Integrative structure of a 10-megadalton eukaryotic pyruvate dehydrogenase complex from native cell extracts

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Abstract

Oxidative decarboxylation of pyruvate is a key metabolic reaction during pyruvate catabolism and a vital step in aerobic metabolism performed by a giant enzymatic complex, the pyruvate dehydrogenase complex (PDHc). PDHc belongs to the α-keto acid/2-oxo-acid dehydrogenase complex (OADH complexes) family together with the 2-oxoglutarate dehydrogenase complex (OGDHc, also known as α-ketoglutarate dehydrogenase complex) and branched-chain keto-acid dehydrogenase complexes (BCKDHc). PDHc components have been characterized in isolation, but its quaternary structure remains elusive due to sheer size, heterogeneity and plasticity. By utilizing mass spectrometry, activity assays, crosslinking, electron microscopy and computational modeling, we identified fully assembled Chaetomium thermophilum α-keto acid dehydrogenase complexes in native cell extracts and characterized their domain arrangements. We reported the cryo-EM structure of the PDHc core and observed unique features of the previously unknown native state. We reconstructed asymmetrically the 10-MDa PDHc and resolved spatial proximity of its components, in agreement with stoichiometric data (60 E2p:12 E3BP:~20 E1p:≤12 E3), and proposed a minimum reaction path among component enzymes. PDHc shows the presence of a dynamic pyruvate oxidation compartment, organized by core and peripheral protein species. Our data provide a framework for further understanding PDHc and α-keto acid dehydrogenase complex structure and function.

Introduction

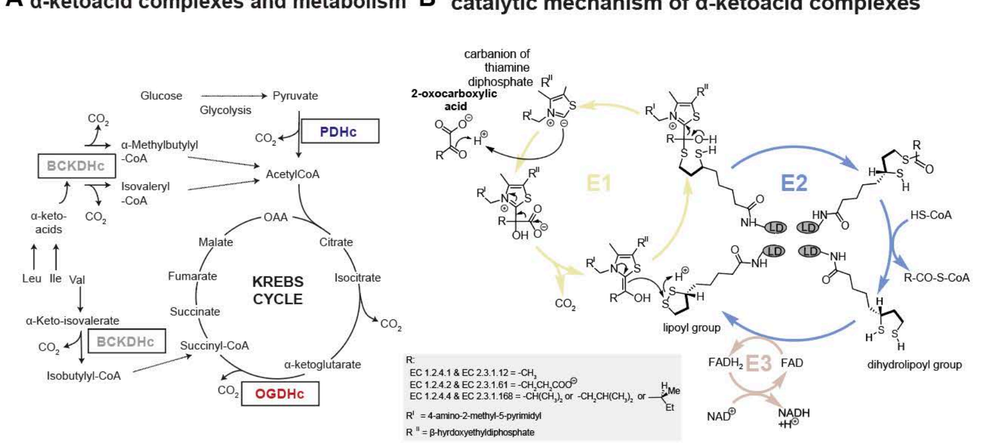

Figure 1: Pathways involving α-keto acid dehydrogenase complexes.
(A) Biochemical pathway with PDHc, OGDHc and BCKDHc as major participants.
(B) Reaction mechanism performed by the E1, E2 and E3 enzymes of the α-keto acid dehydrogenase complexes.

