The thermoacidophilic methanotroph *Methylacidiphilum fumariolicum* SolV oxidizes subatmospheric H₂ with a highaffinity [NiFe] hydrogenase

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The trace amounts (0.53 ppmv) of atmospheric H₂ can be utilized by microorganisms to persist during dormancy. This process is catalyzed by certain Actinobacteria, Acidobacteria and Chloroflexi, and is estimated to convert 75 Tg H₂ annually, which is half of the total atmospheric H_2 . This rapid atmospheric H_2 turnover is hypothesized to be catalyzed by high-affinity [NiFe] hydrogenases. However, apparent high-affinity H₂ oxidation has only been shown in whole cells, rather than for the purified enzyme. Here, we show that the membrane-associated hydrogenase from the thermoacidophilic methanotroph Methylacidiphilum fumariolicum SolV possesses a high apparent affinity $(K_{m(app)} = 140 \text{ nM})$ for H₂ and that methanotrophs can oxidize subatmospheric H₂. Our findings add to the evidence that the group 1h [NiFe] hydrogenase is accountable for atmospheric H_2 oxidation and that it therefore could be a strong controlling factor in the global H₂ cycle. We show that the isolated enzyme possesses a lower affinity ($K_m = 300 \text{ nM}$) for H₂ than the membrane-associated enzyme. Hence, the membrane association seems essential for a high affinity for H₂. The enzyme is extremely thermostable and remains folded up to 95 °C. The ability to conserve energy from H₂ could increase fitness of verrucomicrobial methanotrophs in geothermal ecosystems with varying CH4 fluxes. Group 1h [NiFe] hydrogenases could therefore contribute to mitigation of global warming, since CH₄ is an important and extremely potent greenhouse gas.