

Contents

Chapter 1

Electron Transfer in Natural Proteins: Theory and Design

Christopher C. Moser, Christopher C. Page, Xiaoxi Chen, and
P. Leslie Dutton

1. Introduction	1
2. Basic Electron Tunneling Theory	2
2.1. Classical Free Energy and Temperature Dependence of Tunneling	3
2.2. Quantum Free Energy and Temperature Dependence	7
2.3. A Generic Protein Tunneling Rate Expression	8
3. Protein Structural Heterogeneity	9
3.1. Monitoring Heterogeneity via Packing Density	10
3.2. Natural Tunneling Distances	13
4. Chains and Robust Electron Transfer Protein Design	14
5. Electron Transfer Clusters.....	17
5.1. Cluster Examples	19
5.2. Control of Electron Transfer Through Escapement Mechanisms	21
6. Caution and Hope.....	23
7. References	24

Chapter 2

Flavin Electron Transfer Proteins

F. Scott Mathews, Louise Cunane, and Rosemary C. E. Durley

1. Introduction	29
2. Background and Structural Properties.....	32

2.1. NADPH-Cytochrome P450 Reductase (CPR)	32
2.2. Flavocytochrome P450BM3	38
2.3. Flavocytochrome b ₂ (FCB2) ..	42
2.4. <i>p</i> -Cresol Methylhydroxylase (PCMH)	45
2.5. Flavocytochrome <i>c</i> Sulfide Dehydrogenase (FCSD) . . .	47
2.6. Trimethylamine Dehydrogenase (TMADH)	48
2.7. Phthalate Dioxygenase Reductase (PDR)	50
2.8. Fumarate Reductase (FUM)	52
3. Electron Transfer	55
3.1. General Aspects	55
3.2. Electronic Coupling	59
3.2.1. BMP/FMN	59
3.2.2. FCB2	61
3.2.3. PCMH	61
3.2.4. FCSD	63
3.2.5. FUM	65
4. Conclusions	65
4.1. Domain Interactions	65
4.2. Features of Electron Transfer	67
5. References	68

Chapter 3

Methanol Dehydrogenase, a PQQ-Containing Quinoprotein Dehydrogenase

Christopher Anthony

1. Introduction ...	73
2. General Enzymology	75
2.1. The Determination of MDH Activity	75
2.2. The Substrate Specificity of MDH	76
3. The Kinetics of Methanol Dehydrogenase	77
3.1. The Reaction Cycle of MDH	77
3.2. The Activation of MDH by Ammonia and Amines ...	78
3.3. The Low <i>in vitro</i> Rate of Cytochrome <i>c</i> _L Reduction by MDH	80
4. The Absorption Spectra of Methanol Dehydrogenase	80
5. Pyrrolo-Quinoline Quinone (PQQ): The Prosthetic Group of Methanol Dehydrogenase	84
6. The Reaction Mechanism of Methanol Dehydrogenase	88
6.1. The Reductive Half-Reaction of MDH	88
6.2. The Role of Ammonia in the Reductive Half-Reaction Mechanism of MDH	94

Contents	xvii
6.3. The Oxidative Half-Reaction of MDH	94
6.3.1. The "Methanol Oxidase" Electron Transport Chain	94
6.3.2. The Interaction of MDH with Cytochrome <i>c</i> _L . .	95
6.3.3. The Oxidation of Reduced PQQ in MDH	97
7. The Structure of Methanol Dehydrogenase	97
7.1. The Structure of the α -Subunit	100
7.2. The Tryptophan-Docking Interactions in the α -Subunit	102
7.3. Structure of the β -Subunit	105
7.4. The Active Site of Methanol Dehydrogenase	105
7.4.1. The Novel Disulphide Ring Structure in the Active Site	105
7.4.2. The Bonding of PQQ in the Active Site	107
7.4.3. The Location of Substrate in the Active Site . . .	109
8. Processing and Assembly of Methanol Dehydrogenase	110
9. References	112

