Metals in Biology
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Lecture - 29
Methane monooxygenase

Hi, welcome back, today we will discuss Methane monooxygenase, it is a fascinating enzyme and actually it is a class of enzyme. A series of enzyme are having similar activities and these enzymes are quite fascinating perhaps as fascinating in their activity as we have seen for cytochrome P450. These are non heme iron enzyme unlike cytochrome P450, we will follow the books of Principle by of Bio-inorganic Chemistry by Lippard and Berg and also class notes and online available materials by Professor Lippard. So, methane monooxygenase has a diiron center.

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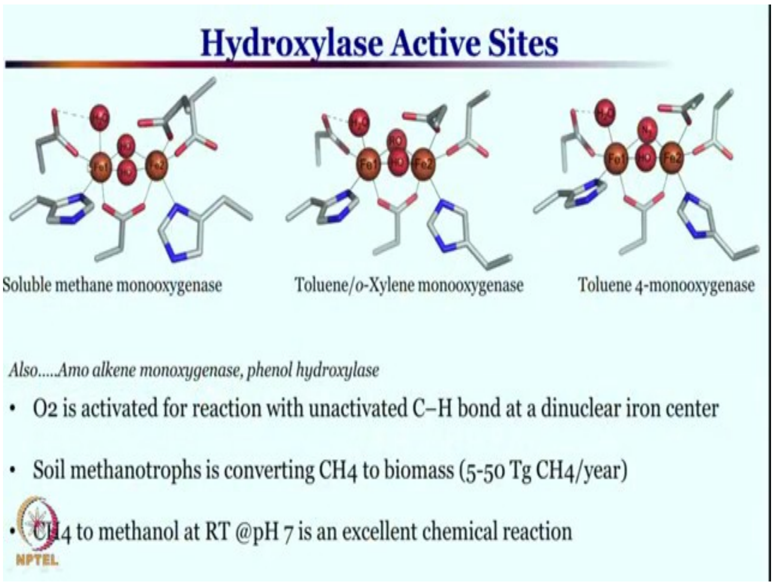

Hydroxylase Active Sites

Soluble methane monooxygenase Toluene/o-Xylene monooxygenase Toluene 4-monooxygenase

Also.....Amo alkene monoxygenase, phenol hydroxylase

- O2 is activated for reaction with unactivated C–H bond at a dinuclear iron center
- Soil methanotrophs is converting CH_4 to biomass (5-50 Tg CH_4/year)
- CH_4 to methanol at RT @pH 7 is an excellent chemical reaction

As you can see over here 2 iron centers are bridged by 2 hydroxy moiety. Of course, they are also linked by these carboxylate group, each of the iron center is having a histidine unit as you see on iron 1 and iron 2. In addition there is also carboxylate linkages as you see over here, clearly these 2 iron sites are unsymmetrical, we have seen such unsymmetrical iron sites in hemerythrin. So, this is responsible for methane monooxygenase; that means, methane is converted to methanol at this site. As you know methane to methanol transformation is the most difficult transformation one can perhaps think of and that is precisely due to the fact that methane is having 104 kcal per mole bond dissociation energy.

This class of compounds or the class of enzymes are not limited for converting methane to methanol, you can have a series of other reaction, even aromatic hydroxylation reaction for instance one can think of taking toluene and convert it at ortho toluene or hydroxylate ok. So, ortho hydroxo toluene can be synthesized, also meta hydroxyl toluene can be synthesized, para hydroxyl toluene can also be synthesized. So, this sort of toluene or xylene as a substrate if the monooxygenase of toluene or xylene is thought of this is the similar active site where in these toluene or xylene will be hydroxylated ok.

Of course let us say for toluene you can have ortho hydroxylation, meta hydroxylation or even para hydroxylation. So, this is the enzyme active site for the toluene para hydroxylation or toluene 4 monooxygenase. Essentially these hydroxylases a group of enzyme which are capable of hydroxylating the organic substrate, it could be aliphatic substrate, it could very well be the aromatic substrate. As you see here these are the crystal structure of different monooxygenase and very little differences are existing among these different crystal structures.