Workflow

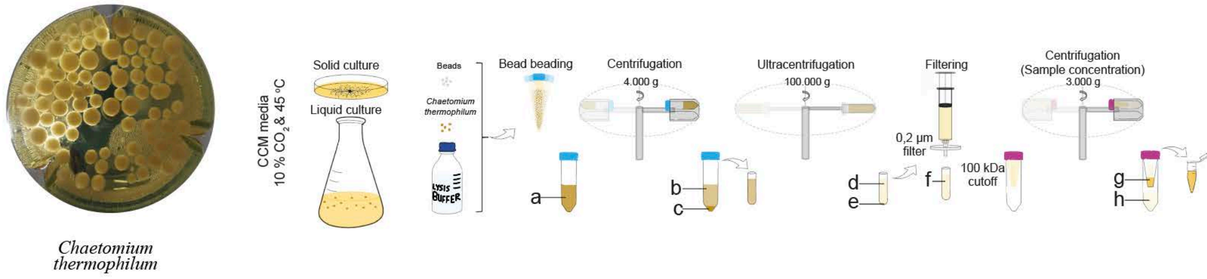

Figure 2: Experimental workflow for cell extracts fractionation. (A) Model organism used in the experiments (C. thermophilum); Bottom view of a liquid culture flask (B) The experiment until the injection of the cell extract is shown with different steps of purification

