Parasite Biochemistry

Lecture 3

Nucleic acid metabolism

Pyrimidine biosynthesis

- Unlike some flagellate protozoa (e.g. Trichomonas), *Plasmodium* is able to synthesise pyrimidines *de novo*, although the erythrocyte is unable to do so.
- Salvage mechanism is unnecessary for survival

Purine salvage

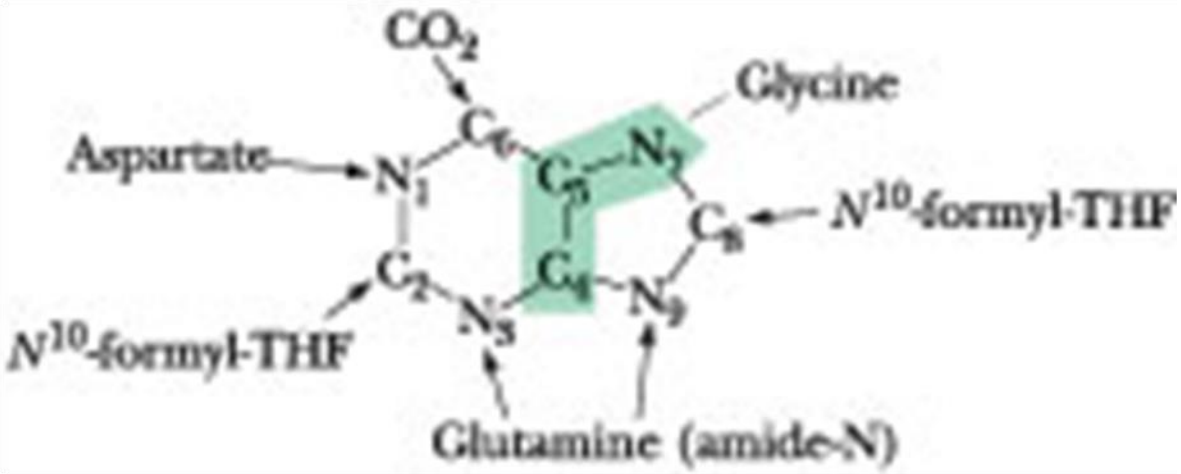
- A functional *de novo* biosynthesis pathway for purines has not been identified in any *Plasmodium* species
- A purine salvage pathway operates
- The purine preferred by *P. falciparum* is hypoxanthine

Purines, Pyrimidines and their salvage

- There is almost no evidence to suggest that either the parasitic protozoa or the parasitic helminths are capable of synthesizing the purine ring out of simple precursors (i.e. are not able to synthesize purines *de novo*).
- The precursors of purine synthesis in organisms that do have the capacity are: CO_2, formate, glycine, aspartate and glutamine.
- In the absence of de novo synthesis, it is not very surprising to discover that parasites have elaborate systems for securing and interconverting purine derivatives of host origin.
- The reactions that result in the interconversion of purine derivatives are collectively known as salvage pathways.
- Analysis of extracts of parasites (studies in malaria parasites and schistosomes) show that they contain the same range of purines and purine derivatives that are found in free-living organisms.

- The processes of purine salvage and the loss of *de novo* purine synthetic activity are parasitic adaptations that release the organism from the necessity of maintaining a large suite of enzymes to sustain a highly energy demanding synthetic pathway.
- Most parasitic protozoa and all helminths seem to be capable of synthesizing pyrimidines *de novo* from carbamoyl phosphate and aspartate
- Carbamoyl phosphate synthase is a key enzyme in pyrimidine synthesis.