There are also amo alkene monooxygenase which can convert an olefin into epoxide there are phenol hydroxylase which can convert phenol into catechol, but all of them once again will have a di iron active site and their active sites are quite similar with respect to each other. In all these cases oxygen is activated and they are often involved in these very difficult transformations. For the case of methane monooxygenase these oxygen activation is coupled with the unactivated C-H bond activation as well as corresponding hydroxylation chemistry right.

Well what is the source of this di iron center what is well we have soil methanotrophs which is converting methane to biomass and that is a that is one of the source for this methane monooxygenase. By utilizing such soil methanotrophs or there is a huge amount of methane there is huge amount of methane that is converted to methanol. This is 5 to 50 T g methane per year which is overall let us say 1 to 10 percent of methane in the air is converted to methanol by utilizing this method.

There is methane to methanol formation at room temperature under you know under pH 7, let us say on under neutral condition I think this is got to be one of the best reaction perhaps you can see in biological system or anywhere in synthetic chemistry set up. So, we are going to see how methane is converted into methanol.

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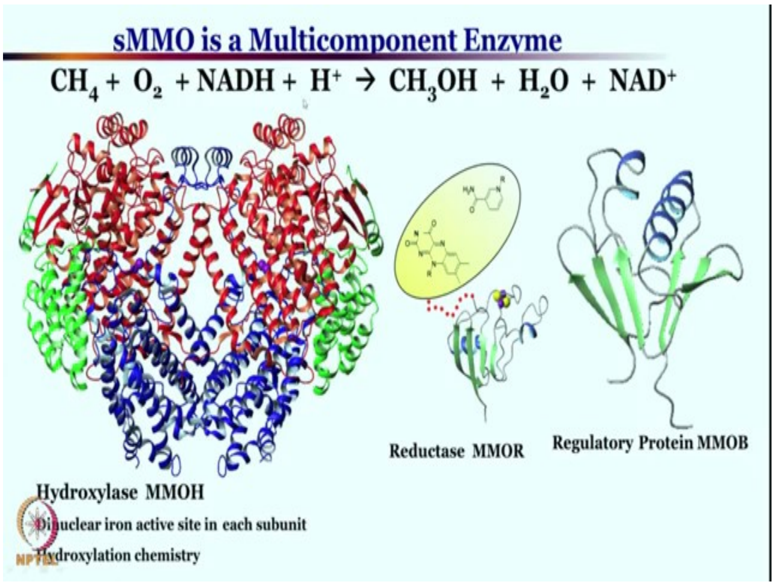

sMMO is a Multicomponent Enzyme

$CH_4 + O_2 + NADH + H^+ \rightarrow CH_3OH + H_2O + NAD^+$

Hydroxylase MMOH

Dinuclear iron active site in each subunit

Hydroxylation chemistry

Methane would require oxygen NADH H+ to form the methanol this is the reaction over here, this is a multi component enzyme the components are Hydroxylase MMOH, Reductase MMOR, Regularity Protien MMOB. If you see at this protein this is a gigantic protein it is having many subunit alpha, beta, gamma, subunits in each of those alpha subunit there is this di iron center which is responsible for converting the methane into methanol; that means, the hydroxylation chemistry is happening ok.

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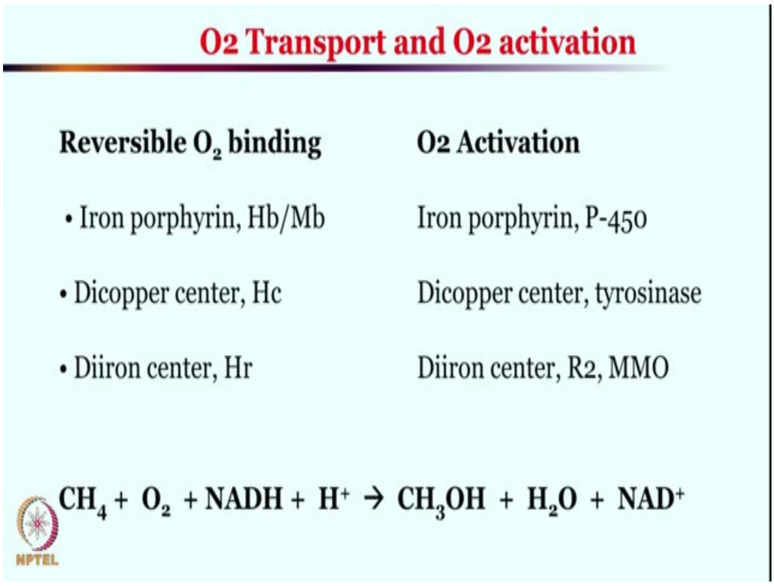

