ScienceDirect

International Journal of Biological Macromolecules

Volume 259, Part 1, February 2024, 129226

# Structural and functional properties of uridine 5′-monophosphate synthase from *Coffea arabica*

Alexis Hinojosa-Cruz [a], Ángel G. Díaz-Sánchez [b], Adelaida Díaz-Vilchis [c], Lilian González-Segura [a]

Show more

Share Cite

https://doi.org/10.1016/j.ijbiomac.2024.129226 ↗



## Abstract

In higher eukaryotes and plants, the last two sequential steps in the *de novo* biosynthesis of uridine 5′-monophosphate (UMP) are catalyzed by a bifunctional natural chimeric protein called UMP synthase (UMPS). In higher plants, UMPS consists of two naturally fused enzymes: orotate phosphoribosyltransferase (OPRTase) at N-terminal and orotidine-5′-monophosphate decarboxylase (ODCase) at C-terminal. In this work, we obtained the full functional recombinant protein UMPS from Coffea arabica (*Ca*UMPS) and studied its structure-function relationships. A biochemical and structural characterization of a plant UMPS with its two functional domains is described together with the presentation of the first crystal structure of a plant ODCase at 1.4Å resolution. The kinetic parameters measured of *Ca*OPRTase and *Ca*ODCase domains were comparable to those reported. The crystallographic structure revealed that *Ca*ODCase is a dimer that conserves the typical fold observed in other ODCases from prokaryote and eukaryote with a 1-deoxy-ribofuranose-5′-phosphate molecule bound in the active site of one subunit induced a closed conformation.

Our results add to the knowledge of one of the key enzymes of the *de novo* biosynthesis of pyrimidines in plant metabolism and open the door to future applications.

## Introduction

Purine and pyrimidine nucleotides are essential cellular compounds that participate in many critical biochemical processes. Pyrimidine nucleotides are fundamental biomolecules in all life forms, not only as RNA and DNA precursors, but are also required as cofactors and regulatory molecules in the energetic metabolism, as well for continuous biosynthesis of carbohydrates, phospholipids, and glycolipids [[1], [2], [3], [4]]. The *de novo* pyrimidine biosynthetic pathway is defined as the formation of uridine 5′-monophosphate (UMP) from carbamoyl phosphate. Although, in almost all organisms this is a highly conserved six catalyzed reactions pathway, in higher eukaryotes, these activities are encoded by only three fused genes, resulting in the fusion of catalytic modules into protein chimeras. In higher eukaryotes and plants, the last two steps of this pathway are encoded by a single gene that produces a chimeric polypeptide called UMP synthase (UMPS). This macromolecule is a bifunctional protein that possesses two catalytic activities: orotate phosphoribosyltransferase (OPRTase) and orotidine-5′-monophosphate decarboxylase (ODCase) [[5], [6], [7], [8], [9]]. The OPRTase catalyzes the $Mg^{2+}$-dependent condensation of orotate (OA) with 5-α-D-phosporibosyl 1-diphosphate (PRPP) to yield pyrophosphate (PPi) and orotidine 5′-monophosphate (OMP), which is subsequently decarboxylated by ODCase, leading to UMP, the entry nucleotide in the biosynthesis of all pyrimidine nucleotides. One of the advantages of fusing catalytic modules into a single polypeptide chain is the channeling of intermediaries between the active sites of the modules without diffusion of intermediaries from the enzyme complexes. Intermediate channeling increases the flux of metabolic pathways, changes regulatory patterns, and prevents the breakdown of relatively unstable intermediates by parallel reactions. The efficiency of reactions catalyzed by UMPS are improved by substrate channeling, where the product of the first reaction is apparently channeled to the second catalytic module without dissociation from the enzyme complex. In most organisms the N-terminal portion of this bifunctional polypeptide corresponds to OPRTase, while the C-terminal region to ODCase [6,10,11]. UMPS is one of the key enzymes of the *de novo* biosynthesis of pyrimidines and is the rate limiting step of the pathway in mammals [12] and plants [13]. In higher plants, few studies have been carried out on the synthesis and metabolism of purines and pyrimidines compared to those carried out in microorganisms and animals. The role of UMPS in plants has been examined in different studies. In *Nicotiana tabacum*, mutants with reduced levels of UMPS from haploid cells were isolated and characterized [13]. Studies carried out in *Nicotiana plumbaginifolia* found that

UMPS is transcriptionally regulated by pyrimidine levels [7]. In transgenic *Solanum tuberosum* tubers plants with decreased expression of UMPS, it was found that this leads to a compensatory stimulation of the pyrimidine salvage pathways, increasing uridine nucleotide pool levels in tubers and improving biosynthetic performance [14].

Recently DNA-based methods have been developed for the reliable and efficient identification of *N. tabacum* in several tobacco samples and its derived products using the sequence of UMPS as a target [15].