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Results

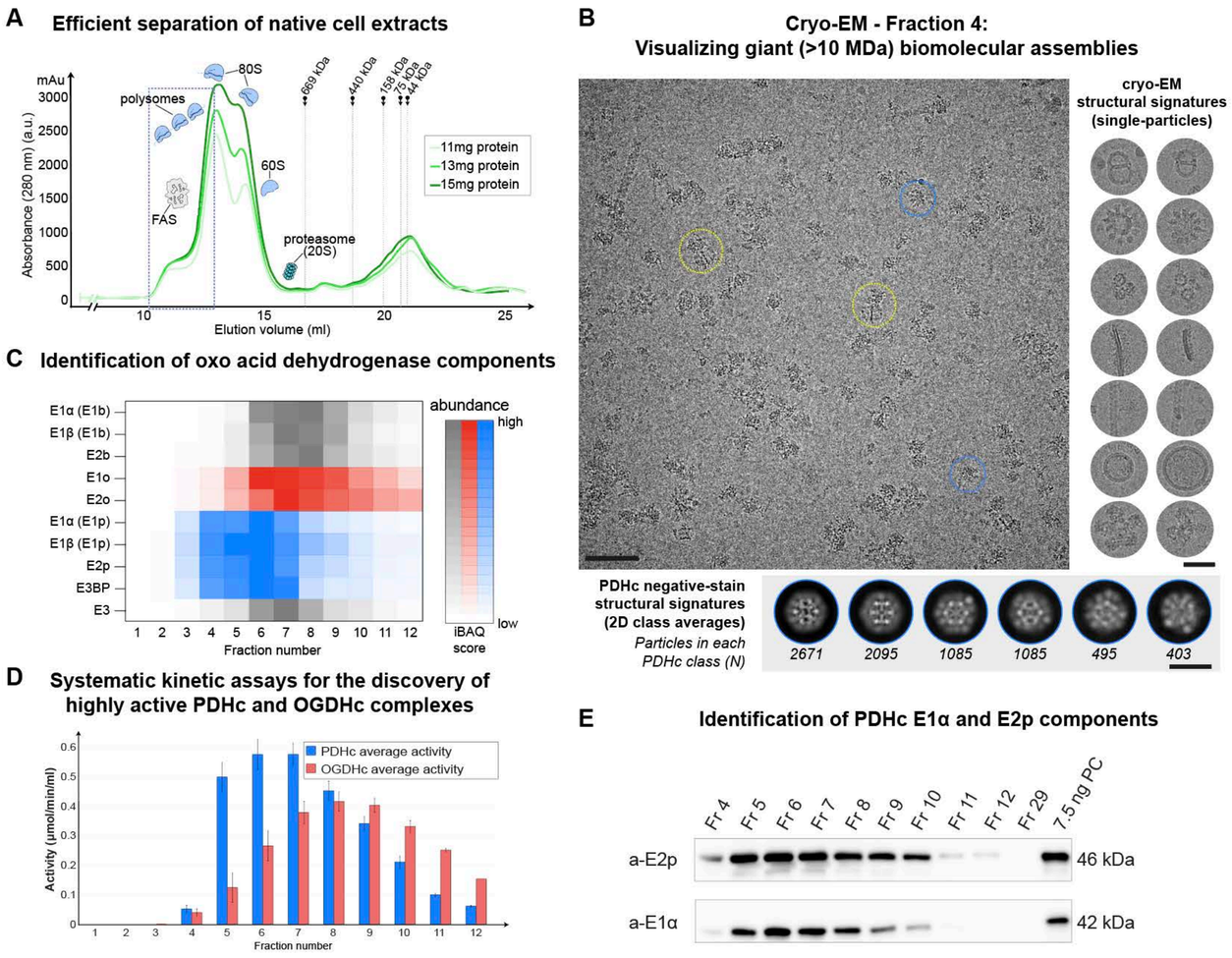

Figure 3. Biochemical characterization of native cell extracts aiming to capture α-keto acid dehydrogenase complexes. (A) SEC profile of *C. thermophilum* native extract; (B) Cryo-EM of fraction 4 (>10 MDa complexes); Insert shows: The metabolon of FAS with the bound carboxylase and PDHc particles shown in yellow and blue circles, respectively; On the right, single particles described in STAR methods; On the bottom, class averages of PDHc derived from NS. Scale bars: insert: 100nm; extracted single-particles and PDHc class averages: 60 nm. (C) MS data show abundance of α-keto acid dehydrogenase subunits. (D) Activity assays for PDHc (blue) and OGDHc (red) E1. (E) Immunodetection of E2p and E1α in cell extracts; NC and PC stand for negative and positive controls.

