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An organism-independent unified model for activity of orotate phosphoribosyltransferases for orotidine monophosphate synthesis

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HIGHLIGHTS

- We propose a mechanistic model for the action of orotate phosphoribosyltransferase.
- The model encompassed the features of ping-pong, ordered and random kinetic model.
- The model proposed was organism-independent.
- The model was tested to describe the kinetics of orotidine monophosphate synthesis.

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ABSTRACT

A unified model for orotate phosphoribosyltransferase-action has been developed describing the enzymatic synthesis of orotidine monophosphate, an important intermediate for nucleotide synthesis. The reaction, prevalent in micro-organisms, participates in the *de novo* nucleotide synthesis pathway, and is a popular target for inhibition to check the growth of *Mycobacterium tuberculosis*. Features of three enzymatic mechanisms, viz., ping-pong bi-bi mechanism, random kinetic mechanism and sequential kinetic mechanism were incorporated in the unified model to increase the range of successful applicability of the model and to make it organism-independent. The model could successfully describe the kinetics of reaction for the reported data. Kinetics of the reaction with enzyme derived from *Mycobacterium tuberculosis*, *Saccharomyces cerevisiae*, *Salmonella typhimurium* and *Plasmodium falciparum* was tested and the unified model was found to be valid with a change in the organism from which the enzyme has been derived. The model can serve as a reliable model to describe the kinetics of the reaction with enzyme derived from new organisms without having to do the mechanistic determination owing to organism-independent nature of the model.

1. Introduction

Orotate phosphoribosyltransferases (OPRT; EC 2.4.2.10) catalyze the reaction of α-D-5-phosphoribosyl-1-pyrophosphate (PRPP) and orotate (OA) to yield orotidine 5′−monophosphate (OMP) and pyrophosphate (PP_i), a reaction important for *de novo* synthesis of pyrimidines in several micro-organisms and higher species (Zhang et al., 2013; West, 2014). Orotate binds to the ribose moiety subsequently giving pyrimidines through a series of conversions. The pyrimidine biosynthesis pathway has been described in detail by Schultheisz et al. (2011). We briefly highlight the pathway below.

Glucose is converted to PRPP through glusose-6-phosphate, 6-phosphogluconic acid, ribulose-5-phosphate and ribose-5-phosphate. This series of conversions is carried out by hexokinase, glucose-6-phosphate dehydrogenase, phosphoribose isomerase, and PRPP synthase, respectively. Similarly, OA is synthesized by a series of conversions involving bicarbonate ion and ammonium ion to give carbamoyl phosphate in the presence of carbamoyl phosphate synthase, conversion of carbamoyl phosphate to aspartate by aspartate carbamoyl transferase, conversion of carbamoyl aspartate to dihydro-orotate by dihydro-orotase and finally to OA by dihydro-orotate dehydrogenase. The two branches in the biosynthesis pathway merge with the reaction of PRPP and OA in the presence of OPRT to give OMP and PP_i through N-glycosidic bond formation (Berti and McCann, 2006). The reaction is shown below.

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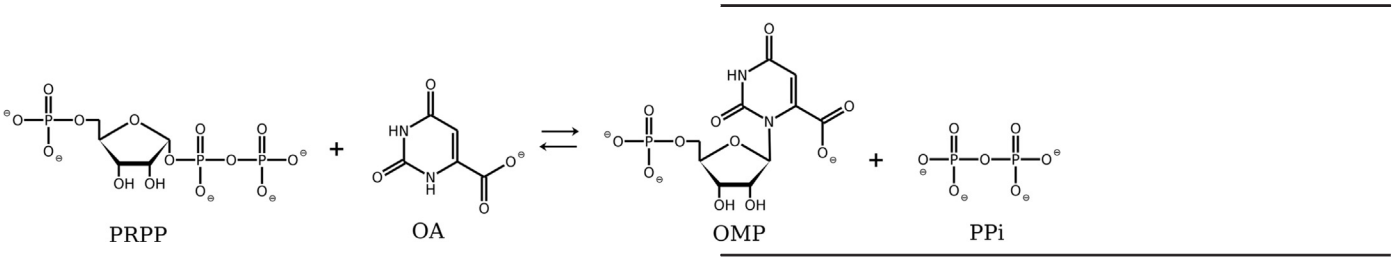

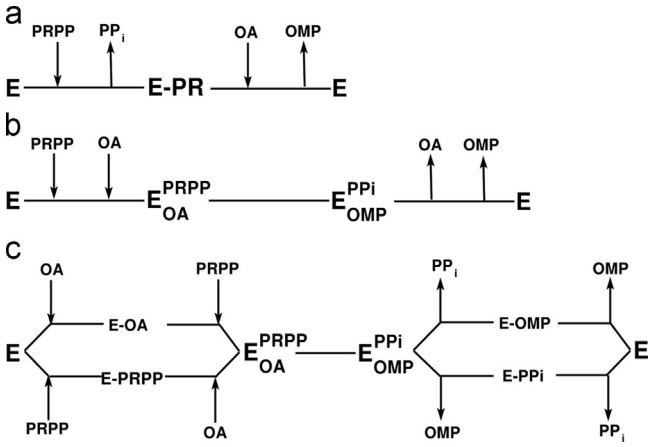

Fig. 1. Different mechanisms available in literature for OPRT-action, (a) ping-pong bi-bi mechanism, (b) ordered mechanism and (c) random-kinetic mechanism (reproduced from Witte et al., 1999).

