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Enzyme Active Site Loop Revealed as Gatekeeper for Cofactor Flip by Targeted Molecular Dynamics Simulations and FRET-kinetics

Sophie Rahuel-Clermont¹, Raphaël Bchini¹, Sophie Barbe², Isabelle André², François Talfournier⁴
¹ Laboratoire IMoPA, Equipe Enzymologie Moléculaire et Structurale, UMR 7365 CNRS-Université de Lorraine, Vandœuvre-lès-Nancy France; ² Molecular Modelling & Design, Catalysis & Enzyme Molecular Engineering, Laboratoire d’Ingénierie des Systèmes Biologiques et des Procédés (LISBP), UMR INSA - CNRS 5504 - INRA 792, Institut National des Sciences Appliquées, Toulouse, FRANCE

The context
Aldehyde dehydrogenases: NAD(P)-dependent dehydrogenases
Involved in detoxification and metabolic pathways (retinoic acid biosynthesis)

The question
Reduced nicotinamide moiety (NMNH) flipping is required for Glu-268 to activate the water molecule in the deacylation step

The strategy
Reduced nicotinamide moiety (NMNH) flipping
Impact on catalysis?
→ Kinetic approach
→ Intrinsic Trp/NADH FRET probe

 NMNH conformation monitoring by FRET

Rapid kinetic monitoring of the flip using stopped flow

RALDH2 WT

\[ \text{FLIP} \]
+ LDH/pyruvate

Acylation = NAD⁺ formation in hydride transfer conformation

NAD⁺ released from active site

Decylation = NAD⁺ released from active site

Excitation 295 nm

Emission 330 nm

Fluorescence intensity

Conformational transition = FLIP

Hypothesis Role of E268LG271 loop in flip control?

Mutation Gly271Ala

G271A mutation in ELGG loop drastically reduces the flip kinetics

<table>
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<th>( k_{\text{eq}} ) (s⁻¹)</th>
<th>( k_{\text{cat}} ) (s⁻¹)</th>
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<tr>
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</table>

CONCLUSION

- Flip/catalytic efficiency relies on ELGG loop flexibility
- Cofactor flipping extends to all ALDH family including CoA-dependent aldehyde dehydrogenases

References
Rahuel-Clermont, S., Bohill, R., Barbe, S., Bouabien, S., André, I., and Talfournier, F. (2019) Enzyme Active Site Loop Revealed as a Gatekeeper for Cofactor Flip by Targeted Molecular Dynamics Simulations and FRET-kinetics. ACS Catal. 9, 1327–1346

Targeted Molecular Dynamics: GAPN

Conserved ELGG loop as a gatekeeper for cofactor flipping

E268 loop

G271 loop

NADPH in hydride transfer conformation

No significant change of the protein structure

NADPH in hydrolysis conformation