Archives of Biochemistry and Biophysics 542 (2014) 28–38

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Catalytic site interactions in yeast OMP synthase ☆

Michael Riis Hansen [a,1], Eric W. Barr [b], Kaj Frank Jensen [a], Martin Willemoës [a], Charles Grubmeyer [b,*], Jakob R. Winther [a]

[a] Department of Biology, University of Copenhagen, Ole Maaløes Vej 5, DK-2200 Copenhagen N, Denmark
[b] Department of Biochemistry, Temple University School of Medicine, 3307 N Broad St., Philadelphia, PA 19140, USA

ARTICLE INFO

Article history:
Received 26 August 2013
and in revised form 4 November 2013
Available online 18 November 2013

Keywords:
Catalytic site interactions
Orotate phosphoribosyltransferase
Kinetic mechanism
Heterodimer
Ligand binding
Stopped-flow

ABSTRACT

The enigmatic kinetics, half-of-the-sites binding, and structural asymmetry of the homodimeric microbial OMP synthases (orotate phosphoribosyltransferase, EC 2.4.2.10) have been proposed to result from an alternating site mechanism in these domain-swapped enzymes [R.W. McClard et al., Biochemistry 45 (2006) 5330–5342]. This behavior was investigated in the yeast enzyme by mutations in the conserved catalytic loop and 5-phosphoribosyl-1-diphosphate (PRPP) binding motif. Although the reaction is mechanistically sequential, the wild-type (WT) enzyme shows parallel lines in double reciprocal initial velocity plots. Replacement of Lys106, the postulated intersubunit communication device, produced intersecting lines in kinetic plots with a 2-fold reduction of k_{cat}. Loop (R105G K109S H111G) and PRPP-binding motif (D131N D132N) mutant proteins, each without detectable enzymatic activity and ablated ability to bind PRPP, complemented to produce a heterodimer with a single fully functional active site showing intersecting initial velocity plots. Equilibrium binding of PRPP and orotidine 5′-monophosphate showed a single class of two binding sites per dimer in WT and K106S enzymes. Evidence here shows that the enzyme does not follow half-of-the-sites cooperativity; that interplay between catalytic sites is not an essential feature of the catalytic mechanism; and that parallel lines in steady-state kinetics probably arise from tight substrate binding.

Introduction

OMP synthase[2] (orotate phosphoribosyltransferase, EC 2.4.2.10) presents unusual and contradictory features in structure, kinetics and mechanism. The enzyme performs the fifth step in *de novo* pyrimidine nucleotide synthesis, the fully reversible (K_{eq} = 0.1 [2]) Mg^{2+}-dependent transfer of the 5-phosphoribosyl group from 5-phosphoribosyl-1-diphosphate (PRPP) to the base orotate (OA), forming the UMP precursor orotidine 5′-monophosphate (OMP) and expelling pyrophosphate (PP_i). The enzyme has no known regulation of activity, other than through gene expression [3].

Structurally, OMP synthases from most microbes (bacteria, yeast, eukaryotic parasites, archaea) are small (180–225 residues), homodimeric and monofunctional (Fig. 1A). Like other members of the type I PRTase family the enzyme contains a Rossmann fold-based nucleotide binding α/β core, with catalytic residues atop the twisted β-sheet [4,5]. Two additional major structural elements are seen: a stable amino-terminal β-stranded "hood" distal from the subunit interface, and a highly flexible loop (also called the catalytic loop) that arises from the interface itself. The hood encloses the base binding sub-site, whereas the closed loop provides several PRPP-binding and catalytically essential residues (Fig. 1B) [1,6].

Uniquely among type I PRTases, OMP synthase is domain-swapped [7,8], in the sense that the catalytic loop from one subunit extends to act in and cover the active site formed by the neighboring subunit. The functional significance of this swap has previously been demonstrated for the *Salmonella typhimurium* enzyme by complementation experiments [9]. The striking appearance of the enzyme structure, with each catalytic loop participating in catalysis by the adjacent site, appears to demand an important active site interaction that could not be achieved by conventional type I PRTase architecture. It is thus tempting to hypothesize that the active sites may interact during the catalytic cycle to lower free energy barriers toward the rate-limiting [10] opening of the active site, or its partition between re-closing or product release.

