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Education

Ph.D. Degree, March 1995
Department of Chemistry, University of Utah, Salt Lake City, Utah, USA
B.S Degree, June 1986
Department of Chemistry, National Cheng Kung University, Tainan, Taiwan

Professional Experience

2010-present	Associate Professor, Dept. of Biochemistry, Kaohsiung Medical University.
2018-2024	Director, Dept. of Biochemistry, Kaohsiung Medical University.
2021-2024	Board of The Taiwan Society for Biochemistry and Molecular Biology
2021-present	Topic Editor of Editorial Board of Catalysts, MDPI, Switzerland
2018-2021	Leader, General Affairs of College of Medicine, Kaohsiung Medical University.
2012-2018	Leader, Research and Development of College of Medicine, Kaohsiung Medical University.
2001-2010	Assistant Professor, Dept. of Biochemistry, Kaohsiung Medical University.
2000-2001	Research Chemist, ScinoPharm Taiwan, LTD.
1999	Postdoctoral Science Associate, University of Oklahoma Health Sciences Center, Department of Biochemistry and Molecular Biology.
1996-1999	Postdoctoral Science Associate, University of Oklahoma, Department of Chemistry and Biochemistry
1995-1996	Postdoctoral Science Associate, University of North Texas Health Science Center at Fort Worth, Department of Biochemistry and Molecular Biology

Awards

2005 Kaohsiung Medical University, The Best Impact Factor Award
2007 Kaohsiung Medical University, The Best Impact Factor Award

Academic Societies

The Taiwan Society for Biochemistry and Molecular Biology
The Biophysical Society of Taiwan

Research Area

A. Enzyme catalysis and mechanism

B. The energetics of protein stability

C. Protein engineering in cofactor specificity

Enzyme is a powerful catalyst with highly substrate specificity. The utilization of enzymes as green and sustainable biocatalysts is applied for pharmaceutical and industrial. Research has focused on the study of mechanism of enzyme-catalyzed reaction, protein stability and protein engineering. We have been studied 3α-hydroxysteroid dehydrogenase/carbonyl reductase (3 α -HSD/CR) catalyzed reaction to elucidate the catalytic mechanisms and the structural conformational changes related to substrate specificity and catalysis and its application in protein engineering for enhancing the thermal stability, substrate variety, and developing a non-canonical redox cofactor system. To elucidate the mechanism of enzyme-catalyzed reaction, the functional groups involved in the ligand binding and catalysis are characterized. Based on the sequence alignment and crystal structure, the groups involved in the catalysis are mutated through site-directed mutagenesis, and characterized through the steady-state kinetic studies, kinetic and solvent isotope effect studies, pH studies and the use of spectroscopic probes such as fluorescence, circular dichroism.

3α-HSD/CR from *Comamonas testosteroni* belonging to short chain dehydrogenase/reductase (SDR) superfamily catalyzes the stereospecific oxidoreductive reaction of androsterone with NAD^+ to form androstanedione and NADH. The functions of HSDs are involved in the biosynthesis of steroid hormones as well as the detoxification of xenobiotic carbonyl compounds. The kinetic mechanism of 3α-HSD/CR is ordered bi bi with NAD^+ bound first and NADH released last with the rate-limiting step on the release of NADH. 3α-HSD/CR utilizes the acid-base catalysis with Y155 acted as a general base and proton shuttle through the proton relay system involving the Y155, 2'-OH of the nicotinamide ribose, K159 and N86 to the bulk solvent for facilitating the reaction. The substrate binding loop is unresolved. Molecular modeling shown the unresolved substrate-binding loop forms the helix-turn-helix when androsterone is bound, suggesting the conformational changes of loop may participate in enzyme catalysis. Meanwhile, the remote binding energy contributed to the enzyme catalysis. We elucidated that the remote non-reacted sites of androsterone induces the conformational change of the substrate binding loop with slight entropy cost for better interaction with transition state to decrease the activation of enthalpy, resulting in significant increases in k_{cat}/K_m.