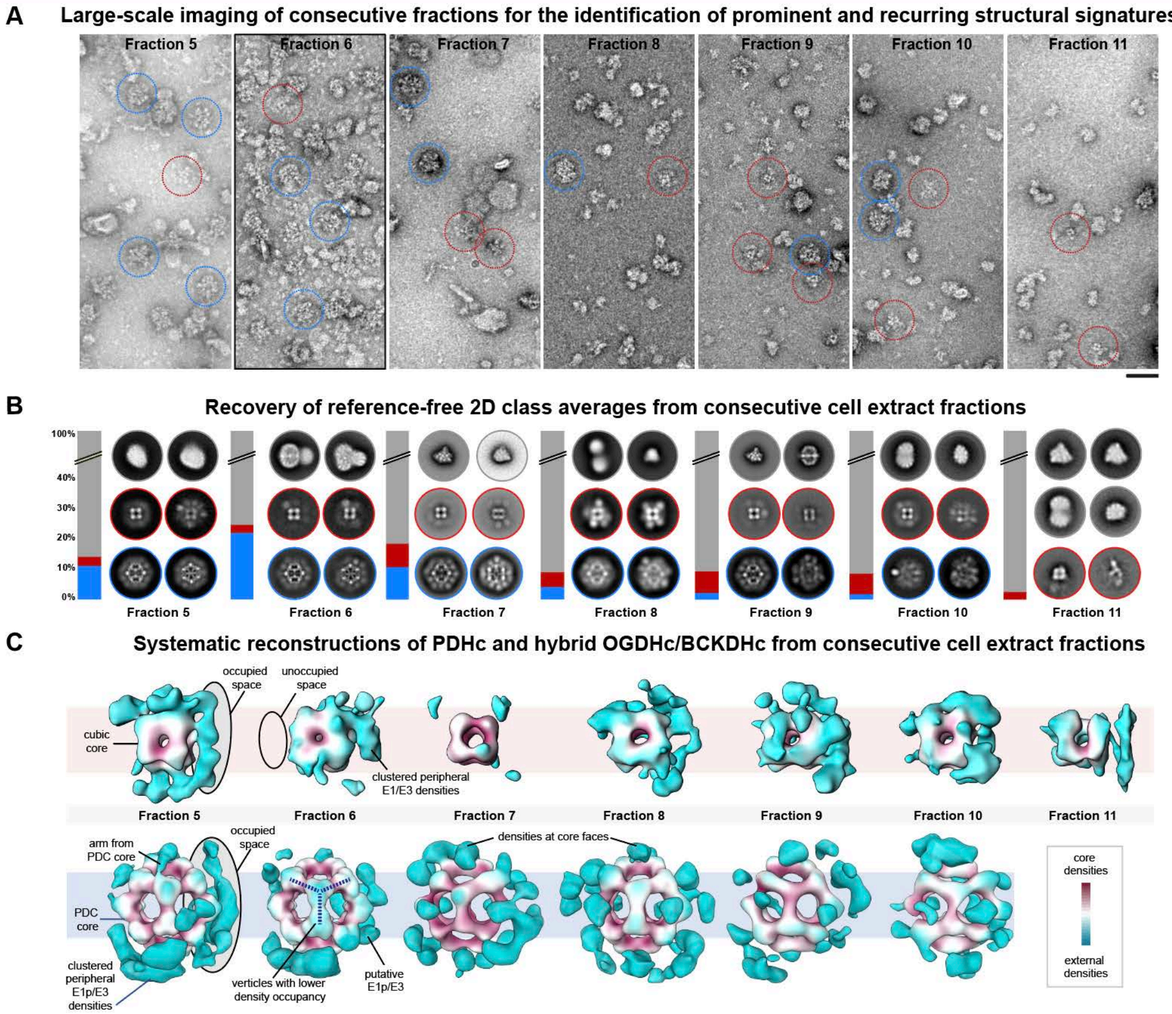

Figure 4. Identification of OADH complexes in native cell extracts by electron microscopy. (A) Typical micrographs showing distinct single particles for fractions 5-11. Blue and red circles correspond to prominent signatures of particles with dodecahedral and octahedral cores, respectively. Scale bar: 60 nm. (B) Quantification of particles resulting in class averages showing characteristics of dodecahedral (blue), octahedral (red) and other types of structures (grey). Insert class averages show a typical class average from each category of molecules. (C) 3D reconstructions with C1 symmetry of complexes with cubic (top) and dodecahedral cores (bottom).

