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PROFESSOR: Good morning everybody. Today we're going to continue our journey in looking at the 10 sort of basic types of reactions that you will encounter when studying basic metabolism. And they're all found on your vitamin bottle.

So here are the Flintstones vitamins. I like Flintstones vitamins because you don't have to swallow them. You can chew them. And today what I'm going to start talking about is redox chemistry. What are the two redox active cofactors?

And what we will focus on today is vitamin B2, which is riboflavin, and vitamin B3 which is niacin. So we're going to talk about oxidation and reduction. And what the cofactors are that involved in these transformations in all primary metabolic pathways.

So the first thing I want to do is introduce you to these two cofactors, the structure of the cofactors. Then what I'll do is focus on a comparison of their properties. And then I want to focus on the mechanism of how these cofactors work. And once you master these concepts, you really will encounter them over and over again throughout your journey in metabolism. And you'll be familiar with the kinds of chemical transformations that are occurring.

So the first cofactor comes from niacin. And it is nicotinamide adenine dinucleotide in the reduced state. Or nicotinamide adenine dinucleotide in the oxidized state. And as with all cofactors, the vitamins are a precursor to the cofactor, and they have to be converted inside the cell to the active form of the cofactor.

So if you look at NADH-- let me draw that structure for you, and then I'm going to show you that while the structure is quite complex, most of it is not involved in the chemical transformations. And so we'll put a big R group on most of the cofactor. And you don't have to memorize the structures of any of these cofactors. And that you will be given the structures whenever you have any kind of exercise to carry out.

So the form of the vitamin is actually a nicotinamide ring. And then it needs to be metabolized to the structure I'm drawing now which has the nicotinamide ring attached to a ribose ring,

which is then attached to ADP, which you're all familiar with. And I'm just going to abbreviate the adenosine part of a molecule with an AD.

So this part of the molecule we're going to call for the rest of this exercise today the R group, because it's not involved in the chemistry. And really, all of the chemistry happens in this part of the molecule. This is the business end, and this is the reduced form of the cofactor, NADH.

So in addition to the reduced form, since we're doing oxidation and reduction, you need to know what the oxidized form is. And the oxidized form is the following where you need to lose a couple of electrons and a couple of protons out of this ring to generate this nicotinamide ring. And again, this part of the molecule is indicated as R, and then you retain a positive charge. So again, this is the part of the molecule we're going to be focusing on. And this is the oxidized form of the cofactor.

So what I want to do now is show you how this compares to the second form of the organic cofactor that you encounter over and over again in metabolism. And this is FADH₂, or FMN_{H₂}. And that's the reduced form, versus FAD or FMN. So this is one of the most complicated of all the cofactors. And you'll see that it has a very complex ring structure called an isoalloxazine ring-- which I'm drawing now-- that most of you have likely never encountered before.

And when you look at the structure of this molecule, you probably will have no idea about where the chemical reactivity is. And I'll show you where the chemical reactivity is and how we know that in a few minutes. So attached to this nitrogen is more complex structure with a sugar where x can be phosphate. This is called the flavinmononucleotide, reduced. This is the reduced form.

And if x is equal to ADP, this is flavinadenine nucleotide reduced. And I want to point out that ADP is present in both NADH and FADH₂, as it is present actually, in coenzyme A and in ATP. So ADP plays a central role in almost all the chemical transformations, the 10 reactions we actually talk about that are central to all of primary metabolism. However in this case, again, this part of the molecule we're going to call R, is not involved in the chemistry. So the business end of the molecule is indicated here.

And so this is the business end, in this is the reduced state. So this cofactor has to be able to toggle between the reduced state and the oxidized state. And if you look at the oxidized state-- so I'm going to indicate this whole part of the molecule is R-- you could see a change in this

part of the isoalloxazine ring. That is you've lost two protons and two electrons to form the oxidized form of this cofactor.

Now I also want to give you the nomenclature for the isoalloxazine ring because I'm going to tell you where the chemical reactive positions are. And so you need to know the numbering of the ring. And so this is the N1 position, this is 2, 3, 4.

This carbon between these two positions is called the C4A. This is carbon C4. And this is the N5 position. And we'll see that C4 and N5 become important in the overall chemistry of the transformation. So these are the two cofactors.