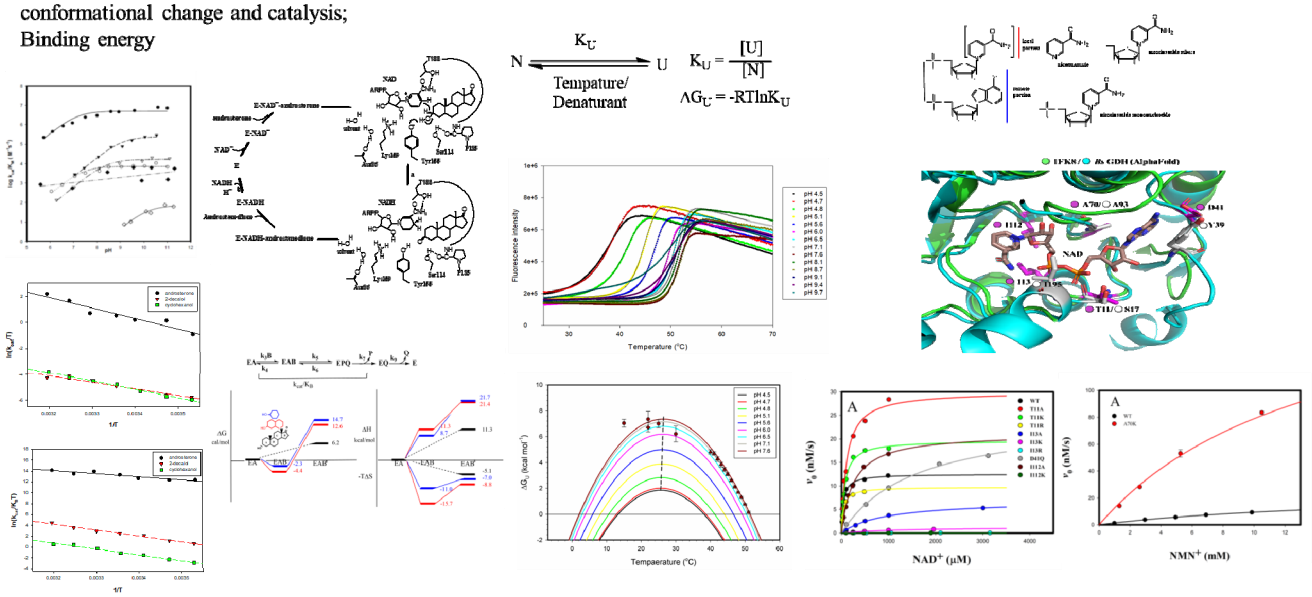
Meanwhile, the platform is setup for evaluating the protein stability by differential scanning fluorimetry (DSF). The protein stability as a function of pH, denaturants and temperature are characterized by intrinsic fluorescence and DSF measurements.

We then perform the rational design of protein engineering of 3α-HSD/CR based on the structure and catalytic mechanism for developing a non-canonical redox cofactor system. Oxidoreductases are the largest group of enzymes in the Enzyme Commission number. We demonstrated that A70K mutant switch the cofactor specificity from NAD^+ toward nicotinamide mononucleotide (NMN^+) by 10^5-fold. However, the disadvantages of A70K mutant are low catalytic efficiency for NMN^+ and less stable than wild-type enzyme. Meanwhile, I112K mutant significantly increases the thermal stability but no activity when acting on NMN^+.

A. Enzyme Catalysis and Mechanism:
Kinetic mechanism; Acid-base chemical mechanism;The proton relay system; Role of the substrate binding loop-conformational change and catalysis; Binding energy

B. The energetics of protein stability:
The protein stability as a function of pH, denaturants and temperature by intrinsic fluorescence and DSF measurements

C. Protein engineering in cofactor specificity:
Dehydrogenases are used to biotechnologically perform various reactions with high specificity, efficiency, and enantioselectivity.



