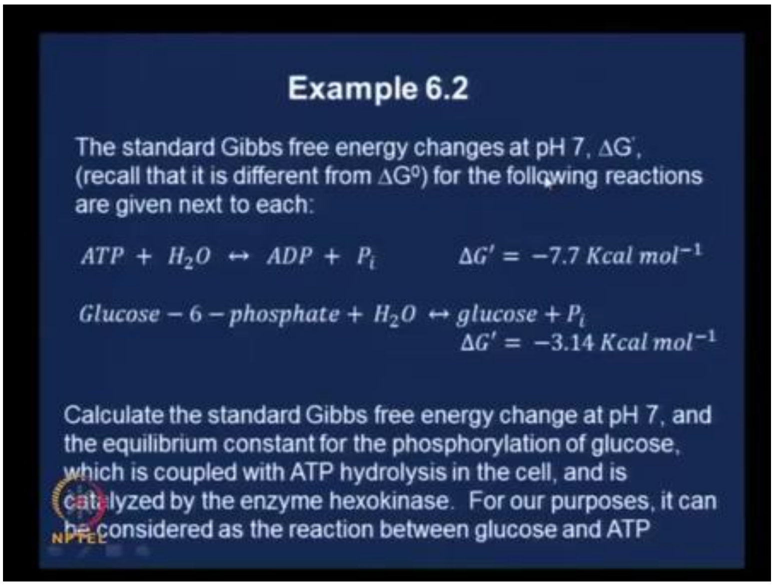
Thermodynamics for Biological Systems:
Classical and Statistical Aspects
Prof. G.K. Suraishkumar
Department of Biotechnology
Indian institute of Technology - Madras

Lecture – 51
Free Energy Changes for Some Bioreactions

Welcome back!

We are looking at reaction equilibria, and we are working out some examples to make sure that we are all in the same plane, in terms of comfort in dealing with the quantities related to reactions. For this, we have looked at some of the concepts that were learnt in the earlier classes. And we are in the middle of working out a set of three examples. We worked out one in the last class, we will work out two, or you want to work it out I am going to give you enough time to work it out in this class.

(Refer Slide Time: 01:01)



Example 6.2

The standard Gibbs free energy changes at pH 7, $\Delta G'$, (recall that it is different from ΔG^0) for the following reactions are given next to each:

$ATP + H_2O \leftrightarrow ADP + P_i \qquad \Delta G' = -7.7\ Kcal\ mol^{-1}$

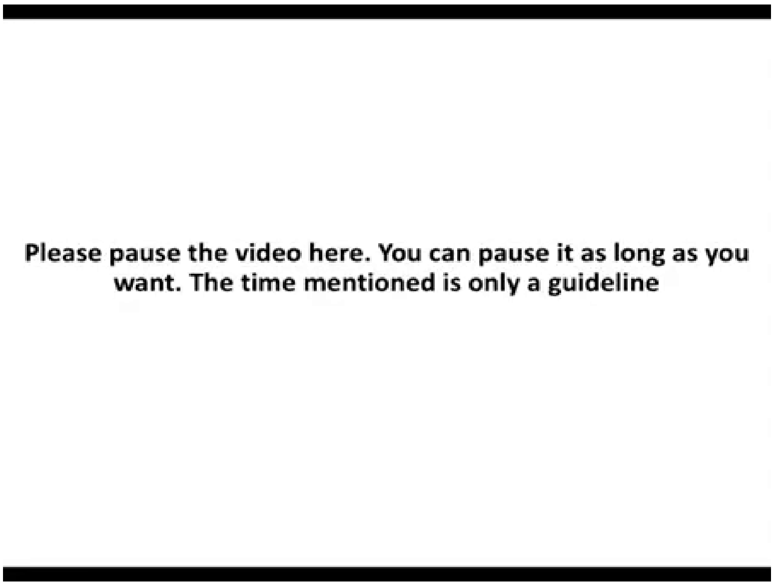
$Glucose - 6 - phosphate + H_2O \leftrightarrow glucose + P_i \qquad \Delta G' = -3.14\ Kcal\ mol^{-1}$