Again, this one is considerably more complex than this one. But what I want to do, and you'll see both of these cofactors used over and over again in break down or biosynthesis. The sugars breakdown or biosynthesis of fatty acids. The question is when are they used and what is the distinction between these cofactors?

So what I want to do is compare the properties of these two cofactors and then specifically give you an example of how each of these cofactors work. So on one side I'll have NAD/NADH, and the other side will have FAD, again, the oxidized form, and FADH₂, the reduced form. So we have NAD/NADH FAD/FADH₂.

And I want to compare these two cofactors. And the first thing I want to compare is the chemistry. And what we will see is NAD/NADH always does 2 electron chemistry all of the time. On the other hand, FAD/FADH₂ can do either 1 electron chemistry or 2 electron chemistry. And in fact, function of flavins inside the cell is the major mediator between something that can donate 2 electrons and can something that can accept only a single electron.

So something they could donate 2 electrons would be NAD/NADH, which I'll show you in a minute, and something that can accept only a single electron, or like metals. Like, if you think back to hemoglobin, and you think about the ion 3 state going to the ion 2 state. So there's a big distinction between the two.

The second thing is since were doing oxidation and reduction, we need to think about the reduction potentials. And if you think about the reduction potential of NAD NADH, it turns out to be minus 320 millivolts. And what you need to recall from freshman chemistry is that the more positive the number, the easier it is to reduce. So the fact that this is minus 320 millivolts tells you that NAD likes to be in the oxidized state, the NAD state.

On the other hand, FAD/FADH₂ can vary between minus 450 millivolts to plus 150 millivolts. So this is a huge difference in reduction potential, 600 millivolts. So why is that true? It turns out that if you look at the hundreds of enzyme structures that we now have-- we've gone through a structural revolution in the last 15 years-- all of the structures where NAD/NADH bind are homologous to each other. And in fact, these structures don't have anything designed into the structure where they perturb the redox potential.

The redox potential remains the same, regardless of what enzyme time it's bound to. On the other hand, flavins don't maintain a single type of binding site. They have five or six different kinds of binding sites. And that's because the flavin chemistry, as you can see from this isoalloxazine ring I introduced to you just a minute ago, does much more diverse chemistry. And in fact, the active site can tune the redox potential so it facilitates either 2 electron chemistry or 1 electron chemistry or a combination of the two.

So the next thing that's really important in thinking about the two cofactors and their differences is that NAD/NADH can act as a substrate. That is, NAD/NADH is any D, for example would act as an oxidant of some second substrate. Every time you have an oxidant, you have to have something that's going to be oxidized. And during turn over of oxidized reduced states, the substrates and products must come on and off the enzyme. So they dissociate in every single turn over.

FAD/FADH₂ on the other hand, is always tightly bound or covalently bound to the substrate. And so that means that if FAD gets reduced to FADH₂, it doesn't disassociate. So you need to have a way in the active site of the enzyme where the FADH₂ can be reoxidized. And again, that's quite distinct from NAD/NADH.

The fourth thing I want to talk about just briefly-- and this is what I'm going to focus the rest of this little section on-- is the mechanisms by which these cofactors work. And so out of all of the cofactors you're going to be introduced to in this little this section in general on the 10 basic reactions, this is probably the simplest. And NAD/NADH involves hydride transfer and proton transfer. So hydride is a hydrogen with a pair of electrons and a proton. And we'll see that in a minute.

With FAD/FADH₂ the chemistry is considerably more complicated. But what I will show you is that it can also do hydride transfer. And when it does hydride transfer, it does it to the N5 position. So if we go back for a second and look at the flavin in the nomenclature, this is the

N5 position.

I also pointed out you can do C4A chemistry, which is in your lexicon, but we're not going to discuss further today. So you can do N5 chemistry by hydride transfer. And you can also do C4A chemistry, which you will see later on when you start talking about primary metabolic pathways.

So what I want to do now is focus on a specific example and show you what the basic chemistry is with these two systems focusing on hydride transfer of chemistry. So an example I've chosen to briefly look at is what I would call an ending to the glycolysis pathway. Glycolysis, as you already know, is breakdown of sugar under anaerobic conditions.

