

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**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.**

AARON JOHN DOBBS

March, 1995

574.192454
Dob

2020

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 molischanum* 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 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.

CONTENTS

	Page
ABSTRACT	ii
ABBREVIATIONS	ix
LIST OF FIGURES	xii
LIST OF TABLES	xv

Chapter One

INTRODUCTION

	Page
1.1	1
1.1.1	1
1.1.2	5
1.2	5
1.2.1	5
1.2.2	6
1.2.3	10
1.3	11
1.3.1	11
1.3.2	12
1.3.3	14
1.3.4	17
1.3.5	17
1.4	18

Chapter Two

CRYSTALLISATION, DATA COLLECTION AND PROCESSING

2.1	Purification of cytochrome c'	19
2.2	Crystallisation of cytochrome c' from <i>Alcaligenes</i> sp and <i>Alcaligenes denitrificans</i>	19
2.3	Data collection and processing	21
2.3.1	Photon factory data set - <i>Alcaligenes</i> sp native data	21
	<i>Data collection</i>	
	<i>Data processing</i>	

2.3.2	Anomalous data from <i>Alcaligenes</i> sp	27
	<i>Data collection</i>	
	<i>Data processing</i>	
2.3.3	Heavy atom data from <i>Alcaligenes</i> sp	30
	<i>Data collection</i>	
	<i>Data processing</i>	
	<i>Scaling with Alcaligenes sp native data</i>	
2.3.4	R-Axis data from <i>Alcaligenes denitrificans</i>	36
	<i>Data collection</i>	
	<i>Data processing</i>	

Chapter Three

MOLECULAR REPLACEMENT

3.1	The rotation function	40
3.2	The translation function	41
3.3	Computational methods	43
3.3.1	ALMN	43
3.3.2	TFFC	45
3.3.3	X-PLOR	45
	<i>Rotation program</i>	
	<i>PC refinement</i>	
	<i>Translation program</i>	
3.3.4	AMORE	47
	<i>Data processing</i>	
	<i>Rotation program</i>	
	<i>Translation program</i>	
	<i>Rigid body refinement</i>	
3.4	Search models	48
3.5	Results	51
3.5.1	Results using ALMN/TFFC	51
	<i>Rotation program (ALMN)</i>	
	<i>Translation program (TFFC)</i>	
3.5.2	Results using X-PLOR	57
	<i>Rotation program</i>	
	<i>Translation program</i>	
3.5.3	Results from AMORE	61
3.6	Retrospective comparison of molecular replacement results	64

Chapter Four
PHASE DETERMINATION BY ISOMORPHOUS REPLACEMENT AND
ANOMALOUS SCATTERING

4.1	Introduction	66
4.2	Structure factor and phase relationships	67
4.2.1	Isomorphous replacement	68
	<i>Single isomorphous replacement (SIR)</i>	
	<i>Multiple isomorphous replacement (MIR)</i>	
4.2.2	Incorporation of anomalous scattering	70
	<i>Single isomorphous replacement with anomalous scattering (SIRAS)</i>	
	<i>Multiple isomorphous replacement with anomalous scattering (MIRAS)</i>	
4.3	Practical considerations	75
4.4	Determination of heavy atom positions	76
4.4.1	Use of the Patterson function	76
4.4.2	Use of difference Fourier	77
4.4.3	Refinement of the heavy atom positions	77
4.4.4	Treatment of errors	78
4.5	Results	79
4.5.1	Heavy atom soaking trials	79
4.5.2	Heavy atom site determination	81
4.5.3	Phase calculations	82
4.5.4	Determination of iron sites	83
4.5.5	Phasing from anomalous scattering	84

Chapter Five
STRUCTURE DETERMINATION

5.1	Density modification	86
5.2	Improvement of anomalous scattering phases and their use in determining the correct molecular replacement solution	86
5.2.1	Improvement of SIR phases using solvent flattening	90
5.2.2	Improvement of SIR phases using molecular averaging	91
5.2.3	Combining SIR phases with anomalous scattering	

	phases using SIGMAA	92
5.2.4	Combining SIR phases with anomalous scattering phases using OVERLAPMAP	93
5.3	Refinement methods	96
5.3.1	Least squares refinement with TNT	96
5.3.2	Refinement with X-PLOR	97
5.3.3	Model rebuilding	97
5.3.4	Inclusion of solvent	98
5.4	Progress of refinement	99
5.4.1	Refinement of <i>Alcaligenes</i> sp cytochrome c'	99
5.4.2	Refinement of <i>Alcaligenes denitrificans</i> cytochrome c'	103
5.5	Quality of the final models	105
5.5.1	Accuracy of the model for <i>Alcaligenes</i> sp cytochrome c'	105
5.5.2	Accuracy of the model for <i>Alcaligenes denitrificans</i> cytochrome c'	109
5.5.3	Conformational angles	112
	<i>Mainchain torsion angles (ϕ, ψ)</i>	
	<i>Sidechain torsion angles ($\chi_1 - \chi_5$)</i>	

