

BL/CH401 Lecture 16B -- Enzyme Mechanism - Examples

Part IV. A. Electron Transfers to/from NADH/NADPH

We discussed dehydrogenases as having two domains one for NADH binding and one for other substrate in lecture 10. These enzymes often have the ability to make stereospecific products like L-malate and L-lactate. How is stereospecificity achieved in dehydrogenases? For lactate dehydrogenase (LDH), Arg-171 holds the carboxylate group of pyruvate ($\text{CH}_3\text{-CO-COO}^-$) while His-195 provides a proton to the carbonyl on carbon-2 to make the stereospecific product.

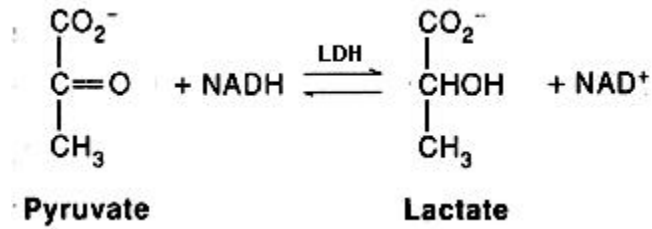


Figure 12. Interconversion of Pyruvate/NADH and Lactate/NAD⁺/H⁺ catalyzed by lactate dehydrogenase (LDH).

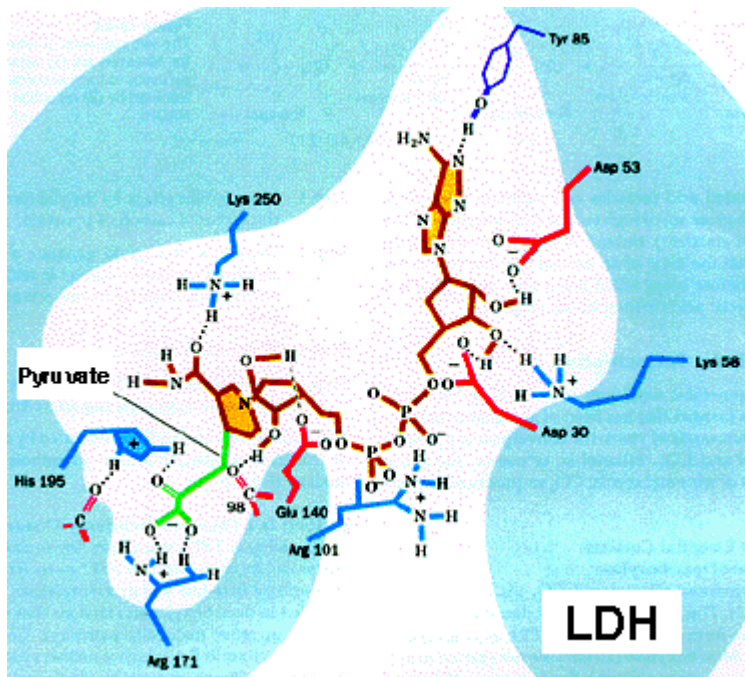


Figure 13. The active site of LDH showing NADH and pyruvate bound by various amino acid side chains.

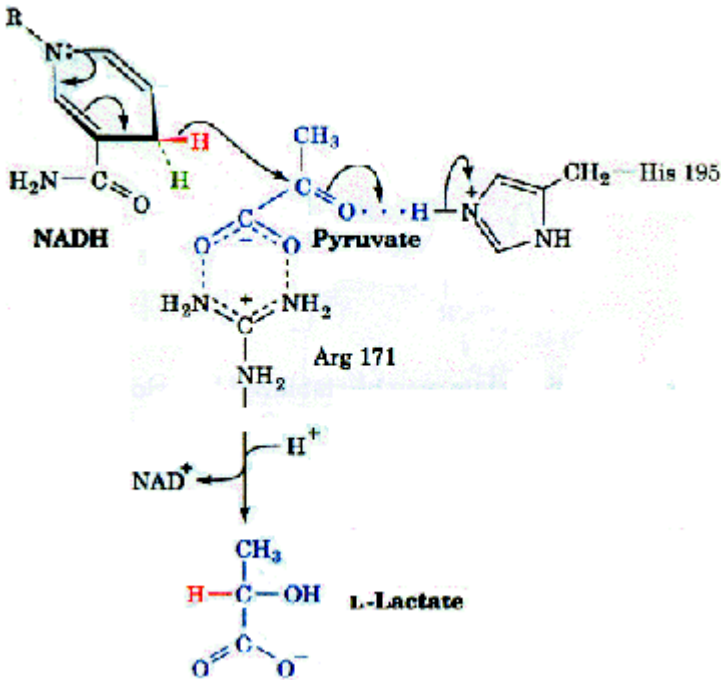


Figure 14. Model of LDH Catalytic Mechanism showing stereospecific transfer of hydride anion from NADH to pyruvate which is held in position by Arg-171 side chain.

