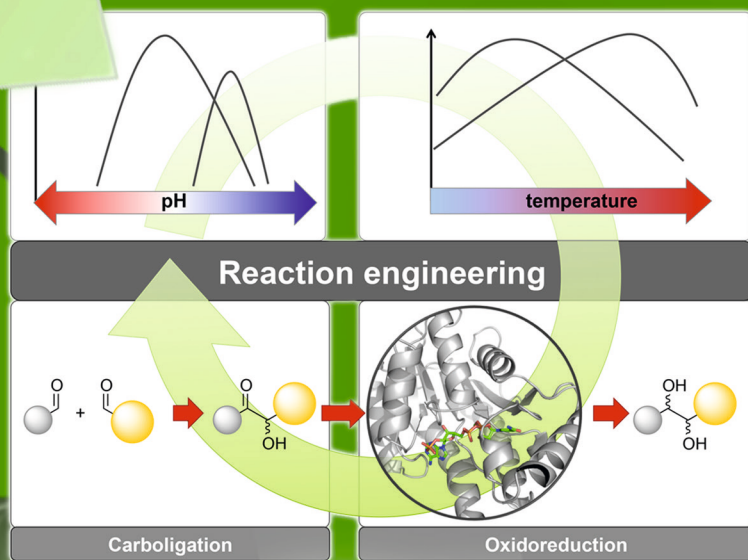




Stereoselective synthesis of vicinal diols with enzymatic cascade reactions

Justyna Katarzyna Kulig

Forschungszentrum Jülich GmbH
Institute of Bio- and Geosciences (IBG)
Biotechnology (IBG-1)

Stereoselective synthesis of vicinal diols with enzymatic cascade reactions

Justyna Katarzyna Kulig

Schriften des Forschungszentrums Jülich
Reihe Schlüsseltechnologien / Key Technologies