Calculate the standard Gibbs free energy change at pH 7, and the equilibrium constant for the phosphorylation of glucose, which is coupled with ATP hydrolysis in the cell, and is catalyzed by the enzyme hexokinase. For our purposes, it can be considered as the reaction between glucose and ATP

In this class, we will just look at these two examples. Example 6.2 reads as, the standard Gibbs free energy changes at pH 7, delta G dash, … recall that it is a different from delta G naught, because the physiological pH is pH 7; delta G naught is defined under conditions of pH 0, which is irrelevant. So, we use delta G dash; this is what we again recalled in the previous solution. So, the delta G dash values for the following reactions are given next to each; this is again ATP hydrolysis, ATP plus water giving you ADP and inorganic phosphate. As we had seen delta G dash, the standard value was minus 7.7 kilo calories per mole. … Glucose 6 phosphate plus water, you know the hydrolysis of glucose 6 phosphate, to give you glucose plus inorganic phosphate, the delta G dash value for that happens to be minus 3.14 kilo calories per mole. Somebody has very carefully determined the values of delta G dash for these reactions, thousands of reactions.

Now, the question here is, or the need here is calculate the standard Gibbs free energy change at pH 7, and the equilibrium constant for the phosphorylation of glucose, which is coupled with ATP hydrolysis in the cell. It is a coupled reaction; the phosphorylation of glucose and ATP. If you recall that is one of the first reactions in glycolysis, and is catalyzed by the enzyme hexokinase. For our purposes it can be considered as the reaction between glucose and ATP; just take it as a reaction between glucose and ATP.

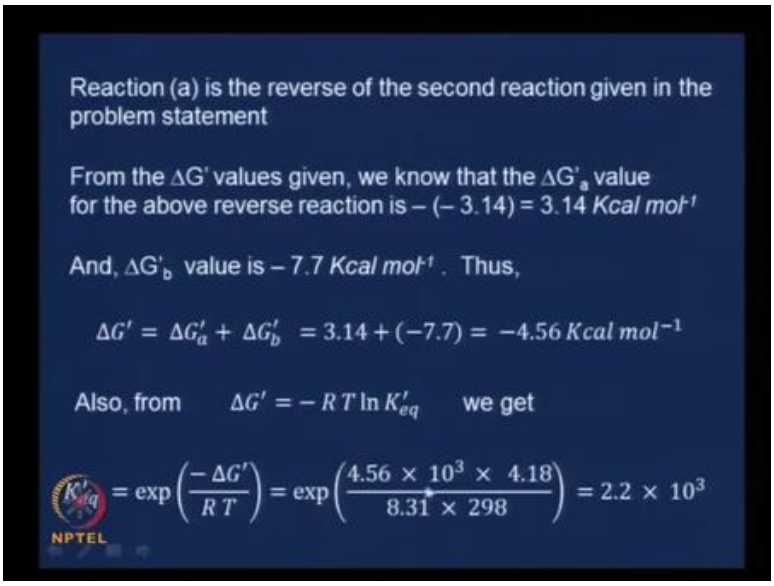
(Refer Slide Time: 03:00)



Please pause the video here. You can pause it as long as you want. The time mentioned is only a guideline

Before I present the solution, let me give you about 20 minutes to work this out. It is going to involve a few things and only thing is that, we have seen these reactions in the context of biological systems. Go ahead please; 20 minutes

(Refer Slide Time: 03:18)



Reaction (a) is the reverse of the second reaction given in the problem statement

From the $\Delta G'$ values given, we know that the $\Delta G'_a$ value for the above reverse reaction is $-(-3.14) = 3.14$ *Kcal mol*$^{-1}$

And, $\Delta G'_b$ value is -7.7 *Kcal mol*$^{-1}$. Thus,

$$\Delta G' = \Delta G'_a + \Delta G'_b = 3.14 + (-7.7) = -4.56\, Kcal\, mol^{-1}$$