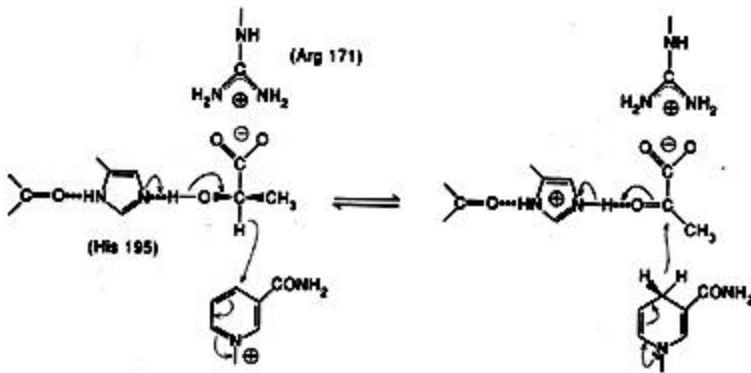
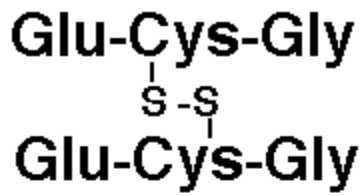


Figure 15. More general view of the LDH Catalytic Mechanism showing the role of specific amino acid side chains.

Part IV. B. Electron Transfers to/from NADH/NADPH

Can an enzyme's disulfide bond be redox active? A much more complicated redox enzyme is glutathione reductase, which uses NADPH to reduce oxidized glutathione in your cells. Glutathione is a 3 AA peptide (Glu-Cys-Gly) and two molecules are linked together by a disulfide bond in the oxidized form.



Glutathione disulfide (GSSG)

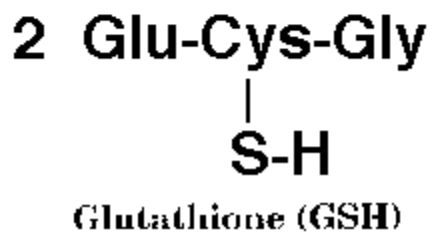
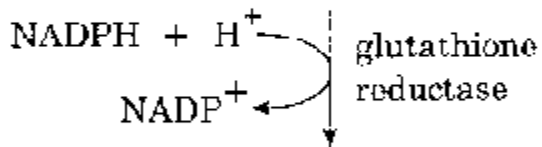


Figure 16. Reduction of glutathione by NADPH catalyzed by glutathione reductase.

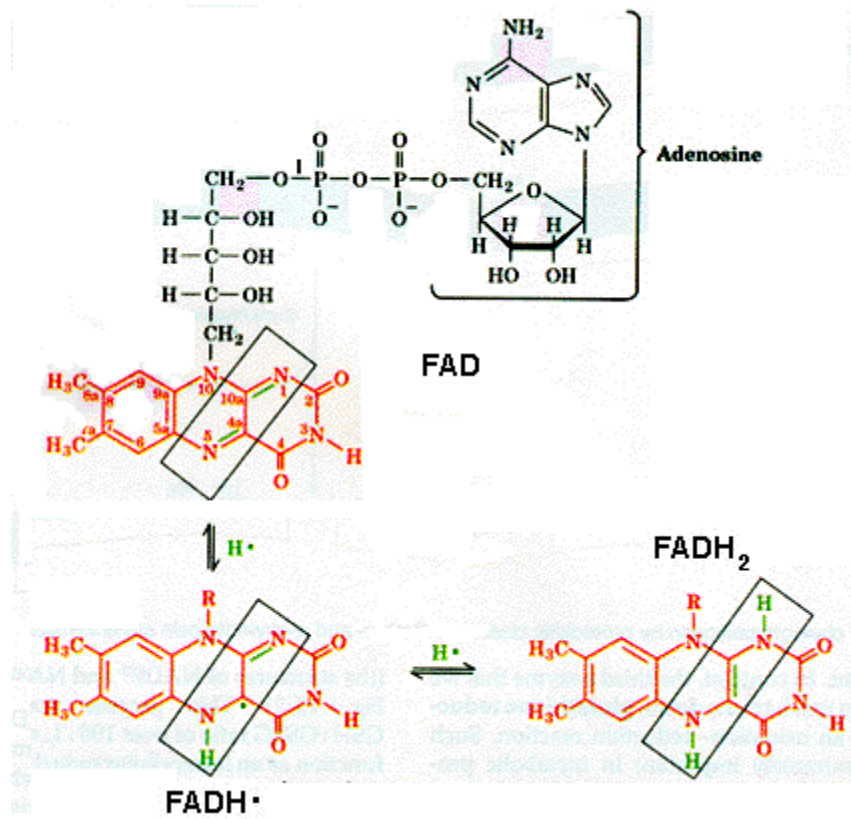


Figure 17. Structural Model of Glutathione Reductase showing the domains for binding the

internal flavin molecule (FAD) and the reducing substrate, NADPH, and the interface domain for joining the two subunits. The active site where glutathione is bound is between the two subunits and it is here where the redox active disulfide of the enzyme is located.

To achieve reduction of GSSG to GSH glutathione reductase must split the two electrons provided by NADPH and give them one at a time to each of the sulfurs of the GSSG. To do this, glutathione reductase contains FAD and it also uses a redox active enzyme disulfide. FAD differs from NADH/NADPH in that it can accept 2 electrons and pass them one at a time.

