A metal enzyme that can cleave benzene rings

Aromatic rings are extremely stable and very difficult to break apart. The Birch reduction of aromatic compounds is a common reaction in organic chemistry and requires conditions that even chemists regard as drastic. Prof. Dr. Matthias Boll from the University of Freiburg's Faculty of Biology and his team work with Geobacter metallireducens, a bacterium that can completely degrade aromatic compounds under strictly anaerobic conditions. While the biological degradation of aromatic hydrocarbons is of global relevance, the chemical resulting from the reduction of benzene rings could also be of pharmaceutical interest.

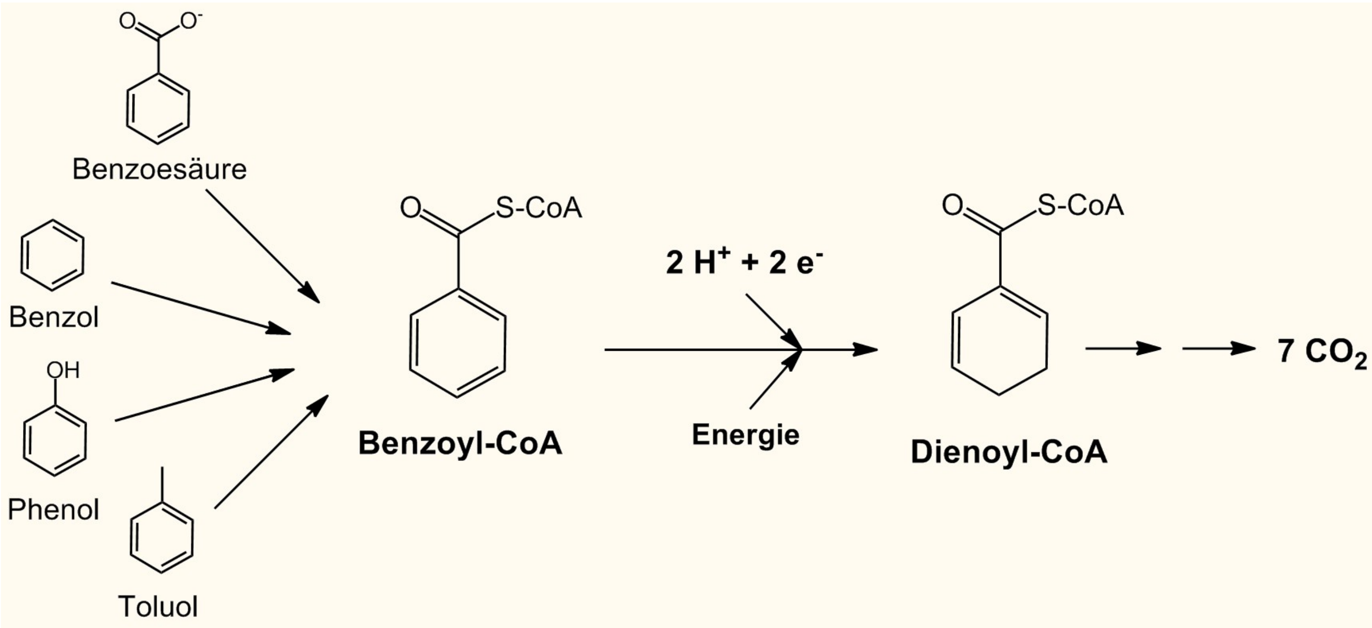
Approximately a quarter of biologically synthesised carbon compounds at terrestrial sites contain benzene rings. Woody plants are the major producers of aromatic compounds; they form lignin, which consists of many benzene rings. Benzene rings are present in aromatic amino acids, and hence every organism. Compounds such as benzene, toluene and xylene are important man-made solvents and as such are present in polymers, paints and pesticides, to name but a few examples. Up until a decade ago, it was thought that aromatic compounds could only be degraded under aerobic conditions. That said, anaerobic conditions prevail in the sediment layers of oceans and lakes where much of the biomass is broken down, suggesting that pathways for degrading aromatic compounds under anaerobic conditions must also exist. However, the catalytic mechanisms that break apart aromatic rings have remained hidden until recently.