Also, from $\Delta G' = -RT \ln K'_{eq}$ we get

$$K'_{eq} = \exp\left(\frac{-\Delta G'}{RT}\right) = \exp\left(\frac{4.56 \times 10^3 \times 4.18}{8.31 \times 298}\right) = 2.2 \times 10^3$$

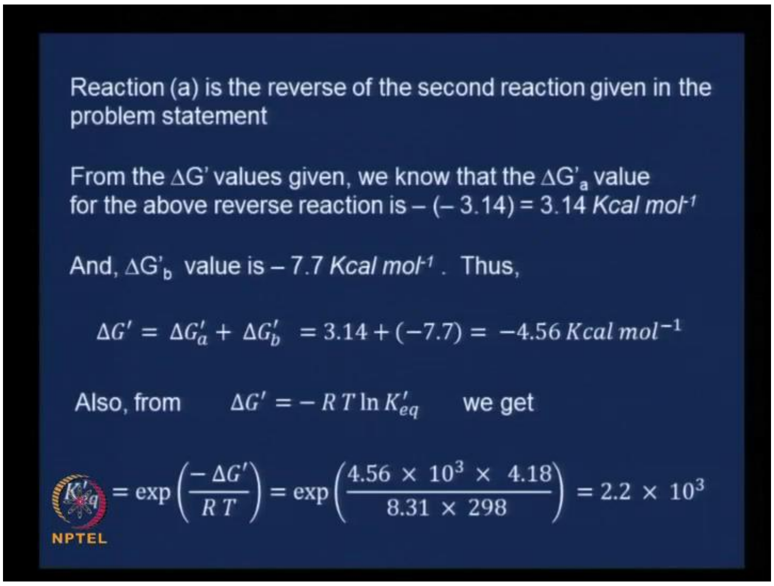
Hopefully, you experienced some deja vu moments and clarified some of the concepts that you had learnt earlier. The solution is as follows: the coupled process that we are interested in, first the reaction that we are interested in the process. As we suggested in the problem statement itself, glucose plus ATP giving you glucose 6 phosphate plus ADP. If you recall we are looking at the phosphorylation of glucose, which is coupled to ATP and, we have written that reaction as glucose plus ATP giving you glucose 6 phosphate plus ADP.

This particular reaction can be obtained by the two reactions, or from the two reactions given in the problem statement itself. Just by adding them appropriately, you know the glucose plus phosphate giving you glucose 6 phosphate plus water is reaction (a) this … of course … was not given as it is written here in the problem statement. And ATP plus water the hydrolysis of ATP to give you ADP plus P i. If you add these two, it can be easy to see that you get glucose plus ATP because water water here will cancel out; p i p i here will cancel out; and what we are left with is, glucose plus ATP giving you glucose 6 phosphate plus ADP … right here.

So, this addition of these two will be fine. And, this directly comes from, what is known in the earlier classes. that, … that the delta G dash values are additive. Therefore, t

$$\Delta G' = \Delta G'_a + \Delta G'_b = 3.14 + (-7.7) = -4.56\, Kcal\, mol^{-1}$$

he needed delta G dash value is nothing but the delta G dash of a reaction (a) added to the delta G dash value of the reaction (b).



Reaction (a) is the reverse of the second reaction given in the problem statement

From the $\Delta G'$ values given, we know that the $\Delta G'_a$ value for the above reverse reaction is – (– 3.14) = 3.14 *Kcal mol⁻¹*

And, $\Delta G'_b$ value is – 7.7 *Kcal mol⁻¹* . Thus,

$$\Delta G' = \Delta G'_a + \Delta G'_b = 3.14 + (-7.7) = -4.56\, Kcal\, mol^{-1}$$

Also, from $\Delta G' = -\, R\, T \ln K'_{eq}$ we get

$$K'_{eq} = \exp\left(\frac{-\,\Delta G'}{R\, T}\right) = \exp\left(\frac{4.56 \times 10^3 \times 4.18}{8.31 \times 298}\right) = 2.2 \times 10^3$$

So, if we have delta G dash of (a) and delta G dash of (b), we are done. We already have delta G dash of (b) in the problem statement but delta G dash of (a) we do not have. What was given in the problem statement is the reverse of the reaction that we need, or reaction (a) is the reverse of the second reaction that was given in the problem statement.