Kinetically, the yeast enzyme displays parallel lines in Lineweaver–Burk plots of initial velocity data indicative of ping–pong

☆ Funding was provided by the Danish Agency for Science, Technology and Innovation (09-065032) to JRW. Eric W. Barr was supported by grants from the National Science Foundation to CTG.

* Corresponding author.
E-mail address: ctg@temple.edu (C. Grubmeyer).
[1] Current address: Department of Biochemistry, Temple University School of Medicine, 3307 N Broad St., Philadelphia, PA 19140, USA.

[2] *Abbreviations used*: OMP synthase, orotate phosphoribosyltransferase; OMP, orotidine 5′-monophosphate; PRTase, phosphoribosyltransferase; PRPP, 5-phosphoribosyl-1-diphosphate; PP_i, pyrophosphate; OA, orotic acid; WT, wild-type; ITC, isothermal titration calorimetry.

0003-9861/$ - see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.abb.2013.11.004

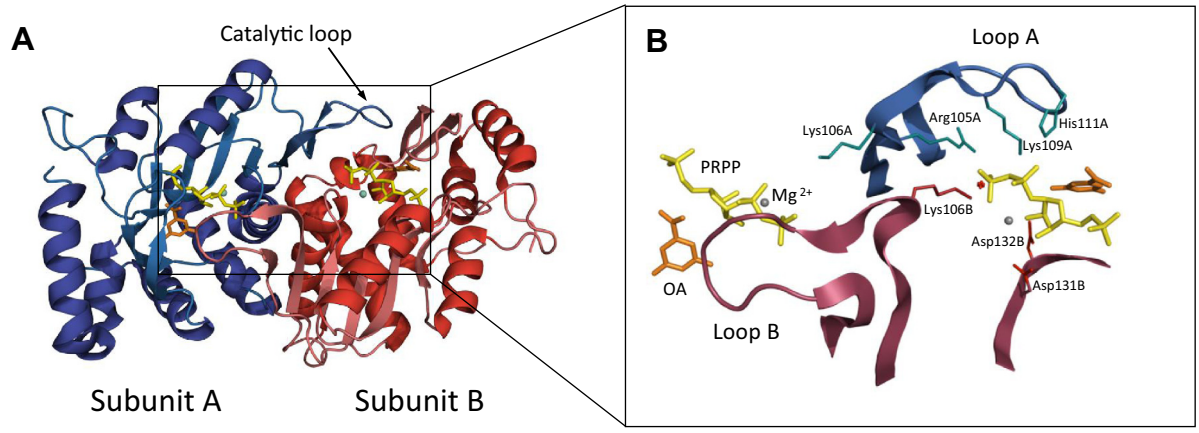

Fig. 1. Structure of homodimeric loop-closed yeast OMP synthase (PDB: 2PS1 [1]) with bound PRPP (yellow), Mg^{2+} (cyan) and OA (orange) in both subunits (A). The highly flexible catalytic loop is indicated for subunit A. (B) A close-up of the active site of subunit B. Loop side chains from adjacent subunit A (Arg105A, Lys109A and His111A) form H-bonds to the pyrophosphoryl moiety in PRPP, whereas Lys106B forms a H-bond to the pyrophosphoryl moiety in PRPP through a water molecule (H_2O 789B). The carboxyl groups of Asp131B and Asp132B from the PRPP binding motif form H-bonds to O3′ of PRPP, and O2′ of PRPP and O2 of OA, respectively. For clarity, H-bonds are not shown. For detailed binding interactions of the highly similar *S. typhimurium* OMP synthase structure see Grubmeyer et al. [6]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

