Aspartate carbamoyl Transferase (ATCase)

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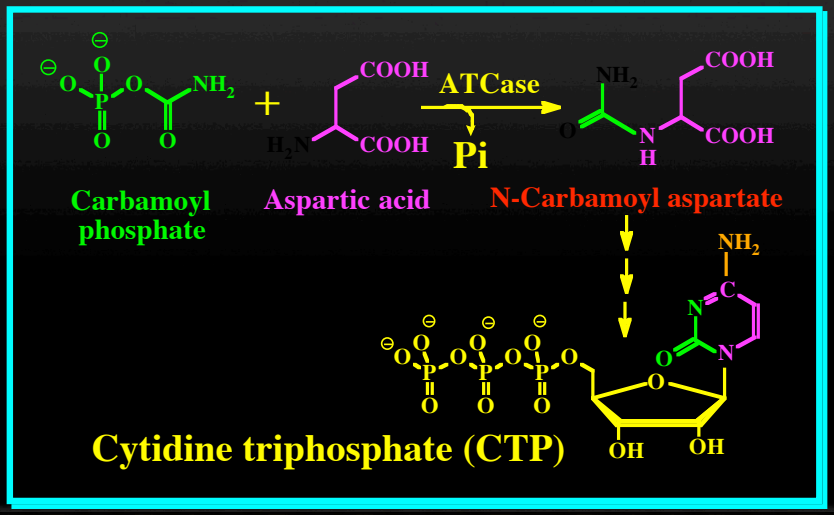
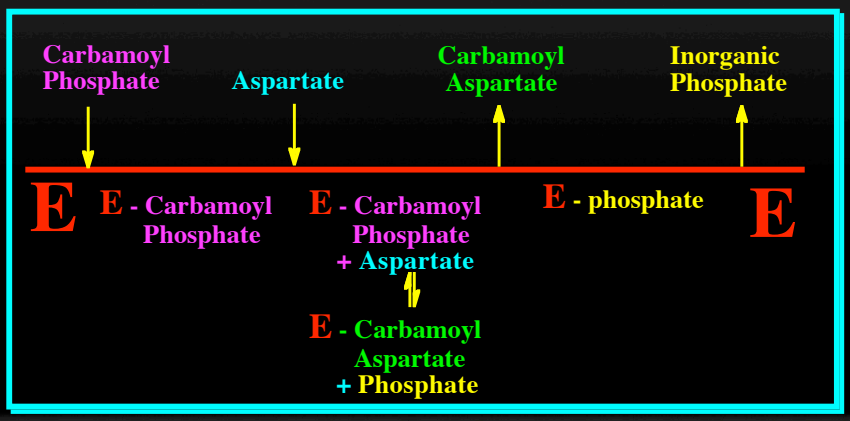
ATCase is the key enzyme responsible for the biosynthesis of pyrimidines