O2 Transport and O2 activation

Reversible O_2 binding	O2 Activation
• Iron porphyrin, Hb/Mb	Iron porphyrin, P-450
• Dicopper center, Hc	Dicopper center, tyrosinase
• Diiron center, Hr	Diiron center, R2, MMO

$$CH_4 + O_2 + NADH + H^+ \rightarrow CH_3OH + H_2O + NAD^+$$

Just to keep it in the perspective you have seen previously the oxygen transport and oxygen activation for with iron pophyrin, we have hemoglobin, myoglobin, which is responsible for reversible oxygen binding right, but utilizing the same iron porphyrin unit with cytochrome P450 we have seen the substrate activation as well as oxygenation chemistry. So, seemingly similar or almost similar active site, but doing completely 2 different jobs, we have seen the dinuclear site such as dinuclear dicopper center in hemocyanin which can activate oxygen or bind it in a reversible fashion and deliver the oxygen in desirable position.

But the same dicopper center is also capable of converting your phenol into catechol or catechol into the quinone. So, those tyrosinase activity by utilizing exactly same species as in hemocyanin we can that dinuclear peroxo side on peroxo species forming in both the cases and in one case it is used for reversible oxygen binding, in another case we have seen oxygen activation as well as substrate hydroxylation chemistry.

For the hemerythrin case we have seen that to diiron II iron centers are involved for oxygen binding and over there in hydro peroxo species is formed, but most interestingly these are non heme diiron center. Similar non heme diiron center can be found as you see in case of methane monooxygenase, but structural similarities are also there these are all dinuclear or di this iron containing active site these are unsymmetrical iron centers.

But in one case as you have seen hemerythrin is capable of converting oxygen into your oxygen into hydro peroxide during the process, but of these bindings are reversible in nature, but in case of diiron center of methane monooxygenase as we will see it will be able to convert oxygen into an useful entity by converting methane to methanol. So, this is what I think are the similarities or the contrast between the reversible oxygen binding as well as oxygen activation.

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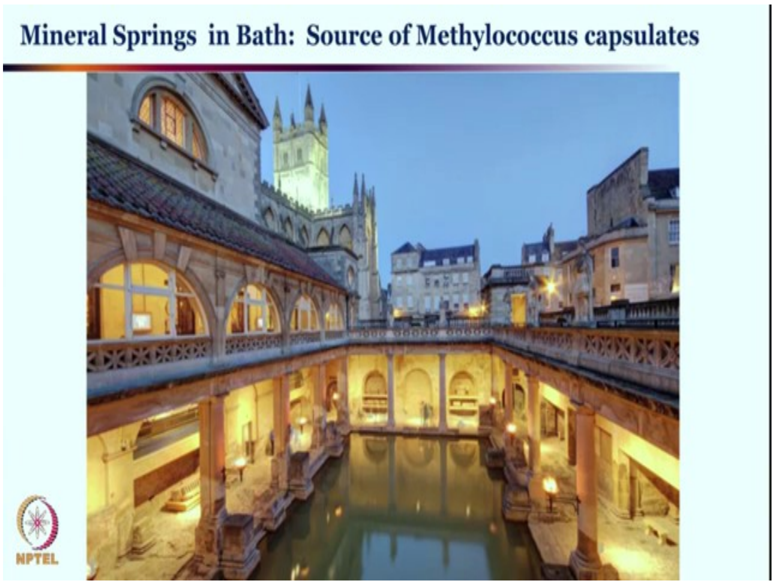

Mineral Springs in Bath: Source of Methylococcus capsulates

Let us look at the resources or source of methylococcus capsulates which is responsible for this methane to methanol formation. These are beautiful location these are the pictures from internet from an England Bath these are the places where people can go and take a bath and these waters are really holy and can convert and can be responsible for curing many skin diseases right. So, this is one of the one picture.

