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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 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

Driving force?
➢ Kinetic approach
➢ Intrinsic Trp/NADH FRET probe

Conserved ELGG loop as a gatekeeper for cofactor flipping

NMNH conformation monitoring by FRET

Emission 330 nm

Excitation 295 nm

Rapid kinetic monitoring of the flip using stopped flow

RALDH2 WT

Deacylation
NADH released from active site

Acylation = NADH formation in hydride transfer conformation

+ LDH/pyruvate coupled assay

➢ simplifies kinetics by bleaching the free NADH

(|FlIP| + glycerol 20%)

G271A mutation in ELGG loop drastically reduces the flip kinetics

G271A mutation in ELGG loop drastically reduces the flip kinetics

Trp177 is the specific probe of cofactor flipping

Rate-limiting FLIP

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., Bchina, R., Barbe, S., Boudewin, S., Andrieu, I., and Talfournier, F. (2019) Enzyme Active Site Loop Revealed as a Gatekeeper for Cofactor Flip by Targeted Molecular Dynamics Simulations and FRET-Based Kinetics. ACS Catal. 9, 1327–1334