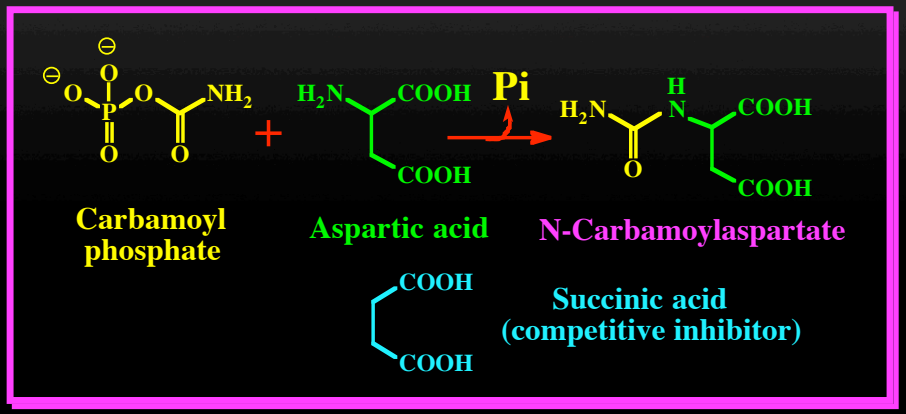


ATCase follows ordered Bi Bi reaction

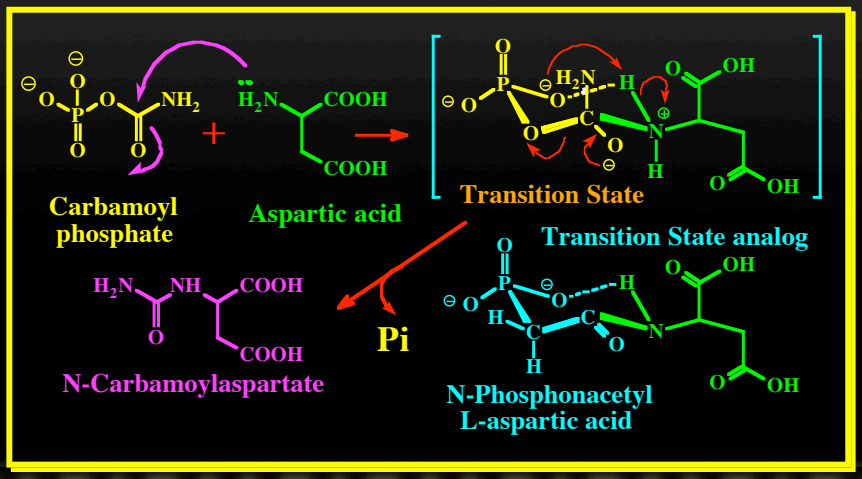
Carbamoyl phosphate binds to the enzyme first. Aspartate then comes and attacks the carbonyl carbon of carbamoyl phosphate. This results in a tetrahedral intermediate formation. The transition state then breaks down to produce carbamoyl aspartate (which then comes out of the enzyme first) and inorganic phosphate.



Succinic acid is a competitive inhibitor (against aspartate) for the enzyme.



Consistent with the reaction mechanism, N-(phosphanacetyl)-L-aspartic acid, the bisubstrate, transition state analog of the enzyme, serves as a potent inhibitor for ATCase (Ki = 10^{-8} M).



Biosynthesis of CTP

Glutamine + 2 ATP + O=C=O ---> Glutamate + 2 ADP + Pi + Carbamoyl phosphate

1

2 + Aspartate

N-Carbamoyl aspartate

3 H_2O

Dihydroorotate

4 NAD / NADH

Orotate

5 PRPP / PPi

Orotidylate (OMP)

6 CO_2

Uridylate (UMP)