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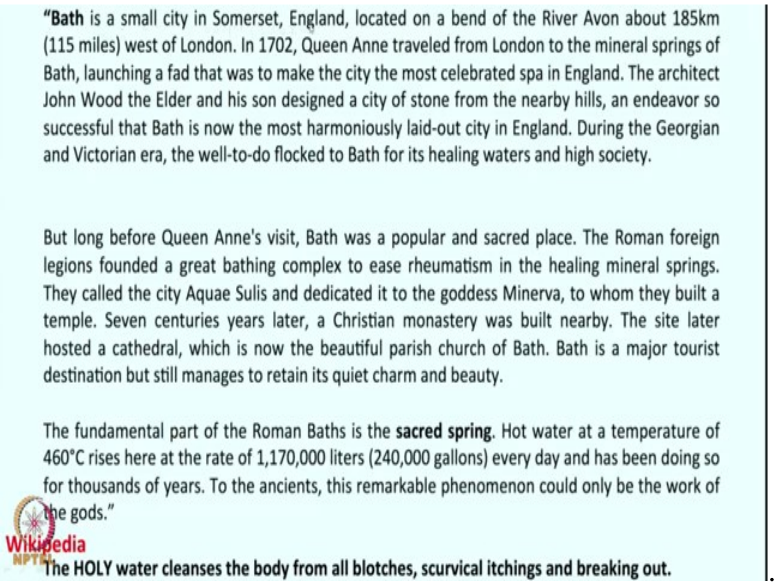

“**Bath** is a small city in Somerset, England, located on a bend of the River Avon about 185km (115 miles) west of London. In 1702, Queen Anne traveled from London to the mineral springs of Bath, launching a fad that was to make the city the most celebrated spa in England. The architect John Wood the Elder and his son designed a city of stone from the nearby hills, an endeavor so successful that Bath is now the most harmoniously laid-out city in England. During the Georgian and Victorian era, the well-to-do flocked to Bath for its healing waters and high society.

But long before Queen Anne's visit, Bath was a popular and sacred place. The Roman foreign legions founded a great bathing complex to ease rheumatism in the healing mineral springs. They called the city Aquae Sulis and dedicated it to the goddess Minerva, to whom they built a temple. Seven centuries years later, a Christian monastery was built nearby. The site later hosted a cathedral, which is now the beautiful parish church of Bath. Bath is a major tourist destination but still manages to retain its quiet charm and beauty.

The fundamental part of the Roman Baths is the **sacred spring**. Hot water at a temperature of 460°C rises here at the rate of 1,170,000 liters (240,000 gallons) every day and has been doing so for thousands of years. To the ancients, this remarkable phenomenon could only be the work of the gods.”

Wikipedia

The HOLY water cleanses the body from all blotches, scurvical itchings and breaking out.

So, Bath this is a small city in Somerset, England, located on a bend of the river Avon about 185 kilometer west of London, but most importantly this is a very nice touristic place, well as you can read from the Wikipedia or any other sources this is a beautiful place where many tourist visits and spend quality time with friends and family.

The fundamental part of these Roman baths which are existing in England bath Somerset are the sacred springs right. Hot water at a temperature of 460 degree C rises here in this sacred spring at the rate of this 24000 gallons every day and has been doing. So, for thousands of years right to the ancients this remarkable phenomenon could only be the work of gods. So, if you are believing in the holy man or sacred water this is the sacred water you can think of and there is no wonder that these are the sources for methylococcus capsulates which is responsible for the methane to methanol conversion.

This holy water cleanses the body from all blotches scurvical itchings and breaking out. So, any sort of skin disease can be recovered or can be can be cured by this holy water. So, if you see that ancient time or even present time the holy water sprinkle on your on someones body and then miraculously the patients got cured or the skin disease or other problem got cured and this is due to the great water which can perhaps have this methylococcus capsulates there is a strong science behind it and you we are going to see what is these active sites and how active they are and how they are functioning. Few more pictures from the mineral springs in Bath England.