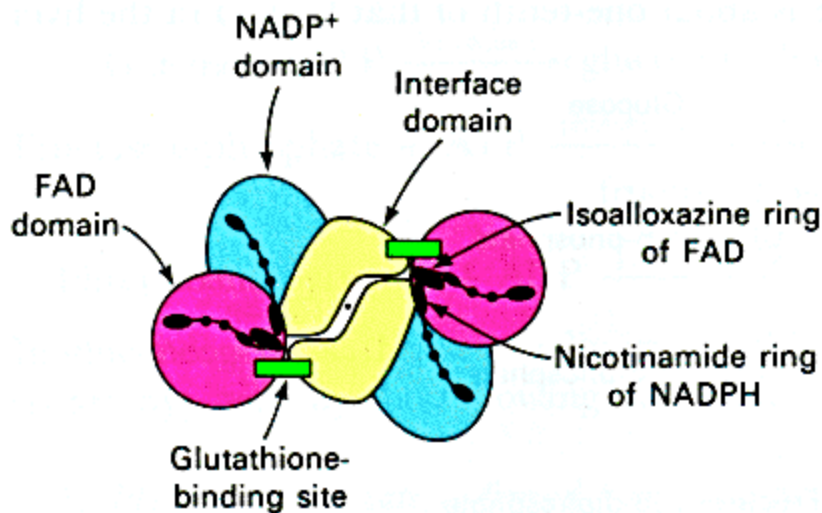


Figure 18. Structure of flavin adenine dinucleotide (FAD) and how it can be reduced in 2 one-electron transfers. It can also be oxidized in two one-electron transfers, which allows it to split the electrons of NADPH to use for reduction of glutathione in two steps each involving the transfer of 1 electron.

Glutathione reductase acts in two steps:

First, NADPH reduces the enzyme and second the reduces GSSG to form 2 GSH.

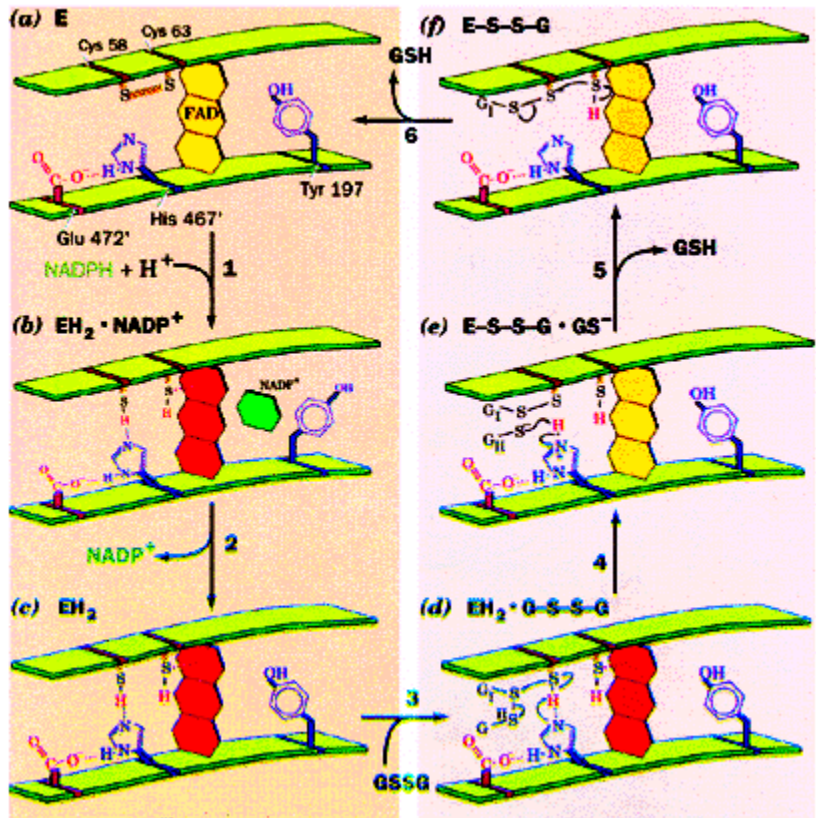


Figure 19. The steps in Glutathione Reductase catalytic mechanisms showing the reduction of the enzyme by NADPH (Tyr 197 is involved in binding NADPH) where the FAD is converted to FADH₂. Next reduced FAD reacts with the internal disulfide of the enzyme where one -SH group is stabilized by interaction with the FAD while the other is stabilized by His 467 in combination with Glu 272. Next glutathione (G-S-S-G) reacts with the -SH group bound to the His-467 leading to one molecule of GSH (reduced glutathione being released) and a mixed disulfide formed between the second glutathione and the Enzyme's thiol (G-S-S-Enzyme). Finally, the enzyme's -SH-FAD complex attacks the G-S-S-Enzyme to displace the second GSH (which is released) and regenerating the enzyme's disulfide (E-S-S-E).