Benzene: stable and carcinogenic

Benzene has one ring and no functional groups attached to the six carbon atoms, making it the simplest aromatic hydrocarbon compound on earth. The six carbon atoms are joined together by three conjugated double bonds, which means that the electrons move around the whole system. This is why the aromatic compounds are so stable. "Human beings are unable to make or break down aromatic rings. Only microorganisms are able to do this," says Prof. Dr. Matthias Boll from the University of Freiburg. "However, aromatic compounds are abundant on earth, and their microbial degradation therefore plays a key role in the global carbon cycle."

Man-made aromatic solvents are quite toxic, especially because they are highly soluble in fat and can therefore effectively destroy membrane structures. "Benzene rings are planar, which makes them carcinogenic," says Boll. "Their flat structure means that benzene rings can intercalate in DNA and thus impair correct transcription." Aerobic bacteria can break up aromatic rings with oxygenases in a reaction known as oxidative dearomatisation. Such reactions have an excellent thermodynamic, and oxygen atoms from the air are incorporated into the resulting products. Aromatic compounds with no functional groups attached to them are difficult to cleave enzymatically. "It gets easier once oxygenase enzymes have added hydroxyl groups to the ring," says Boll. "Attaching two hydroxyl groups enables enzymes to break up the ring relatively easily." But what happens where there is no oxygen, such as in water or oil deposits?

Birch in anaerobic bacteria

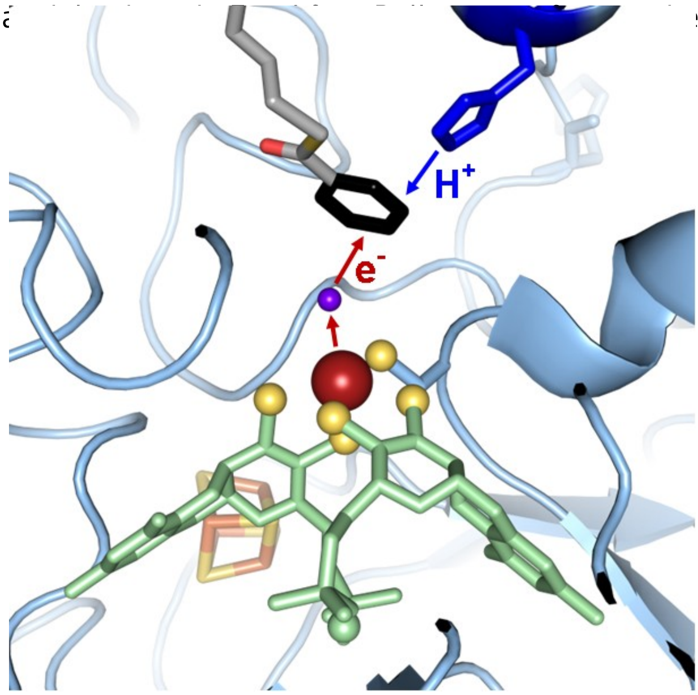


Anaerobic degradation of monocyclic aromatic compounds; intermediary benzoyl-CoA is converted by reductase enzymes into cyclic dienoyl-CoA, which subsequently generates carbon dioxide via biochemical pathways that are characteristic of the degradation of fatty acids.