Invited Speech:

Date	Meeting /Place	Title
2002/4	Department of Biochemistry, National Defense Medical School	Mechanism of O-acetylserine Sulfhydrase Catalyzed Reaction
2006/12	Cheng Shiu University	The catalytic mechanism of 3α-hydroxysteroid dehydrogenase/carbonyl reductase
2008/7	Trends in Enzymology/ St. Malo, France	Characterization of the proton relay system in the 3α-hydroxysteroid dehydrogenase/carbonyl reductase from *Comamonas testosteroni*: the function of Lys 159 and Asn 86 in proton transfer
2009/05	National Yang-Ming University	The catalytic mechanism of 3α-hydroxysteroid dehydrogenase/carbonyl reductase from *Comamonas testosteroni*
2009/12	2009 Annual Meeting of Chinese Chemical Society	Mechanism of 3α-Hydroxysteroid Dehydrogenase/Carbonyl Reductase: Insight into the Roles of N86, S114, Y155 and K159 in Cofactor Binding, Conformational Change, and Catalysis
2009/12	2009 Taiwan enzyme mechanism	Mechanistic roles of the catalytic tetrad N86, S114,

	conference/ *Academia Sinica*	Y155 and K159 in 3α-hydroxysteroid dehydrogenase/carbonyl reductase
2012/7	Enzymology and Molecular Biology of Carbonyl Metabolism, Sixteenth International Meeting/ Plön, Germany	Catalytic role of the flexible substrate binding loop in 3alpha-hydroxysteroid dehydrogenase/carbonyl reductase
2012/9/21	Department of Biochemistry Kaohsiung Medical University	Kinetic and catalytic mechanisms of 3α-hydroxysteroid dehydrogenase/carbonyl reductase
2013/06/28	The 18th Biophysics Conference/ Institute of BioMedical Sciences, Academia Sinica/Taipei	Kinetic and catalytic mechanisms of 3α-hydroxysteroid dehydrogenase/carbonyl reductase
2016/7	18th International Workshop on the Enzymology and Molecular Biology of Carbonyl Metabolism/Sant Feliu de Guíxols, Spain	Differential binding energy contributed to 3α-hydroxysteroid dehydrogenase/carbonyl reductase catalysis
2018/7/20	19th International Workshop on the Enzymology and Molecular Biology of Carbonyl Metabolism/ Breckenridge, Colorado, USA	Thermodynamic study of remote substrate binding to catalysis in 3α-hydroxysteroid dehydrogenase/carbonyl reductase

Publications

A. 3α-Hydroxysteroid dehydrogenase/carbonyl reductase related papers:

1. Chou, Y.-H., Hsieh, C-L., Chen, Y.-L., Wang, T.-P., Wu, W.-J., **Hwang, C.-C**.* (2023) "Characterization of the pH-dependent protein stability of 3α-hydroxysteroid dehydrogenase/carbonyl reductase by differential scanning fluorimetry" Protein Science. 2023;32(8):e4710

2. Chen, Y.-L., Chou, Y.-H., Hsieh, C.-L., Chiou, S.-J., Wang, T.-P., **Hwang, C.-C**.* (2022) "Rational Engineering of 3α-Hydroxysteroid Dehydrogenase/Carbonyl Reductase for a Biomimetic Nicotinamide Mononucleotide Cofactor" Catalysts 12, 1094.

3. Hwang, C.-C. *, Chang, P.-R., Hsieh, C.-L., Chou, Y.-H., and Wang, T.-P. (2019) "Thermodynamic analysis of remote substrate binding energy in 3α-hydroxysteroid dehydrogenase/carbonyl reductase catalysis" Chemico-Biological Interactions **302**,183-189

4. Hwang, C.-C.*, Chang, P.-R., and Wang, T.-P. (2017) "Contribution of remote substrate binding energy to the enzymatic rate acceleration for 3α-hydroxysteroid dehydrogenase/carbonyl reductase" *Chemico-Biological Interactions*, **276**, 133-140