Part V. A. Beta Barrel Enzymes

In Lecture 10, I told you that many enzymes use the beta barrel shape to form their active site. Let's look at two examples of those type of enzymes. First, Triose-P Isomerase (TIM), which catalyzes the conversion of an aldehyde to a ketone. How does the 'perfect enzyme' work? Aldehydes are converted to ketones via an endiol intermediate. First, recall the structure of TIM from lecture 10.

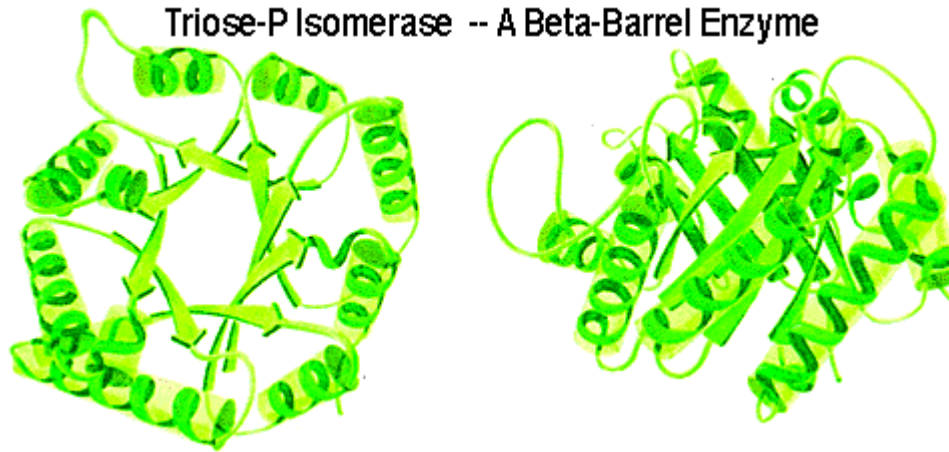


Figure 20. Beta-barrel structure of TIM. The active site of TIM is at the top of the barrel where the loops joining the beta strands to the alpha-helix has the amino acid side chains which hold the triose-P substrate and catalyzes the interconversion between its aldose and ketose forms (ie the isomerization reaction).

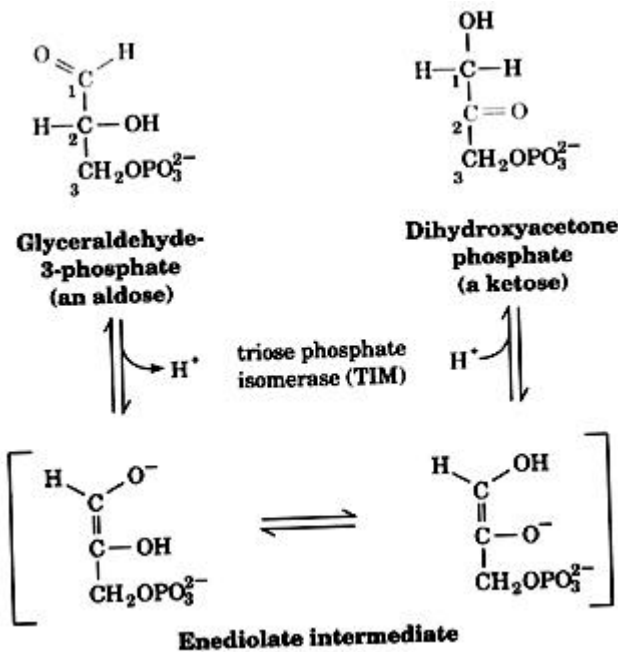


Figure 21. The reaction catalyzed by TIM showing the ENE-diolate intermediate.

TIM has 3 AA's to catalyze the isomerization:

Glu-164, His-95, and Lys-13

The catalytic mechanism is illustrated below by conversion of ketone to aldehyde, which is OK since TIM catalyzes the interconversion equally well in either direction.

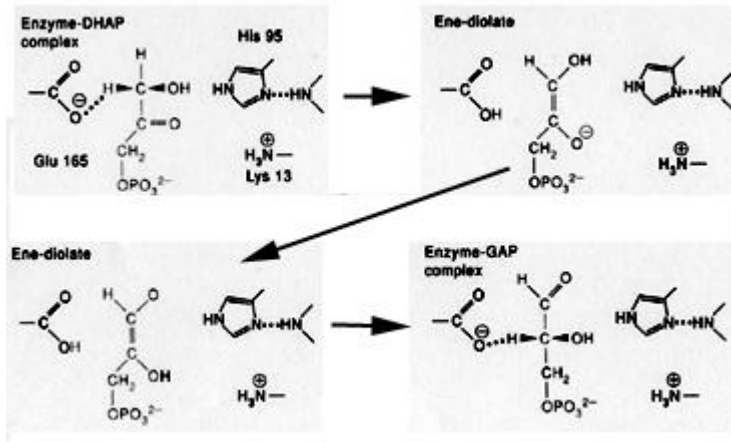


Figure 22. Catalytic mechanism of TIM.