So under aerobic conditions, what happens is when all this energy is given off when you break down your sugar, it's stored in chemical bonds, NADH. The NADH then goes into the respiratory chain. And the respiratory chain ultimately transfers this energy to convert oxygen into water, which really releases a huge amount of energy, which is then trapped to make the energy currency of the cell, ATP.

So we're under anaerobic conditions. And we need to be able to recycle and keep. Because the NAD/NADH comes off and on the enzyme at every single turnover, you really need to be able to recycle the reduced form back to the oxidized form. So glycolysis can actually continue.

So what happens at the ending, one anaerobic ending of glycolysis, is you need a proton plus NADH, so that's the reduced form that's generated during glycolysis, plus the molecule called pyruvate. This is a molecule you're going to see over and over again over the course of the semester whose structure you need to remember. So this is the reduced state and this is the oxidized state. And NADH is going to reduce this ketone to an alcohol. And it itself becomes oxidized.

NADH loses a hydron and a proton to form NAD, and you generate lactic acid where this hydrogen from the NAD as a hydride is going to get transferred to the carbon of the carbonyl. And this proton in some way comes from solution, and has picked up a proton from the active site of the enzyme.

Now another point I wanted to make, which you will encounter again over the course of thinking about coupling reactions in metabolism to make them favorable, is that with NAD/NADH cofactor, you always have to think about the proton. So you need a proton to have

a balanced equation. That means that if you're at pH 6 versus pH 8, the concentration of the species changes dramatically. And that can affect where the equilibrium of the overall reaction lies. So you need to keep in the back of your mind there's a proton with NAD/NADH systems.

And so this is NAD, but it turns out that flavins can also catalyze the same type of reaction. So in the case of this particular enzyme, it's found in eukaryotic systems, it's found also in prokaryotic systems. But in the case of the flavin dependent enzymes, it's only found in bacterial systems. And so their reaction goes more favorably in the direction I'm writing it now, but again, it's an equilibrium reaction where you take lactate-- so this is now the reduced state. And so you now have FMN.

So this is the form of the cofactor that's used with this specific enzyme, but the chemistry is same with FMN and FAD. And so this needs to get oxidized. And it gets oxidized to form pyruvate. And the FMN gets reduced to form FMNH₂.

So both of these are capable of catalyzing the same redox chemistry. And this is consistent with what I've told you before about the redox potential. So NAD/NADH is always in this region. But you can see that FAD/FADH₂ also covers that region as well.

So many of the chemistries between these two cofactors can be overlapping. And you'll see this in for example, fatty acid oxidation and fatty acid biosynthesis. And it really is not clear at all which one of the redox chemistry you could use. Nature, in fact could use different cofactors in different organisms. But we know what the cofactors are in human systems because we purified all the enzymes and study the cofactor requirements.

So this is the reaction I want to focus on. And what I forgot to tell you to maybe make this more relevant to some of you I guess, since this is what you do when you go to college nowadays, is most of you have heard about yeast, that under anaerobic conditions make you a friend, to make you happy and cheery when you're having tough times at MIT, you want to drink some ethanol. Yeast are able to convert glucose all the way down under anaerobic conditions into ethanol, and in fact uses the cofactor NADH to in two steps, convert pyruvate into a molecule called acid aldehyde. And then the NADH converts the acid aldehyde into ethanol, which is the major ingredient of beer and wine.

So what I want to do now is very briefly then focus on the chemistry of how NADH actually works. So if we're now going to look at the chemistry of this and the mechanism, let's start with pyruvate. And you need to go back and you need to think about the carbonyl chemistry you've

been introduced to when talking about the claisen reaction and the aldol reaction. And we spent a lot of time on carbonyl chemistry because it's central to everything in biochemistry.

And so you remember that a carbonyl is polarized where the electrons like to result on the oxygen, leaving a deficiency of electrons on the carbon of the carbonyl. And so one can draw a resonant structure. This is the predominate structure, but these resonance structures often tell you something about where the molecule is most reactive.

And so what we've done is oxygen is electro negative and likes to can accommodate electrons in its orbitals. So it forms a resonance structure like this leaving behind electron deficiency at the carbon. And so we want to reduce this carbonyl to an alcohol. And this is done with the reduced form of the cofactor. So again, in every redox chemistry, this is the oxidized form, this is the reduced form.

