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Breaking methane

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Abstract

The most powerful oxidant found in nature is compound Q, an enzymatic intermediate that oxidizes methane. New spectroscopic data have resolved the long-running controversy about Q's chemical structure.

Bacteria that consume methane gas (CH₄) to produce methanol (CH₃OH) using dioxygen (O₂) must break two chemical bonds: that holding the two oxygen atoms together, and one of the extremely strong carbon–hydrogen (C–H) bonds in methane. Knowing how these bonds are broken is central to the development of biological processes for converting methane into liquid fuels. Such processes offer a possible way of dealing with the methane that is wastefully burned or leaked to the atmosphere as a result of the worldwide hydraulic fracturing (fracking) boom. In a paper published on *Nature*'s website today, Banerjee *et al.*¹ report the chemical structure of the molecular species that reacts with methane in the active site of one of the enzymes that converts methane to methanol, soluble methane monooxygenase (sMMO).

Abundant and cheap natural gas is composed primarily of methane, and is a crucial source of fuel and chemicals. Unfortunately, large quantities of natural gas extracted together with oil are burned at some fracking sites to the tune of gas worth US\$100 million being wasted each month². Moreover, some of this methane is vented into the atmosphere, where it acts as a potent greenhouse gas. The problem could be alleviated by converting the wasted gas into liquid fuel at fracking sites, but gas-to-liquid (GTL) conversion of methane requires large-scale, expensive 'Fischer–Tropsch' facilities that are not easily established.

An alternative that has recently attracted much attention is biological GTL conversion using either bacteria that oxidize methane or isolated forms of the bacteria's primary metabolic enzyme, methane monooxygenase³ (MMO). Small-scale biological GTL facilities could be deployed at remote or temporary locations, and offer advantages over Fischer–Tropsch plants because GTL conversion occurs at ambient temperature and pressure; by contrast, Fischer–Tropsch chemistry requires high temperatures and pressures. But substantial increases in the rates of MMO reactions, as well as in the fractions of the carbon and energy present in methane that are converted to product (the carbon and energy efficiencies respectively) are necessary to create a viable technology³. Understanding the details of how MMOs work is germane to making such improvements.

There are two types of MMO, a membrane-bound, copper-containing enzyme⁴ (known as pMMO) and a soluble, iron-containing enzyme (sMMO). The latter belongs to a large

family of bacterial multicomponent monooxygenases that use a pair of iron ions (a dinuclear iron centre) to oxidize hydrocarbons, but it is the only member that can oxidize methane⁵. Extensive studies⁶ over the past 20 years have worked out many details of the catalytic cycle of sMMO. First, the iron ions are reduced from the +3 oxidation state to the +2 state by a reductase protein. The dinuclear iron(II) centre then reacts with dioxygen in the presence of an essential regulatory protein to form peroxodiiron(III) intermediates. Next comes the key step: the oxygen–oxygen bond is cleaved, resulting in the formation of an intermediate called compound Q, which reacts with methane to break a C–H bond. Compound Q is then converted into a complex denoted T.

Compound Q has been investigated using a range of spectroscopic and computational approaches since it was first reported more than 20 years ago⁷. In 1997, Q was assigned a ‘diamond core’ structure consisting of two iron ions bridged symmetrically by two single oxygen atoms⁸. This structure was proposed on the basis of data acquired using a technique called ⁵⁷Fe-Mössbauer spectroscopy, which indicated the presence of two iron(IV) ions occupying similar electronic and geometric environments, as well as X-ray absorption spectroscopic data that showed an unusually short iron–iron (Fe–Fe) distance (2.46 ångströms). However, computational work and studies of synthetic model compounds suggested longer Fe–Fe distances⁶ (2.6 to 2.8 Å), casting doubt on the proposed structure. By the late 2000s, the tide began to turn toward another possible structure, an ‘open core’ containing a terminal Fe(IV)=O unit — a motif found in model compounds that can oxidize C–H bonds rapidly⁹, although not those in methane.

In principle, the true nature of Q could be determined by resonance Raman spectroscopy, which can detect molecular vibrations from the stretching of iron–oxygen bonds; the frequencies of such vibrations provide a fingerprint for how the iron and oxygen atoms are bonded. This experiment is challenging for several reasons. First, intermediate Q forms only transiently, so the spectrum must be acquired in a time-resolved fashion. Second, signals from Q are expected to be weak because solutions of sMMO can be prepared at only low concentrations for analysis, and because of other experimental difficulties.

Banerjee *et al.* overcame these obstacles using a specially designed and optimized Raman instrument. In their set-up, a continuous stream of the diiron(II) enzyme was mixed with a second continuous stream of dioxygen-saturated buffer, and spectra were then acquired at different time points to capture the largest possible quantity of the short-lived Q. By comparing the spectra generated when both atoms in dioxygen were oxygen-18 isotopes (¹⁸O₂) with those obtained using two oxygen-16 isotopes (¹⁶O₂), they were able to isolate Q’s vibration from the sea of other signals. A comparison of the frequency of this vibration with those observed for various iron–oxygen species in model complexes and enzymes gives only one match: the diamond core (Fig. 1). Importantly, the vibration does not correspond to a terminal Fe^{IV}=O species, as would be expected for an open core structure.

To probe how the apparent diamond core forms, Banerjee and colleagues conducted experiments using a mixed isotopic form of dioxygen (¹⁶O–¹⁸O). They observed a new frequency in the spectrum of Q, which can be explained only by a diamond core that contains one ¹⁶O and one ¹⁸O atom, and which indicates that both dioxygen atoms end up in

Q. The spectra also reveal a vibration attributable to the product complex T, which contains one of the dioxygen atoms as a single unprotonated oxygen (an oxygen without a hydrogen atom attached) bridging the two iron ions. Further consideration of the results sheds light on how sMMO breaks the O–O bond to form intermediate Q. The data are most consistent with a mechanism in which the two electrons of the bond are distributed one to each oxygen atom (homolytic cleavage), although it is not possible to completely rule out a mechanism in which both electrons go to the same oxygen atom (heterolytic cleavage).

Further verification of the Q structure is now desirable, and might be obtained from high-level computational studies and additional spectroscopic work. Diiron diamond cores have been previously observed in model complexes that cannot oxidize methane¹⁰, so what is it about Q that enables methane oxidation? One possibility suggested by Banerjee *et al.* is that a different arrangement of the valence electrons of the iron(IV) ions in Q (a high spin state) confers increased reactivity, compared to the low spin state of synthetic complexes. This difference is probably just one of many ways that the enzyme micro-manages the oxidation chemistry to ensure Q's potency.

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Figure 1. The structure of Q

Banerjee *et al.*¹ report the structure of compound Q, a key intermediate in the conversion of methane to methanol by the enzyme soluble methane monooxygenase. Their results indicate that Q's structure contains a 'diamond core' (red) in which two iron ions in the +4 oxidation state (Fe^{IV}) are bridged by oxygen atoms. The numbered groups in black surrounding the diamond core are the side chains of amino-acid residues; H, histidine residues, E, glutamate residues.