Band / Volume 73

ISSN 1866-1807

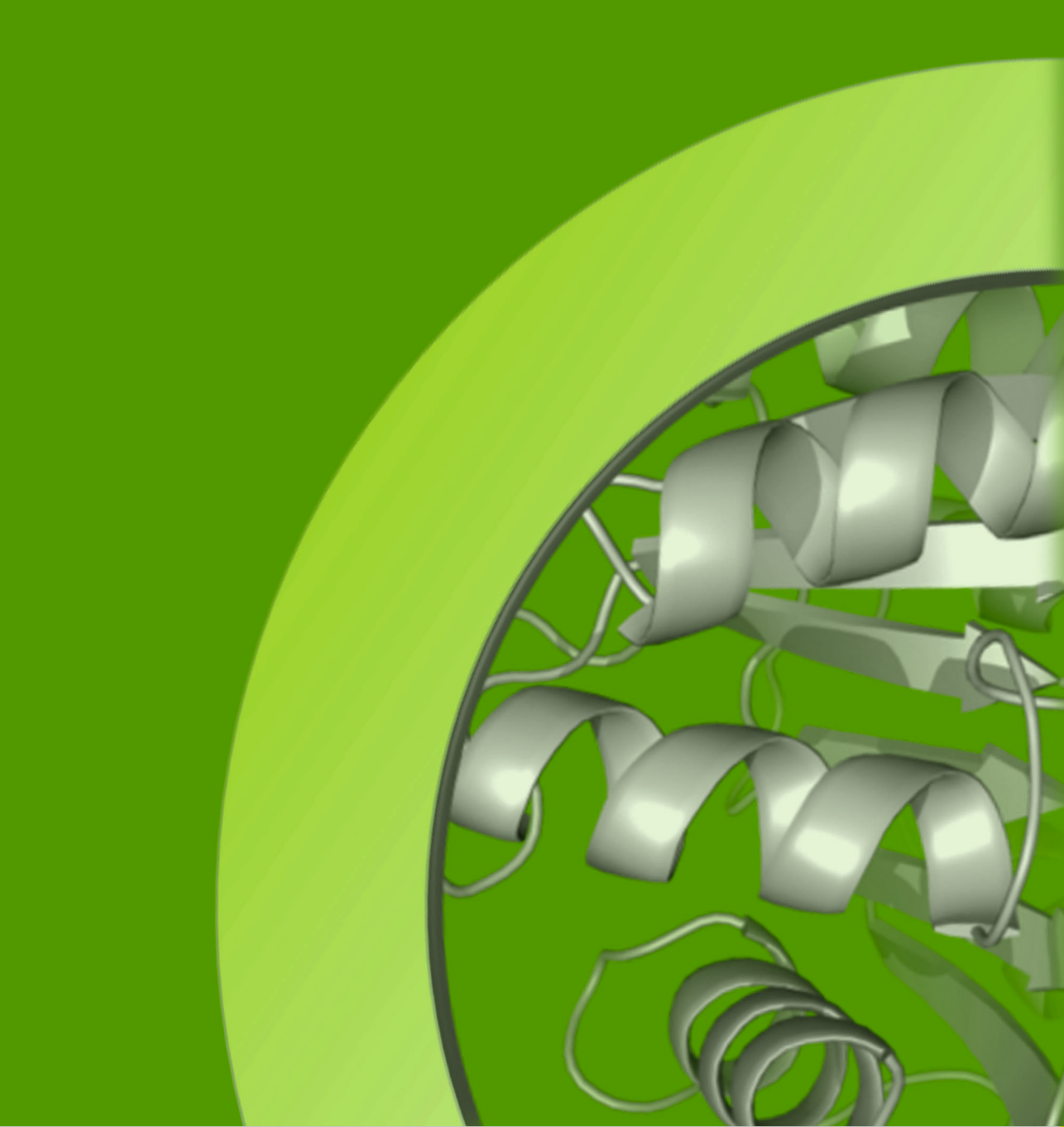
ISBN 978-3-89336-911-9

Table of content

1. INTRODUCTION	1
1.1. Concept of Green Chemistry	2
1.2. Industrial biotechnology	3
1.3. Enzymes in biocatalysis	5
1.4. Classification of enzymes	7
1.5. Oxidoreductases classification	8
1.6. Alcohol dehydrogenases/Ketoreductases	9
1.7. Cofactor regeneration	10
1.7.1. Substrate-coupled approach	11
1.7.2. Enzyme-coupled approach	11
1.8. Mechanism of alcohol dehydrogenases	12
1.9. Chiral pharmaceuticals	15
1.10. Chiral 1,2-diols as building blocks	17
1.11. Production of 1,2-diols	19
1.11.1. Chemical methods	19
1.11.2. Enzymatic methods	22
1.11.2.1. First step: carbonylation by ThDP-dependent enzymes	24
1.11.2.1.1. Benzaldehyde lyase	26
1.11.2.1.2. Benzoylformate decarboxylase	26
1.11.2.1.3. Acetohydroxyacid synthase	26
1.11.2.1.4. Pyruvate decarboxylase	27
1.11.2.2. Second step: oxidation by alcohol dehydrogenases	27
1.11.2.2.1. Carbonyl reductase from <i>Candida parapsilosis</i>	27
1.11.2.2.2. ADH from <i>Flavobacterium frigidimaris</i>	28
1.11.2.2.3. ADH from horse liver	28
1.11.2.2.4. ADH from <i>Lactobacillus brevis</i>	29
1.11.2.2.5. ADH from <i>Ralstonia</i> sp.	29
1.11.2.2.6. ADH from <i>Sphingobium yanoikuyae</i>	29
1.11.2.2.7. ADH from <i>Thermoanaerobacter brockii</i>	30
1.11.2.2.8. ADH from <i>Thermoanaerobacter</i> sp.	30
1.11.2.2.9. ADH from <i>Thermus</i> sp.	31
1.12. Synthetic cascade reactions	31
1.13. Reaction/Process engineering	33

2. PUBLICATIONS	36
Publication I	37
Stereoselective synthesis of bulky 1,2-diols with alcohol dehydrogenases	
Publication II	55
Biochemical characterization of an alcohol dehydrogenase from <i>Ralstonia</i> sp.	
Publication III	79
Stereoselective synthesis of 1,2-diols with synthetic enzyme cascades including <i>in situ</i> cofactor regeneration and co-product recycling	
Publication IV	101
Structures of alcohol dehydrogenases from <i>Ralstonia</i> and <i>Sphingobium</i> spp. reveal the molecular basis for their recognition of 'bulky-bulky' ketones	
 3. DISCUSSION	 119
3.1. Overview of publications	119
3.2. Alcohol dehydrogenases as versatile catalysts	121
3.3. Stereoselectivity of ADHs	125
3.4. Biochemical characterisation of ADH from <i>Ralstonia</i> sp.	128
3.4.1. Purification and storage stability	128
3.4.2. Effect of salts	129
3.4.3. Determination of the native molecular weight of RADH	130
3.4.4. pH- and temperature optima	130
3.4.5. Substrate range for oxidation and reduction	131
3.4.6. Steady-state kinetic parameters	131
3.4.7. Activity in organic solvents	133
3.5. Preparations to crystallise RADH	134
3.5.1. Cloning RADH into a vector with cleavable tag	134
3.5.2. Expression and purification of His-RADH	134
3.5.3. Crystallisation and structure analysis of RADH	136
3.6. Reaction engineering	139
3.6.1. Optimisation of the cofactor regeneration system	139
3.6.1.1. Substrate-coupled approach	140
3.6.1.2. Enzyme-coupled approach	140
3.6.2. Synthetic cascade reaction	141
3.6.2.1. Cascade reaction using substrate-coupled cofactor regeneration	141
3.6.2.2. Cascade reaction using enzyme-coupled cofactor regeneration	145

3.6.2.3. Comparison of cascade reactions using the substrate- and enzyme coupled approach	146
3.6.2.4. Further cascade reactions	146
4. CONCLUSIONS AND FUTURE PERSPECTIVES	147
5. APPENDIX	149
5.1. DNA sequence of RADH (without His-tag)	150
5.2. Protein sequence of RADH (without His-tag)	150
5.3. Primers for RADH subcloning	151
5.4. DNA sequence of His-RADH	151
5.5. Protein sequence of His-RADH	151
5.6. Protein sequence of His-RADH: after His-tag cleavage	152
5.7. General procedure for subcloning	152
5.8. Thermocycler program for LIC-cloning	153
5.9. Protocol for His-RADH purification using IMAC	153
5.10. DNA sequence of strep-SADH	154
5.11. Protein sequence of strep-SADH	155
6. REFERENCES	156



Schlüsseltechnologien / Key Technologies
Band / Volume 73
ISBN 978-3-89336-911-9

