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

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The context

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

The strategy

Reduced nicotinamide moiety (NMNH) flipping

Impact on catalysis?

→ Kinetic approach

→ Intrinsic Trp/NADH FRET probe

Driving force?

→ Identification of molecular factors allowing cofactor movement

→ Targeted Molecular Dynamics

Conserved ELGG loop as a gatekeeper for cofactor flipping

NMNH conformation monitoring by FRET

Rapid kinetic monitoring of the flip using stopped flow

The question

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

Driving force? Pathway?

Targeted Molecular Dynamics: GAPN

Hypothesis Role of E268LGG271 loop in flip control?

Mutation Gly271Ala

G271A mutation in ELGG loop drastically reduces the flip kinetics

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., Bchini, R., Barbe, S., Boudewin, S., André, I., and Talfournier, F. (2019) Enzyme Active Site Loop Revealed as Gatekeeper for Cofactor Flip by Targeted Molecular Dynamics Simulations and FRET-Kinetics. ACS Catal. 9, 1327-1336