Boll is working on ways to break up aromatic rings using bacterial enzymes. "If oxidative pathways do not work, reductive pathways will," is his simple solution to the problem. It takes a great deal of effort to overcome the stable system. In 1944, the Australian organic chemist Arthur Birch discovered what is known as the Birch reduction of aromatic compounds, which has since become a major synthetic reaction in organic chemistry. The Birch reduction only reduces an aromatic ring into dienes, rather than right down to cyclohexanes. Dienes are important building blocks for chemical syntheses, which makes the Birch reduction particularly useful in organic chemistry, in steroid synthesis for example. However, the conversion requires relatively harsh reaction conditions, including cryogenic temperatures, alkali metals as reduction agents, and ammonia. If ammonia is not added to the reaction mixture, protons in aqueous solutions would be reduced. "The conditions are far from physiological and much too reactive for biological systems," says Boll who is working on finding alternatives to the Birch reaction which requires the use of elementary sodium in liquid ammonia as extremely low-potential electron donors. This leads to solvated electrons, which are so reactive that they are transferred to the aromatic ring, thereby reducing it. "The enzyme we use does this in an aqueous solution at temperatures of 30°C and a pH of 7. Chemists do not really believe that an enzyme that could survive such conditions exists in nature," says Boll, smiling. Geobacter metallireducens, a soil organism that can oxidise organic compounds with iron oxides as electron acceptors under strictly anaerobic conditions, has a suitable enzyme. However, until recently, the way it works has remained elusive.

Benzoyl-CoA reductase does not require so harsh reaction conditions

In cooperation with researchers from the Max Pla rs discovered that an analogous reaction is catalysed by benzoyl-CoA reductases that play a key role in the microbial degradation of aromatic compounds in anaerobic environments. The researchers isolated and crystallised the two subunits of the enzyme, thus gaining insights into the structure of the enzyme's active centre. They discovered that the enzyme contained a tungsten cofactor that transfers electrons to the benzene.



In the active centre of the enzyme benzoyl-CoA reductase, the tungsten atom (red) is bound to organic cofactor and protein by sulphur atoms (yellow). The electrons are transferred from tungsten to the benzoyl-CoA benzene ring (black) by an as yet unknown tungsten ligand. The protons (H+) stem from a histidine residue on the opposite side.

Tungsten, which is the hardest metal in the natural world, had previously only been known from certain archaea bacteria. It does not occur in humans. The element's redox characteristics make it particularly suitable for transferring electrons at extremely negative redox potentials. The enzyme uses activated benzoic acid benzoyl-CoA with an aromatic ring as substrate. It is an important metabolic intermediate in bacteria that degrade monocyclic aromatic compounds. Boll's enzyme occurs in two conformations, closed involving zinc, and open involving benzoyl-CoA. Boll discovered that these two conformations needed to be antagonistic to keep the enzyme in a closed conformation when no substrate was available. An open conformation would be fatal for the enzyme in an aqueous environment because water molecules would destroy its active centre. "The enzyme opens up when the substrate it requires is present. Substrate binding then induces proton transfer from the solvent to the enzyme's active site by expelling Zn^{2+}," explains the microbiologist.

A particularity of the Geobacter enzyme is the energetic coupling of the reaction, which presents a thermodynamic challenge. Whereas facultative anaerobes, which have a relatively simple enzyme, use ATP as an energy currency to pay for the reduction, complex Geobacter enzymes require much less energy. This is because a block and tackle principle in which the delicate transfer of the electrons from donor to aromatic ring is coupled to an energy-saving, more thermodynamically favourable process. "This process is known as electron bifurcation and is a new energy coupling mechanism in biological systems," says Boll.

From molecular to global scale

Boll was formerly coordinator of the DFG priority programme "Biological transformation of hydrocarbons without oxygen", which specifically focused on cracking aromatic compounds in the absence of oxygen. The more information that becomes available on the anaerobic degradation of aromatic compounds in global carbon cycles, the better the methods for removing pollutants from contaminated soils, groundwater or oil spills will become. "Wastewater treatment plants will in future probably be operated under purely anaerobic conditions," says Boll. This process also has the potential to be used for pharmaceutical applications, for example the enzymatic production of synthetic building blocks for new antibiotics and other medicines. "The enzyme is easy to work with," says Boll, adding, "I am sure that the industry and chemists would be very happy to have a biological

Birch catalyst at their disposal."

Article

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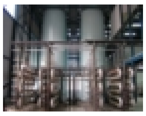

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