X-RAY CRYSTALLOGRAPHIC ANALYSES OF THE STRUCTURES OF TWO HEME PROTEINS

by

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ABSTRACT

During human development three embryonic hemoglobins are synthesised prior to formation of the placenta. These hemoglobins function to scavenge oxygen from the mother's interstitial fluid enabling embryonic respiration. The human Gower II embryonic haemoglobin ($\alpha_2\varepsilon_2$) has been crystallized in its carbonmonoxy form, and its structure determined by X-ray crystallography. The structure was solved by molecular replacement and refined at 2.9 Å. The Gower II hemoglobin tetramer is intermediate between the adult hemoglobin R and R2 states, though closer to R2. The tertiary structure of the α subunit is essentially identical when compared to that found in the adult ($\alpha_2\beta_2$) and fetal ($\alpha_2\gamma_2$) hemoglobins. The embryonic ε subunit has a very similar structure to the homologous adult β and fetal γ subunits, although with small differences at the N-terminus and in the A helix. Amino acid substitutions can be identified that may play a role in the altered response of the Gower II haemoglobin to allosteric effectors, in particular chloride ions.

Nitrite reductase from *Pseudomonas stutzeri* is a periplasmic heme enzyme responsible for the reduction of nitrite to nitric oxide. This reaction is the second step in the bacterial denitrification pathway, during which nitrate acts as the terminal electron acceptor for anaerobic respiration and is consequently reduced to nitrogen gas. Nitrite reductase from *Pseudomonas stutzeri* JM300 has been crystallized in the oxidised state and X-ray diffraction data collected to a resolution of 2.8 Å. The structure has been solved by the method of molecular replacement. The structure of the enzyme is dimeric, with each monomer comprised of two domains. The smaller N-terminal domain covalently binds a *c* heme group within an all α-helical fold similar to that of the class I *c*-type cytochromes. The larger C-terminal domain consists of an eight-bladed β-propeller structure that coordinates a d_1 heme, a cofactor unique to this class of enzyme. The relative positions of the two domains, and hence the orientations of the bound heme groups are markedly different compared to homologous enzymes from other species.

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ABBREVIATIONS

2,3BPG 2,3-bisphosphoglycerate
ATP Adenosine triphosphate
BIS-TRIS bis(2-hydroxyethyl)imino-tris(hydroxymethyl)methane
cDNA Copy deoxyribonucleic acid
CM cellulose Carboxymethyl cellulose
C-terminal Carboxy-terminal
DEAE Diethylaminoethyl
DMSO Dimethylsulphoxide
EPR Electron paramagnetic resonance
$FADH_2$ Reduced flavin adenine dinucleotide
FMN Flavin mononucleotide
Hb Hemoglobin
Hb A Human adult hemoglobin
Hb F Human foetal hemoglobin
HEM *b* heme
HEPES N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]
HMC *c* heme
HMD d_1 heme
LSQR Least squares refinement
MGD Molybdopterin guanine dinucleotide
MLR Maximum likelihood refinement
MME-PEG Polyethylene glycol monomethyl ether
MOPS 3-[N-Morpholino]propanesulfonic acid
MPD 2-Methyl-2,3-pentanediol
MR Molecular replacement
mRNA Messenger ribonucleic acid
Mw Molecular weight
NAc N-terminal acetylated
NADH Reduced nicotinamide adenine dinucleotide
NAR Nitrate reductase
NCS Non-crystallographic symmetry
NIR Nitrite reductase
NMR Nuclear magnetic resonance
NOR Nitric oxide reductase
NOS Nitrous oxide reductase
N-terminal Amino-terminal
PCR Polymerase chain reaction
PDB Protein Data Bank
PEG Polyethylene glycol
P_{O2} Partial pressure of oxygen
PQQ Pyrrolo-quinoline quinone
RBR Rigid body refinement
rms Root mean square
RT Room temperature
SAR Simulated annealing refinement
SDS-PAGE Sodium dodecyl sulphate - polyacrylamide gel electrophoresis
TAPS N-tris[Hydroxymethyl]methyl-3-aminopropanesulfonic acid

TRIS Tris(hydroxymethyl)aminomethane
UV Ultraviolet
Ala Alanine
Arg Arginine
Asn Asparagine
Asp Aspartic acid
Cys Cysteine
Gln Glutamine
Glu Glutamic acid
Gly Glycine
His Histidine
Ile Isoleucine
Leu Leucine
Lys Lysine
Met Methionine
Phe Phenylalanine
Pro Proline
Ser Serine
Thr Threonine
Trp Tryptophan
Tyr Tyrosine
Val Valine

RELATED PUBLICATION

Some of the material presented in this thesis has been accepted for publication.

Sutherland-Smith, A. J., Baker, H. M., Hofmann, O. M., Brittain T., and Baker, E. N. (1998). Crystal structure of a human embryonic haemoglobin: the carbonmonoxy form of Gower II ($\alpha_2\varepsilon_2$) haemoglobin at 2.9 Å resolution. *Journal of Molecular Biology*. In press.