Part V. B. Beta Barrel Enzymes

The most abundant enzyme on earth is Ribulose bis-phosphate carboxylase (RuBisCO), the carbon dioxide (CO₂) fixing enzyme of green plants. RuBisCO has some special interest on this campus because it was discovered by Melvin Calvin, who did his undergraduate B.S. on this campus. Calvin was awarded the Nobel Prize for discovery of RuBisCO and the understanding of the mechanism by which plants fix carbon dioxide. So Calvin is our only Nobel Prize winner and we should take a look at the enzyme which he discovered. Part of the 3-D structure of RuBisCO is a beta-barrel structure like TIM.

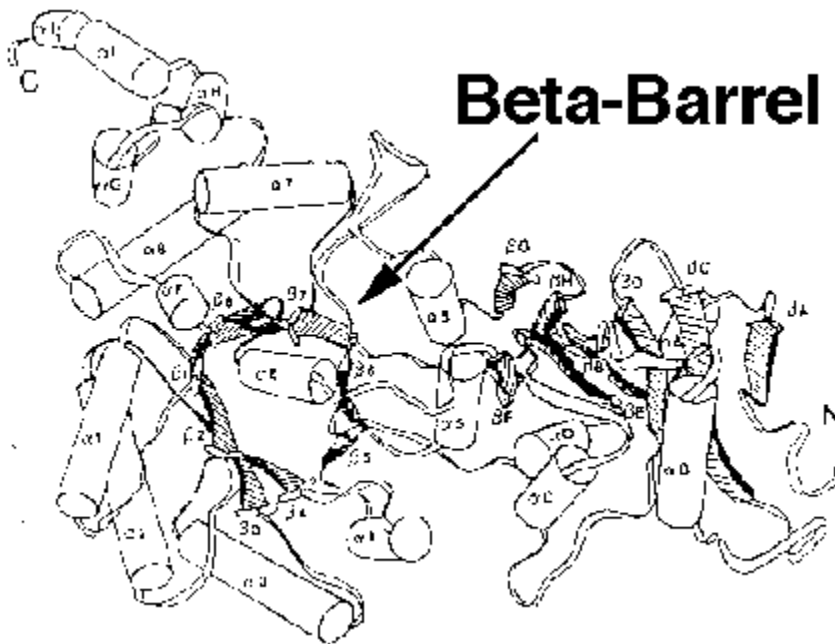


Figure 23. Structure of RuBisCO catalytic subunit.

How does the most abundant enzyme on earth work? The 5-carbon molecule Ribulose bis-P

(RuBP) is the CO₂ acceptor and binds to the enzyme first in the active site at the top of the beta barrel. RuBP binding to the active site has been studied using an inhibitor (CABP) which resembles the RuBP with CO₂ attached to it. Mg²⁺ is directly involved in RuBP binding via a carboxylated form of Lys-201.

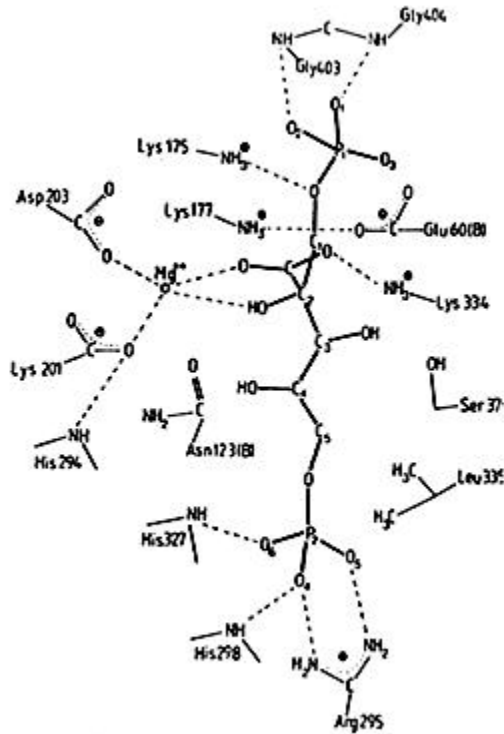


Figure 24. RuBP model molecule bound in the active site of RuBisCO with various key amino acid side chains identified.

I have made a diagram of the top of the beta barrel to show the positions of the amino acids involved in RuBP and carbon dioxide binding. This shows how the beta barrel brings together the important AA side chains of the active site.

