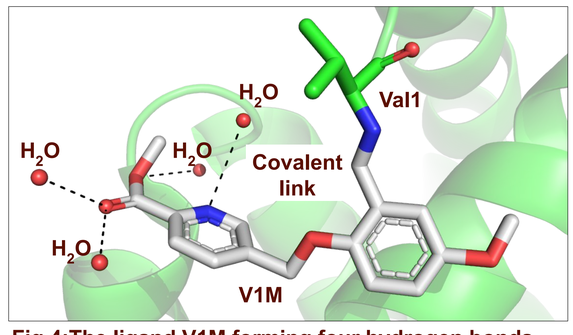

Fig.4:The ligand V1M forming four hydrogen bonds with waters and is covalently linked to protein at Val1.

RESULTS & DISCUSSION

The difference between R-Free and R-Work of the protein carbonmonoxyhemoglobin (PDB ID: 6XDT) is 0.05 and the R-Work value is roughly equal to 1/10th of the resolution(0.19) which tells us that the structure is of good quality. The protein is a tetramer with 2 hemoglobin alpha subunits and 2 hemoglobin beta subunits. The structure of carbonmonoxyhemoglobin contains 20 alpha-helices and 0 beta-strands. The ligand HEM forms 4 hydrogen bonds of which three are with water molecules and one with His45 and the ligand V1M forms 4 hydrogen bonds with water molecules as shown in Figures 3 and 4 respectively. The aromatic aldehydes in complex with carbonmonoxyhemoglobin strengthened the effects of sickling inhibition, Hb modification and the molecular interaction between Hb and O_2. These compounds showed notable results which can be used as antisickling agents for the therapy of sickle cell disease.

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