Chapter 4

Methylamine Dehydrogenase: Structure and Function of Electron Transfer Complexes

Victor L. Davidson

1. Methylamine Dehydrogenase	119
1.1. Structure and Function	120
1.2. The TTQ Prosthetic Group	121
1.3. Catalytic Reaction Mechanism	121
1.4. Spectral and Redox Properties	124
2. Amicyanin	125
2.1. Physical Properties	125
2.2. Interactions with Methylamine Dehydrogenase	126
3. Methylamine Dehydrogenase-Amicyanin-Cytochrome <i>c</i> -551i Complex	128
3.1. Structure of the MADH-Amicyanin-Cytochrome <i>c</i> -551i Complex	128
3.2. Interactions between Amicyanin and Cytochrome <i>c</i> -551i	128
3.3. Pathways Analysis of the Electron Transfer Protein Complex	129
4. Electron Transfer Reactions in Methylamine Dehydrogenase Complexes	131

4.1. Electron Transfer Theory	131
4.2. Kinetic Complexity of Protein Electron Transfer Reactions	132
4.2.1. True Electron Transfer	132
4.2.2. Coupled Electron Transfer	133
4.2.3. Gated Electron Transfer	133
4.3. Electron Transfer from TTQ to Copper	134
4.3.1. Non-Adiabatic Electron Transfer Reactions	134
4.3.2. Mutation of Amicyanin Alters the H_{AB} for Electron Transfer from MADH	134
4.3.3. Gated Electron Transfer Reactions	137
4.4. Electron Transfer from Copper to Heme	138
5. Application of Marcus Theory to other Protein Electron Transfer Reactions	138
6. Conclusions	139
7. References	140

Chapter 5

Trimethylamine Dehydrogenase and Electron Transferring Flavoprotein

Nigel S. Scrutton and Michael J. Sutcliffe

1. Electron Transfer Proteins in Methylophilic Bacteria	145
2. Prosthetic Groups and Structure of TMADH	148
2.1. Identification of the Prosthetic Groups	148
2.2. Structure of TMADH	149
2.2.1. Evolution of the Crystal Structure of TMADH	149
2.2.2. Domain and Quaternary Structure	150
2.2.3. Large Domain and Active Site Structure	150
2.2.4. The Medium and Small Domains	153
3. Electron Flow in TMADH Static Titrations, Reduction Potentials and Inactivation Studies.	154
3.1. Static Titrations	154
3.2. Reduction Potentials and Selective Inactivation.	155
4. Single Turnover Stopped-Flow Studies of Electron Transfer	156
4.1. Early Stopped-Flow Investigations	156
4.2. pH-Dependence of the Reductive Half-Reaction with Diethylmethylamine	158
4.3. pH-Dependence of the Reductive Half-Reaction with Trimethylamine: Native and Mutant Enzyme Studies	159
4.4. Quantum Tunneling of Hydrogen	163

5. Control of Intramolecular Electron Transfer: pH-Jump Stopped-Flow Studies	164
6. The Oxidative Half-Reaction and TMADH-ETF Complex Assembly	167
6.1. Stopped-Flow Studies	167
6.2. A Model for the Electron Transfer Complex	168
7. Enzyme Over-Reduction and Substrate Inhibition: Multiple Turnover Studies	170
8. Cofactor Assembly and the Role of the 6-S-CysteinyI FMN	173
9. Summary and Future Prospects	176
10. References	177

Chapter 6

Amine Oxidases and Galactose Oxidase

Malcolm Halcrow, Simon Phillips, and Peter Knowles

1. Introduction	183
2. Galactose Oxidase	184
2.1. Structure	185
2.1.1. General Structural Properties	185
2.1.2. Primary Structure	185
2.1.3. Secondary and Tertiary Structure	186
2.1.4. Structure of the Active Site	186
2.2. Catalytic Mechanism	189
2.2.1. Substrate Binding	191
2.2.2. Substrate Activation	191
2.2.3. Hydrogen Abstraction from Substrate	191
2.2.4. Formation of E _{reduced}	192
2.2.5. Reoxidation of E _{reduced}	192
2.3. Biogenesis of the Thio-Ether Bond and other Processing Events	192
2.4. Model Studies	193
2.5. Biological Role of Galactose Oxidase	196
3. Amine Oxidases	197
3.1. Structure	199
3.1.1. General Structural Properties	199
3.1.2. Protein Structure	199
3.1.3. Active Site Structure	205
3.1.3.1. The Copper Site	205
3.1.3.2. The TPQ Site	205
3.1.3.3. Substrate Access Channels to the Active Site of Amine Oxidases	207

