CryoEM snapshots of a native lysate provide structural insights into a metabolon-embedded transacetylase reaction

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Abstract

Found across all kingdoms of life, 2-keto acid dehydrogenase complexes possess prominent metabolic roles and form major regulatory sites. Although their component structures are known, their higher-order organization is highly heterogeneous, not only across species or tissues but also even within a single cell. Here, we report a cryoEM structure of the fully active Chaetomium thermophilum pyruvate dehydrogenase complex (PDHc) core scaffold at 3.85 Å resolution (FSC=0.143) from native cell extracts. By combining cryoEM with macromolecular docking and molecular dynamics simulations, we resolve all PDHc core scaffold interfaces and dissect the residing transacetylase reaction. Electrostatics attract the lipoyl domain to the transacetylase active site and stabilize the coenzyme A, while apolar interactions position the lipoate in its binding cleft. Our results have direct implications on the structural determinants of the transacetylase reaction and the role of flexible regions in the context of the overall 10 MDa PDHc metabolon architecture.

Introduction

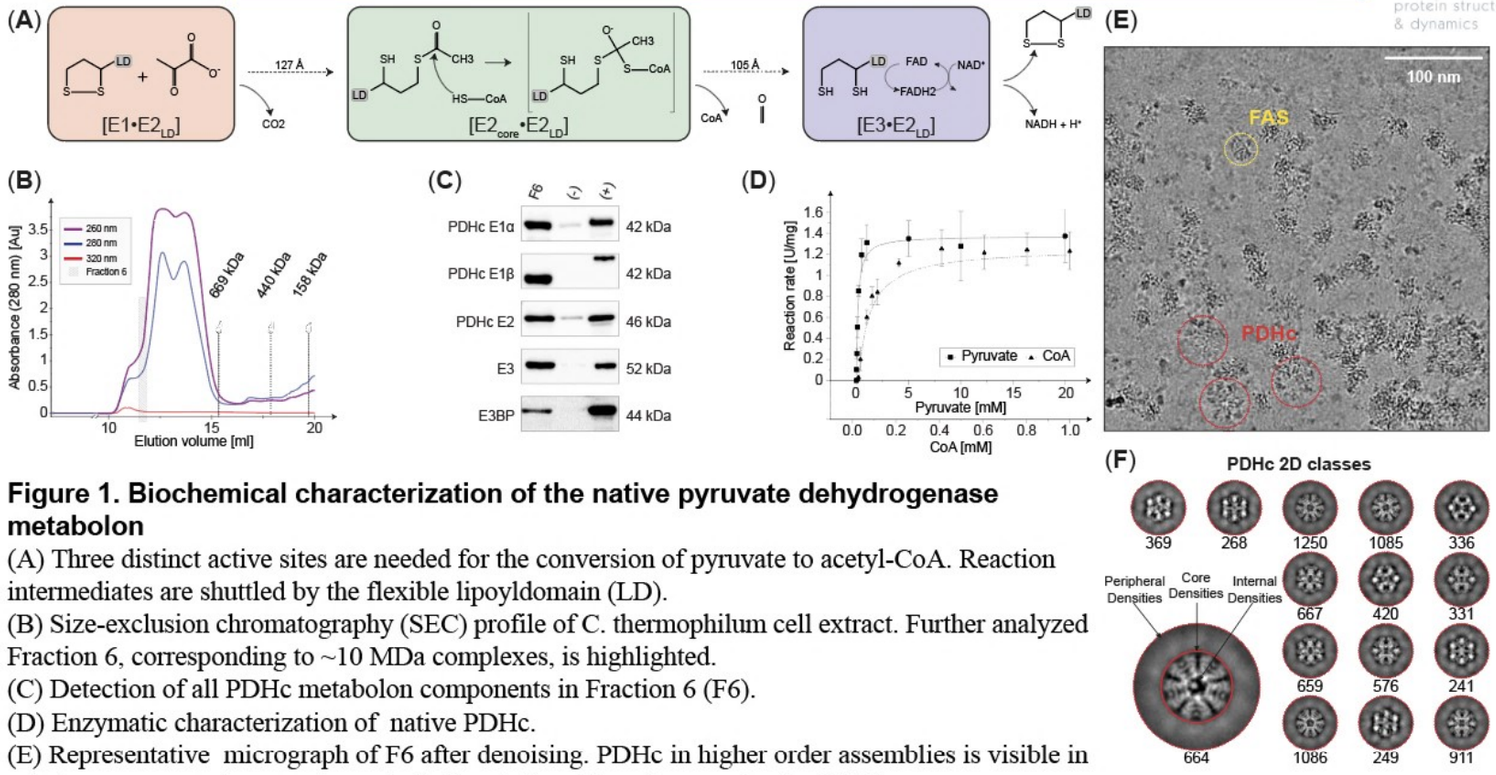

Figure 1. Biochemical characterization of the native pyruvate dehydrogenase metabolon

(A) Three distinct active sites are needed for the conversion of pyruvate to acetyl-CoA. Reaction intermediates are shuttled by the flexible lipoyldomain (LD).
(B) Size-exclusion chromatography (SEC) profile of C. thermophilum cell extract. Further analyzed Fraction 6, corresponding to ~10 MDa complexes, is highlighted.
(C) Detection of all PDHc metabolon components in Fraction 6 (F6).
(D) Enzymatic characterization of native PDHc.
(E) Representative micrograph of F6 after denoising. PDHc in higher order assemblies is visible in a heterogenous environment, e.g., including fatty acid synthase molecules (FAS).
(F) 2D classes of PDHc retrieved from cryoEM single-particles. External, core and internal densities are clearly distinguishable.