Origins of atoms in the purine ring

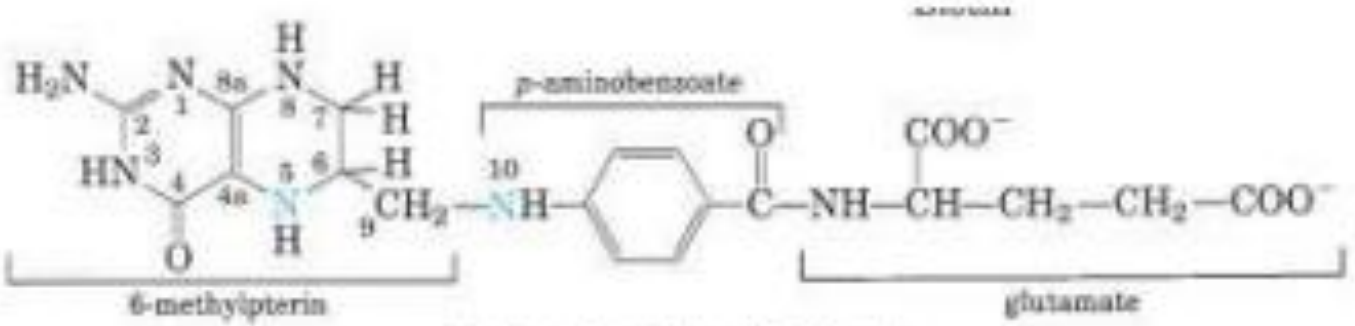


Metabolism of cofactors
Folic Acid Metabolism

- Folic acid is very important in metabolism because it is involved in transfer of one carbon group from one compound to another during biosynthetic processes.
- The one carbon groups carried include: methyl, methylene, methenyl, formyl and formimino groups.

Structure

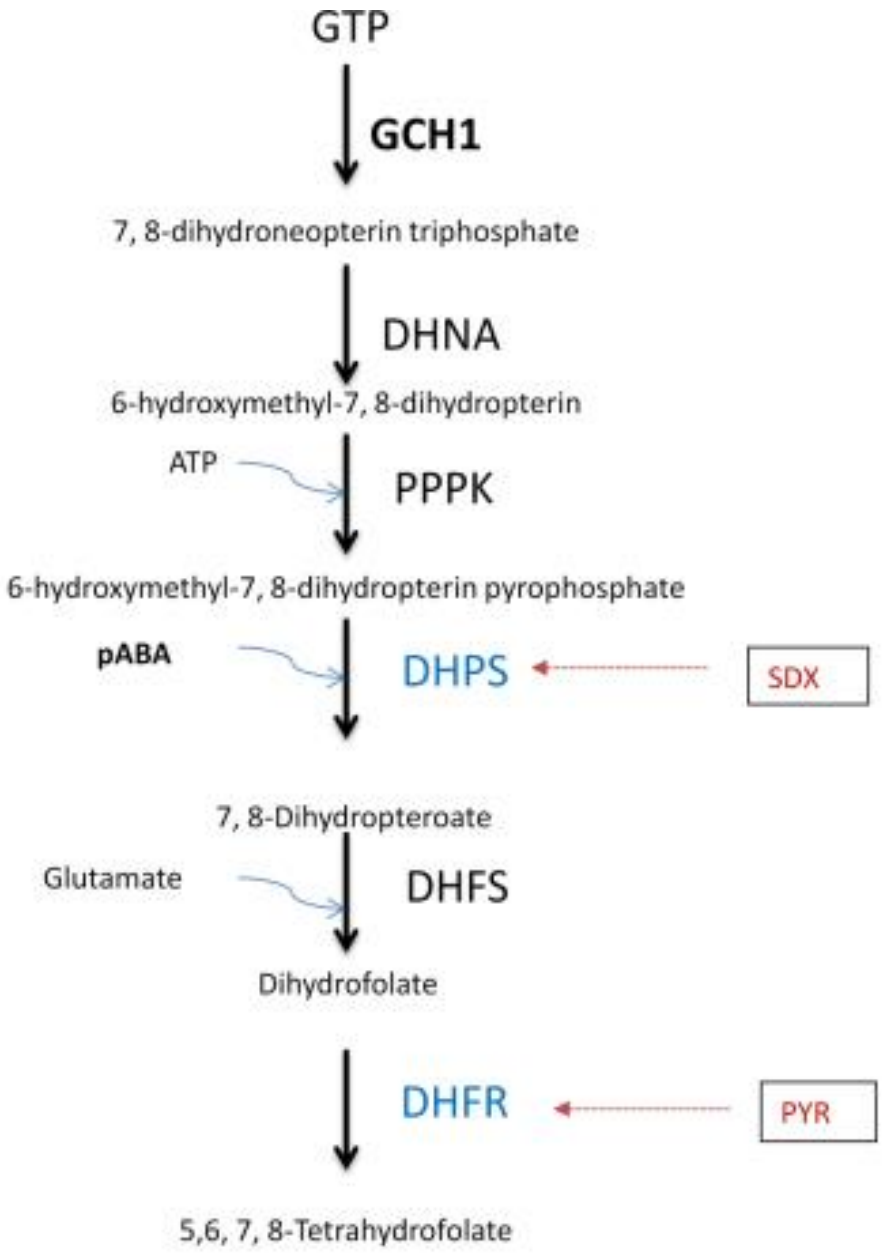
Tetrahydrofolate (also called tetrahydropteroylglutamate), consists of three groups: a substituted pteridine, p-aminobenzoate, and a glutamate residue.