3.1.3.4. Other Structural Features	207
3.2. Catalytic Mechanism	208
3.2.1. Reductive Half Cycle.	208
3.2.1.1. Substrate Binding/Substrate Schiff-Base	209
3.2.1.2. Product Schiff-Base	211
3.2.1.3. Reduced Forms of the Enzyme	213
3.2.2. Oxidative Half Cycle	214
3.3. Biogenesis of TPQ and Related Cofactors	217
3.4. Model Studies	219
3.5. Biological Roles of Amine Oxidases.	219
3.5.1. Microorganisms	219
3.5.2. Plants	219
3.5.3. Mammals	220
4. Comparisons between Galactose Oxidase and Amine Oxidases	221
5. Future Directions	222
6. References	223

Chapter 7

Electron Transfer and Radical Forming Reactions in Methane Monooxygenase

Brian J. Brazeau and John D. Lipscomb

1. Introduction	233
2. Components	237
2.1. MMOH	237
2.1.1. X-Ray Crystallography	237
2.1.2. Spectroscopy	241
2.1.2.1. Diferric MMOH	241
2.1.2.2. Mixed Valence MMOH	242
2.1.2.3. Diferrous MMOH	243
2.2. MMOB	243
2.2.1. NMR Solution Structure	244
2.3. MMOR	244
3. Component Complexes	245
4. Oxidation-Reduction Potentials	246
5. Electron Transfer Kinetics	248
6. Turnover Systems	250
7. Reaction Cycle Intermediates	252
7.1. Transient Intermediates of the Reaction Cycle of MMOH	252

7.2. Structures of the Intermediates	256
7.3. The Mechanism of Oxygen Cleavage	259
8. Mechanism of C α H Bond Cleavage and Oxygen Insertion	261
8.1. Radical Rebound Mechanism	262
8.2. Isotope Effects	262
8.3. Radical Clock Substrates	264
8.4. Modified Radical Rebound Mechanism	266
8.5. Concerted Oxygen Insertion Mechanisms	267
8.6. Mechanistic Theory based on Calculations	268
9. Conclusions	270
10. References	270

Chapter 8

Flavocytochrome b_2

Christopher G. Mowat and Stephen K. Chapman

1. Introduction	279
2. Flavin Reduction and Substrate Oxidation	282
3. Flavin to Heme Electron Transfer	285
4. The Flavocytochrome b_2 : Cytochrome c Interaction	286
5. Engineering Substrate Specificity in Flavocytochrome b_2	290
6. Conclusions	292
7. References	292

Chapter 9

Flavocytochrome P450 BM3 Substrate Selectivity and Electron Transfer in a Model Cytochrome P450

Andrew W. Munro, Michael A. Noble, Tobias W. B. Ost,
Amanda J. Green, Kirsty J. MacLean, Laura Robledo,
Carolyn S. Miles, Jane Murdoch, and Stephen K. Chapman

1. Introduction	297
2. Bacterial Model P450 Systems	302
3. P450 BM3 Structure and Mechanism	304
4. Electron Transfer and its Control	307
5. Site-Directed Mutagenesis in the Study of Substrate Selectivity and Electron Transfer	309
6. Conclusions	312
7. References	312

*Chapter 10***Peroxidase-Catalysed Oxidation of Ascorbate: Structural, Spectroscopic and Mechanistic Correlations in Ascorbate Peroxidase**

