The structural basis for energy extraction from air by Mycobacterium smegmatis

Ashleigh Kropp[1], Hari Venugopal[2], Chris Greening[1], Rhys Grinter[3]
[1]Department of Microbiology, Monash Biomedicine Discovery Institute, Monash University, Clayton, Australia, [2]Ramaciotti Centre for Cryo-Electron Microscopy, Monash University, Clayton, Australia, [3]Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Australia

Background
Mycobacterium smegmatis is a heterotrophic, obligately aerobic soil-dwelling bacterium that requires organic carbon for growth. Due to fierce competition for resources in the soil, the bacterium often experiences deprivation of both oxygen and organic carbon sources. To cope with this, M. smegmatis has evolved to persist through metabolic flexibility, upregulating enzymes, such as the high-affinity [NiFe] hydrogenase Huc, that enable it to utilise electron donors and acceptors other than organic carbon and molecular oxygen. Huc provides electrons from H_2 to the cytochrome bcc-aa3 oxidase super complex via the quinone pool, which results in the generation of the proton motive force. The molecular details underpinning hydrogen oxidation in M. smegmatis were unknown. We aimed to characterise how Huc oxidises hydrogen and how the electrons liberated from this process are transferred to the respiratory chain.

Methods
In this work, we purified Huc natively from an M. smegmatis strain and visualised it by cryo-electron microscopy (cryo-EM). We used a wide range of techniques to characterise the enzymatic capabilities of this unique enzyme. Utilising an H_2 sensing electrode and gas chromatography, we quantitated the kinetics of hydrogen oxidation by Huc and the specificity of Huc to different electron acceptor molecules. Further molecular dynamics simulations and mass spectrometry enabled us to identify the specific electron acceptor substrate of Huc. To characterise how this enzyme differs from other low-affinity hydrogenases, we employed spectroscopic and electrochemical techniques.

Results
Our cryo-EM maps of Huc revealed an impressive enzymatic structure with electron density maps at a maximum resolution of 1.52 Å. Uniquely, Huc is an octamer of the large and small enzymatic subunits, with an additional novel membrane-associated central stalk. We showed that this stalk, called HucM, is critical for transporting the electron acceptor molecule menaquinone directly from the bacterial membrane to the enzyme. This long-range quinone transport is a unique feature of Huc. Further, we demonstrated for the first time a very high-affinity purified enzyme capable of oxidising atmospheric H_2 in isolation from the respiratory chain. In addition, we demonstrated that, unlike many other [NiFe] hydrogenases, Huc is insensitive to inhibition by oxygen.

Conclusions
This work represents the first molecular characterisation of a member of a novel family of hydrogenases capable of scavenging H_2 at atmospheric concentrations. This discovery will provide insights for the investigation and development of hydrogenase-based fuel cells that can operate at atmospheric levels of oxygen and use very small amounts of hydrogen to produce energy.

Keywords:

CryoEM, microbiology, enzymes, metabolism

Reference:

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