7 ATP / ADP

UDP

8 ATP / ADP

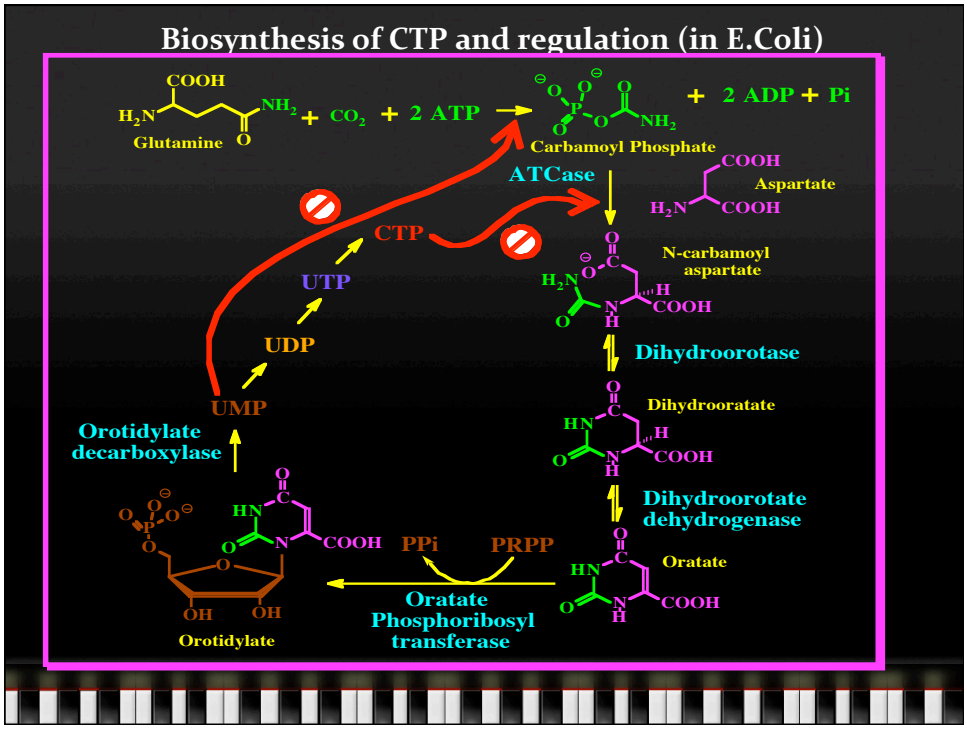
UTP

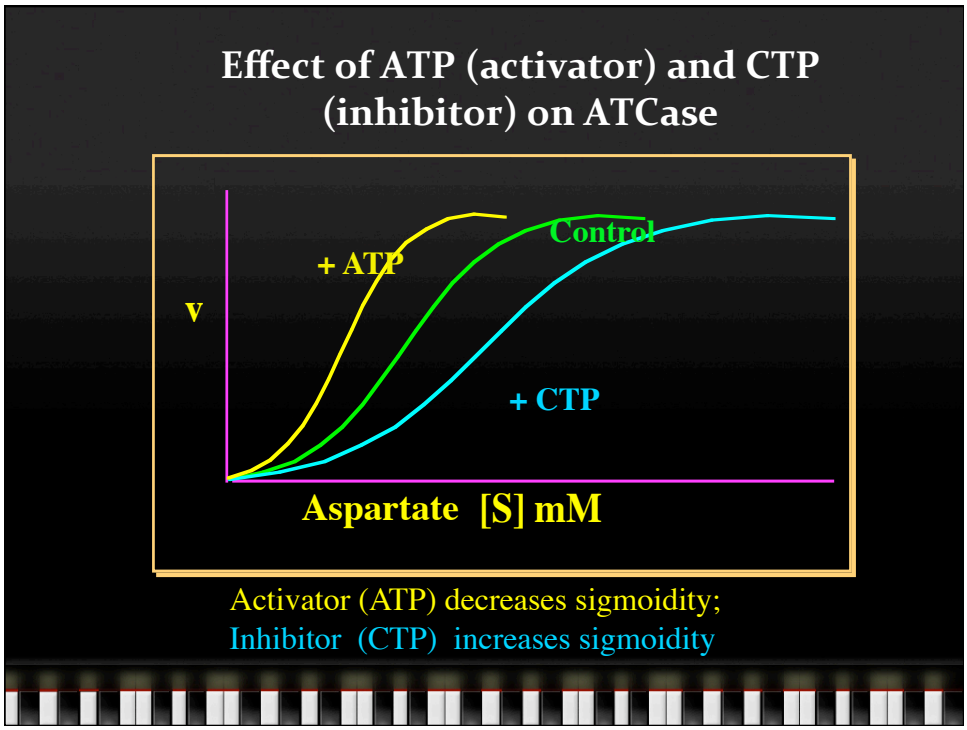
9 Gln + ATP / Glu + ADP

CTP

Enzymes:

1. Carbamoyl phosphate synthetase
2. Aspartate transcarbamoylase
3. Dihydroorotase
4. Dihydroorotate dehydrogenase
5. Orotate Phosphoribosyl transferase
6. Orotidylate decarboxylase
7. Nucleoside monophosphate kinase
8. Nucleotide diphosphate kinase
9. CTP synthetase

