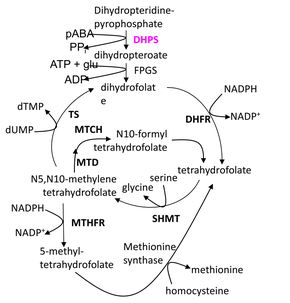
The effect of osmolytes on ligand binding to dihydropteroate synthase

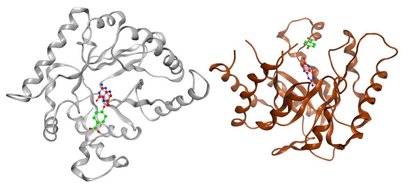
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Abstract

Folic Acid, or Vitamin B_9, is required for the synthesis of amino acids and nucleotide precursors. Bacteria can synthesize their own folic acid, unlike humans. This allows for drugs to target bacterial folate synthesis enzymes.
Dihydropteroate synthase (DHPS) is one of the enzymes in the folate synthesis pathway. DHPS is responsible for the production of dihydropteroate and pyrophosphate from 6-hydroxymethyl-7, 8-dihydropterin pyrophosphate (DHPP) and para-aminobenzoic acid (pABA). The reaction is an ordered reaction with DHPP binding to the enzyme first, which then allows pABA to bind.

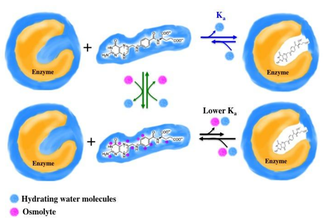


Folate cycle



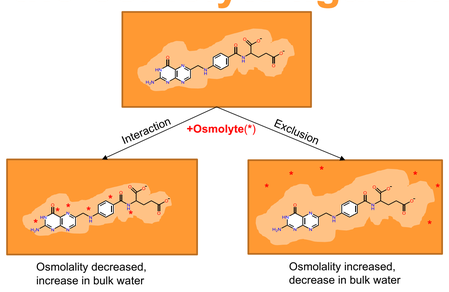
Dihydropteroate synthase
DHPS is a homodimer with a molecular weight of 61952Da. The ligands, pterdine pyrophosphate (PtPP, an analog of natural ligand DHPP) is highlighted in red and p-amino-benzoic acid (pABA) is highlighted in green.

Previous studies in our lab have shown that osmolytes weakly interact with folate at particular functional groups. Osmolytes are small molecules involved in osmoregulation in living cells.
During an enzyme ligand complex formation, binding occurs when the water molecules are removed from both the ligand and enzyme as shown in the top panel of the figure to the right. In cases wherein osmolytes are introduced in the system and osmolytes bind weakly with the ligand, then there has to be removal of not only the water molecules but also the osmolytes in order for the enzyme ligand complex to form. When osmolytes interact with the ligand, the K_a decreases and equilibrium shifts to the left.
We hypothesize that osmolytes will weakly interact with DHPP and pABA and the ligands of DHPS will prevent them from associating with the DHPS enzyme.



Model of the preferential interaction of an osmolyte with the ligand

Preferential interaction coefficients for the osmolyte ligand interactions



Model of the interaction of an osmolyte with a compound

Enzyme	Ligand/cofactor	Betaine μ_{23}/RT (m^{-1})	Trehalose μ_{23}/RT (m^{-1})
Dihydropteroate Synthase	DHPP	0.648	-1.15
	PABA	-0.471	-0.096

Table 1 Predicted coefficients for preferential interaction between folate ligands with betaine and trehalose. A negative number indicates preferential interaction and a positive number indicates preferential exclusion.

Protein Expression and Purification

Bacillus anthracis DHPS gene cloned in pET plasmid and transformed in *Escherichia coli* was procured from St. Jude's hospital

A starter culture of 10mL was grown overnight and added to 1L TB. Culture was shaken at 37˚C until the OD reached 0.6-0.8 at 600nm

Cells were then induced with 1mM IPTG then shaken at 18˚C for 24 hours

Cells were centrifuged at 5000 rpm for 10 minutes and cell pellet was used for protein purification

Pellet was mixed in Buffer A (50mM Tris, 500mM NaCl, 5mM Imidazole)

Lysozyme and protease inhibitor cocktail was added to the cells for 30 minutes and incubated in a cold room