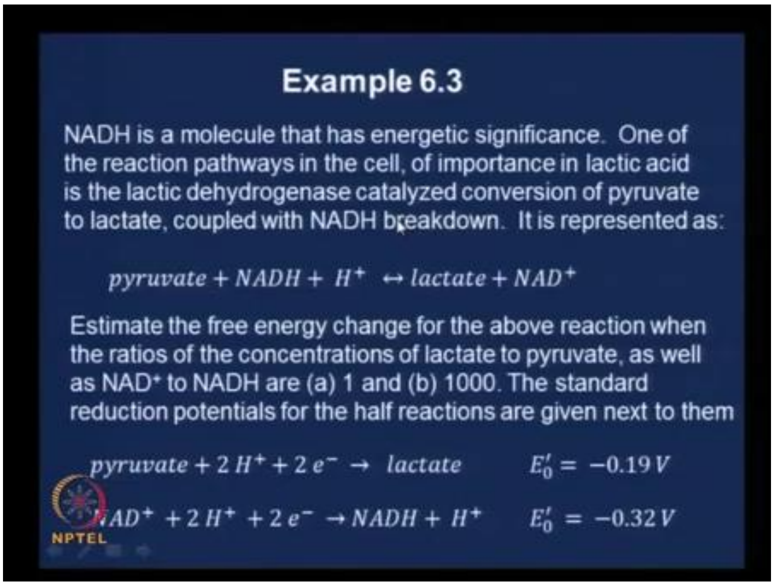
So, from the delta G dash values that were given, we know that delta G dash (a) value for the above reaction is minus of minus 3.14 which is plus 3.14 kilo calories per mole, you know it is a reverse. Therefore, we had put in a minus there. And delta G dash (b) value was minus 7.7 kilo calories per mole. And therefore, delta G dash of the reaction that we are looking for, which is the ATP catalyzed phosphorylation of glucose, and we have already established that is being equal to delta G (a) dash plus delta G (b) dash. Just adding these two together we get, minus 4.56 kilo calories per mole. Also, from

$$\Delta G' = -\, R\, T \ln K'_{eq}$$

… this again we derived in this particular course, or we saw in the earlier one of the earlier lectures, this relationship. … We had used this equation extensively, subsequently. But this equation itself was known to you from earlier classes. From this, we can get the equilibrium constant that we are looking for, as

$$K'_{eq} = \exp\left(\frac{-\Delta G'}{R\,T}\right) = \exp\left(\frac{4.56 \times 10^3 \times 4.18}{8.31 \times 298}\right) = 2.2 \times 10^3$$

(Refer Slide Time: 08:15)



Example 6.3

NADH is a molecule that has energetic significance. One of the reaction pathways in the cell, of importance in lactic acid is the lactic dehydrogenase catalyzed conversion of pyruvate to lactate, coupled with NADH breakdown. It is represented as:

$$pyruvate + NADH + H^+ \leftrightarrow lactate + NAD^+$$

Estimate the free energy change for the above reaction when the ratios of the concentrations of lactate to pyruvate, as well as NAD⁺ to NADH are (a) 1 and (b) 1000. The standard reduction potentials for the half reactions are given next to them

