STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF CYANOGLOBIN: A PERIPHERAL MEMBRANE HEMOGLOBIN IN *NOSTOC COMMUNE* UTEX 584 (CYANOBACTERIA)

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ABSTRACT

Investigations of the nitrogen fixing (*nif*) genes in the cyanobacterium *Nostoc commune* UTEX 584 revealed a gene encoding a hemoprotein, named cyanoglobin. The cyanoglobin gene was isolated and subcloned into *Escherichia coli* previously. Cyanoglobin possesses a high oxygen affinity. The study presented here investigated the functional role of cyanoglobin, and encompassed the determination of the kinetic basis for the high oxygen affinity of cyanoglobin through kinetic studies utilizing stopped-flow spectrophotometry and flash photolysis. In addition, studies of cyanoglobin, in the presence of a variety of ligands, employed as structural probes of the distal pocket architecture, are presented. These data are interpreted in terms of structural models of cyanoglobin produced by homology modelling and hemoglobins with known crystal structures. Cyanoglobin coordinated oxygen and a variety of ligands with high rates of association, which explained the high oxygen affinity of cyanoglobin. Cyanoglobin possessed high rates of autoxidation and hemin loss. The ligand binding behavior of cyanoglobin was more similar to leghemoglobin than to sperm whale myoglobin. The ligand binding behavior of cyanoglobin is explained in terms of a highly reactive, and solvent exposed, heme-iron. The 5' region of *glbN* interacted with NtcA, the global regulator of nitrogen metabolism in cyanobacteria, which may provide an indication of the nitrogen deprivation signal required for cyanoglobin expression *in vivo*. Finally, the isolation and N-terminal sequencing of a potential cyanoglobin homolog in *Anabaena* sp. strain PCC 7120 is presented. Collectively, the data obtained in this study may support the model of cyanoglobin function described by Hill, et al., that cyanoglobin sequesters oxygen, and presents it to, or is a part of, a terminal cytochrome oxidase complex in

Nostoc commune UTEX 584 under microaerobic conditions, when nitrogen fixation, and thus ATP demand, is maximal.

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The work presented here is dedicated to my father and his model of integrity and industry; to believe that work is good, labor is better. My father was the philosopher, who encouraged me to be curious about life and nature. For this, I owe him a great debt.

List of Abbreviations

EDTA - ethylenediaminetetraacetate
DTT - dithiothreitol
MWCO - molecular weight cut-off
PVDF - polyvinylidene difluoride
PCR - polymerase chain reaction
nif - nitrogen fixing gene(s)
FixL - hemoprotein of *Rhizobium meliloti*
FixJ - response regulator of *Rhizobium meliloti*
DNA - deoxyribonucleic acid
mRNA - messenger ribonucleic acid
vhb - *Vitreoscilla* hemoglobin gene
Hmp - hemoprotein of *E. coli* K-12
NMR- Nuclear magnetic resonance
EPR - electron paramagnetic resonance
TAE - Tris/acetate/EDTA buffer
TE - Tris/EDTA buffer
TBST - Tris-buffered saline with 0.05% Tween 20
IPTG - isopropylthio-B-D-galactopyranoside
CAPS - (3-[cyclohexylamino]-1-propanesulfonic acid)
MES - [2-(N-morpholino)]ethanesulfonic acid
Tris.HCl - Tris(hydroxymethyl)aminomethane hydrochloride
SDS-PAGE - sodium dodecyl sulfate-polyacrylamide gel electrophoresis
FPLC - Fast protein liquid chromatography
HPLC - High pressure liquid chromatography
NtcA - Nitrogen control protein in cyanobacteria
FNR - Fumarate and nitrate reductase regulator protein
PDB - Protein Data Bank

O_2 - oxygen
CO - carbon monoxide
NO - nitric oxide
R-NC - alkylisonitrile
ORF - open reading frame
WT - wild type
LB - Luria-Bertani media
ATP - adenosine triphosphate
kD - kilodalton
glbN - cyanoglobin gene
CD - circular dichroism
PITC - phenylisothiocyanate
k_{obs} - observed rate constant
k'_x - association rate constant of X
k_x - dissociation rate constant of X
K_x - calculated affinity of X (k'_x / k_x)
TBS - Tris-buffered saline

Table of Contents

List of Figures

List of Tables