5. **Hwang, C.-C.*,** Chang, Y.-H., Lee, H.-J., Wang, T.-P., Su, Y.-M., Chen, H.-W., Liang, P.-H. (2013) "The Catalytic Roles of P185 and T188 and Substrate-Binding Loop Flexibility in 3α-Hydroxysteroid

Dehydrogenase/Carbonyl Reductase from *Comamonas testosteroni*" *PLoS ONE* **8**(5): e63594.
6. Chang, Y.–H., Wang, Y.-L., Lin, J.–Y., Chuang, L.-Y., and **Hwang, C.-C.*** (2010) "Expression, purification, and characterization of a human recombinant 17β-hydroxysteroid dehydrogenase type 1 in *E. coli*" *Mol. Biotechnol.* 44, 133-139.
7. Chang, Y.–H., Wang, C.-Z., Chiu, C.-C., Chuang, L.-Y., and **Hwang, C.-C.*** (2010) "Contributions of Active Site Residues to Cofactor Binding and Catalysis of 3α-Hydroxysteroid Dehydrogenase/Carbonyl Reductase" *Biochim. Biophys. Acta.* **1804**, 235-241.
8. Hwang, C.-C.*, Hsu, C.-N., Huang, T.-J., Chiou, S.-J., and Hong, Y.-R. (2009) "Interactions across the interface contribute the stability of homodimeric 3α-hydroxysteroid dehydrogenase/carbonyl reductase"*Arch. Biochem. Biophys.* **480**, 36-41.
9. Chang, Y.-H., Huang, T.-J., Chuang, L.-Y., and **Hwang, C.-C.*** (2009) "Role of S114 in the NADH-induced conformational change and catalysis of 3α-hydroxysteroid dehydrogenase/carbonyl reductase from *Comamonas testosteroni*" *Biochim. Biophys. Acta* **1794**, 1459–1466.
10. Chang, Y.-H., Chuang, L.-Y., and **Hwang, C.-C.*** (2007) "Mechanism of Proton Transfer in the 3α-Hydroxysteroid Dehydrogenase/Carbonyl Reductase from *Comamonas testosteroni*" *J. Biol. Chem.* **282**, 34306-34314.
11. Hwang, C.-C.*, Chang, Y.-H., Hsu, C.-N., Hsu, H.-H., Li, C.-W., & Pon, H.-I. (2005) "Mechanistic Roles of Ser114, Tyr155 and Lys159 in 3α-Hydroxysteroid Dehydrogenase/Carbonyl Reductase from *Comamonas testosterone*" *J. Biol. Chem*, **280**, 3522-3528.

