

SUMMARY

Three decades ago, respiration with a solid electron donor or acceptor would have made great science fiction. Now we know that “electroactive” microorganisms perform this on a daily basis in various anaerobic habitats. These electroactive microorganisms are classified by their ability to utilize solid electron donors or acceptors outside of the cell in connection to intracellular redox reactions. Among them, an ecologically and economically relevant group is the methanogenic archaea, which are often the playing the role of terminal electron acceptors in anaerobic environments. Methanogens are capable of direct extracellular electron uptake from solid electron donors, but the mechanisms remain elusive. This knowledge gap was the motivation for this thesis. We examined direct electron uptake by methanogens from an electron donating microorganism and from a poised cathode in a bioelectrochemical system. We identified five new electroactive methanogens of the order *Methanosarcinales*, which received electrons directly from an electron donating *Geobacter metallireducens*. However, the investigation of electron uptake in a bioelectrochemical system, showed that one of three methanogens tested (*M. horonobensis*) was unable to take up electrons from a cathode although it did from a *Geobacter*. This was attributed to the ability of *Geobacter* to modulate the expression of its extracellular electron transfer proteins (multiheme c-type cytochromes/MHC) to match the redox potential of electron accepting molecules on the surface of the partner methanogen. Expression of multiheme cytochromes in *Geobacter* co-cultivated with *M. horonobensis* was dissimilar to that of *Geobacter* co-cultured with *Ms. barkeri*, but similar if the *Geobacter* was co-cultured with *Mtx. harundinacea*. This is suggestive of comparable electron uptake conduits in *Ms. horonobensis* and *Mtx. harundinacea*. Until now, extracellular electron transfer has been mainly studied in model organisms like *Geobacter* where it involved pili and multiheme c-type cytochromes. Therefore, similar strategies for electron transfer were expected for methanogens. However, only two out of seven electroactive methanogens have such cytochromes showing that MHCs are not ubiquitous among methanogens capable of direct electron uptake. To confirm or disconfirm involvement of MHCs in direct electron uptake, we selected a *M. mazei* which had an available MHC mutant. *M. mazei* retrieved extracellular electrons independent of its multiheme cytochrome confirming that MHCs are not necessary for extracellular electron uptake in *Methanosarcina*. Previously, the transient electrochemical stimulation of methanogens has been proposed as a biogas upgrading strategy to match the discontinuous production of green energy. We therefore investigated the impact of transient and continuous electrochemical stimulation on a *Geobacter-Methanosarcina* consortium and observed that both microorganisms were negatively affected. This informs future biotechnological strategies that implicate such consortia. These unprecedented insights in the electroactivity of methanogenic archaea are valuable for better understanding their role in the environment and also in the design of future electrochemical biogas upgrading technologies. My studies have thus revealed hydrogen independent electron uptake from a cathode and electrogenic partners in several *Methanosarcina* species by a mechanism which was cytochrome independent and put forward a novel hypothesis for electron uptake in *Methanosarcinales*.