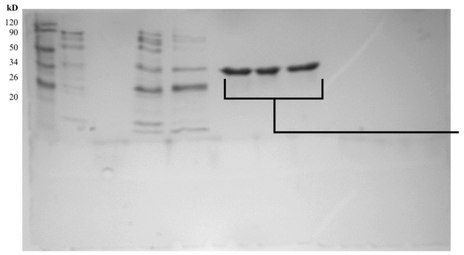
The cell lysate was sonicated, then centrifuged for 10 mins at 14000 rpm. Process was then repeated and supernatant was added to a Nickel-NTA column

The column was washed with Buffer A and the protein was eluted with an Imidazole gradient (5-500mM)

SDS gel electrophoresis was conducted to confirm the presence of DHPS in the fractions.

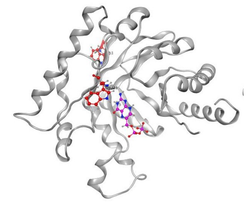
Fractions with the protein were dialyzed in snakeskin dialysis bag against buffer (50mM Hepes pH 7.6 and 150mM NaCl)

BCA assay was conducted to calculate concentration and extinction coefficient of the protein (extinction coefficient = 0.85mL/mg)

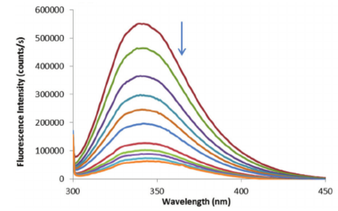


SDS Page of DHPS protein prep
DHPS ~ 32 kD

Fluorescence spectroscopy can be used to detect PtPP ligand binding to DHPS

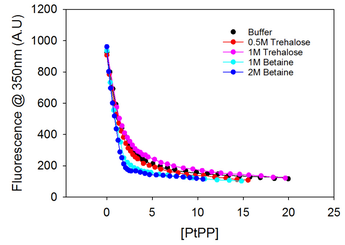


Monomer of DHPS (1TWW)
There are two tryptophans present in a monomer of DHPS. The ligand PtPP is labelled in pink and the tryptophan residues are labelled in red.

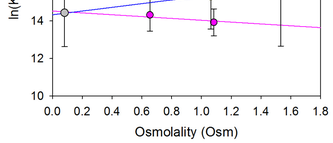


Representative graph of fluorescence titration with addition of increasing concentration of PtPP
As increasing concentrations of PtPP are added to DHPS, the fluorescence intensity of tryptophan decreases because the binding of PtPP to the enzyme affects the environment of tryptophan. This causes the emission of tryptophan to decrease. Fluorescence spectroscopy was conducted at an excitation of 295nm and emission of 350nm.

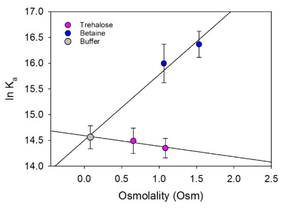
Addition of osmolytes affects the ligand PtPP binding to the enzyme



Change in fluorescence with increasing concentrations of PtPP binding to enzyme in presence of osmolytes.
Changes in fluorescence were seen when the ligand PtPP was titrated into DHPS in absence and in presence of the osmolytes trehalose and betaine.



Differential effects on binding of PtPP to DHPS were seen in presence of betaine and trehalose
The binding of the ligand PtPP to the DHPS enzyme decreases in the presence of trehalose and increases in the presence of betaine. We have obtained a negative preferential interaction coefficient for PtPP and trehalose as shown in the table. Trehalose preferentially interacts with certain functional groups of PtPP, which inhibits the ligand from binding to the protein. The preferential interaction coefficient of PtPP with betaine is positive. Betaine is excluded from PtPP which allows the ligand to bind to the protein tighter.



Isothermal Titration Calorimetry studies of ligand binding to DHPS in the presence of betaine and trehalose. Pteridine pyrophosphate binding to DHPS. The association of PtPP to DHPS is reduced in presence of trehalose whereas the presence of betaine tightens the association.

Conclusions

Fluorescence spectroscopy can be used to monitor ligand PtPP binding to the enzyme DHPS.
Trehalose preferentially interacts with PtPP and prevents its association with enzymes DHPS.
Betaine is preferentially excluded from PtPP and allows PtPP to bind to enzyme DHPS tightly.

Acknowledgements

We thank Dr. Richard Lee at St. Jude's Hospital for kindly supplying the plasmid for this project.

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