Tetrahydrofolate (H_4 folate)

Role

- Folic acid is required for purine and pyrimidine metabolism
- Folate metabolism is extremely important for metazoans and is highly conserved in all metazoans.
- An important difference in folic acid metabolism and mammalian host is in Filariae.
- Filaria can oxidise 5-methyl Tetrahydrofolate to 5,10 Methylene Tetrahydrofolate. This reaction doesn’t occur in the host.
- This may be an adaptation with the parasite to provide them with an additional substrate other than folate.



GCH1, GTP cyclohydrolase I ;
DHNA, dihydroneopterin aldolase;
PPPK, hydroxymethyldihydropterin pyrophosphokinase ;
DHPS, dihydropteroate synthase ;
DHFS, dihydrofolate synthase ;
DHFR, dihydrofolate reductase.

- 5,10 Methylene Tetrahydrofolate is a substrate for thymidylate synthetase, which is involved in the conversion of dUMP to dTMP
- dTMP can be converted to dTTP required for nucleic acid synthesis.
- The reaction is very important for the parasites that cant synthesise the nucleotide *de novo* or salvage it.
- However, parasites employing de novo purine synthesis require 5,10 Methylene Tetrahydrofolate and such parasites include Filariae.
- Because of this importance of purine metabolism, interruption of folate supply in metabolism will inhibit growth and reproduction in parasites.
- Intracellular protozoa and pathogenic bacteria synthesise Tetrahydrofolate *de novo* from GTP, PABA and glutamate, whereas mammalian host don’t have this pathway.

- Mammals get folate from their diet which they directly reduce to tetrahydrofolate.
- This substantial difference in the source of tetrahydrofolate has proved useful in chemotherapy since analogs of PABA competitively inhibit the enzyme dihydropteroate synthase (DHPS) and this diminishes the rate of tetrahydrofolate synthesis.
- Sulfonamides e.g. sulfathiazole, sulfaquinidine, sulfanilamide, sulfadoxine etc and sulfones e.g. dapsone, will inhibit the enzyme in Plasmodia spp as well as other intracellular species of sporozoans such as Toxoplasma and Eimeria.
- Another important chemotherapeutic target in folic acid metabolism is the enzyme dihydrofolate reductase.
- Methotrexate which is a structural analog of pteridine derivative inhibits dihydrofolate reductase (DHFR).
- Methotrexate is used in cancer chemotherapy. Since the drug is moderately selective it causes toxicity problems in the host.

