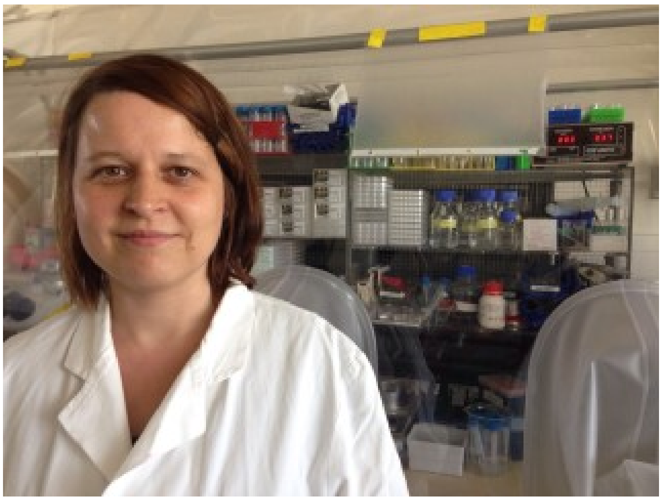
Bacterial MccA is better than other enzymes when it comes to reducing sulphites

Bacteria have many metabolic capacities that make them important players in the global ecosystem and climate. Dr. Bianca Hermann from the University of Freiburg specialises in multi-haem enzymes, and investigates the enzymes' structure and reaction mechanisms. She has clarified the enzymes' crystal structure and reaction mechanisms and found out why the bacterial MccA enzyme complex can reduce sulphur-containing substances such as sulphites up to a hundred times faster than other enzymes. These findings open up the possibility of using specific microorganisms for simplifying or accelerating industrial desulphurisation.

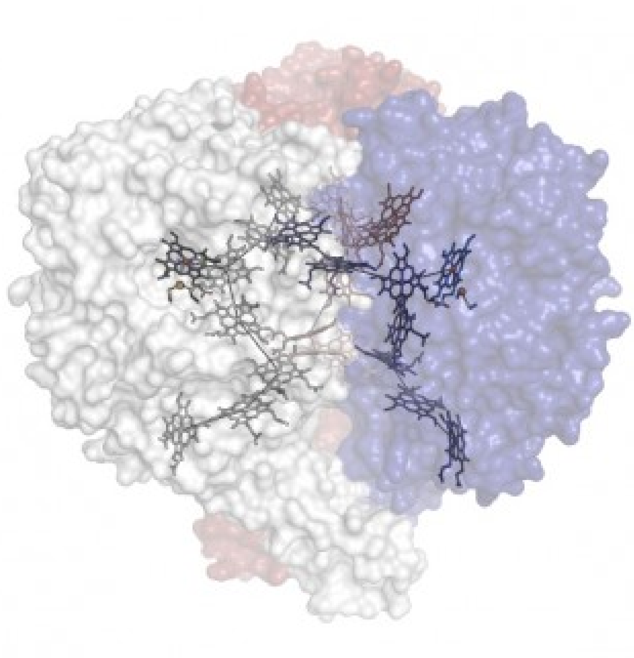


Multi-haem proteins are chemist Dr. Bianca Hermann's speciality. © Dr. Bianca Hermann, University of Freiburg

While sulphur-containing substances may already be toxic at small concentrations for certain bacteria, yeasts and fungi, other species are able to effectively metabolise such compounds. Sulphites are the salts and esters of sulphurous acid and contain a negatively charged sulphite ion (SO_3^{2-}). Sulphites can inhibit the growth of fungi, yeasts and bacteria due to their extraordinary reactivity to proteins, nucleic acids and lipids. They can turn into sulphates by "consuming" atmospheric oxygen. This enables them to slow down undesired oxidation processes in food, which would otherwise ruin the food's taste and colour and destroy its vitamin content. Sulphites have been used as preservatives in industrial foods since the 18th century; they are also added to wine although wines naturally contain sulphur dioxide in various forms (i.e. sulphites).