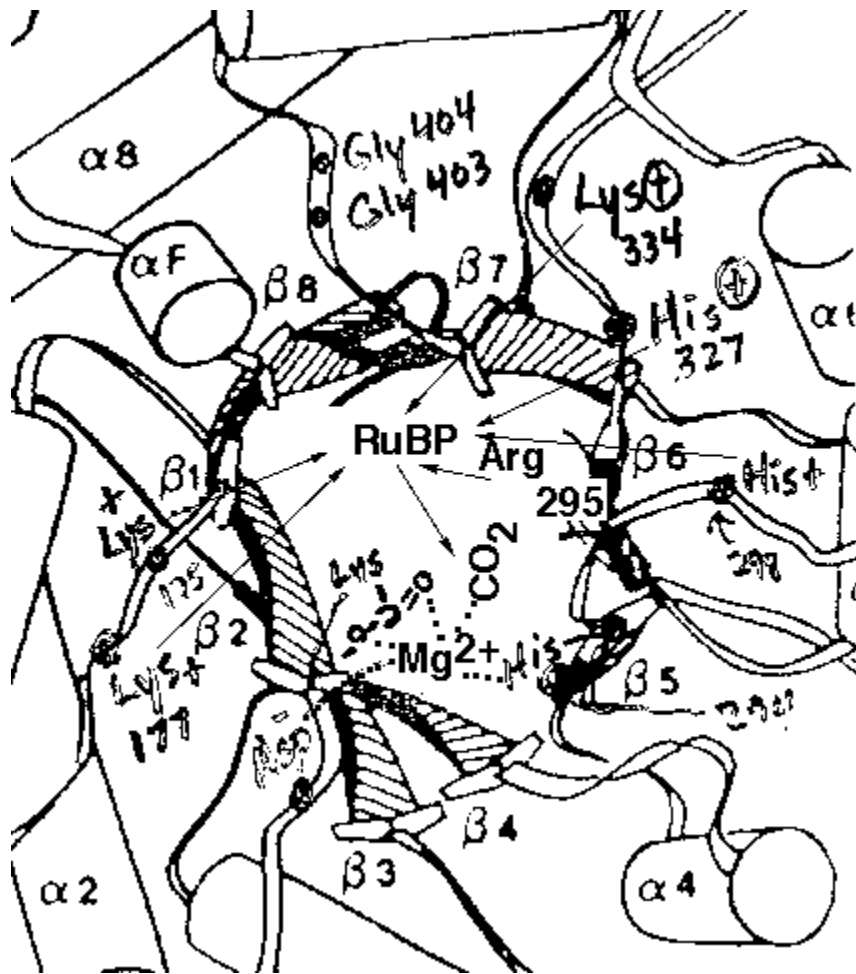


Figure 25. Close-up of the active site of RuBisCO with key amino acids shown interacting with RuBP and CO₂.

As it turns out, RuBP also forms an enediol like the triose did in the TIM reaction. This RuBP enediol is the highly reactive form of the substrate that can react with CO₂ to form the 6 carbon intermediate that breaks down into 2 molecules of triose, which are used by the plant chloroplast to make sugars.

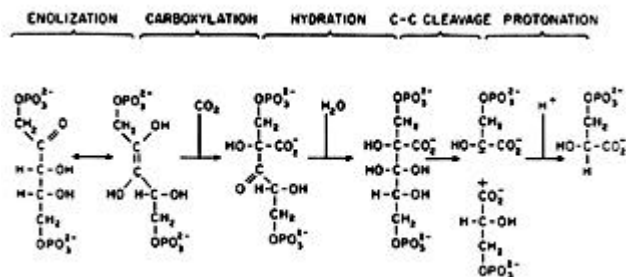


Figure 26. Reaction of RuBP in the active site of RuBisCO proposed to account for its reactivity with carbon dioxide.

Since the active form of RuBP is very reactive when bound to the RuBisCO's active site, it can also react with oxygen rather than CO₂. This discovery explained how oxygen inhibits photosynthesis. So in the process of green plants filling the earth's atmosphere with oxygen, they produced a strong inhibitor of their most basic reaction. Of course, the oxygenation of the earth's atmosphere led to the rise of animals, which depend on plants for their food. So the plants really got a raw deal out of making oxygen.

Part VI. An Enzyme from a Thermophilic Organism

How do enzymes function at high temperature? My final enzyme mechanism example is of a comparison of a protein factor involved in protein synthesis called elongation factor (EF-Tu). 3-D structures are available for EF-Tu from *E. Coli* and a thermophile, *Thermus aquaticus*. EF-Tu binds GTP and a t-RNA/AA molecule before it binds to the ribosome. After it binds to the ribosome, GTP is hydrolyzed to GDP and the EF-Tu/GDP complex is released leaving behind the t-RNA/AA to add an amino acid to the growing polypeptide chain on the ribosome.