$$pyruvate + 2\,H^+ + 2\,e^- \rightarrow lactate \qquad E'_0 = -0.19\,V$$

$$NAD^+ + 2\,H^+ + 2\,e^- \rightarrow NADH + H^+ \qquad E'_0 = -0.32\,V$$

We will work out one more problem to become better comfortable with these concepts, which is this; example 6.3. NADH, nicotinamide adenine dinucleotide, the hydrogenated form of that, NADH, is a molecule that has energetic significance. You can recall this, you know NADH molecules result from the glycolysis pathway, the TCA cycle, and they are the electron carriers between these reactions and the oxidative phosphorylation pathway in the cell. If you recall, the metabolism of the cell, the primary metabolism … from your biochemistry course; you would have done this quite in detail. One of the reaction pathways in the cell … of importance in lactic acid production is the lactic acid dehydrogenase catalyzed conversion of pyruvate to lactate. Remember, the end point of glycolysis is considered to be pyruvate, and that goes to lactate by this lactate dehydrogenase reaction. That is how you get lactate. *Lactococcus lactis* essentially has this pathway completely channelized, and therefore, such organisms are used for production of lactic acid, and that is essentially what produces your curd from milk.

So, that is represented as … or let me complete this … of importance in lactic acid production is the lactic acid lactic dehydrogenase catalyzed conversion of pyruvate to lactate, coupled with

NADH breakdown. This NADH breakdown also happens there and also lactate could be replaced with ethanol in some cases, in some organisms and there also you have an NADH breakdown. This is represented as, pyruvate plus NADH plus H plus giving you lactate plus NAD. What is needed is, to estimate the free energy change for the above reaction, when the ratios of concentrations of lactate to pyruvate; as well as NAD plus to NADH. You know NADH, when it gives out a hydrogen ion, becomes NAD plus and the ratios of lactate to pyruvate; as well as … NAD plus to NAD H are in the first instance one, and the second instance, thousand.

The standard reduction potentials for the half reactions – recall what half reactions are – are given next to them.

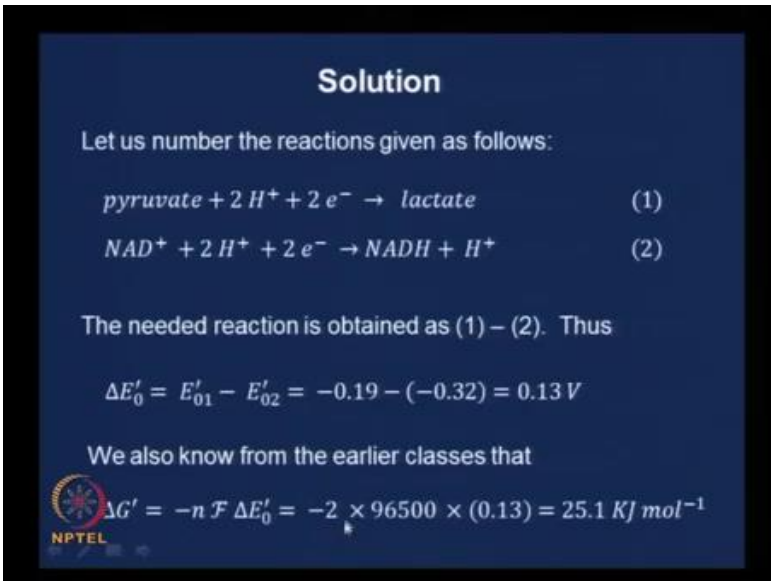
$$pyruvate + 2\,H^{+} + 2\,e^{-} \rightarrow \;\; lactate \qquad E_0' = \; -0.19\,V$$

$$NAD^{+} \; + 2\,H^{+} \; + 2\,e^{-} \; \rightarrow NADH + \; H^{+} \qquad E_0' \; = \; -0.32\,V$$

again, the dash refers to a pH 7 condition I am sorry, this is a standard reduction potential here … NAD plus, plus 2 H plus, plus 2 e minus giving you NADH, plus H plus; and here the standard reduction potential of this half reaction is, minus 0.32 volts. Take about 25 minutes and work this out. Go ahead, please.

You hopefully, would have recalled something to do with half reactions, reduction potentials and so on.