Mechanistic models of chemical and biochemical processes help in quantification of experimental data and facilitate the comparison of different sets of experimental observations. With a reliable mechanistic model available for describing the kinetics of an enzymatic reaction, it becomes feasible to compare and contrast the effects of different process conditions, including the environment and inhibitors, on the reaction under study. A good mechanistic model, therefore, can help in identification and screening of inhibitors for drug development through quantification of the kinetic data. Therefore, a detailed understanding of kinetics and mechanism of OPRT-action is important as the inhibition of this reaction in organisms like *Mycobacterium tuberculosis* can have implications on development of drugs for cure of tuberculosis (Breda et al., 2012a,b).

Due to the biological and medical implications, OPRT-action has been a matter of investigation for a long time. There are several issues concerning the mechanism of the reaction which remain unresolved till date and require attention. Experimental studies have been carried out probing the mechanism of OPRT-action (Victor et al., 1979a,b; Syed et al., 1987; Bhatia et al., 1990; Tao et al., 1996; Witte et al., 1999; McClard et al., 2006; Krungkrai et al., 2004). Surprisingly, different studies either report different mechanisms for the reaction which are not in agreement or the mechanisms are suspected to be organism-dependent thereby making the mechanism of the reaction doubtful. Three important mechanisms reported for the reaction are ping-pong bi-bi mechanism, ordered kinetic mechanism and random kinetic mechanism. A schematic of these mechanisms for OPRT-action is shown in Fig. 1.

Victor et al. (1979a,b) have carried out the mechanistic investigations of OPRT-action with enzyme derived from yeast. Isotope exchange, initial velocity kinetics and product inhibition studies were conducted to identify the mechanism of the reaction. All of their studies confirm the ping-pong bi-bi mechanism for the formation of OMP. To further confirm the ping-pong bi-bi mechanism, the authors conducted isotope exchange experiments which showed independent half-reactions and OMP as the last species to leave the site. Bhatia et al. (1990) have suggested two possible mechanisms for the reaction, viz., direct transfer mechanism in which nucleophilic attack by OA on PRPP takes place followed by displacement of pyrophoshate, and a two-step mechanism in which elimination of pyrophosphate from enzyme-bound PRPP takes place followed by the attack of OA to yield OMP. Formation of carboxonium-like intermediate is proposed in the first step. The steady-state kinetic analysis and isotope exchange studies conducted by the authors strongly favour the random kinetic mechanism and ping-pong bi-bi mechanism was discarded.

The non-existence of ping-pong bi-bi mechanism proposed by Bhatia et al. was further supported by a study by Witte et al. (1999). The authors observed that it was not possible to carry out the reaction as two independent half reactions, a requirement for ping-pong bi-bi mechanism. The experiments by the authors showed the ordered sequential mechanism. The authors also investigated the life-time of oxocarbocation and found that the life-time was too short for the species to be observed kinetically. However, in a later study by the same group, the investigators found the possibility of ping-pong bi-bi mechanism and established the mechanism as a variant of alternating site catalysis, indistinguishable from ping-pong bi-bi mechanism (McClard et al., 2006). Random kinetic mechanism has also been observed in the studies by Krungkrai et al. (2004).

It can be seen from the above discussion that no consensus has been reached for a single conclusive mechanism of the reaction. Therefore, for every new observation made for OPRT-action, a series of kinetic studies and tests of different kinetic models are to be done every time. The aim of this study is to develop a generic mechanistic model for the activity of OPRT for catalyzing the reaction. We propose a detailed reaction pathway for OMP formation catalyzed by OPRT which encompasses the features of all three mechanisms. The model was rigourously tested with kinetic data reported in the literature for OPRT-action with OPRT derived from different organisms and our unified mechanism was able to successfully describe all the test data without any additional assumption. The model will prove to be valuable for assessment of experimental kinetic studies without making any reference to the organism from which OPRT has been derived.

2. Model development

There are two major problems associated with the kinetic models that are described in the previous Section. Firstly, the mechanisms described by the models are not conclusive, i.e., more than one model developed on the basis of different mechanisms describe the kinetics of the reaction under different conditions. Secondly, the mechanism, and therefore the models in turn, depend upon the organism containing OPRT. Therefore, we intend to develop a model for the kinetics of PRPP-action which can describe the activity of the enzyme for a range of organisms