However, it is important that sulphur dioxides, which arise from the combustion of sulphur-containing substances, are not emitted into the air. From 1974 onwards, German coal and ignite-fired power stations have been legally required to put in place processes for desulphurising flue gases. Around a hundred different desulphurisation processes exist that can remove more than 90% of the sulphur. The most popular method is lime scrubbing, a process that turns sulphur dioxide into gypsum.

Sulphur respiration in Wolinella



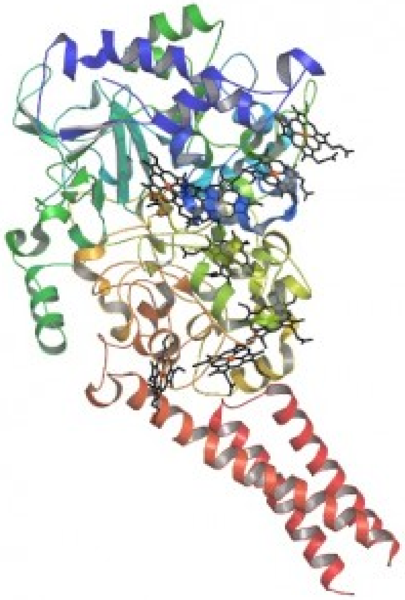
MccA, a homotrimeric Wolinella enzyme, reduces sulphite fast. The photo shows a surface model of the enzyme. © Dr. Bianca Hermann, University of Freiburg

Wolinella succinogenes can relatively easily do something that we are unable to do. Wolinella succinogenes is a bacterium that uses nitrates and sulphites as an energy source, and it is Dr. Bianca Hermann's model bacterium. Hermann and Prof. Dr. Oliver Einsle from the Institute of Biochemistry at the University of Freiburg, are working with Prof. Dr. Jörg Simon from Technische Universität Darmstadt to try and elucidate the function of multi-haem enzymes and complexes (metalloenzymes with several haem groups). Wolinella belongs to the proteobacteria group. It lives under anaerobic conditions in the rumen of cows where it has no access to oxygen from which it could generate energy. "Normally, Wolinella grows by nitrate or nitrite respiration, but when it has no access to either of these, it switches to sulphite respiration," says Hermann explaining the mechanism that has been conserved during evolution to ensure the bacterium's survival. Rather than metabolising sugar, the sulphite reductase enzyme synthesises ATP through anaerobic respiration where it uses sulphite as terminal electron acceptor. During this reaction, sulphide (S^{2-}) is formed.

The team of scientists from Freiburg and Darmstadt wanted to find out why the Wolinella enzyme works much faster than other known sulphite reductases. Hermann analysed

Wolinella's sulphite-reducing enzyme and managed to clarify its three-dimensional structure. She found a homotrimer with an unprecedented fold and haem arrangement. The metalloprotein multi-haem cytochrome C (MccA) is located between the cytoplasmic membrane and the outer membrane where it forms homotrimeric complexes. Each of the three monomers consists of eight covalently attached haem groups with iron ions that are also arranged in a characteristic way. In each monomer, one of these haem groups (haem 2) forms the active site.

Isolation method uncovers molecular details

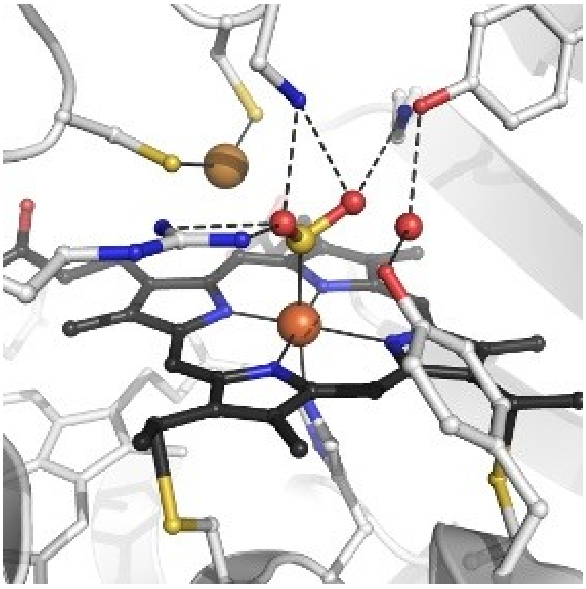


One of the three monomers of the enzyme complex MccA with its eight haem groups. © Dr. Bianca Hermann, University of Freiburg

