BIOTRANS
LA ROCHELLE 2023

N°668 / OC
TOPIC(s) : Enzyme discovery and engineering

Tungsten aldehyde oxidoreductase/ hydrogenase forms an enzymatic decorated protein nanowire

AUTHORS

Maciej SZALENIEC / JERZY HABER INSTITUTE OF CATALYSIS AND SURFACE CHEMISTRY POLISH ACADEMY OF SCIENCE, NIEZAPOMINAJEK 8, KRAKÓW

PURPOSE OF THE ABSTRACT

Tungsten aldehyde oxidoreductase/ hydrogenase forms an enzymatic decorated protein nanowire

Agnieszka Winiarska1, Fidel Ramírez-Amador2, Gabriela Pacek1, Ivana Aleksic1, Dominik Hege3, Yvonne Gemmecker3, Simone Prinz4, Georg Hochberg5, Johann Heider*2,3, Jan Michael Schuller*2, Maciej Szaleniec*1
1 - Jerzy Haber Institute of Catalysis and Surface Chemistry Polish Academy of Sciences
2 - SYNMIKRO Research Center and Department of Chemistry, University of Marburg, Marburg, Germany.
3 - Faculty of Biology, Philipps-Universität Marburg, Marburg, Germany
4 - Max Planck Institute of Biophysics, Frankfurt am Main, Germany
5 - Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

The multi-subunit aldehyde oxidoreductase from the denitrifying betaproteobacterium Aromatoleum aromaticum (AORAa) catalyzes the reversible oxidation of aldehydes to carboxylic acids with electron acceptors like NAD+ or viologens (1). Recently we showed the ability of AORAa to use hydrogen as an electron donor for acid or NAD+ reduction, which was not shown before for any tungsten enzyme and qualifies AOR as a new type of hydrogenase (2). Although the catalytic W-containing subunit (AorB) is highly similar to AOR from Pyrococcus furiosus (pdb code 1AOR), AORAa also contains an additional FeS cluster-rich polyferredoxin-like subunit (AorA) and a FAD-containing subunit (AorC).

Recently, we resolved the first molecular structure of AORAa, with a global resolution of 3.22 Å using cryo-electron microscopy (3). The study resulted in the molecular structure of an Aor(AB)2C complex, although the subunit stoichiometry of AORAa varies and additional electron density depicting an Aor(AB)3C complex was obtained. These results, supported by mass spectrophotometry, revealed that the polyferredoxin-like subunit AorA oligomerizes from a single AorC subunit to form an electron-conducting nanowire that is decorated with enzymatically active and W-cofactor (W-co) containing AorB subunits (Fig. 1). This supermolecular structure model of AORAa was additionally supported by disrupting the interface between AorAB protomers via mutagenesis, which led to a simple AorABC complex.

The structure of the AorB subunit revealed the binding mode of the substrate benzoate in the active site. This, together with EXAFS spectrometry and QM:MM modelling of the W-co, enabled the proposal of a catalytic mechanism describing the oxidation of aldehydes and the H2-dependent reduction of carboxylic acids. We characterized the catalytic activity of AORAa in H2-dependent reductions. A series of reactors proved that an enzymatic cascade from carboxylic acids to alcohols can be catalyzed at both acidic and neutral pH, ruling out an electron bifurcation mechanism. We have also demonstrated that whole-cells with recombinant AOR can be applied for substrate conversion.

Acknowledgement: This project has received funding from the European Research Council (ERC) under the

European Union's Horizon 2020 research and innovation programme (Two-CO2-One, grant agreement no. 101075992) (JMS); European Molecular Biology Organization Scientific Exchange Grant 9048 (AW); EU Project POWR.03.02.00-00-I004/16 (AW); National Science Centre, Poland grant PRELUDIUM 2017/27/N/ST4/02676 (AW); German Research Foundation grant He2190/15-1 (JH); Emmy Noether grant SCHU 3364/1-1 (JMS); We gratefully acknowledge Poland's high-performance computing infrastructure PLGrid (HPC Centers: ACK Cyfronet AGH) for providing computer facilities and support within computational grant no. PLG/2022/015584

FIGURES

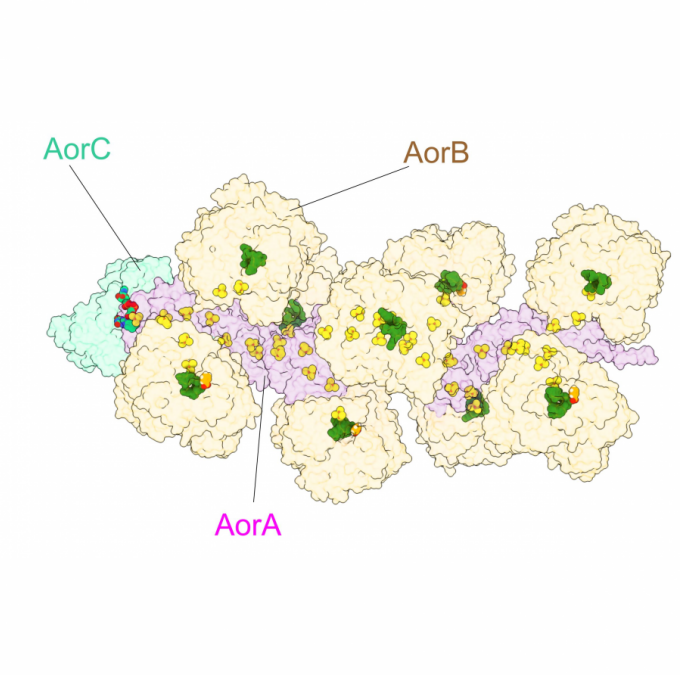

FIGURE 1

Fig. 1.

Filament formation in the AORAa complex - modelled nanowire complex of composition Aor(AB)5C;

FIGURE 2

KEYWORDS

tungsten enzyme | hydrogenase | aldehyde oxidoreductase | cryo-EM

BIBLIOGRAPHY

[1] F. Arndt, et al. , Characterization of an Aldehyde Oxidoreductase From the Mesophilic Bacterium Aromatoleum aromaticum EbN1, a Member of a New Subfamily of Tungsten-Containing Enzymes. Front Microbiol. 10 (2019), doi:10.3389/fmicb.2019.00071.

[2] A. Winiarska, et al., Tungsten Enzyme Using Hydrogen as an Electron Donor to Reduce Carboxylic Acids and NAD +. ACS Catal. 12, (2022) 8707-8717.

[3] A. Winiarska, et al., A bacterial tungsten-containing aldehyde oxidoreductase forms an enzymatic decorated protein nanowire, BioRxiv (2023) doi: 10.1101/2023.01.23.525143