Results

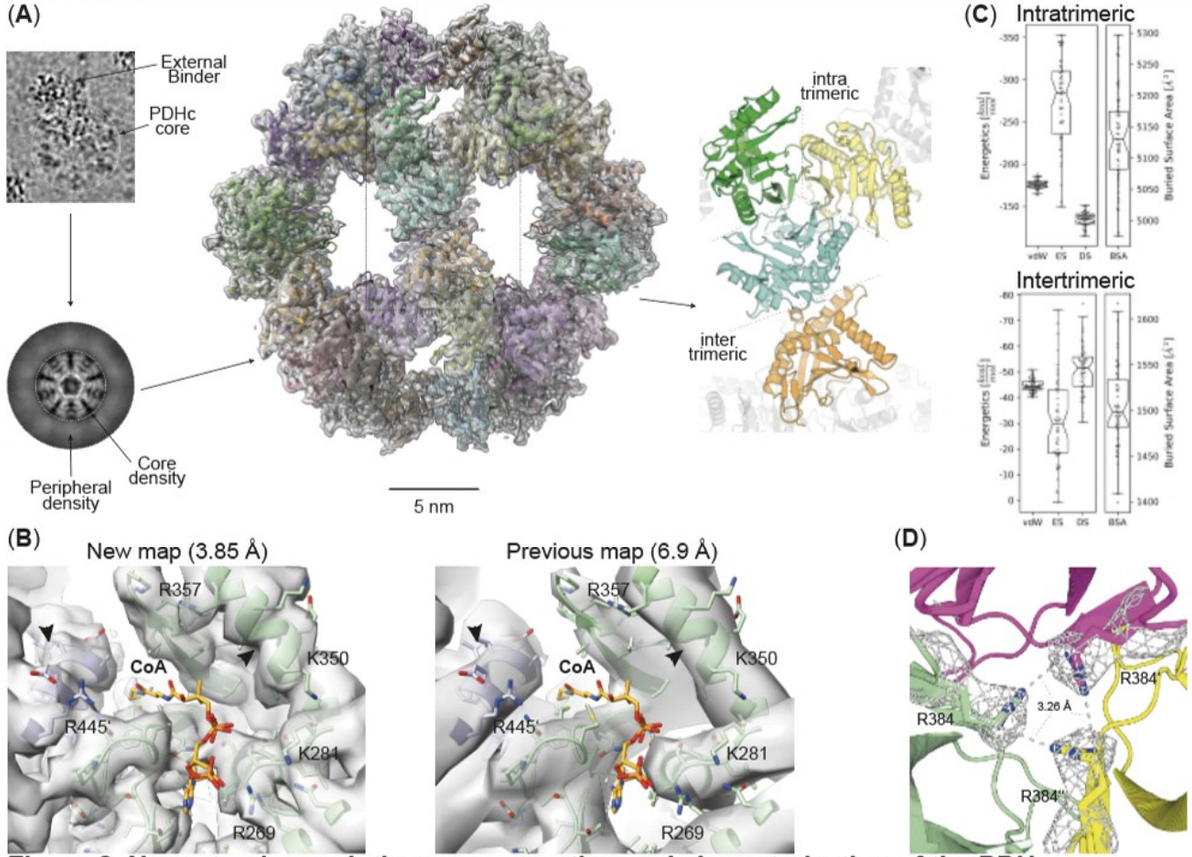

Figure 2. Near atomic resolution reconstruction and characterization of the PDHc core

(A) Icosahedrally-averaged cryoEM reconstruction of PDHc core. Densities corresponding to PDHc higher-order binders, as well as external PDHc densities, are averaged to retrieve the high-resolution structure.

(B) Comparison of improvement in resolution, compared to previous data [1]. CoA molecule is computationally placed and refined within the binding site. Side chains in the CoA binding pocket of the active site are distinguishable. Helical pitch (arrowhead) allows now unambiguous placement of the backbone.

(C) Energetic calculations within the E2 core structure. The intra-trimeric building block with an extensive buried surface area (BSA) is stabilized by electrostatics (ES) and van der Waals (vdW) energies. In the dimeric inter trimeric interaction, DS energy plays the major role, whereas vdW and ES energies have decreasing contributions.

(D) Potential Arginine cluster in the intra-trimeric interface. Arg384 of each subunit are in close contact, thereby contributing to the electrostatic binding energy.