Although UMPS is one of the key enzymes of the *de novo* biosynthesis of pyrimidines, in plants has not been characterized neither biochemically nor structurally. In this work we presented the biochemical and structural information of the recombinant UMPS from *Coffea arabica* (*Ca*UMPS) obtained by means of circular dichroism, thermofluorescence assays, enzyme kinetics, *in silico* modeling as well as X-ray-crystallography. To the best of our knowledge, here is presented the first crystallographic structure of a plant ODCase and the characterization of a plant UMPS. This study contributes to the elucidation of the mechanism of action of a class of chimeric enzymes critical for plant metabolism and adaptation.



## Section snippets

## Materials

Reagents (kanamycin, chloramphenicol, arabinose, isopropyl 1-thio-β-D-galactopyranoside IPTG, glycerol, imidazole, ATP, sinapinic acid, acetonitrile, trifluoroacetic acid and PEG 10000), culture media Luria Bertani (LB), salts (NaCl, phenylmethylsulfonyl fluoride PMSF, and $MgCl_2$), buffers (HEPES and Tris), OA, PRPP pentasodium salt, OMP trisodium salt were purchased in Sigma-Aldrich unless otherwise stated. All the chemicals and reagents were of the highest quality commercially available. Gene …

## Preparation of the recombinant CaUMPS protein

The recombinant *Ca*UMPS protein was purified to homogeneity using affinity and size-exclusion chromatography (SEC) which yielding about 0.73 mg of pure and active protein per liter of bacterial culture. The *Ca*UMPS comprises two domains, OPRTase at the N-terminal with 208 residues and ODCase at the C-terminal from 220 to 482 residues (Supplementary Fig. S1). The subunit molecular mass of the recombinant *Ca*UMPS estimated by SDS-PAGE was 53 kDa (Fig. 1A), corresponding to the predicted molecular …

## Conclusions

The UMPS enzyme from the *C. arabica* plant can be obtained recombinant, soluble and in its native state with correct folding. The thermal stability of *Ca*UMPS suggests that it is a stable enzyme. Both catalytic domains present biological activity comparable to that observed in other chimeric enzymes and that of individual proteins. The native *Ca*UMPS molecule is a dimer, where each polypeptide chain consists of a OPRTase domain and another ODCase domain. The data from the initial velocity study of …

## CRediT authorship contribution statement

**Alexis Hinojosa-Cruz:** Formal analysis, Investigation, Writing – review & editing. **Ángel G. Díaz-Sánchez:** Formal analysis, Resources, Writing – review & editing. **Adelaida Díaz-Vilchis:** Investigation, Resources, Writing – review & editing. **Lilian González-Segura:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft. …

## Declaration of competing interest

The authors declare no potential competing interests. …

## Acknowledgments

This work was supported by grants from FQ-5000-9129 and UNAM-PAPIIT (IN227920 and IN211223 to LGS). AHC is a recipient of a CONACYT scholarship. Crystallographic results shown in this report are derived from work performed at Argonne National Laboratory, Structural Biology Center at the Advanced Photon Source, operated by UChicago Argonne, LLC, for the U.S. Department of Energy, Office of Biological and Environmental Research under contract DE-AC02-06CH11357. We are grateful to the staff of the …

Recommended articles

## References (39)

C.W. Ross
Biosynthesis of nucleotides

T.W. Traut *et al.*
Uracil metabolism—UMP synthesis from orotic acid or uridine and conversion of uracil to beta-alanine: enzymes and cDNAs
Prog. Nucleic Acid Res. Mol. Biol. (1996)

C. Stasolla *et al.*
Purine and pyrimidine nucleotide metabolism in higher plants
J. Plant Physiol. (2003)

T. Nara *et al.*
Evolutionary implications of the mosaic pyrimidine-biosynthetic pathway in eukaryotes
Gene (2000)

S. Schoeber *et al.*
Sequence of the cDNA encoding bovine uridine monophosphate synthase
Gene (1993)

T.W. Traut *et al.*
Inhibitors of orotate phosphoribosyltransferase and orotidine-5′-phosphate decarboxylase from mouse Ehrlich ascites cells: a procedure for analyzing the inhibition of a multi-enzyme complex
Biochem. Pharmacol. (1977)

J.G. Wittmann *et al.*
Structures of the human orotidine-5′-monophosphate decarboxylase support a covalent mechanism and provide a framework for drug design
Structure (2008)

S.R. Krungkrai *et al.*
Human malaria parasite orotate phosphoribosyltransferase: functional expression, characterization of kinetic reaction mechanism and inhibition profile
Mol

Biochem. Parasitol. (2004)

M.J. Yablonski *et al.*

Intrinsic activity and stability of bifunctional human UMP synthase and its two separate catalytic domains, orotate phosphoribosyltransferase and orotidine-5′-phosphate decarboxylase

J. Biol. Chem. (1996)

N. Wu *et al.*

Crystal structure of inhibitor complexes reveal an alternate binding mode in orotidine-5′-monophosphate decarboxylase

J. Biol. Chem. (2002)

View more references

## Cited by (0)

View full text