Emma Lloyd Raven

1. Introduction	318
2. cDNA Sequences and Bacterial Expression of Recombinant APXs	319
3. Isolation and Characterisation of APXs	320
4. General Properties	321
5. Structural Studies	321
6. Spectroscopic and Spin-State Considerations	328
7. Catalytic Mechanisms	329
7.1. Steady-State Kinetics	329
7.2. Pre-Steady-State Kinetics	331
8. Radical Chemistry	332
8.1. Fate of the Monodehydroascorbate Radical	332
8.2. Nature of the Intermediates	333
8.2.1. Compound I	333
8.2.2. Compound II	333
8.2.3. Role of the Proximal Metal Ion	334
8.2.4. Role of Trp 179	335
9. Substrate Recognition	335
10. Redox Properties	337
11. Inactivation	339
11.1. Inactivation by Cyanid	339
11.2. Inactivation by Sulfhydryl Reagents	339
11.3. Inactivation in Ascorbate-Depleted Media	340
11.4. Inactivation by Hydrogen Peroxide	341
11.5. Inactivation by Salicylic Acid	341
12. References	342

*Chapter 11***Adenosylcobalamin-Dependent Enzymes**

E. Neil G. Marsh and Daniel E. Holloway

1. Introduction	351
1.1. Structure and Reactivity of Cobalamins	352
1.2. Methylcobalamin-Dependent Enzymes	354

Contents	xxiii
1.3. Adenosylcobalamin-Dependent Enzymes	355
1.4. Outline Mechanism of Adenosylcobalamin-Dependent Rearrangements	357
1.5. Ribonucleotide Reductase	358
1.6. Carbon-Centered Radicals in Enzyme Reactions	359
1.7. Longstanding Questions	360
2. Strucural Features of Adenosylcobalamin-Dependent Enzymes	361
2.1. Cobalamin Binding by Enzymes Containing the D-x-H-x-x-G Motif	362
2.2. Substrate Binding and Initiation of Catalysis	369
2.3. Structure of Diol Dehydrase	371
3. Mechanistic Aspects of Adenosylcobalamin-Mediated Catalysis	375
3.1. Homolysis of Adenosylcobalamin and the Formation of Substrate Radicals	375
3.1.1. EPR Studies on Adenosylcobalamin Enzymes .	375
3.1.2. Stopped-Flow Studies of Adenosylcobalamin Homolysis	377
3.1.3. Magnetic Field Effects on Adenosylcobalamin- Dependent Reactions	381
3.1.4. Resonance Raman Experiments	382
3.1.5. Role of the Axial Ligand in Catalysis	384
3.2. Rearrangement of Substrate Radicals	386
3.2.1. Mechanistic Studies on Methylmalonyl- CoA Mutase	390
3.2.2. Mechanistic Studies on Glutamate Mutase	391
4. Perspective	394
5. References	397

Chapter 12

Ribonucleotide Reductaseóa Virtual Playground for Electron Transfer Reactions

Margareta Sahlin and Britt-Marie Sjöberg

1. Introduction	405
2. Three Different Ribonucleotide Reductase Classes	406
3. Reaction Mechanism	410
3.1. Radical Chemistry at Work	410
3.2. Substrate Analogues	412

3.3. Studies in Active Site Mutant Enzymes	413
4. Class I ribonucleotide ReductaseóThe Radical Transfer Pathway	415
4.1. Protein R1	418
4.2. Protein R2	418
4.2.1. The Tyrosyl Radical and the Iron Centre	418
4.2.2. A Hydrogen-Bonded Triad	420
4.2.3. The Flexible C-Terminal Domain	421
4.2.4. Is the Tyrosyl Radical H-Bonded to the Radical Transfer Pathway?	422
4.3. Theoretical Considerations on Radical Transfer and Protein Dynamics	422
5. Generation of the Stable Tyrosyl Radical in Protein R2	424
5.1. Radical Generation Involves the Radical Transfer Pathway	424
5.2. Interactions between Metal Sites	428
5.3. Non-Native Radicals and Secondary Radical Transfer Pathways Observed in Mutant R2 Proteins.	429
5.4. Unexpected Hydroxylation Reactions	431
6. Stability of the Tyrosyl Radical	431
6.1. Different Tyrosyl Radical Conformers	431
6.2. The Tyrosyl Radical Environment	433
6.3. Radical Stability during Catalysis	433
7. Radical Transfer Reactions in Class II and III Ribonucleotide Reductases	434
7.1. Class II Ribonucleotide Reductases	434
7.2. Class III Ribonucleotide Reductases	434
8. References	436

