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# A study of *Bos taurus* muscle specific enolase; biochemical characterization, homology modelling and investigation of molecular interaction using molecular docking and dynamics simulations

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ABSTRACT

Tropical theileriosis caused by *Theileria annulata* obligate parasite that infect ruminant animals, including *Bos taurus*. The disease results massive economic losses in livestock production worldwide. Here we describe cloning, expression and both biochemical and structural characterization of beta enolase from *Bos taurus in vitro and in silico*. The interconversion of 2 phosphoglycerate to phosphoenolpyruvate was catalyzed by enolase is a metalloenzyme in glycolytic pathway and gluconeogenesis. Enolase from *Bos taurus* was cloned, expressed and the protein was purified at 95% purity using cobalt column by affinity chromatography. The optimum enzymatic activity was calculated at pH 6.5. For the first time in the literature, the kinetic parameters of the enzyme, *Vmax* and *Km*, were measured as 0.1141 mM/min and 0.514 mM, respectively. Besides, *Bos taurus* enolase 3-dimensional structure was built by homology modelling to be used *in silico* analyses. The interactions of the enzyme-substrate complex were elucidated by molecular dynamics simulations for 100 ns. These interactions were found to be the same as experimentally determined interactions in yeast. These results would enable further structure based drug design studies with the biochemical characterization of the host organism *Bos taurus* enolase enzyme *in vitro* and the elucidation of behavior of enzyme-substrate complex *in silico*.



## 1. Introduction

Tropical theileriosis is one of the most widespread diseases among thick-borne cases, causing serious losses in livestock production in especially North Africa, the Mediterranean coastal area, South Europe and Asia [1,2]. *Theileria annulata* is an obligate intracellular protozoan parasite transmitted by *Hyalomma* genus tick vectors and causes the disease in ruminant animals especially *Bos taurus* and *Bos indicus* [2,3]. Buparvaquone has been the most effective antitheilerial drug used for the treatment of tropical theileriosis but resistance of *T. annulata* against buparvaquone have been reported since 2010 [4–6]. Because of this reported resistance and long time required to drug development process, need for discovering alternative drugs has greatly increased.

Advances in genomics, computational chemistry and biology give a chance to design new structure based drugs. In structure based drug design, metabolic enzymes are mostly favorable targets [7]. Glucose catabolism in *Theileria* macroschizonts predominantly consist of lactic acid production pathway and therefore glycolytic enzyme enolase has an important role in glucose catabolism [8–10]. Enolase is a key enzyme that catalyzes the interconversion of 2 phosphoglycerate to phosphoenolpyruvate in glycolytic pathway and gluconeogenesis [10,11]. The enzyme is also responsible for non-glycolytic functions such as contributing regulation of the cytoskeletal filaments [10,12]. In addition, enolase is plasminogen binding receptor, located at cell surface and this plasminogen activation mediates pathogen invasion [10,13,14]. Taken together, enolase would be a crucial target protein that these functions of the parasite could be inhibited.

The isofunctional host enzyme should be analyzed in parallel with the parasite enzyme according to structure based drug design approach in order to evaluate the selectivity and possible toxicity [15]. Therefore the gene encoding *Bos taurus* muscle enolase (*Bt*Eno3) which is counterpart of *T. annulata* enolase, was cloned, expressed, purified and biochemically characterized for the first time in the literature. As no empirically defined structure was solved, *Bt*Eno3 was modelled to predict 3-dimensional (3D) protein structure of the enzyme. The substrate 2 phosphoglycerate (2PG) was docked into enzyme and molecular dynamics simulation of the enzyme-ligand complex was carried out to predict binding behavior of the substrate on the enzyme. These *in vitro* and *in silico* analyses on *Bt*Eno3 were performed to enlighten

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