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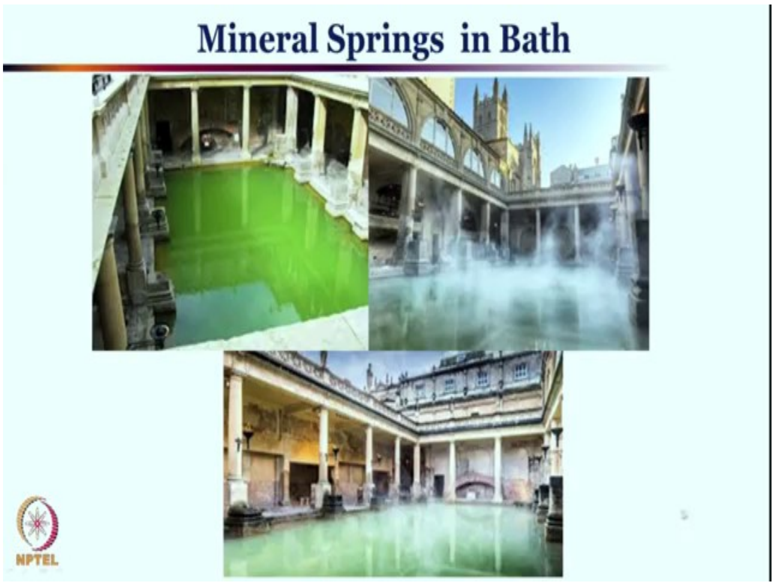

Mineral Springs in Bath

So, this is as you can see really you know wonderful atmosphere being created it is great to take bath in these places and have nice time over there, as well as it has extreme benefits of the mid and have you know medicinal value ok. So, if you are looking for a great time and wants to solve some of your skin diseases perhaps this is the place to visit and do enjoy your time all right. Another quite exciting stuff that is included or that we can discuss about this methanotrophs bacteria is their ability for bioremediation.

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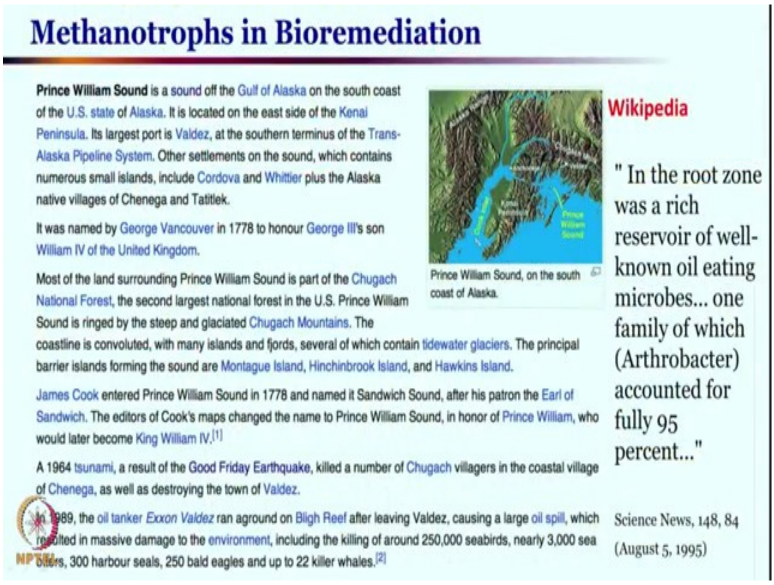

Methanotrophs in Bioremediation

Prince William Sound is a sound off the Gulf of Alaska on the south coast of the U.S. state of Alaska. It is located on the east side of the Kenai Peninsula. Its largest port is Valdez, at the southern terminus of the Trans-Alaska Pipeline System. Other settlements on the sound, which contains numerous small islands, include Cordova and Whittier plus the Alaska native villages of Chenega and Tatitlek.

It was named by George Vancouver in 1778 to honour George III's son William IV of the United Kingdom.

Most of the land surrounding Prince William Sound is part of the Chugach National Forest, the second largest national forest in the U.S. Prince William Sound is ringed by the steep and glaciated Chugach Mountains. The coastline is convoluted, with many islands and fjords, several of which contain tidewater glaciers. The principal barrier islands forming the sound are Montague Island, Hinchinbrook Island, and Hawkins Island.