(Refer Slide Time: 12:09)



Solution

Let us number the reactions given as follows:

$$pyruvate + 2\,H^{+} + 2\,e^{-} \rightarrow \; lactate \qquad (1)$$

$$NAD^{+} \; + 2\,H^{+} \; + 2\,e^{-} \rightarrow NADH + \; H^{+} \qquad (2)$$

The needed reaction is obtained as (1) – (2). Thus

$$\Delta E_0' = E_{01}' - E_{02}' = -0.19 - (-0.32) = 0.13\,V$$

We also know from the earlier classes that

$$\Delta G' = -n\,\mathcal{F}\,\Delta E_0' = -2 \times 96500 \times (0.13) = 25.1\,KJ\,mol^{-1}$$

If you did not, I will show you how we go about it. And, also in this case, we are looking at two concentration ratios. So let us go through the solution; let us number the reactions as follows,

$$pyruvate + 2\,H^+ + 2\,e^- \rightarrow \; lactate \qquad (1)$$

$$NAD^+ \; + 2\,H^+ \; + 2\,e^- \rightarrow NADH + \; H^+ \qquad (2)$$

The needed reaction that we require in the problem statement is obtained as, 1 minus 2; … why don't you work it out; 1 minus 2, you would have done this already. Check whether, 1 minus 2 gives you the reaction that we are looking for.

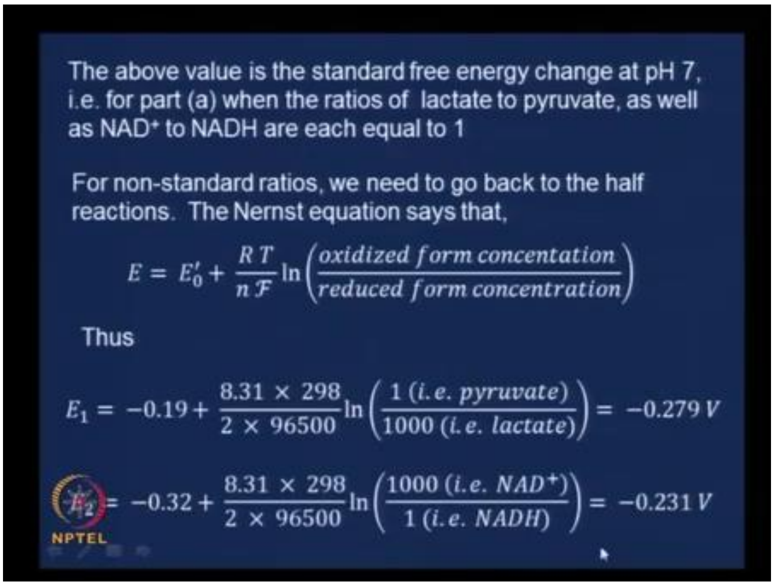
So, delta E naught dash equals E naught 1 dash minus E naught 2 dash; which is … taking the values from the problem statement,

$$\Delta E_0' = \; E_{01}' - \; E_{02}' = \; -0.19 - (-0.32) = 0.13\,V$$

We know from earlier classes the relationship between delta G and the difference in the reduction potentials, that is, delta G dash equals minus n F, the Faraday's constant, times delta E naught dash. And n, in this case, the number of electrons transferred happens to be 2; therefore, minus 2 into 92500 is the Faraday's constant in the required units, times 0.13 volts, we get 25.1 kilo joules per mole. Recall that Faraday's constant is essentially a conversion factor between energy units in normal energy, kilo joules, and in electrical energy units.

$$\Delta G' = \; -n\,\mathcal{F}\,\Delta E_0' = \; -2 \; \times 96500 \; \times (0.13) = 25.1\,KJ\,mol^{-1}$$

(Refer Slide Time: 14:06)



The above value is the standard free energy change at pH 7, i.e. for part (a) when the ratios of lactate to pyruvate, as well as NAD^+ to NADH are each equal to 1

For non-standard ratios, we need to go back to the half reactions. The Nernst equation says that,

$$E = E_0' + \frac{R\,T}{n\,\mathcal{F}} \ln\left(\frac{oxidized\ form\ concentation}{reduced\ form\ concentration}\right)$$