Chapter Six

STRUCTURE DESCRIPTION

6.1	The overall fold	118
6.2	Hydrogen bonding details	121
6.2.1	Mainchain...mainchain hydrogen bonding	122
6.2.2	Mainchain...sidechain hydrogen bonding	127
6.2.3	Sidechain...sidechain hydrogen bonding	132
6.2.4	Haem hydrogen bonding	134
6.3	Loops and turns	139
6.4	Modified N-terminus	142
6.5	Analysis of the haem geometry	143
6.6	Analysis of the haem environment	148
6.7	Dimer interface	151
6.8	Thermal parameters	155
6.9	Solvent structure	157
6.10	Crystal packing	163

Chapter Seven
STRUCTURAL COMPARISONS WITH OTHER CYTOCHROMES C'

7.1	Sequence alignment	167
7.2	Structural superposition	169
7.3	Secondary structure	171
7.4	Helix orientations	173
7.5	Connecting loops	178
7.6	Sidechain hydrogen bonding	181
	7.6.1 Sidechain...mainchain hydrogen bonding	181
	7.6.2 Sidechain...sidechain hydrogen bonding	183
	7.6.3 Haem hydrogen bonding	185
7.7	Haem geometry and environment	188
	7.7.1 Haem stereochemistry	188
	7.7.2 Haem environment	192
7.8	The dimer interface	198
7.9	Solvent structure	200

Chapter Eight
GENERAL DISCUSSION

8.1	Structure determination	205
8.2	Structural aspects	210
8.3	Future work	220
APPENDIX I		222
APPENDIX II		232
BIBLIOGRAPHY		247

ABBREVIATIONS

NO ₃ ⁻	Nitrate ion
A ₂₈₀	Absorbance at 280nm
CM-cellulose	Carboxymethyl cellulose
<i>R. rubrum</i>	<i>Rhodospirillum rubrum</i>
<i>R. molischanum</i>	<i>Rhodospirillum molischanum</i>
Cys	Cysteine
His	Histidine
a.a	amino acid
NH ₃	ammonia
NADP/H	phosphorylated NAD
NO	Nitric oxide
N ₂ O	Nitrous oxide
NO ₂	Nitrogen dioxide
NO ₂ ⁻	Nitrite
<i>Rps. viridis</i>	<i>Rhodopseudomonas viridis</i>
<i>Rps. palustrus</i>	<i>Rhodopseudomonas palustrus</i>
<i>Rps. capsulata</i>	<i>Rhodopseudomonas capsulata</i>
<i>Rps. sphaeroides</i>	<i>Rhodopseudomonas sphaeroides</i>
pI	Isoelectric point
<i>Chr. Vinosum</i>	<i>Chromatium vinosum</i>
M	mol L ⁻¹
IP	Image plate
Asp	<i>Alcaligenes</i> sp
Ade	<i>Alcaligenes denitrificans</i>
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-benzoxazin-2-one
Da	Dalton
ASCC	<i>Alcaligenes</i> sp cytochrome c'
ADCC	<i>Alcaligenes denitrificans</i> cytochrome c'
CVCC	<i>Chr. vinosum</i> cytochrome c'
RMCC	<i>R. molischianum</i> cytochrome c'
RRCC	<i>R. rubrum</i> cytochrome c'
VDW	van der Waals
PCMB	<i>para</i> -chloromecuribenzoate
PHMB	<i>para</i> -hydroxymecuribenzoate