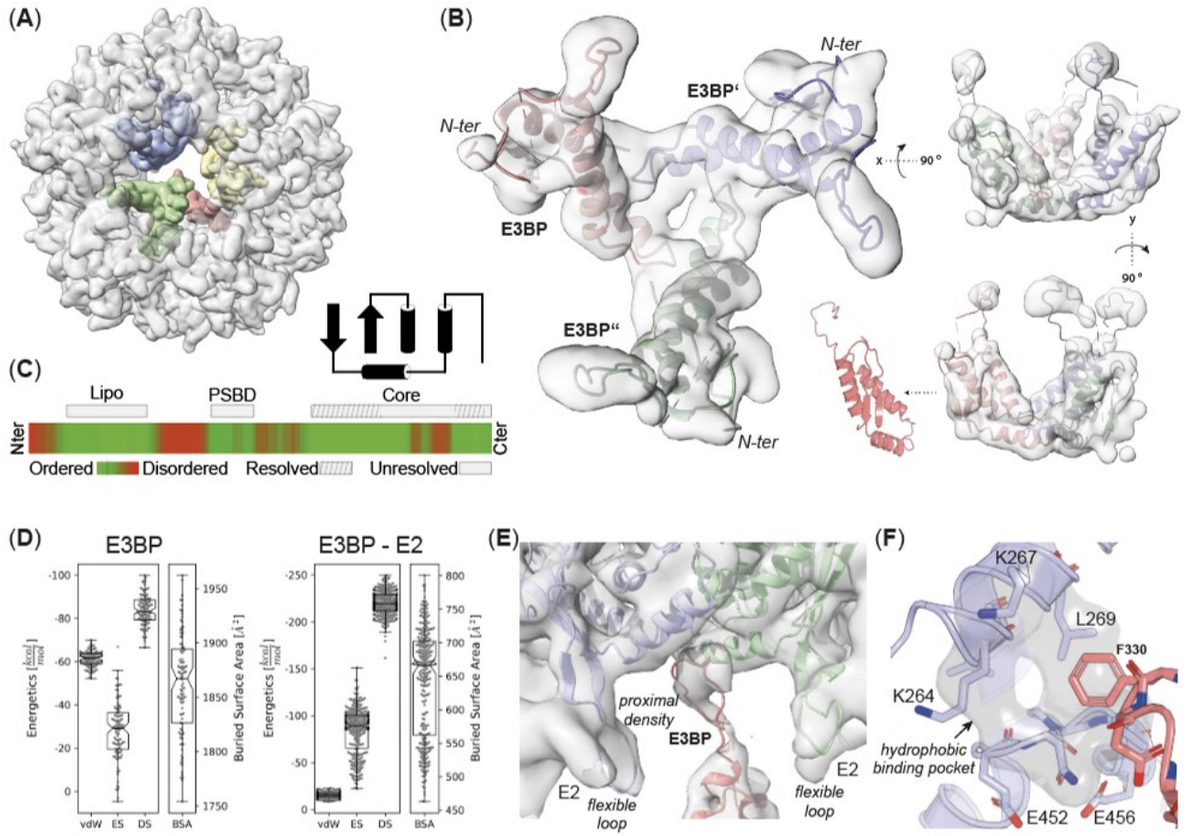

Figure 3. Localization of E3BP assembling the PDHc core scaffold.

(A) Localization of four distinct densities inside the core structure in the C2 reconstructed cryoEM map.

(B) Atomic model of the E3BP trimer in one of the four recapitulated densities shows a prominent fit.

(C) Domain organization of C. thermophilum E3BP. The primary sequence is strikingly similar to E2, consisting of an LD, a PSBD and the core region, separated by flexible linkers predicted as disordered.

(D) Energetic calculations of the trimeric E3BP. E3BP is mainly stabilized by DS, and the E2-E3BP interaction, composed of two E2s and one E3BP is primarily stabilized by DS.

(E) Interaction interface of E3BP and E2. A proximal density near the inter-trimeric interface of the core, identified as an extension of E3BP is located between two flexible loops of two E2s from two different trimeric building blocks.

(F) Interaction interface formed by E3BP and E2. Phe330 of E3BP is buried in a hydrophobic pocket formed by E2 apolar residues.

