PhD thesis title : Study of catalytic and inhibition properties of pyruvate ferredoxin oxidoreductases from different organisms.

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Team : Hydrogen metabolism

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Study background

Pyruvate:ferredoxin oxidoreductase (PFOR) is an essential enzyme in the metabolism of various anaerobic bacteria and parasites. It is a metalloenzyme that reversibly catalyses the following reaction:

$CoA + pyruvate + Fd_{ox} \rightleftarrows Acetyl\text{-}coA + CO_2 + Fd_{red}$ (Fd= ferredoxin).

The catalytic reaction takes place at a thiamine pyrophosphate (TPP) active site, and three FeS clusters serve as electron relays between the active site and the redox partner.

In the field of health, the inhibition of this enzyme is used as a therapeutic treatment against infections by anaerobic (or microaerobic) bacteria or parasites such as *Clostridium difficile*, *Giardia intestinalis* or *Heliobacter pylori* [1]. This enzyme is a central en to anaerobic metabolism and is not present in mammals. Food and Drug Administration approved inhibitors including nitazoxanide (NTZ) or metronidazole. It is proposed that these inhibitors act on the active site of thiamine pyrophosphate (TPP) [1]. However, our preliminary studies indicate that these inhibitors act as an oxidant of the artificial or physiological redox partner of PFOR, such as benzyl viologen or ferredoxin. Thus, the inhibition by these compounds is based on a non-specific reaction with the redox partner, and not on an active site-specific inhibition and the mechanism of inhibition by the commercial drugs is often only speculative. It is therefore possible that these drugs may also disrupt the redox balance of human cells. On the other hand, phosphonates are analogues of pyruvate and have been proposed as inhibitors of other TPP active site enzymes (pyruvate dehydrogenase, lactate dehydrogenase) [2-3], so phosphonates could also be PFOR inhibitors.

We propose the rational design of phosphonic inhibitor, compound from this family was shown to be competitive inhibitors of other TPP enzymes. The determination of inhibition constant for PFOR from 4 different micro-organisms will allow to strength our confidence on the inhibition properties of the compounds we will synthetized, on PFOR of various bacteria and parasites.

PhD project description

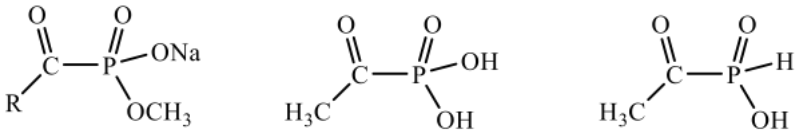
The objective of the thesis is to purify PFOR from several different micro-organisms, to determine their enzymatic properties, to synthesise phosphonate derivatives in order to determine the inhibition properties of these compounds on the different PFOR. To achieve this objective, the studies will be divided into two phases:

1) Production, purification and enzymatic characterization of PFORs:

Escherichia coli strains allowing the production of PFORs from 4 organisms (*Desulfocurvibacter africanus, Solidesulfovibrio fructosovorans, Escherichia coli* and *Chlamydomonas reinhardtii*) are available in the team. As all PFORs are very sensitive to oxygen, the entire purification protocol will be carried out completely anaerobically (in a recently acquired glove box). The purification can be done in one step on an affinity column. PFOR from *C. reinhardtii* and from *D. africanus* have been homologously produced, purified and studied by biochemical methods

previously [4,5,6]. During the thesis, the student will determine the enzymatic properties (Km for substrates, optimal pH and temperature) of *D. africanus* PFOR produced in *E. coli* and compare them to those already published to verify that heterologous expression does not influence the catalytic behavior of the enzyme. PFORs from *Solidesulfovibrio fructosovorans* and *Escherichia coli* have never been studied. The student will purify them and determine their enzymatic and biochemical properties (oligomerisation state, metal content). The comparison of the properties of the different enzymes will allow us to define the molecular determinants of catalysis by these enzymes, especially the effect of inhibitor of the catalytic site environment varies.

2) Synthesis of alkyl monophosphonic acids and determination of the inhibition properties of the compounds on the enzymatic activity of PFOR.
In collaboration with the BioSciences team of ISM2, the student will synthesize phosphonate derivatives and evaluate their inhibitory properties towards different PFORs (Figure below).



The 3D structure of *D. africanus* PFOR is avalaible [7], and it can be used as a template for structural model for the 3 other PFORs. Putting together the inhibition constant that will be determined with the analysis of these structures, the structural parameters likely to modulate the inhibition activities will be identify. This step will allow the design and synthesis of phosphonate analogues that could have better inhibition properties of PFOR enzymatic activity. This part of the study is based on back-and-forth synthesis of new compounds whose structure will be rationalized according to the results of the inhibition tests.
In a last step, the effect of the best inhibitors on the growth of anaerobic du micro-organisms will be tested.

Bibliography

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