- The drug is not generally taken up by most bacteria or protozoa because this organisms don't have a specific transport mechanism for the drug.
- However this drug is a potent inhibitor of *P. berghei* DHFR. It will also inhibit the growth of Leishmania species *in vitro*.
- Pyrimethamine is a derivative of 2, 4 diamino pyrimidinyl moiety of methotrexate. It is a powerful antimalarial which is highly selective and therefore widely used.
- Plasmodial DHFR is a 1000× more sensitive to inhibition to this compound compared to mammalian enzyme. However, Leishmanial enzyme is less sensitive.
- Other inhibitors of DHFR used against Plasmodia include: Trimethoprim, Proguanil, Clociguanil etc
- Trimethoprim is also active against trypanosomes *in vitro* but not in vivo. Because trypanosomes in vivo seemingly have a higher concentration of DHFR is their cells.

- Suramin inhibits DHFR of *T. brucei brucei*.
- The enzyme from filaria worms *O. volvulus* appears to be different among metazoans because it is particularly sensitive to suramin.
- Combination of agents that attack both of these enzymes in the folate metabolism act synergistically because pathway inhibition is achieved by inhibiting two sequential sites in a reaction series.
- Important malarial treatments employing this principle are: Maloprim (pyrimethamine and dapsone), Fansidar (Pyrimethamine and sulfadoxine) and Metakelfin.
- Toxoplasmosis has also been effectively treated with high concentrations of pyrimethamine and sulfonamide. In this case the host is protected from inhibition of the DHFR by supplementation of Leucovorin (reduced folate)
- Leucovorin cant be utilised by the toxoplasma but can only be utilised by mammalian cells.

Potential Drug Targets

- Parasitic protozoa multiply rapidly in the host. Most undergo differentiation and maturation in the host.
- Metabolism associated with cell division e.g. Nucleic acid metabolism and compounds involved in other metabolism e.g. polyamines hence these are potential targets.
- Most helminths invade the host after their last stage of reproduction hence no proliferation.
- In helminths proliferation takes place in specialized reproductive tissues and inhibiting such proliferation would not necessarily affect the disease process, though it may interrupt the life cycle.
- Some useful targets in helminths are like muscular activity and neuromuscular coordination, nutrient uptake.
- Other targets include: interparasite communication, essential energy metabolism etc.

- Helminths are close to humans than in protozoa hence protozoa will be having more unique receptors than helminths

Drug Targets in Parasites

- In a broad sense the type of drug targets can be categorised according to the kind of metabolism that can be targeted.
- Complication is that many compounds affect more than one process. e.g. Suramin has multi-targets
- In addition many compounds attack different taxonomic groups hence no justification that they will work in the same way in all the groups.

Drug targets in energy metabolism

- Because of heavy reliance in glycolysis for the provision of energy, the enzymes involved are different.
- Hexokinase and PFK are rate limiting steps and feedback inhibition targets them but in *T. brucei* there are no such feedback inhibitions.