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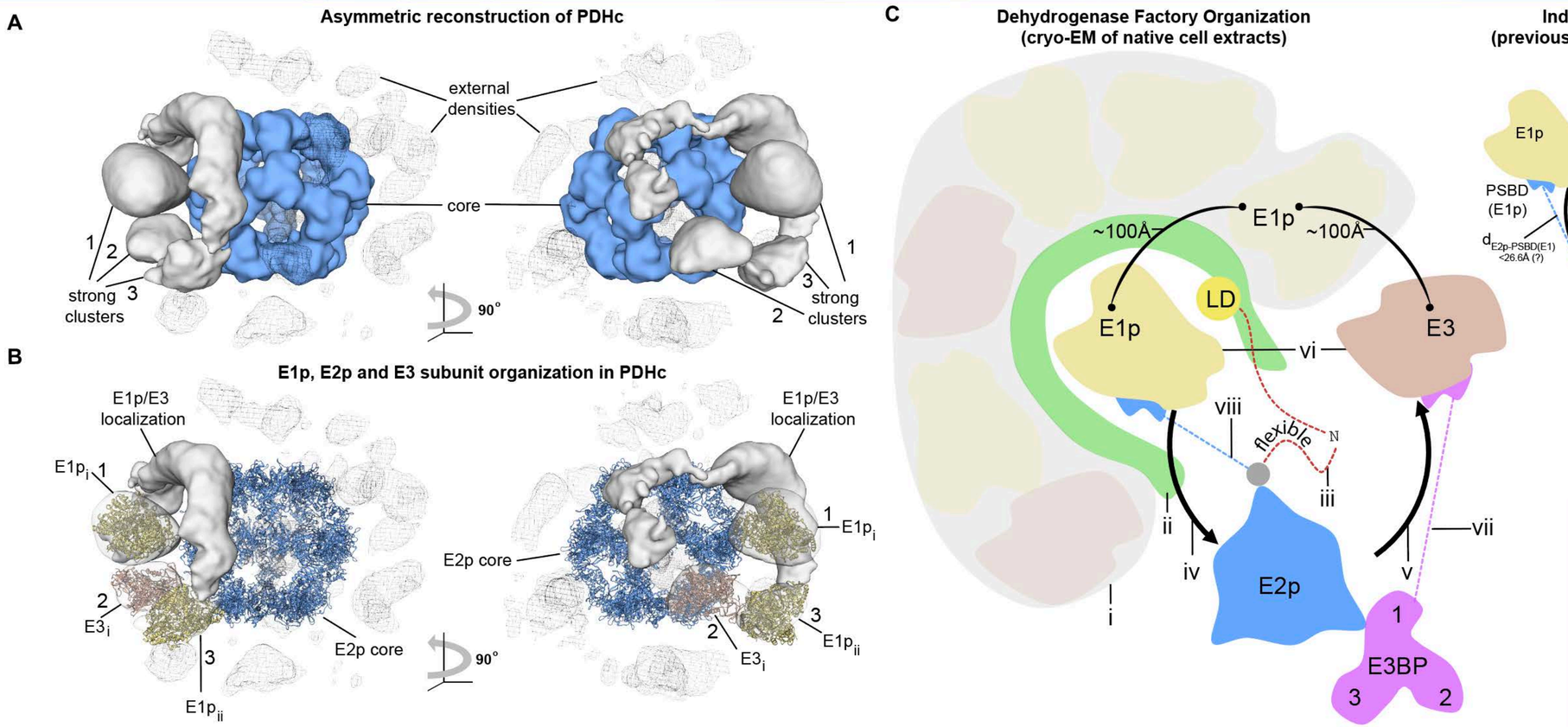

Figure 6. Asymmetric reconstruction of the full PDHc from native cell extracts. (A) The PDHc map was segmented with Segger (Pintilie and Chiu, 2012); localized densities (wire), strong clusters of densities (silver); and E2p core (blue) are shown. Outer density clusters are denoted as 1, 2 and 3. (B) Top fits are shown for E1p, E2p and E3; The rest of the densities are not fitted due to ambiguity in localizing the enzymes. (C) On the right, the model of PDHc is shown where the LD Cter is stably attached to the E2p core, covering the binding site of the lipoyl. The arm transfers intermediates from E1p to E2p and then to E3. E3BP is described inside the E2p, but its intra-molecular interface is unknown. On the left, the pyruvate dehydrogenase factory model observed with our integrative methodology.

Conclusions

1. Diverse biochemical and biophysical methods combined to probe **native cell extract** organization
2. An **asymmetric** reconstruction of the **active**, **native** pyruvate dehydrogenase complex (PDHc) is reported
3. Spatial relationships among protein components in the **dynamic assembly** are determined
4. Enzyme clusters forming a transient catalytic nanocompartment are identified and defined as the **"pyruvate dehydrogenase factory"**

Publication

Kyrilis et al., 2021, Cell Reports *34*, 108727, February 9, 2021 © 2021 The Author(s). https://doi.org/10.1016/j.celrep.2021.108727

Acknowledgements

This work was funded by the Federal Ministry for Education and Research (BMBF, ZIK program: 02Z22HN23) the European Regional Development Funds for Saxony-Anhalt (EFRE: ZS/2016/04/78115) and the Martin Luther University Halle-Wittenberg.

Funding sources

ZENTREN FÜR INNOVATIONSKOMPETENZ UNTERNEHMEN REGION Die BMBF-Innovationsinitiative Neue Länder

Bundesministerium für Bildung und Forschung

MARTIN-LUTHER UNIVERSITÄT HALLE-WITTENBERG

SACHSEN-ANHALT

European Union
European Regional Development Fund