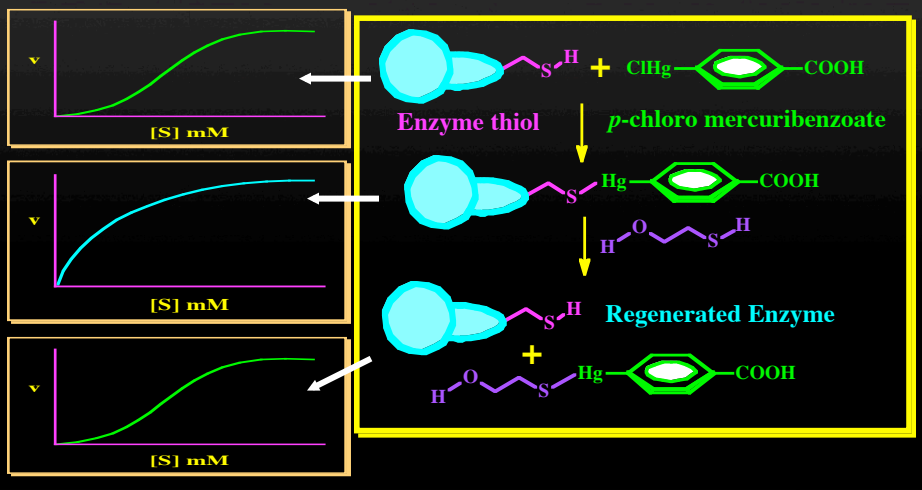

Biosynthesis of CTP and regulation (in E.Coli)
Glutamine
+ CO2 + 2 ATP
Carbamoyl Phosphate
+ 2 ADP + Pi
ATCase
Aspartate
N-carbamoyl aspartate
Dihydroorotase
Dihydrooratate
Dihydroorotate dehydrogenase
Oratate
Oratate Phosphoribosyl transferase
PPi
PRPP
Orotidylate
Orotidylate decarboxylase
UMP
UDP
UTP
CTP


Effect of ATP (activator) and CTP (inhibitor) on ATCase
V
+ ATP
Control
+ CTP
Aspartate [S] mM
Activator (ATP) decreases sigmoidity;
Inhibitor (CTP) increases sigmoidity

The significance of CTP/ATP regulation

- CTP (end product inhibition) is essential to turn off the synthetic pathway when excess CTP accumulates.
- When high concentration of purines accumulate and energy rich environment exist, ATP activation helps to produce enough pyrimidines for DNA replication.

ATCase can be modified by pcmb. Modified ATCase exhibits Michaelis Menten type kinetics and is not responsive to either ATP or CTP.

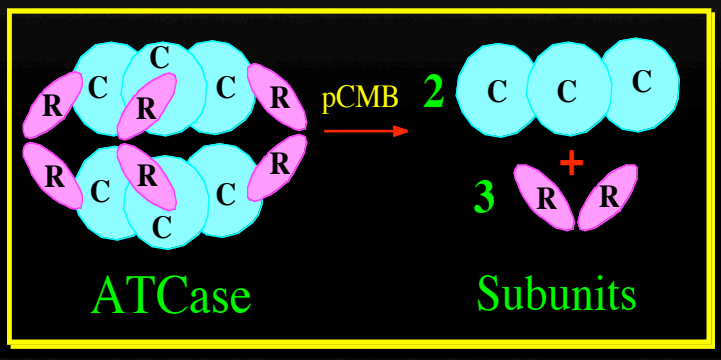


Catalytic and regulatory sites are located on different subunits of ATCase.

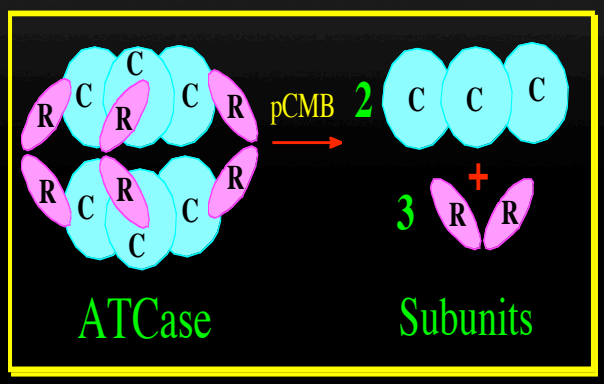
- p-hydroxymercuribenzoate dissociates ATCase into catalytic subunits (C_3) and regulatory subunits (R_2).
- C_3 and R_2 can be separated by centrifugation and/or ion exchange chromatography.
- Isolated C_3 shows ATCase activity, but exhibits only typical Michaelis Menten kinetics. It is also non responsible to allosteric effectors - ATP and/or CTP.
- Regulatory subunit binds to ATP and CTP but does not have any detectable ATCase activity.
- Reconstituion of regulatory and catalytic subunits (after the removal of mercurial) generates the fully fiunctional ATCase.

