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Comparison of hemoglobin sequences of normoxic species to hypoxia-resistant *Heterocephalus glaber* (naked mole rat)

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Motivation and Aim: Red blood cells containing hemoglobin are carriers of oxygen from the lungs to all tissues of our body. Hemoglobin binds oxygen in the lungs and releases it to peripheral tissues. Hemoglobin is very tightly packed in the erythrocyte and is surrounded by a layer of bound water [1, 2]. The binding and release of oxygen is cooperative in nature (Borh effect) [3]. In different species, hemoglobin may have different affinities for oxygen, may have a feature of oxygen release in tissues, and may also have different sensitivity to oxidative stress developing during hypoxia. Most animals live and breed at a normal level of atmospheric oxygen of about 20 % and experience hypoxia when it decreases. Under conditions of hypoxia, oxidative stress develops in tissues, which leads to oxidative modifications of proteins and cell damage. However, not all animals are equally resistant to lack of oxygen. There are hypoxia resistant animals, such as the naked mole rat, which not only show extreme resistance to hypoxia (5 % O_2) [4], but are also capable of reproduction. The search for molecular reasons for such stability was the goal of this work. We attempted to find the structural determinants that determine the resistance of the naked digger to oxidative stress by comparing the sequences and structures of hemoglobins from different species.

Methods and Algorithms: Each hemoglobin molecule normally consists of four subunits, in humans two of them are formed by alpha chains, and two by beta chains. We searched the Uniprot database and compiled two sets of amino acid sequences separately for hemoglobin alpha and beta chains. The sequences of the corresponding hemoglobins of normoxic animals were assigned to the first group. The second group included hemoglobin sequences of the naked mole rat, which easily tolerate hypoxic conditions and are able to live and reproduce under such conditions. We built multiple alignments in each group by Uniprot (https://www.uniprot.org/) and TCoffee (https://tcoffee.crg.eu) servers to find conserved amino acid residues in both cases and compared them between themselves. In addition, we mapped the variability map to the structure of hemoglobin to search for molecular causes of hypoxic resistance by the ConSurf server (https://consurf.tau.ac.il/).

Results: The naked mole rat, unlike many other mammals, contains several types of hemoglobin. So, in addition to two variants of the alpha chain, one of which contains two cysteine residues instead of one, it has four variants of the zeta chain close to it and the theta-1 chain similar to them. The beta chain has three different versions, in addition, there are epsilon-1 and gamma chains that are similar to it, and in all the cysteine residue 112 is replaced by valine. If within these groups the sequences of hemoglobin chains are quite similar to each other, then the naked mole rat has another hemoglobin alpha chain, which differs greatly from both the first and second groups by the presence of a deletion of the first 32 amino acids from the N-terminus, an insertion of five amino acids TSGGA between amino acids 96 and 97 and a 27 amino acid insert QAGPYPCPLCLRPGSPPLPTVHLCIFE at the C-terminus. These substitutions, in addition to structural changes, introduce three additional cysteine residues into the structure of hemoglobin.

Conclusion: We believe that the diversity of different types of hemoglobin chains, as well as the replacement of the cysteine 112 residue by valine in all beta chains, contribute to the hypoxic resistance of Heterocephalus glaber, since cysteines are actively involved in the regulation of proteins through post-translational modifications of oxidative stress, such as glutathionylation, nitrosylation, or sulfhydrogenation. The biological role of the Cys112Val substitution remains to be elucidated.

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