X-RAY CRYSTALLOGRAPHIC ANALYSIS OF CYTOCHROME C' FROM TWO BACTERIAL SPECIES

This thesis is submitted as partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry at Massey University.

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March, 1995

ABSTRACT

The structure of cytochrome c' from two closely related bacterial species, *Alcaligenes* sp and *Alcaligenes denitrificans*, have been determined from X-ray diffraction data to 1.80 Å and 2.15 Å resolution respectively using the anomalous scattering of the single iron atom in each to identify and refine a weak molecular replacement solution. Molecular replacement studies, with the program AMORE, used two isomorphous data sets (from the two species), two independent search models (the cytochromes c' from *Rhodospirillum molischianum* and *Rhodospirillum rubrum*), both with and without sidechains, and two different resolution ranges (10.0-4.0 Å and 15.0-3.5 Å) to generate a large number of potential solutions. No single solution stood out and none appeared consistently. The iron position in each structure was then determined from its anomalous scattering contribution and all molecular replacement solutions were discarded which did not (i) place the iron correctly and (ii) orient the molecule such that a crystallographic 2-fold axis generated a dimer like those of the two search models. Finally, electron density maps phased solely by the iron anomalous scattering were calculated. As these were combined and subjected to solvent flattening and histogram matching (program SQUASH), correlation with the remaining molecular replacement solutions identified one as correct and enabled it to be improved and subjected to preliminary refinement. The correctness of the solution was confirmed by parallel isomorphous replacement studies. Both crystal structures have been refined using least-squares methods, to give final R-factors of 0.184 for the *Alcaligenes* sp cytochrome c' (953 protein atoms and 89 solvent molecules) and 0.167 for the *Alcaligenes denitrificans* cytochrome c' (950 protein atoms and 75 solvent molecules). Analysis and comparisons of these structures with three cytochrome c' structures previously determined show that despite the low level of sequence conservation among the family members the gross overall structure is maintained (four-α-helix bundle). The configuration of the iron ligands and of the surrounding haem environment are very similar indicating that although residues are not conserved they are replaced by residues which fulfil a similar function. There are relatively few sidechain...sidechain interactions in cytochromes c' which probably reflects the fact that the majority of stabilising $i^{interactions}$ are hydrophobic in nature. This hydrophobic packing could also explain the lack of sequence identity between the different species of cytochrome c' as strict conservation of the residues is not required. All the structures do have a similar haem stabilisation framework, with an approximately ten residue piece of structure situated in the BC loop involved in both haem stabilisation and interhelix stabilisation in all of the cytochromes c'

determined. The overall four-helix structure is also discussed in terms of its parameters with respect to the current interest in the *de novo* design of proteins.

ACKNOWLEDGEMENTS

Many people have given me their time and advice during my thesis and if I do not mention your name please don't be offended as I do thank you.

The biggest acknowledgement goes to my supervisors Prof. Ted N. Baker and Dr. Bryan F. Anderson who gave endless support and guidance throughout a long and difficult Ph.D. Their contribution was immeasurable in both knowledge and encouragement.

Also Mrs Heather M. Baker for her adept crystallisation skills and kindly ear when required. Dr. Gill E. Norris for providing me the crystals for this study plus a place to stay when finishing off this thesis.

Dr. Hale. H. Nicholson and the University of Oregon for the collection of data sets involved in this thesis.

The post-docs such as Drs. David Thomas, Hale Nicholson, Colin Groom, Rick Faber and Maria Bewley who all provided me with different ideas and techniques in macromolecular crystallography .

The other members of the crystallography research group (past and present) should not get away without mention, so thank you..

Two very good friends, Michael "you should be humanitarian ambassador" Nairn, who unfortunately for the general population has a very similar sense of humour to mine and Dean "pirate" Severinsen both of whom got me through this thesis and life in general.

I am grateful to Massey University for the position of Graduate Assistant to make this study possible and to the Howard Hughes Medical Institute grant for further funding.

Lastly I would like to thank my family who still are not quite sure about what I do but gave me their support throughout my time at Massey University.

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ABBREVIATIONS

NO_3^-	Nitrate ion
A_{280}	Absorbance at 280nm
CM-cellulose	Carboxymethyl cellulose
R. rubrum	*Rhodospirillum rubrum*
R. molischianum	*Rhodospirillum molischianum*
Cys	Cysteine
His	Histidine
a.a	amino acid
NH_3	ammonia
NADP/H	phosphorylated NAD
NO	Nitric oxide
N_2O	Nitrous oxide
NO_2	Nitrogen dioxide
NO_2^-	Nitrite
Rps. viridis	*Rhodopseudomonas viridis*
Rps. palustrus	*Rhodopseudomonas palustrus*
Rps. capsulata	*Rhodopseudomonas capsulata*
Rps. sphaeroides	*Rhodopseudomonas sphaeroides*
pI	Isoelectric point
Chr. Vinosum	*Chromatium vinosum*
M	mol L^{-1}
IP	Image plate
Asp	*Alcaligenes* sp
Ade	*Alcaligenes denitrificans*
Rms	Root-mean-square
MR	Molecular replacement
MIR	Multiple isomorphous replacement
MIRAS	Multiple isomorphous replacement with anomalous scattering
SIR	Single isomorphous replacement
SIRAS	Single isomorphous replacement with anomalous scattering
Asp	Aspartic acid
Gln	Glutamine
Glu	Glutamic acid
Ala	Alanine
Pro	Proline
Asn	Asparagine
Hem	Haem

Val	Valine
Lys	Lysine
Ile	Isoleucine
Leu	Leucine
Thr	Threonine
Met	Methionine
Tyr	Tyrosine
Phe	Phenylalanine
Ser	Serine
AB loop	Loop between helices A and B
BC loop	Loop between helices B and C
CD loop	Loop between helices C and D
DEAE	Diethyl aminoethyl
ALDH	Aldehyde dehydrogenase
FPLC	Fast protein liquid chromatography
NAD/H	Nicotinamide adenine dinucleotide
ATP	Adenosine triphosphate
SDS	Sodium dodecylsulphate
PEG	Polyethylene glycol
DMNB	3,4-dihydro-3-methyl-6-nitro*2H*-1,3-benoxazin-2-one
Da	Dalton
ASCC	*Alcaligenes* sp cytochrome c'
ADCC	*Alcaligenes denitrificans* cytochrome c'
CVCC	*Chr. vinosum* cytochrome c'
RMCC	*R. molischianum* cytochrome c'
RRCC	*R. rubrum* cytochrome c'
VDW	van der Waals
PCMB	*para*-chloromecuribenzoate
PHMB	*para*-hydroxymecuribenzoate

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