The exact structure of the active centre was as yet unknown despite previous crystallographic analyses of the enzymes. Hermann studied the enzyme's atom arrangement and electron density and found these rather strange. This was because W. succinogenes MccA had only been isolated in the presence of oxygen. As W. succinogenes lives in anaerobic conditions in the cow rumen, Hermann therefore decided to isolate the enzyme under exclusion of oxygen. This change of conditions led to the discovery of a second MccA version, which had an additional metal ion in its active centre. Isolation of the enzyme under anoxic conditions also revealed that each monomer had a copper ion bound to two cysteine residues in the close vicinity of one of the haem groups. Oxic isolation always led to the detachment of the copper because copper oxidises easily and loses its binding partners in the presence of oxygen. "The copper ion is always located above the binding site, and acts as a sulphite ion barrier. The reactions thus proceed much faster," says Hermann.

This appears to explain the apparent contradiction: in the absence of copper, the relatively large sulphite ions have enough space to dock to the binding site and are catalysed more slowly by the MccA enzyme. However, if copper is present above the binding site, as is the case in the cow rumen, SO_3^{2-} is no longer able to bind for steric reasons. Instead, sulphur dioxide (SO_2) is able to bind to the active centre because it requires less space. "The copper ion acts as a barrier and the chemical equilibrium directly shifts to SO_2," says Hermann. All subsequent reactions towards sulphide (S^{2-}) proceed much faster because one of the energy-consuming dehydration steps involving transferral of electrons has been skipped. "We have shown that MccA isolated under anoxic conditions has a much higher sulphite reductase activity than enzymes isolated under oxic conditions," says the chemist.

A new heterometallic oxidoreductase type



Reaction site of sulphite reduction: the active centre of MccA with the copper ion (brown, top) above the haem level. SO2 (yellow and red) binds here and is kept in place by hydrogen bonds (blue). The iron is part of the haem group (brown, bottom). © Dr. Bianca Hermann, University of Freiburg

This is the first time a sulphite reductase enzyme with a haem group and a copper molecule has been found. The team discovered the unusual binding motif using mass spectrometry: The eighth haem is attached to a specific motif within the peptide chain, resulting in an extended region. The researchers assume that the loop, which results from the altered position of the eighth haem group, rotates the haem out of plane, presumably to facilitate the interaction with the putative electron donor which binds here and allows the electrons to enter. "It is exciting to see how nature manages to get the electrons to come in at one site and then flow to where they are needed," says Hermann, pointing out the resemblance to small circuits.

This knowledge could be of interest for desulphurising flue gases emitted into the atmosphere. The resulting acid rain is damaging to nature and the environment. Although existing methods are quite sophisticated, their main disadvantage is that they generate gypsum in quantities that greatly exceed demand. Every year, around seven million tons of flue gas desulphurisation (FGD) gypsum accumulate and the construction industry only uses about three million tons. The remaining four million are deposited in landfills. MccA could theoretically also become a smart alternative measurement method (sulphite sensor) in the winemaking process. One idea would be to coat electrodes with MccA, which would then enable the sulphur concentration in the wine to be measured and kept at a desired level. Other researchers have used a similar approach with a related multi-haem enzyme (nitrite reductase) to develop a sensor for measuring nitrite concentrations in water.

The more we understand about these oxygen-sensitive enzymes and the role they play in the environment, the easier it might be to prevent environmental damage such as acid rain.

Article

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Further information

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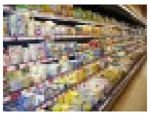

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