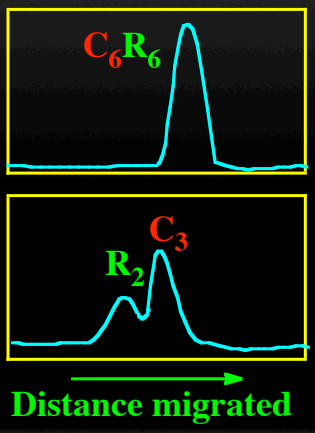
Catalytic and regulatory sites are located on different subunits of ATCase.

- ATCase treated with p-hydroxymercuribenzoate (pCMB) does not exhibit allosteric kinetics. This desensitization is caused by the separation of catalytic subunits (C_3) and regulatory subunits (R_2) by the reaction with mercurials.

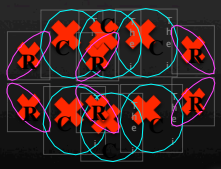


Catlytic subunit C_3 and regulatory subunit R_2 can be separated by centrifugation and/ or ion exchange chromatography.

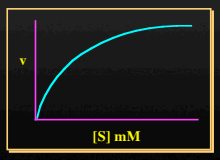


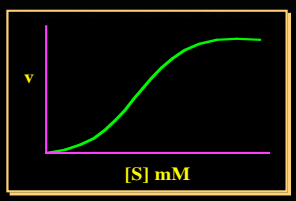


Native ATCase: (C_6R_6) Allosteric kinetics
Catalytic subunit alone : (C_3) Michaelis Menten kinetics
Regulatory subunit: (R_2) No enzyme activity; but binds to ATP or CTP.



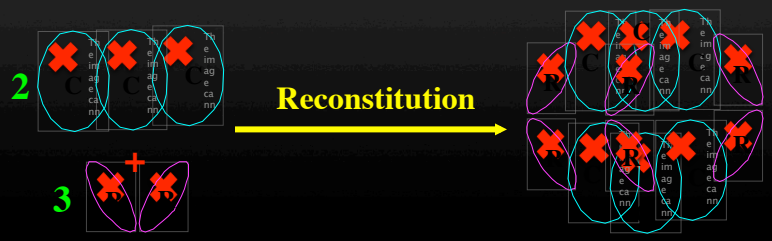
ATCase





Regulatory Subunits bind to ATP/CTP; but no catalytic activity.

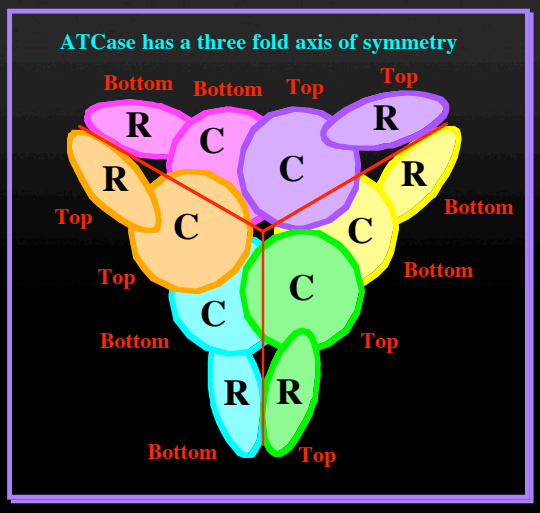
Reconstitution of catalytic and regulatory subunits restores the fully functional ATCase



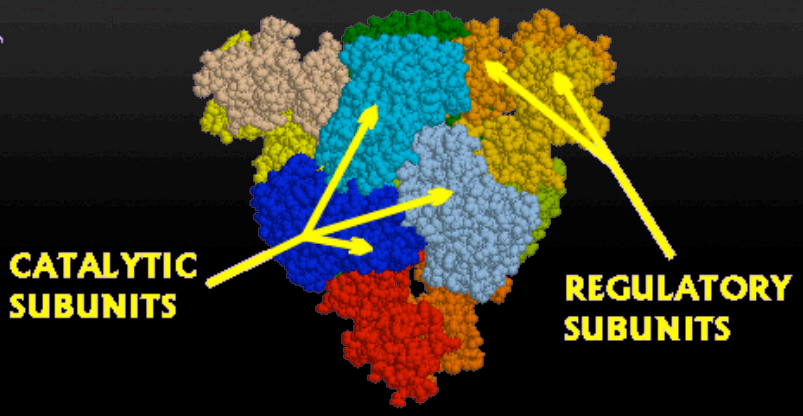
Catalytic subunits show normal M.M. kinetics & insensitive to ATP/CTP

The reconstituted enzyme now shows allosteric properties.