LIST OF FIGURES

Figure		Page
1.1.1.1	Structures of haem groups in cytochromes	2
1.1.1.2	UV-visible absorption spectra of c-type cytochromes	4
1.2.2.1	Ribbon diagrams of various c-type cytochromes	7
1.2.2.2	UV-visible spectra of selected c-type cytochromes	8
1.2.3.1	Terminal reactions in bacterial electron transport systems	10
1.3.2.1	Denitrifying electron transport system of <i>Pa. denitrificans</i>	13
1.3.3.1	Effect of octahedral crystal field on the d-orbital splitting	15
1.3.3.2	Spin states of octahedral Fe(III)	16
2.2.1	Crystals of cytochrome c' from <i>Alcaligenes</i> sp	20
2.3.1.1	% intensities greater than 3σ (Photon factory data set)	26
2.3.1.2	% completeness vs resolution (Photon factory data set)	26
2.3.2.1	% completeness vs resolution (Xuong-Hamlin data set)	29
2.3.2.2	% intensities greater than 3σ (Xuong-Hamlin data set)	29
2.3.3.1	% completeness vs resolution (Heavy atom data set)	33
2.3.3.2	% intensities greater than 3σ (Heavy atom data set)	33
2.3.3.3	R_{iso} vs resolution for platinum derivatives	35
2.3.4.1	% completeness vs resolution (R-Axis data set)	37
2.3.4.2	% intensities greater than 3σ (R-Axis data set)	39
3.3.1.1	Eulerian angle system	44
4.2.1	Argand diagram defining structure factor amplitude and phases	67
4.2.1.1	Harker construction for SIR	68
4.2.1.2	"True" and "wrong" values for SIR structure factors	69
4.2.1.3	Harker construction for MIR	70
4.2.2.1	Relationship between F_P , F_{PH} , and F_H	73
4.2.2.2	SIR with anomalous scattering	75
5.2.1	Haem density from anomalously phased map	88
5.2.2	AB Loop density from anomalously phased map	88
5.2.1.1	Electron density in AB loop after solvent flattening of SIR phases	90
5.2.2.1	Application of molecular averaging	91
5.2.2.2	Electron density in AB loop	92
5.2.3.1	Density in AB loop from combined "SIR" and "Iron" map	93
5.2.4.1	Electron density in AB loop	94
5.2.4.2	Electron density in BC loop	94
5.5.1.1	Electron density from final 2Fo-Fc map in AB loop	106

5.5.1.2	Electron density from final 2Fo-Fc map of haem pyrrole rings	106
5.5.1.3	Luzzati plot for <i>Alcaligenes</i> sp and <i>Alcaligenes denitrificans</i>	107
5.5.1.4	Real space-coefficient plot (mainchain atoms) vs residue number - <i>Alcaligenes</i> sp	108
5.5.1.5	Real space-coefficient plot (sidechain atoms) vs residue number - <i>Alcaligenes</i> sp	108
5.5.1.6	Electron density from final 2Fo-Fc map of Asp75	109
5.5.2.1	Example of ADCC model fitting density in final 2Fo-Fc map	110
5.5.2.2	Real space-coefficient plot (mainchain atoms) vs residue number - <i>Alcaligenes denitrificans</i>	111
5.5.2.3	Real space-coefficient plot (sidechain atoms) vs residue number - <i>Alcaligenes denitrificans</i>	111
5.5.2.4	Density from final 2Fo-Fc map around Arg69 in ADCC	112
5.5.3.1	Ramachandran plot for <i>Alcaligenes</i> sp	114
5.5.3.2	Ramachandran plot for <i>Alcaligenes denitrificans</i>	115
5.5.3.3	Distribution of χ_1 values in ASCC	116
5.5.3.4	Distribution of χ_1 values in ADCC	117
6.1.1	Molscript diagram of monomer	119
6.1.2	C α stereo plot (Molscript) of monomer	120
6.2.1.1	Plot of n...n+4 hydrogen bond lengths in helix A	124
6.2.1.2	Plot of n...n+4 hydrogen bond lengths in helix B	125
6.2.1.3	Plot of n...n+4 hydrogen bond lengths in helix C	125
6.2.1.4	Plot of n...n+4 hydrogen bond lengths in helix D	126
6.2.1.5	Stereoview of mainchain hydrogen bonding (residues 41 - 45)	126
6.2.1.6	Stereoview of n...n+4 hydrogen bonding (residues 91 - 97)	127
6.2.2.1	Stereodiagram of N-cap (residue 37)	130
6.2.2.2	Stereodiagram of backbonding interaction	131
6.2.3.1	Stereoview of "local" sidechain...sidechain interaction	133
6.2.4.1	Hydrogen bonding around propionate sidechain on ring A	135
6.2.4.2	Hydrogen bonding around propionate sidechain on ring B	136
6.2.4.3	Hydrogen bonding of conserved Arg12 residue	138
6.3.1	Type II turn involving residues 60-63	140
6.3.2	Type II' turn involving residues 65-68	141
6.4.1	Electron density of modified N-terminus in <i>Alcaligenes</i> sp	142
6.5.1	Protohaem IX group present in cytochromes c'	143
6.5.2	Coordination of iron atom	144
6.5.3	Stereodiagram of Arg124 sidechain and its environ	146
6.6.1	Molscript diagram showing residues within VDW contact	149