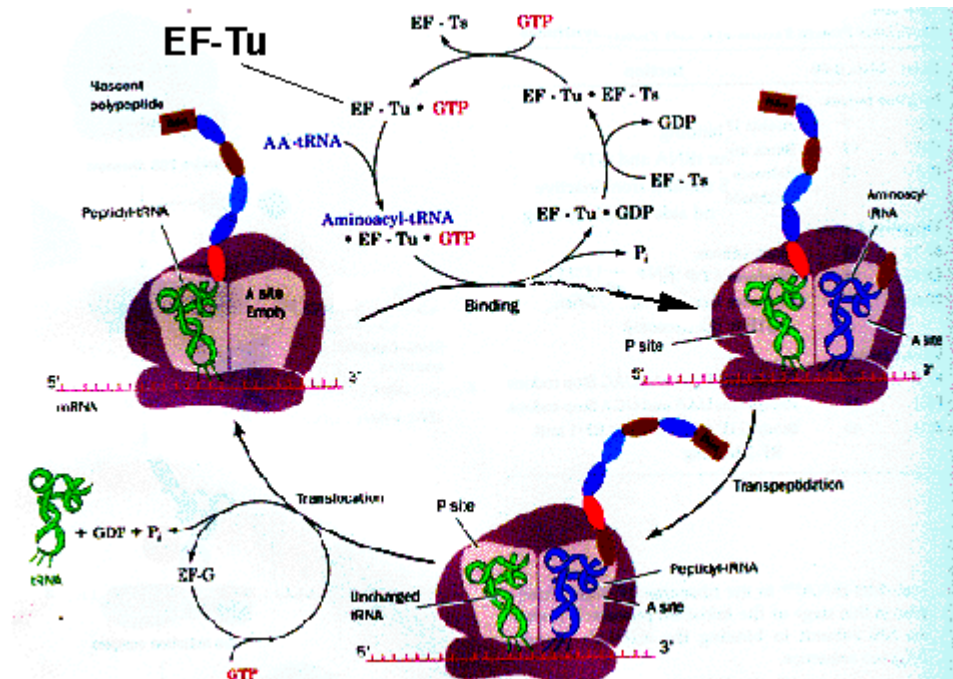


Figure 27. Model of the reactions in protein synthesis catalyzed by the ribosome. The role of EF-Tu is shown here as being involved in binding the t-RNA-AA complex to the ribosome, which involves the hydrolysis of GTP, during elongation of nascent polypeptide chain.

EF-Tu resembles other 'G-proteins' like those involved in cancerous cells.

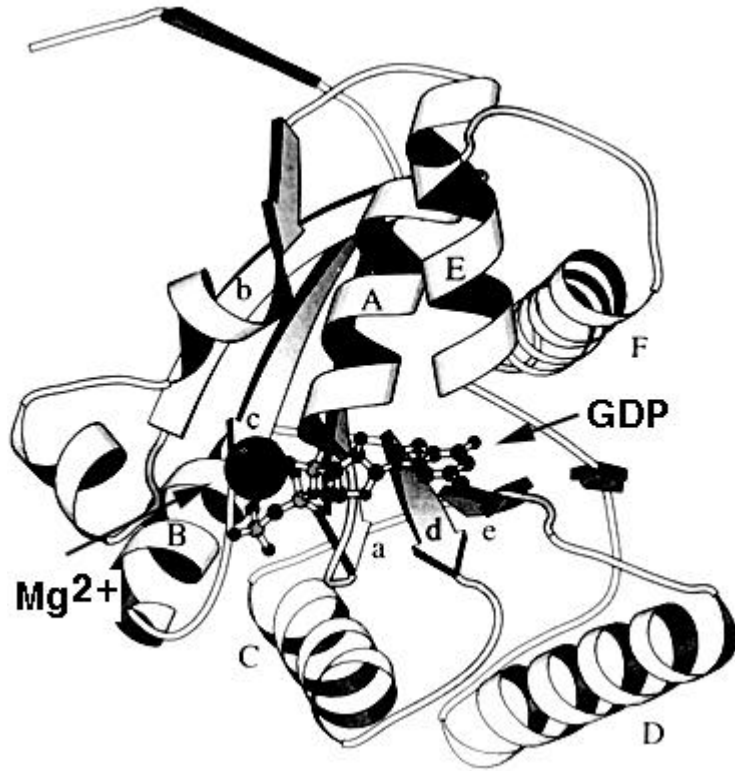


Figure 28. 3-D Model showing EF-Tu domain which resembles a 'G-protein' with GDP bound to it along with the Mg^{2+} which is bound to the GDP.

Interestingly, the GTP hydrolysis catalyzed by EF-Tu involves a catalytic triad very similar to that of serine protease, except that it activates water to hydrolyze GTP to GDP and P_i .

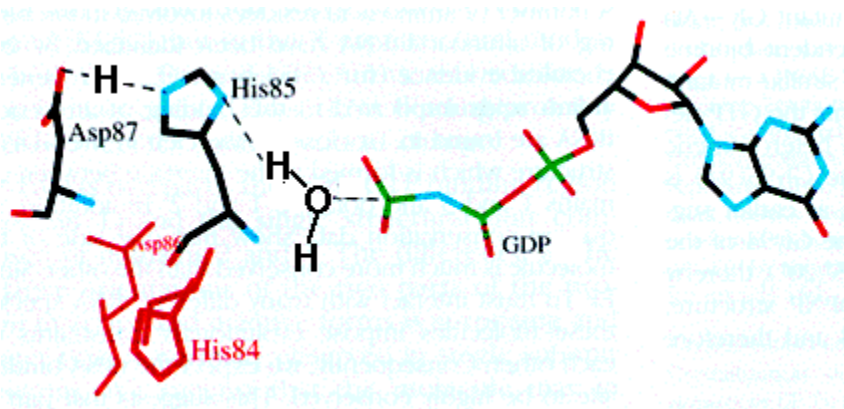


Figure 29. Detail of the binding of the GDP-inhibitor bound in the active site shown the 'catalytic triad' including a water molecule which attacks the GTP molecule leading to its hydrolysis. Figures 28, 29 & 30 are from Structure 1: 35-50 (1993).