Subunit structural arrangement in ATCase



Crystal structure of ATCase



Ligand interaction with ATCase

- Aspartate exhibits positive homotropic interaction.
- ATP shows positive heterotropic interaction.
- CTP shows negative heterotropic interaction.

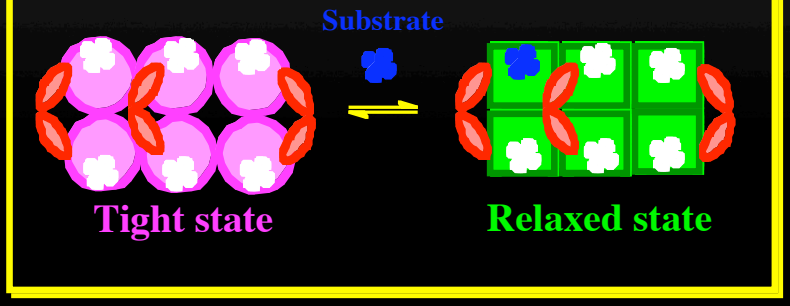
Effects of ATP and CTP

- The end product metabolism, CTP inhibits ATCase reaction allosterically.
- The activator ATP competes to the same site at which CTP binds; but the results are different. While ATP enhances the affinity of ATCase for its substrates; CTP decreases the affinity.

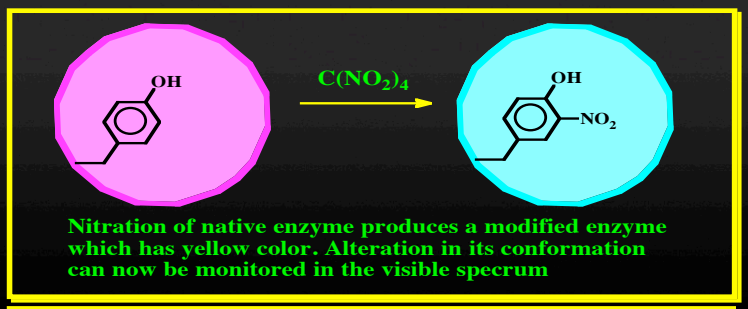
Allosteric effects of ATCase can be explained by concerted model

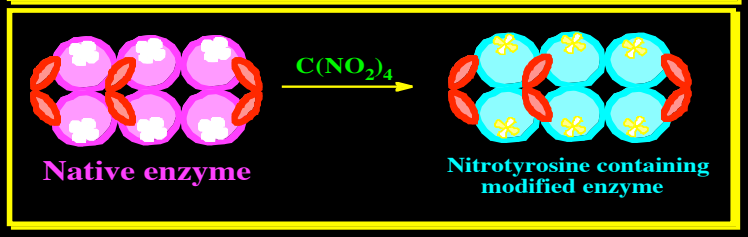
- The T state is more closed (tight). Therefore it is poorly accessible to the substrate.
- The R (relaxed) state is more open and allows the substrate to bind to it freely.

Substrate binding to one subunit causes conformational changes in other subunits also.

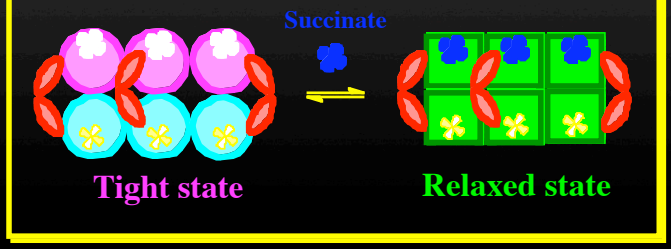


Nitration experiment





Active site is not in direct geometric relation with subunit interaction site.



Reconstitution of normal catalytic trimer with nitrotyrosine containing trimer generates the dodecamer. Succinate binding to the normal catalytic trimer, causes conformation changes in the nitrotyrosine containing trimer also. This confirm that conformational changes in one subunit are communicated to the others even though the second one does not bind to the substrate or its analog.