6.7.1	Molscript diagram of dimer interface	152
6.7.2	Schematic diagram showing shape recognition at interface	153
6.7.3	Electron density at dimer interface	153
6.8.1	Plot of mainchain B-values vs residue number - <i>Alcaligenes</i> sp and <i>Alcaligenes denitrificans</i>	156
6.8.2	Plot of sidechain B-values vs residue number - <i>Alcaligenes</i> sp and <i>Alcaligenes denitrificans</i>	157
6.9.1	"Common" solvent molecules between <i>Alcaligenes</i> sp and <i>Alcaligenes denitrificans</i>	160
6.9.2	Solvent structure in ASCC	161
6.9.3	Solvent structure in ADCC	162
6.10.1	Crystal packing of cytochrome c'	163
6.10.2	Packing down six-fold axis	164
6.10.3	Salt bridge between Arg106 and Asp88	165
7.1.1	Sequence alignment of cytochromes c'	168
7.2.1	<i>Rms</i> deviation as a function of residue number for CVCC	170
7.2.2	<i>Rms</i> deviation as a function of residue number for RMCC	170
7.2.3	<i>Rms</i> deviation as a function of residue number for RRCC	171
7.5.1	Stereodiagram of AB loops superimposed	178
7.5.2	Stereodiagram of BC loops superimposed	179
7.5.3	Stereodiagram of CD loops superimposed	179
7.6.1.1	Hydrogen bonding involving Arg12	182
7.6.3.1	Haem hydrogen bonding	186
7.9.1	"Conserved" solvent positions in ASCC/RMCC	201
7.9.2	"Conserved" solvent positions in ASCC/CVCC	202
7.9.3	"Conserved" solvent positions in ADCC/RMCC	203
7.9.4	"Conserved" solvent positions in ADCC/CVCC	204
8.2.1	Helical wheel representation of residues	216
8.2.2	Correlation between soin state and ligand field strength	218
II.3.2.1	Structure of acetophenone	240
II.3.2.2	Solid matrix of p-hydroxyacetophenone resin	240
II.3.3.1	Native gel of ALDH using revised method	241
II.3.3.2	SDS gel of ALDH using revised method	242
II.4.1.1	Structure of β -octyl glucoside	244
II.4.1.2	Crystals of ALDH under "best" conditions	245
II.4.2.1	Crystals of DMNB modified ALDH	246

LIST OF TABLES

Table	Page	
1.2.2.1	Distinguishing properties of c-type cytochromes	9
1.3.3.1	Properties of some cytochromes c'	14
2.3.1.1	Image plate data collection statistics (c-axis)	21
2.3.1.2	Image plate data collection statistics (a-axis)	22
2.3.1.3	Data collection and processing statistics (c-axis)	23
2.3.1.4	Data collection and processing statistics (a-axis)	24
2.3.1.5	Intav4 statistics	24
2.3.1.6	Statistics for Photon factory data as a function of resolution	25
2.3.2.1	Data processing statistics (Xuong-Hamlin data set)	28
2.3.2.2	Statistics for Xuong-Hamlin data set as a function of resolution	28
2.3.3.1	Data processing statistics (Heavy atom derivative data set)	32
2.3.3.2	Statistics for PT1 data set as a function of resolution	32
2.3.3.3	Scale and R_{iso} values for PT1 derivative data set	34
2.3.3.4	Scale and R_{iso} values for PT2 derivative data set	35
2.3.4.1	Cell dimensions of <i>Alcaligenes</i> sp and <i>Alcaligenes denitrificans</i>	36
2.3.4.2	Data processing statistics (R-Axis ADCC data set)	37
2.3.4.3	Statistics for R-Axis ADCC data set as a function of resolution	38
3.4.1.1	Search models used in ALMN and X-PLOR	49
3.4.1.2	Search models used in AMORE	50
3.5.1.1	Input values for ALMN	51
3.5.1.2	Cross rotation peaks - models derived from <i>R. rubrum</i>	52
3.5.1.3	Cross rotation peaks - models derived from <i>R. molischianum</i>	53
3.5.1.4	Rotation angles used in translation function	55
3.5.1.5	Translation function results - models derived from <i>R. rubrum</i>	55
3.5.1.6	Translation function results - models derived from <i>R. rubrum</i>	56
3.5.1.7	Translation function results - models derived from <i>R. molischianum</i>	56
3.5.1.8	Translation function results - models derived from <i>R. molischianum</i>	57
3.5.2.1	X-PLOR rotation peaks - models derived from <i>R. rubrum</i>	58
3.5.2.2	X-PLOR rotation peaks - models derived from <i>R. molischianum</i>	58
3.5.2.3	Rotation peaks after PC-refinement - models derived from <i>R. rubrum</i>	59
3.5.2.4	Rotation peaks after PC-refinement - models derived from <i>R. molischianum</i>	59