And so now the question is, how does this chemistry happen? So let me point out here-- and this is something you will hear about over and over again in basic metabolism-- but these two hydrogens-- and this is the 1, 2, 3, 4 position of the nicotinamide ring are not equivalent. And why aren't they equivalent? They're not equivalent because all of the chemistry happens in the active site of an enzyme that is chiral. So these hydrogens are prochiral.

And because they sit in a chiral environment, that makes these hydrogens act like they're chiral. So all chemistry with NADH is stereo specific. Depending on the active site, only one of these two protons is involved in hydride transfer.

So if you look at the chemistry now, we're set up to deliver a proton with a pair of electrons, that's a hydride, to this carbon of the carbonyl. See we have a hydride transfer. And now we have the electron density sitting on the oxygen. And so likely then in the active site of the enzyme you have a general acid catalyst which is able to then donate a proton. And remember I told you that NAD/NADH involves hydride transfer and proton transfer.

So what you generate then is OH, where this hydrogen comes from a general acid catalyst in the active site. And you have the hydride transfer coming from the NADH to generate lactic acid. And now what you've generated is the oxidized form of the NAD.

So reduced form oxidized, reduced form oxidized. So this is the general reaction and all NAD/NADH enzymes involves this simple type of [INAUDIBLE], protons, hydrides, and protons.

Now what I want to do is show you one of the many types of reactions that flavins can undergo. And again we're focusing on hydride transfer reactions. And a recall for you that again, the redox potentials of the flavins in the NAD/NADH overlap.

So this is always minus 320. But this has a span of redox potentials. And in fact, what you will see when you look at basic metabolism is often FAD is reduced by NADH. And so these two partner up to do chemistry. And so what I want to do now is show you the chemistry with the flavin and NADH.

If we go back and we look at our oxidized form of the flavin, and again, I'm not going to draw out all the structure. And remember there's a lot of spinach here and there's also some spinach here. This is the business end of the molecule. And remember I told you the business end of the molecule is the N5 and the C4A position. And so now we have our NAD. NADH, sorry.

So this is the oxidized form, this is the reduced form. And so NAD/NADH goes through a hydride proton transfers, does it stereo specifically. And the question is, where does the chemistry happen with the flavin? And the chemistry happens at stereo specifically. So each enzyme will be different.

You can figure out what the stereo chemistry is at the N5 position. So here is again, the N5 position. So you have hydride transfer to the N5 position. And now you can pick up a proton or not at this position.

I'm going to show you the PKA. If you go back and you look at the handout in the lexicon, you'll see the PKA of this nitrogen is under physiological conditions, about 7. So it can be protonated or not protonated.

And so what then happens is you end up with the reduced form where this hydrogen is transferred to the N5 position, and you could pick up a hydrogen here, from AH leaving you with A. So this proton, this may or may not be protonated depending on the active site of the enzyme. And so then what you also have here is the oxidized form of NADH.

Now those of you who were paying attention to what I was just saying with the reduction of pyruvate to lactate, I just went through this whole big spiel about how you wanted to take advantage of the fact that carbonyl is a polarized delta plus delta minus, and that the nucleophile, this case the hydride, is always being transferred to the electron deficient carbon,

not to the oxygen that already has a lot of electron density. So you might be asking yourself well, why doesn't that hydride get transferred to this position? This is a position, if you look at this, and you take it out of context of the isoalloxazine ring. This looks remarkably similar to this carbonyl. And in fact, this is where your chemical intuition falls apart.

And you need to know that you don't have chemical intuition about those people that spent 40 years studying isoalloxazine rings. And this is a complex heterocyclical molecule where I think most chemists, when looking at this for the first time, had no clue as to what the reactive positions were and what the chemistry was. And they spent, in the 1970s and 1980s, spent a decade figuring out the chemistry of flavins. And so I'm telling you what the answer is. There is experimental evidence that supports this that in the case of NADH, hydride is transferred to the N5 position to give you the reduced form and the oxidized form of NAD.

So I hope I've gotten you excited interested about the two redox active cofactors that you're going to encounter over and over again during your journey through primary metabolic pathways, FAD and NAD. And I hope I've taught you something about the chemistry of how these two cofactors work and you'll get plenty of practice with these cofactors over the rest of the semester.