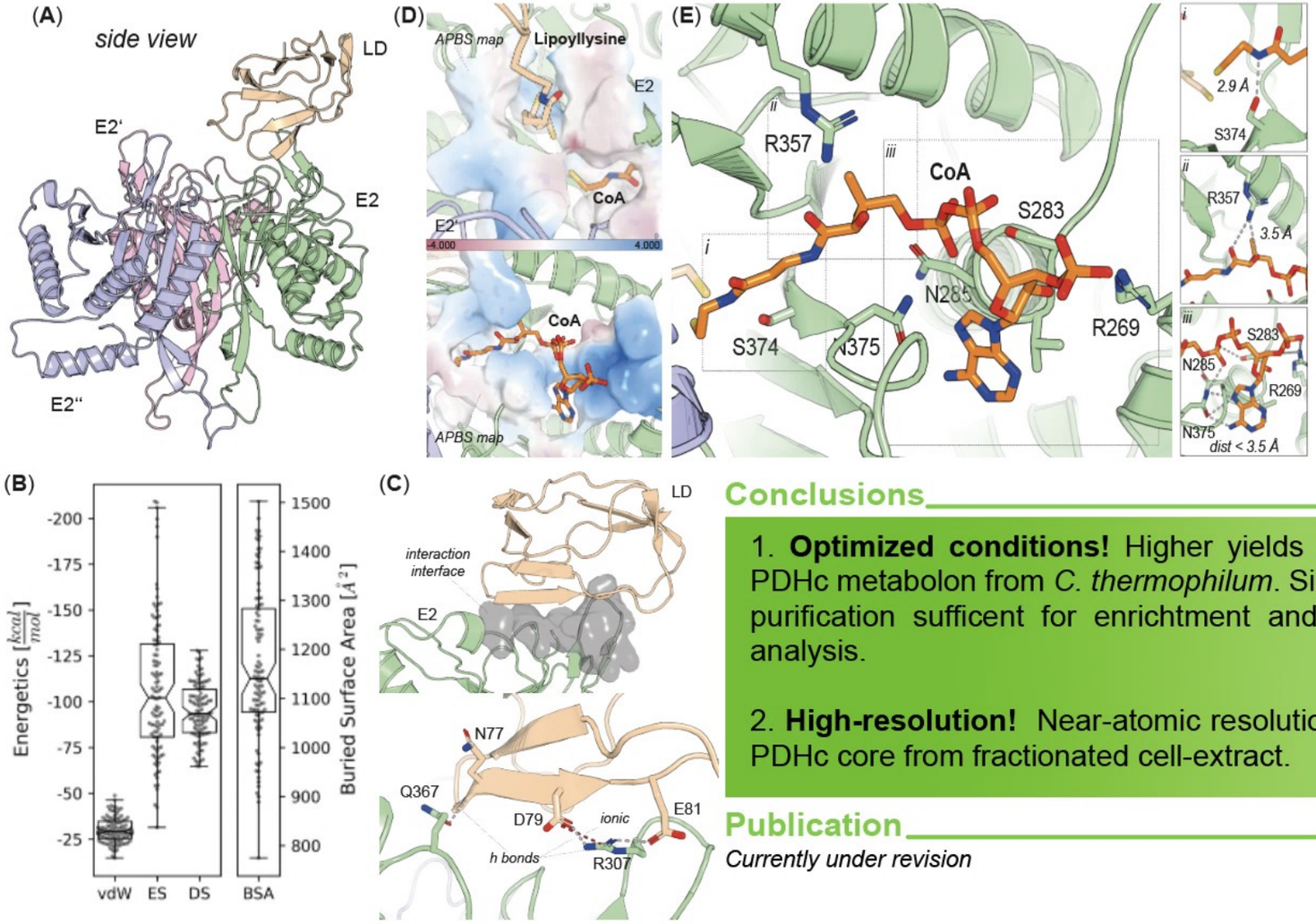

Figure 5. Structure-based analysis of the transacetylase reaction
(A) Top-scoring solution from the top-scoring cluster derived from flexible docking with HADDOCK [2]. The LD is bound by one monomer of the trimeric building block.
(B) HADDOCK energetics of the selected cluster. Binding is mainly mediated by ES and DS, while vdW contribution is decreased.
(C) Interaction interface between LD and E2. The interaction is stabilized by three H-bonds and a very strong ionic interaction between the Arg307 of E2 and the Asp79 of LD.
(D) Electrostatic surface potential maps of the two substrate binding pockets in the active site. The lipoate binding pocket is overall uncharged, whereas the CoA binding pocket stabilizes the negatively charged phosphates.
(E) Binding of the CoA in the active site. Inlets i – iii show the hydrogen bonding networks stabilizing the complete molecule. The lipoate and CoA were derived from docking procedures and refined during MD simulation.

Conclusions

1. **Optimized conditions!** Higher yields of native PDHc metabolon from *C. thermophilum*. Single step purification sufficent for enrichtment and cryoEM analysis.

2. **High-resolution!** Near-atomic resolution for the PDHc core from fractionated cell-extract.

3. **Localication of E3BP!** Unbiased identification and modelling of bound E3BP inside the core, forming the core scaffold.

4. **Structural insights into the transacetylase reaction!** Flexible docking and MD simulations allows analysis of the active site of the E2 core.

Publication

Currently under revision

References

[1] Kyrilis, Fotis L., et al. "Integrative structure of a 10-megadalton eukaryotic pyruvate dehydrogenase complex from native cell extracts." Cell Reports 34.6 (2021): 108727.
[2] Van Zundert, G. C. P., et al. "The HADDOCK2. 2 web server: user-friendly integrative modeling of biomolecular complexes." Journal of molecular biology 428.4 (2016).

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