3.5.2.5	Translation function shifts - models derived from <i>R. rubrum</i>	60
3.5.2.6	Translation function shifts - models derived from <i>R. molischianum</i>	61
3.5.3.1	AMORE molecular replacement solutions	63
3.6.1	Molecular replacement solutions (ALMN/TFFC) using final <i>Alcaligenes</i> sp model	64
3.6.2	Testing AMORE solutions	65
4.2.2.1	Variation of atomic scattering factor with wavelength	71
4.5.1.1	Heavy atom complexes used in trials	80
4.5.2.1	Refined platinum sites	81
4.5.3.1	Phasing statistics for derivatives	82
4.5.4.1	Position of iron sites in unit cell - <i>Alcaligenes</i> sp	83
5.2.1	Electron density map correlations	89
5.2.4.1	Maps used in density combination	95
5.4.1.1	Course of refinement in ASCC	101
5.3.2.1	Course of refinement in ADCC	104
5.5.3.1	ϕ , ψ values in helical regions in ASCC and ADCC	113
6.1.1	α -helix assignments	121
6.2.1.1	Hydrogen bonding in kink found in helix A	124
6.2.2.1	Mainchain...sidechain hydrogen bonding in ASCC	128
6.2.2.2	Mainchain...sidechain hydrogen bonding in ADCC	129
6.2.3.1	Sidechain...sidechain hydrogen bonds in ASCC/ADCC	132
6.2.4.1	Protein...haem, solvent...haem hydrogen bonds	137
6.3.1	Turns present in BC loop	139
6.5.1	Porphyrin stereochemistry and haem ligand binding geometry	145
6.5.2	Deviations from planarity in haem group	147
6.6.1	Haem packing contacts in ASCC/ADCC	150
6.7.1	Dimer interface interactions in ASCC/ADCC	154
6.8.1	Average mainchain and sidechain B-values in ASCC/ADCC	156
6.8.2	Average mainchain and sidechain B-values of the helices in ASCC/ADCC	156
6.9.1	Number of hydrogen bonds and mean B-values	158
6.9.2	Analysis of solvent hydrogen bonds (ASCC)	159
6.9.3	Analysis of solvent hydrogen bonds (ADCC)	159
6.10.1	Intermolecular hydrogen bond contacts	166
7.2.1	<i>Rms</i> deviations and residues used in sequence alignment	169
7.3.1	Residues involved in α -helices in cytochromes c'	172
7.3.2	Residues forming 3_{10} helices in cytochromes c'	173

7.4.1	Residues used in structural superpositions in cytochromes c'	174
7.4.2	<i>Rms</i> deviations of "core" elements	174
7.4.3	<i>Rms</i> differences for individual helices	175
7.4.4	<i>Rms</i> differences between helices and haem arrangement	175
7.4.5	Interhelix angles and distances in cytochromes c'	176
7.4.6	Range of interhelix angles and distances	177
7.5.1	Turns present in cytochromes c'	180
7.6.1.1	Mainchain...sidechain interactions	181
7.6.1.2	Conserved sidechain...sidechain and sidechain...mainchain Hydrogen bonding involving Arg12	183
7.6.2.1	Sidechain...sidechain interactions in cytochromes c'	184
7.6.2.2	"Local" and "cross-linking" interactions	185
7.6.3.1	Haem hydrogen bonding	187
7.7.1.1	Porphyrin stereochemistry and haem ligand binding in RRCC, RMCC and CVCC	189
7.7.1.2	Porphyrin stereochemistry and haem ligand binding in ASCC and ADCC	189
7.7.1.3	Alternate χ_1 and χ_2 conformations at haem binding region	191
7.7.1.4	Comparison of Fe-Fe distance and haem - haem plane angles in cytochromes c'	191
7.7.1.5	Closest contact distance for the "sixth" ligand in cytochromes c'	192
7.7.2.1	Haem packing contacts in ASCC	193
7.7.2.2	Haem packing contacts in RMCC	194
7.7.2.3	Haem packing contacts in CVCC	195
7.7.2.4	Haem packing contacts in RRCC	196
7.7.2.5	"Conserved" residues in haem packing	197
7.8.1	Dimer interface contacts	199