James Cook entered Prince William Sound in 1778 and named it Sandwich Sound, after his patron the Earl of Sandwich. The editors of Cook's maps changed the name to Prince William Sound, in honor of Prince William, who would later become King William IV.[1]

A 1964 tsunami, a result of the Good Friday Earthquake, killed a number of Chugach villagers in the coastal village of Chenega, as well as destroying the town of Valdez.

In 1989, the oil tanker *Exxon Valdez* ran aground on Bligh Reef after leaving Valdez, causing a large oil spill, which resulted in massive damage to the environment, including the killing of around 250,000 seabirds, nearly 3,000 sea otters, 300 harbour seals, 250 bald eagles and up to 22 killer whales.[2]

Prince William Sound, on the south coast of Alaska.

Wikipedia

" In the root zone was a rich reservoir of well-known oil eating microbes... one family of which (Arthrobacter) accounted for fully 95 percent..."

Science News, 148, 84 (August 5, 1995)

Let us look at this Wikipedia information on Prince William Sound, which is off the Gulf of Alaska on the south coast of the US state of Alaska. It is located on the east side of the Kenai Peninsula, it is a largest port of Valdez and you can read more from Wikipedia or from here. In 1989 the oil tanker Exxon Valdez ran aground on Bligh Reef after living Valdez, causing a large oil spill in the Prince William Sound area, which resulted in massive damage to the environment, including the killing of around 250000 seabirds, nearly 3000 sea otters, 300 harbour seals, 250 bald eagles and up to 22 killer whales right.

That is quite amazing number I mean quite frustrating quite devastating number right if you are looking at this number the oil spill that has caused or that has happened in 1989 by e or from this Exxon Valdez this was quite devastating and it has long reaching impact on environment. It has polluted that area quite naturally for long time to come, but if you are looking to for bioremediation I think this is when methanotrophs comes into the picture and these bacteria are grown over there and overall it was possible over time to make this place a suitable place as it was before.

Well, what was found that in the root zone was a rich reservoir of well known oil eating microbes one family of which accounted for fully 95 percent recovery of this oil spill. So, therefore, natural disaster or unnatural disaster like this one as you have just seen little bit perhaps can be taken care of by this methanotrophs bacteria by converting the alkane or oils into the corresponding let us say alcohol products and setting of further degradation over long term. So, this methanotrophs bacteria as you have seen are found in one of the source could be this you know Bath England, but they are having capability of bio remediation which is wonderful right. Now let us look at the active site of these crystal structures ok.

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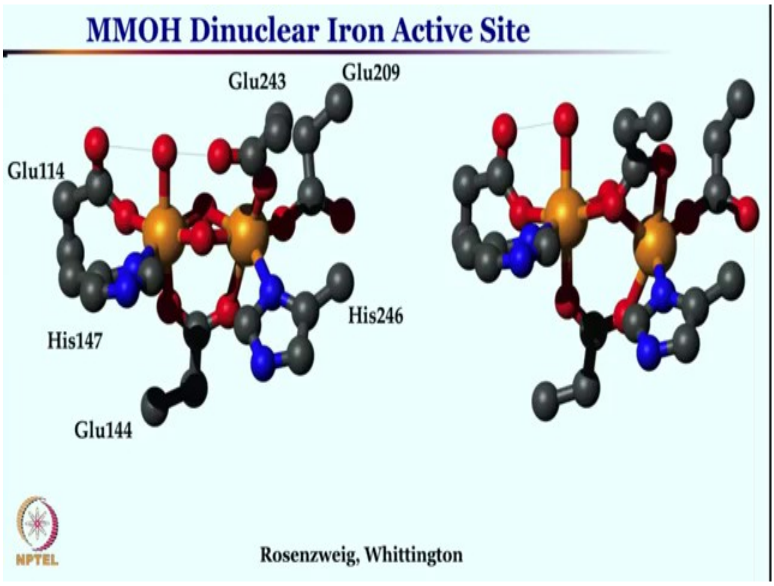

MMOH Dinuclear Iron Active Site

Rosenzweig, Whittington

If you are looking at this active site, you see that there are 2 iron center, they are bridged by these 2 dihydroxo unit, here is a water molecule as you have seen there is a glutamate unit it is a mono coordinated glutamate along with an histidine along with a histidine coordinated with this iron center. This iron center is not exactly same as same as this iron center here you see the 2 terminal glutamate in this case it was just only one glutamate that was present as you see that there is histidine as well and both the iron centers are bridged by this glutamate 144 this is phenomenal and quite fantastic crystal structure right.