kinetics [11], a mechanism defined by the demand that one or more products are released before all substrates are bound [12]. Ping–pong kinetics often result from double displacement chemical mechanisms in which one substrate is used to form a stable enzyme-associated intermediate (i.e. a phosphoribosylated enzyme). However, the OMP synthase reaction proceeds with stereochemical inversion at the anomeric carbon C1 (substrate α-PRPP undergoes inversion to product β-OMP). The enzyme also lacks isotope exchange reactions with substrate-product pairs PRPP/PP_i or OMP/OA showing that OMP synthase does not perform group transfer chemistry until both substrates have bound [2,13]. It has been shown by isotope trapping that the *S. typhimurium* enzyme can form kinetically stable and productive binary complexes with each of its four substrates: PRPP, OMP, PP_i and OA [14]. The enzyme was shown to follow a random mechanism with rapid PRPP and OMP binding in each direction. Following catalysis, OMP and PP_i leave the ternary complex with almost equal rates in the forward direction, whereas OA will dissociate first (85% of the time) in the reverse reaction. Furthermore, X-ray structures show specialized sub-sites for OA and PRPP in fully occupied OMP synthase complexes [1,6] and of transition state analog complexes in homologous type I PRTases [15–17]. Finally, there are several reports in the literature describing PRTases following sequential mechanisms in which the steady-state initial velocity kinetic data show a parallel line pattern in double reciprocal plots [18–20]. It is thus clear that chemical catalysis by yeast OMP synthase does not proceed by a double-displacement mechanism, and that parallel lines in steady-state kinetics must result from unusual rate constants.

Recently, the yeast enzyme has been closely reexamined both structurally and kinetically [1,13,21]. In a novel finding, isothermal titration calorimetry (ITC) results from those studies showed that binding of OMP or PRPP to the apoenzyme were each restricted to a single site per dimer (half-of-the-sites binding), in direct contrast to studies with OMP synthases from other organisms [14,20,22] and recent yeast structures occupied with PRPP•OA (PDB: 2PS1) and OMP (PDB: 2PRZ) in both subunits of the dimeric enzyme [1]. To account for the half-of-the-sites binding results, an alternating-sites type mechanism was proposed whose key feature is that catalytic cycles in the two sites are coupled through the action of the domain-swapped loops. It was proposed that binding of PRPP to one site aids displacement of product OMP in the other site, and that the free enzyme does not occur in the steady-state

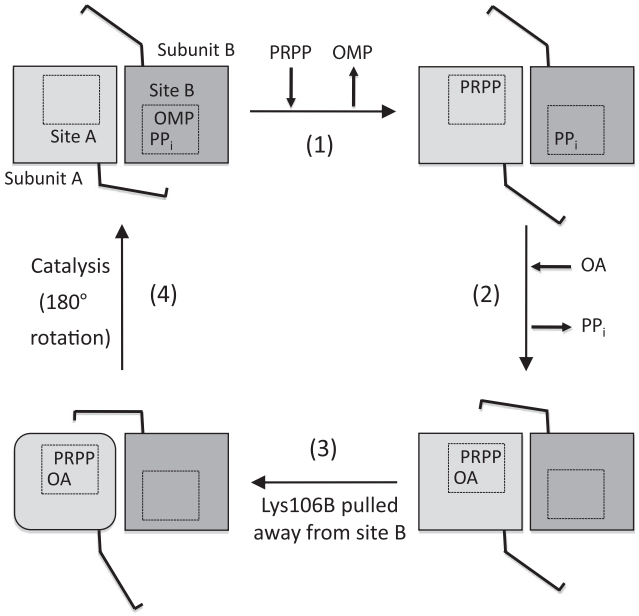

Fig. 2. Alternating site mechanism for yeast OMP synthase modified after [1]. (1) Binding of PRPP in site A causes release of product OMP in site B. PP_i remains bound in site B. (2) PP_i in site B is ejected as OA binds in site A. (3) The catalytic loop from subunit B covers the ternary complex in site A resulting in tightening of subunit A, Lys106B (projecting back into site B from the base of the loop) is pulled from site B preventing PRPP from binding in site B. Lys106A participates in binding in site A and the loop is forced open. (4) Catalysis occurs in site A to produce OMP and PP_i that end the first cycle. The free enzyme does not occur in the catalytic cycle.

process (Fig. 2). Kinetically, the interleaving of reaction sequences fulfills the demands of a ping–pong mechanism in that substrates PRPP and OA, respectively, bind in compulsory order to one site before products OMP and PP_i have left the other. Structurally and dynamically, the mechanism demands that communication between the subunits must occur. The alternating sites proposal receives some support from the recent highly asymmetric *S. typhimurium* enzyme structure in complex with PRPP and OA (PDB: 1LH0 [6]). In that complex, one catalytic loop has descended to cover the adjacent active site, which is filled by PRPP and OA, and is compact in structure. In the other site, only OA is bound, the associated loop remains "open" and disordered, and the overall