Chapter 13

Molybdenum Enzymes

Russ Hille

1. Introduction	445
1.1. Molybdenum Enzymes and the Reactions they Catalyse	445
1.2. Sequence Homologies and Classification of the Molybdenum Enzymes	446
1.3. Structural and Catalytic Variations within the Three Families of Molybdenum Enzymes	451
2. The Molybdenum Hydroxylases	453
2.1. Structural Studies	453

2.2. Mechanistic Aspects	458
3. The Eukaryotic Oxotransferases	465
3.1. Structural Studies	465
3.2. Mechanistic Aspects	468
4. The Prokaryotic Oxotransferases	472
4.1. Structural Studies	472
4.2. Mechanistic Aspects	475
5. Concluding Remarks	477
6. References	478

Chapter 14

Nickel Containing CO Dehydrogenases and Hydrogenases

Stephen W. Ragsdale

1. Background	487
1.1. Importance of CO and H ₂ Metabolism for Anaerobic Microbes	487
1.1.1. Why CO?	487
1.1.2. Where Does the CO Come from and Where Does It Go?	489
1.1.3. Why H ₂ ?	489
1.1.4. Where Does the H ₂ Come from and Where Does It Go?	490
1.1.5. Supercellular Biochemistry: Interspecies H ₂ Transfer	490
1.2. Introduction to the Enzymes	491
1.2.1. CO Dehydrogenase and Acetyl-CoA Synthase	491
1.2.2. Hydrogenase	491
2. CO Oxidation and CO ₂ Reduction by CO Dehydrogenase ..	493
2.1. The Catalytic Redox Machine: a NióFeóS Cluster ...	493
2.2. The Intramolecular Wire	495
2.3. The Intermolecular Wires: How Electrons Enter and Exit CO Dehydrogenase	495
2.4. By Channel or by Sea? How Carbon Enters and Exits CO Dehydrogenase	496
2.5. Coupling CO ₂ Reduction and Acetyl-CoA Synthesis...	496
2.6. Acetyl-CoA Synthase: Another Catalytic NióFeóS Cluster	497
3. H ₂ Oxidation and Proton Reduction by Hydrogenase	499
3.1. The Catalytic Redox Machine: NióFeóS and FeóFeS Clusters	499
3.1.1. The NióFe Hydrogenase	499

3.1.2. The NióFe Regulatory Hydrogenase	504
3.1.3. The Fe-Only Hydrogenase	504
3.1.4. Models of Hydrogenase	505
3.1.5. The Metal-Free Hydrogenase	505
3.2. Proton Transfer Pathway	506
3.3. The Intramolecular Wire	508
3.4. The Intermolecular Wires: How Electrons Enter and Exit Hydrogenase	510
3.5. Tunnel-Diodes and Catalytic Bias	510
4. Summary	511
5. References	512

Chapter 15

Cytochrome *cd*₁ Nitrite Reductase Structure Raises Interesting Mechanistic Questions

Stuart Ferguson and Vilmos F_lp

1. Introduction	519
2. Structure of <i>Paracoccus pantotrophus</i> Cytochrome <i>cd</i> ₁	522
3. Kinetic Studies on <i>P. pantotrophus</i> Cytochrome <i>cd</i> ₁	531
4. Solution Spectroscopy of <i>P. pantotrophus</i> Cytochrome <i>cd</i> ₁	532
5. The Cytochrome <i>cd</i> ₁ from <i>Pseudomonas aeruginosa</i>	533
6. Copper Nitrite Reductase	536
7. Conclusions and Outstanding Issues	537
8. References	538

Chapter 16

Mitochondrial Cytochrome *bc*₁ Complex

ZhaoLei Zhang, Edward A. Berry, Li-Shar Huang, and
Sung-Hou Kim

1. Introduction	541
2. Crystal Structure of a <i>bc</i> ₁ Complex	546
2.1. <i>bc</i> ₁ Complex is a Homodimer as a Functional Unit....	547
2.2. Cytochrome <i>b</i> and Two Haems	549
2.3. Cytochrome <i>c</i> ₁	550
2.4. Alternative Conformations of Rieske Protein and Cross-Transfer of Electrons	553
2.5. The Two Core Proteins and Subunit 9	557
2.6. Other Subunits without Prosthetic Groups	559
3. Quinone Reactions and Electron Transfer by <i>bc</i> ₁ Complex ..	561

