Accessibility of the heme pocket in hexa-coordinate globins

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Abstract: Studies of fluorescence analogues of neuroglobin and cytoglobin to characterize conformational changes upon ligand binding

Neuroglobin (Ngb) and cytoglobin (Cygb) are newly discovered hexacoordinate members of the globin family. Ngb is expressed in nerve cells and the retina and its main role is to protect cells under hypoxic–ischemic conditions. Cygb is expressed almost in all tissues in our body and it have tumor suppression role. Here we report a preparation of fluorescent analogues of Ngb and Cygb by replacing the non-fluorescent Fe protoporphyrin IX with a fluorescent probe, Zn protoporphyrin IX (ZnPPIX). The accessibility of the heme binding pocket in fluorescent analogues of Ngb and Cygb was probed in quenching studies. The quenching of the ZnPPIX steady state emission by methyl viologen indicates an increased accessibility of the heme binding pocket in Ngb with the Stern-Volmer constant of 13.0 M^{-1} whereas the accessibility of the heme pocket in Cygb is restricted as evident by Ksv = 4.0 M^{-1}. The replacement of the distal histidine by glutamine decreases the accessibility of the ZnPPIX in Ngb; Ksv = 9.0 M^{-1}, but slightly increases the accessibility of the fluorophore in Cygb, Ksv = 5.2 M^{-1}. The oxygen migration was probed by monitoring the lifetime of ZnPPIX triplet state. The oxygen quenching of the triplet state of ZnPPIX incorporated in Ngb protein matrix is characterized by a quenching rate constant of $3x10^8$ $M^{-1}s^{-1}$, suggesting that the rate of oxygen migration through the protein is diffusion limited.

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