B. Enzymology related papers:

12. Huang, C.-W., **Hwang, C.-C.**, Chang, Y.-L., Liu, J.-T., Wu, S.-P., Huang, K.-L., Huang, W.-M., Lee, H.-J.* (2021) "Functional role of residues involved in substrate binding of human 4-hydroxyphenylpyruvate dioxygenase" *The Biochemical journal* **478**:12, 2201-2215.
13. Liu, D., **Hwang, C.-C.**, & Cook, P. F. (2002) "Alternative Substrates for Malic Enzyme: Oxidative Decarboxylation of L-Aspartate" *Biochemistry* **41**, 12200-12203.
14. Stanley, T. M., Johnson, W. H., Jr., Burks, E. A., Whitman, C. P., **Hwang, C.-C**., & Cook, P. F. (2000) " Expression and Stereochemical and Isotope Effect Studies of Active 4-Oxalocrotonate Decarboxylase" *Biochemistry* **39**, 718-726.
15 Karsten, W. E., Chooback, L., Liu, D., **Hwang, C.-C.,** Lynch, C., & Cook, P. F. (1999) "Mapping the Active Site Topography of the NAD-Malic Enzyme via Alanine-Scanning Site-Directed Mutagenesis" *Biochemistry* **38**, 10527-10532.
16. Ehrlich, J. I., **Hwang, C.-C.,** Cook, P. F., & Blanchard, J. S. (1999) "Evidence for a Stepwise Mechanism of OMP Decarboxylase" *J. Am. Chem. Soc.* **121**, 6966-6967.
17. Karsten, W. E., **Hwang, C.-C.,** & Cook, P. F. (1999) "α-Secondary Tritium Kinetic Isotope Effects Indicate Hydrogen Tunneling and Coupled Motion in the Oxidation of L-Malate by NAD-Malic Enzyme" *Biochemistry* **38**, 4398-4402.
18. Hwang, C.-C., & Cook, P. F. (1998) "Multiple Isotope Effects as a Probe of Proton and Hydride Transfer in the 6-Phosphogluconate Dehydrogenase Reaction" *Biochemistry* **37**, 15698-15702.
19. Hwang, C.-C., Berdis, A., Karsten, W. E., Cleland, W. W., & Cook, P.F. (1998) "Oxidative Decarboxylation of 6-Phosphogluconate Dehydrogenase Proceeds by a Stepwise Mechanism with NADP and APADP as Oxidants" *Biochemistry* **37**, 12596-12602.
20. Cook, P. F., Tai, C. -H., **Hwang, C.-C.,** Woehl, E. U., Dunn, M. F., & Schnackerz, K. D. (1996) "Substitution of Pyridoxal 5'-Phosphate in the *O*-Acetylserine Sulhydrylase from *Salmonella typhimurium* by Cofactor Analogs Provides a Test of the Mechanism Proposed for Formation of the α-Aminoacrylate Intermediate" *J. Biol. Chem*. **271**, 25842-25849.
21. Hwang, C.-C., Woehl, E. U., Minter, D. E., Dunn, M. F., & Cook, P. F. (1996) "Kinetic Isotope Effects as a Probe of the β-Elimination Reaction Catalyzed by *O*-Acetylserine Sulfhydrylase" *Biochemistry* **35**, 6358-6365.

22. Hwang, C. -C. and Grissom, C. B. (1994) "Unusually Large Isotope Effects in Soybean Lipoxygenase Are Not Caused by a Magnetic Isotope Effect" *J. Am. Chem. Soc.* **116**, 795-796.

C. Others papers:

23. Chiou, S.-J., Ko, H.-J., **Hwang, C,-C.**, Hong, Y.-R. (2021) "The Double-Edged Sword of Beta2-Microglobulin in Antibacterial Properties and Amyloid Fibril-Mediated Cytotoxicity" *Int. J. Mol. Sci.* **22(12)**, 6330.

24. Gong, M-M., Dai, C.-Y., Severance, S., **Hwang, C.-C.**, Fang, B.-K., Lin, H.-B., Huang, C.-H., Ong, C.-W., Wang, J.-J., Lee, P.-L., and Wang, T.-P. (2020) "A Bioorthogonally Synthesized and Disulfide-Containing Fluorescence Turn-On Chemical Probe for Measurements of Butyrylcholinesterase Activity and Inhibition in the Presence of Physiological Glutathione" Catalysts 10 (10), 1169.

25. Wang, T.-P.*, Su, Y.-C., Chen, Y., Severance, S., **Hwang, C.-C.**, Liou, Y.-M., Lu, C.-H., Lin, K.-L., Zhua, R. J. and Wang, E.-C. (2018) "Corroboration of Zn(II)–Mg(II)-tertiary structure interplays essential for the optimal catalysis of a phosphorothiolate thiolesterase ribozyme" *RSC Adv*., **8**, 32775–32793

26. Su, Y.-C., Chen, H.-Y., Ko, N. C., **Hwang, C.-C.,** Wua, M. H., Wang, L.-F., Wang, Y.-M., Chang, S.-N., Wang, E.-C., Wang, T.-P. (2014) "Effective and site-specific phosphoramidation reaction for universally labeling nucleic acids" *Analytical Biochemistry* **449,** 118–128.