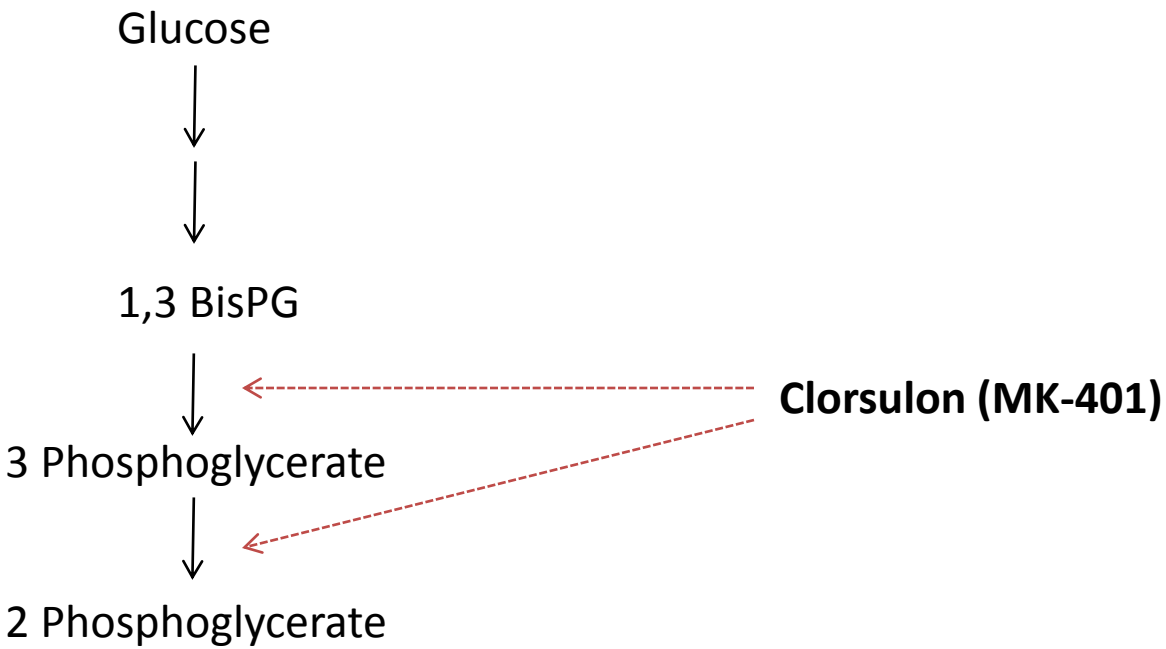
Drug targets in carbohydrate metabolism

- It is possible to design a molecule that specifically inhibits glycolytic pathway in the parasites.
- Since TCA cycle is absent or restricted hence inhibition of glycolytic pathway will be detrimental.
- Rate controlling enzymes (RCE) are very attractive targets for attack.
- In parasites it will be the glucose transport system from the environment or for parasites that store glycogen it will be the 1st step of breakdown of glycogen i.e. glycogen phosphorylase.
- Glycogen phosphorylase is controlled by covalent modification which is affected by external signals.
- A number of chemotherapeutic agents affect the glycogen phosphorylase

e.g. In *schistosoma mansoni*, a drug Niridazole affects glycogen phosphorylase phosphatase (enzyme responsible for inactivating glycogen phosphorylase).

- Because of this inhibition there is disruption of glycogenolysis and this leads to increase in rate of the glycogen breakdown hence the reserves will be depleted hence the parasite starves.
- Levamisole is antihelminthic drug which affects the control of glycogen metabolism. It has disruptive effects on nematode *in vitro.* It is used in treatment in *L. carinii* and *A. suum.*
- Levamisole increases glycogen synthase activity and inhibits glycogen phosphorylase activity. Therefore, the mobilisation of glycogen is affected at a time when ATP levels are diminished as a result of initial muscular paralysis induced by Levamisole.
- The precise molecular site of action has not been identified

- **Clorsulon (MK-401)** is a structural analog of 1,3 Bisphosphoglycerate
- It inhibits 2 enzymes of glycolysis in *F. hepatica***.** The enzymes are phosphoglycerate kinase and phosphoglycerate mutase.



- The net result is the inhibition of glucose utilisation and end product formation.

- In the host MK-401 binds to the carbonic anhydrase in RBC. The blood feeding parasites ingest the RBC and liberate the compounds which are active during digestion hence affected more than the host.
- Glycolytic kinases are particularly sensitive to organic antimony compounds. e.g. 6-phosphofructokinase of *S. mansoni* is a lot more sensitive to two of these antimony compounds i.e. Stibophen and Antimony potassium tartarate.
- Parasite from adult filarial worms is also sensitive to Stibophen.
- Antimony organic compounds, Melarsen and Melarsen oxide inhibit pyruvate kinase of African trypanosomes both *in vivo* and *in vitro*.
- In Leishmania mexicana Hexokinase, pyruvate kinase, PFK and malate DH are inhibited by Melarsen oxide