In cases where it is also possible in the reduced form when one of the hydroxo is missing and then this glutamate one of this glutamate can have the by coordinating nature and also can be relevant in the catalytic cycle these are the resting state of the catalytic cycle. So, as you see over here clearly methane monooxygenase dinuclear active site looking somewhat similar to what you have seen in heme erythrin where these 2 iron centers were supported by the histidine side 3 on one side 2 on other side, but here you have you do not have all the histidine side. But you have the glutamate replacing some of those histidine side, but quite naturally there is similarity between hemerythrin and this methane monooxygenase active site.

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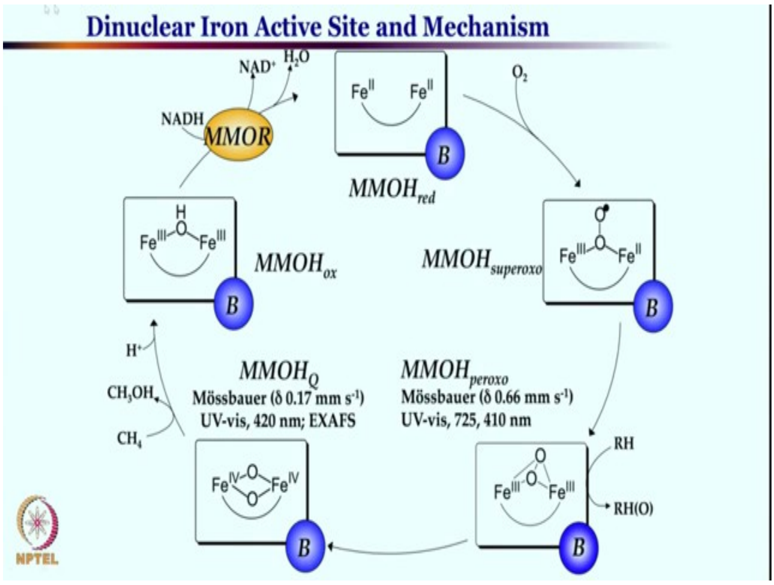

If you look at the overall activity by this active site you will find that this bridging a diiron center which is abbreviated over here in this form where this is the reduced form you start with both the iron center in +2 oxidation state oxygen, molecular oxygen is reacting with this diiron center to give the superoxo species, where one electron reduction from one of these iron center is happening giving rise to the iron III iron II superoxo intermediate.

This oxygen is singly reduced right now so, this is MMOH reduced form, this is MMOH superoxo form. Now from there on you one can think of transferring another electron to this oxygen moiety which is now superoxo upon getting reduced by one electron from the second iron center you will see that both the iron center are now in plus 3 oxidation state and the oxygen moiety is reduced by 2 electron to peroxo. Not too much characteristics spectroscopic character are data are available for this intermediate nonetheless this peroxo intermediate is suitably characterized. For example, Mossbauer spectra are Mossbauer data are quite definitive of these iron III species and UV visible spectra have the characteristic 725 and 410 nanometer once again indicating such a species is existing.

Now this is the species one can think of introducing the substrate 2 and substrate can get hydroxylated by utilizing this peroxo species indeed many studies has been done upon generation of the species and quick study shows that this is capable of converting RH substrates sp3 C-H bond can be hydroxylated to corresponding the hydroxylated product right. Well from this side on peroxo intermediate one can think of or one can characterize this bismi oxo species which is a iron IV, iron IV bismi oxo species here most where spectroscopic data as well as the UV visible and EXAFS data are consistent with this intermediate which is known as intermediate Q, MMOH Q.

So, this intermediate quite interestingly both of the iron are having iron IV as you have seen in this previous case this was iron III this is a mixed valance scenario and have not much proof in terms of the reaction mechanism or reaction intermediate study.