Basically the enzyme from *E. coli* (a mesophilic organism - grows at $37^{\circ}C$) is exactly the same shape as the enzyme from *Thermus aquaticus* (a thermophile which grows at $70^{\circ}C$). A comparison of the amino acid sequence shows they are 70% the same. However, the thermophilic enzyme has 3 insertions of amino acids in the sequence. One of these insertions is 10

AA's long. It seems that these extra AA's are all that is needed to make the thermophilic enzyme stable at high temperature.

T.a	1	AKGEFIRTKPHVNVGTIGHVHGKTTLTAALTYVAAAEENPNVEVKDYGDI	50
T.t	1	AKGEFVRTKPHVNVGTIGHVHGKTTLTAALTYVAAAEENPNVEVKDYGDI	50
E.c	1	SKEKFERTKPHVNVGTIGHVHGKTTLTAATTTVLAKT-YGGAARAFDQI	49
HOM	0	K F RTKPHVNVGTIGHVHGKTTLTA T V A	30
T.a	51	DKAPEERARGITINTAHVEYETAKRHYSHVDCPGHADYIKNMITGAAQMD	100
T.t	51	DKAPEERARGITINTAHVEYETAKRHYSHVDCPGHADYIKNMITGAAQMD	100
E.c	50	DNAPEEKARGITINTSHVEYDTPRHYAHVDCPGHADYVKNMITGAAQMD	99
HOM	31	D APEE ARGITINT HVEY T RHY HVDCPGHADY KNMITGAAQMD	72
T.a	101	GAILVVSAADGPMPTREHILLARQVGVPIVVFHNKYDMYDDPELLOLV	150
T.t	101	GAILVVSAADGPMPTREHILLARQVGVPIVVFHNKYDMYDDPELLOLV	150
E.c	100	GAILVVAATDGPMPOTREHILLGRQVGVPIVVFHNKYDMYDDPELLELV	149
HOM	73	GAILVV A DGPMPOTREHILL RQVGVPI VF NK DMVDD ELL LV	114
T.a	151	EMEVRLLNQYFPGDEVPIRGSALLALEEMHNPKTKRGENEVDKIW	200
T.t	151	EMEVRLLNQYFPGDEVPIRGSALLALEEMHNPKTKRGENEVDKIW	200
E.c	150	EMEVRELLSQYDFPGDDTPIVRSALKALE-----GDAEWEAKIL	189
HOM	115	EMEV R LL QY FPGD P RGSAL ALE ----- G E W KI	141
T.a	201	ELLDAIDEYIPTPRDVKPFLMPVEDVFTITGRGTVATGRIERGKVKVG	250
T.t	201	ELLDAIDEYIPTPRDVKPFLMPVEDVFTITGRGTVATGRIERGKVKVG	250
E.c	190	ELAGFLDSYIPEPERAIDKPFLLPIEDVFSISGRGTVVTGVRGIIKVG	238
HOM	142	EL D YIP P R DKPFL P EDVF I GRGTV TGR ERG KVG	174
T.a	251	DEVEIVGLAPETRRVTVTGVEMMRKTLQEGTAGDNVGLLLRGVSRREEVER	300
T.t	251	DEVEIVGLAPETRRVTVTGVEMMRKTLQEGTAGDNVGLLLRGVSRREEVER	300
E.c	240	EEVEIVGIK-ETQKSTCTGVEMFRKLLDEGRAGENVGLLRGIIKREEIER	288
HOM	174	EVEIVG ET TGVEM RK L EG AG NVG LLRG REE ER	206
T.a	301	GQVLAKPGSITPHTKFEASVYILKKEEGGRHTGFFTGYPQFYFRITDVT	350
T.t	301	GQVLAKPGSITPHTKFEASVYVLLKKEEGGRHTGFFSGYRPFYFRITDVT	350
E.c	289	GQVLAKPGTIPHTKFESEVYVLLSKDEGRHTPFFKGYPQFYFRITDVT	338
HOM	207	GQVLAKPG I PHTKFE VY L K EGGRT FF GYRPFYFRITDVT	247
T.a	351	GVVRLPGVEMVMPGONVFTVELIKPVALEEGLRFAIREGGRTVAGGVV	400
T.t	351	GVVQLPPGVEMVMPGONVFTVELIKPVGLEEGLRFAIREGGRTVAGGVV	400
E.c	339	GTIELPEGVEMVMPGONIKMVVTLIHPIAMDGLRFAIREGGRTVAGGVV	388
HOM	248	G LP GVEVMPGON V LI P GLRFAIREGGRTVAGGVV	282
T.a	401	TKILE	405
T.t	401	TKILE	405
E.c	389	AKVLS	393
HOM	282	K L	284

Figure 30. Amino acid sequence of 2 thermophilic forms of EF-Tu compared to the sequence of EF-Tu from E. coli. The amino acids which are the same in all three sequences are shown on the 4th line of each segment of the sequence. The extra 10 amino acids which is the major difference between the enzyme forms stable at the higher temperatures of the thermophiles is lined above and below.

Part VII. Summary of Lecture 16.

What we have seen in this survey of some known enzyme catalytic mechanisms is that the most common AA's involved with catalysis are:

Ser, His, Asp, Glu, Lys, and Arg

These are the most versatile AA's found in enzyme active sites.