3.1. Quinone Reduction at Q _i Site	561
3.2. Quinone Oxidation at Q _o Site	566
3.3. The 'Domain Shuttle' Mechanism	569
3.4. Bifurcated Reaction at Q _o Site	571
4. Summary and Perspective	572
5. References	573

Chapter 17

Bovine Heart Cytochrome *c* Oxidase

Shinya Yoshikawa

1. Introduction	581
2. Composition of Bovine Heart Cytochrome <i>c</i> Oxidase	582
2.1. Purification	582
2.2. Metal Content	584
2.3. Structures and Spectral Properties of the Redox-Active Metal Sites	585
2.4. Subunit Composition and Amino Acid Sequences.....	589
3. Function of Bovine Heart Cytochrome <i>c</i> Oxidase	590
3.1. Enzymic Activity.....	590
3.2. Reduction of O ₂	592
4. Crystallisation of Bovine Heart Cytochrome <i>c</i> Oxidase	596
4.1. Crystallisation of Membrane Proteins	597
4.2. Crystallisation of Cytochrome <i>c</i> Oxidase	597
5. X-ray Structure of Bovine Heart Cytochrome <i>c</i> Oxidase	599
5.1. Three-Dimensional Structure of the Protein Portion...	599
5.2. Structures and Locations of the Metal Sites	603
6. A Possible O ₂ Reduction Mechanism	607
7. Proton Transfer in Bovine Heart Cytochrome <i>c</i> Oxidase	608
7.1. Possible Proton Transfer Pathways in Membrane Proteins	608
7.2. A Redox Coupled Conformational Change in Cytochrome <i>c</i> Oxidase	611
8. References	616

Chapter 18

Reaction Centres of Purple Bacteria

Marion E. van Brederode and Michael R. Jones

1. Introduction	621
2. Structure of the Bacterial Reaction Centre	622

2.1.	The Structures of the <i>Rhodopseudomonas viridis</i> and <i>Rhodobacter sphaeroides</i> Reaction Centres.	622
2.2.	Recent Structural Information Relating to Function ...	625
2.3.	Structures of Mutant Complexes	626
2.4.	Electronic Structure: The Reaction Centre Absorbance Spectrum	627
3.	The Mechanism of Energy Storage by the Bacterial Reaction Centre	628
3.1.	Primary Photochemistry	631
3.2.	Ubiquinol Formation and Re-Reduction of P ⁺	633
3.3.	Light-Driven Cyclic Electron Transfer Coupled to Proton Translocation	634
4.	Biological Electron Transfer	634
4.1.	Non-Adiabatic Electron Transfer	635
4.2.	Adiabatic Electron Transfer	639
5.	Studies of Ultrafast Electron Transfer in a Light-Activated Protein	640
5.1.	The Role of the B _A Monomeric BChl	641
5.2.	The Asymmetry of Primary Electron Transfer	643
5.2.1.	Evidence for the Asymmetry of Primary Electron Transfer	643
5.2.2.	Origins of the Asymmetry of Primary Electron Transfer	644
5.2.3.	Re-Routing Primary Electron Transfer	646
5.3.	Temperature Dependence of Activationless Reactions ..	650
5.4.	Dispersive Kinetics: Heterogeneity or Protein Dynamics?	651
5.5.	Femtosecond Biology: Coherent Nuclear Dynamics Studied in Populations of Proteins	654
5.6.	Modulation of the Time Constant for Primary Electron Transfer between 200fs and 500ps through Site-Directed Mutagenesis	656
5.6.1.	Tyrosine M210	657
5.6.2.	Hydrogen Bond Mutants	658
5.7.	Parallel Pathways for Primary Electron Transfer	661
6.	Summary	665
7.	References	665
Index		677