Now, this intermediate once it is formed can react undoubtedly with methane to methanol. So, this is this process we are interested in following up too much of course, it has to follow stepwise this is the real active species which will convert methane to methanol and from there on the hydroxo intermediate it generated, MMOH ox which can then in presence of NADH and reducing equivalent can it gives rise to the original compound as we have seen over here. So, these dinuclear iron active sites are quite fascinating as you can see and can convert methane to methanol.

If we are to follow up this procedure or these steps over here we will see that the diiron IV+ oxidation state intermediate will be reacting with methane to form methanol, but there will be an intermediate getting generated into the process.

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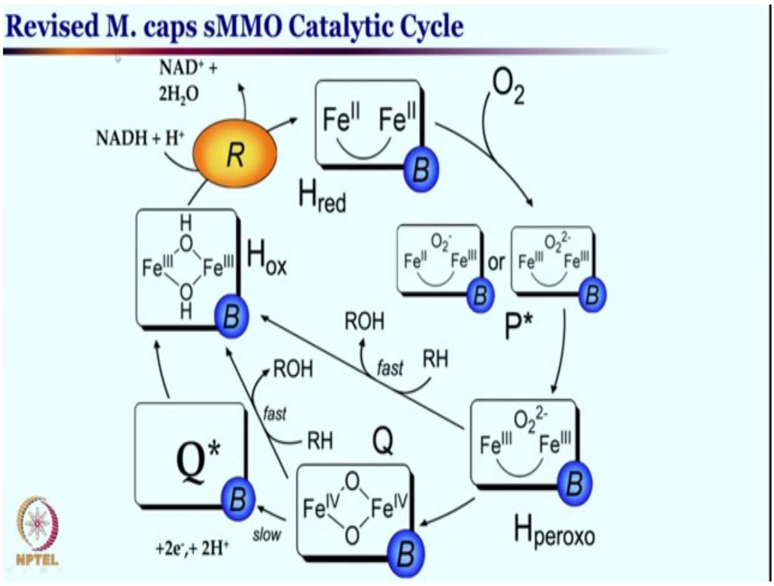

Revised M. caps sMMO Catalytic Cycle

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Let us look at the modified mechanism which has been recently which has been corrected little bit where in you see that almost everything remains similar, where iron II;

iron II reacting with oxygen to give you the iron III peroxo intermediate or a or a or a iron III iron II superoxo intermediate from there on it can undergo further reaction or electron transfer to give you the iron III iron III peroxo intermediate.

So, these are peroxo intermediate can directly give rise to the substrate reactivity from RH to ROH. So, that could be one of the possibilities, but likely another possibility that can become in that can be coming into the picture is the oxygen oxygen bond cleavage of the peroxo to give the iron IV oxo species. Now this iron IV oxo species can then react with substrate methane for example, to give an intermediate the characteristics of which is not let us say known so far subsequently it can go and form the diiron III dihydroxo bridged intermediate. So, some of these intermediate remains same; however, these new intermediates such as Q star is important as well it is important to know that these H peroxo.

These peroxo intermediate can directly react with RH and to give you ROH without perhaps formation of these intermediate. So, far we have seen that these methane monooxygenase enzymes are having the dinuclear iron center and it is supported by this side chain of glutamate and histidine and the bridging ligand such as dihydroxo. These units are capable of doing or converting methane into methanol with the help of the other subunits which are essential part of this catalytic cycle, but most importantly there is this reaction mechanism as you have seen in the previous slide where in this diiron center is reacting with oxygen to give rise to the superoxo then peroxo.

Now, the revised version of this mechanism says that this the peroxo can directly be linked with this MMOH ox intermediate where it is a dihydroxo intermediate from there on the rest of the catalytic cycle can be completed. Alternatively this peroxo intermediate can gives rise to an intermediate diiron IV mu oxo intermediate which we were seeing. This intermediate can further react to give a meta stable intermediate which is once again a very reactive intermediate which can then go on to form the iron III dihydroxo intermediate.

So, in the next class we will try to see how these studies are done to better understand these intermediate that is forming over here this iron IV di dioxo intermediate is quite quite reactive we will see the similarity of the reaction of this diiron IV mu oxo species with that of the copper 3 bismuoxo or mu oxo intermediate. Well, keep studying we will come back soon later on in the next class discussing the mechanism of this reaction and how people have studied this reaction mechanism in greater detail.

Thank you very much.