Thus

$$E_1 = -0.19 + \frac{8.31 \times 298}{2 \times 96500} \ln\left(\frac{1\ (i.e.\ pyruvate)}{1000\ (i.e.\ lactate)}\right) = -0.279\ V$$

$$E_2 = -0.32 + \frac{8.31 \times 298}{2 \times 96500} \ln\left(\frac{1000\ (i.e.\ NAD^+)}{1\ (i.e.\ NADH)}\right) = -0.231\ V$$

Now, the above value is the standard free energy change at pH 7; that is for part a, when the ratios of lactate to pyruvate as well as NAD plus to NADH are equal to 1. Why is that? To appreciate this, the second ratio is given; for non standard ratios, we need to go back to the half reactions itself. To do that, we need to look at the Nernst equation that says,

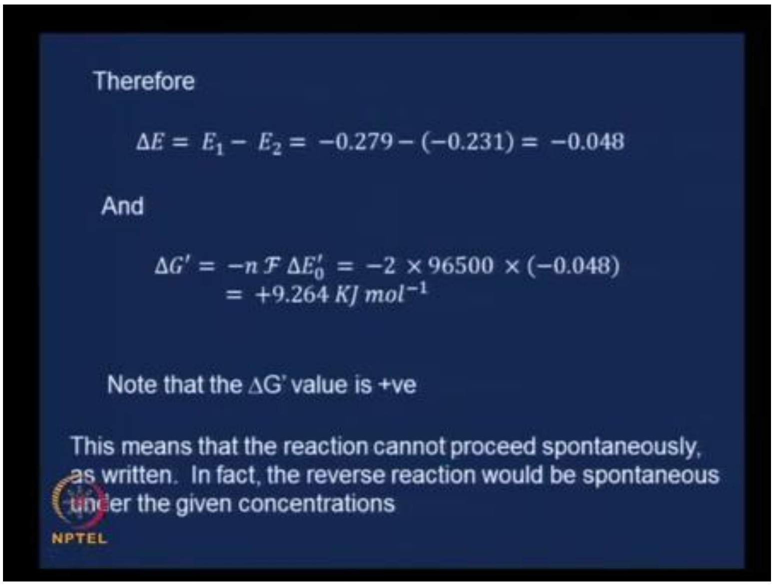
$$E = E_0' + \frac{R\,T}{n\,\mathcal{F}} \ln\left(\frac{oxidized\ form\ concentation}{reduced\ form\ concentration}\right)$$

Therefore,

$$E_1 = -0.19 + \frac{8.31 \times 298}{2 \times 96500} \ln\left(\frac{1\ (i.e.\ pyruvate)}{1000\ (i.e.\ lactate)}\right) = -0.279\ V$$

$$E_2 = -0.32 + \frac{8.31 \times 298}{2 \times 96500} \ln\left(\frac{1000\ (i.e.\ NAD^+)}{1\ (i.e.\ NADH)}\right) = -0.231\ V$$

(Refer Slide Time: 15:18)



Therefore

$$\Delta E = E_1 - E_2 = -0.279 - (-0.231) = -0.048$$

And

$$\Delta G' = -n\,\mathcal{F}\,\Delta E_0' = -2 \times 96500 \times (-0.048)$$
$$= +9.264\ KJ\ mol^{-1}$$

Note that the $\Delta G'$ value is +ve

This means that the reaction cannot proceed spontaneously, as written. In fact, the reverse reaction would be spontaneous under the given concentrations

Therefore,

$$\Delta E = E_1 - E_2 = -0.279 - (-0.231) = -0.048$$

And

$$\Delta G' = -n\,\mathcal{F}\,\Delta E_0' = -2 \times 96500 \times (-0.048) = +9.264\ KJ\ mol^{-1}$$

Now, that the delta G value is positive, and you know from higher secondary school that for a spontaneous reaction, the delta G has to be negative. So what this essentially means is that the reaction as written cannot proceed spontaneously. In fact, the reverse reaction would be the one that is spontaneous under the given concentration ratios of 1000 to 1, whereas the first part was spontaneous as written. So, that was interesting, I suppose. … We are out of time. When we come back, we will begin with something new, which is a formalism to consider electrolytes in biological systems. See you then.