Study of the Enzymatic Activity of COG3236 Protein on Riboflavin Intermediates

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Introduction

The early steps of the riboflavin synthesis produce notoriously labile and reactive intermediates and may be overproduced *in vivo* because riboflavin pathway enzymes lack feedback controls [1]. It is intriguing that a domain of unknown function (COG3236) is fused to two enzymes of the riboflavin pathway in plants and some bacteria (Fig. 1).

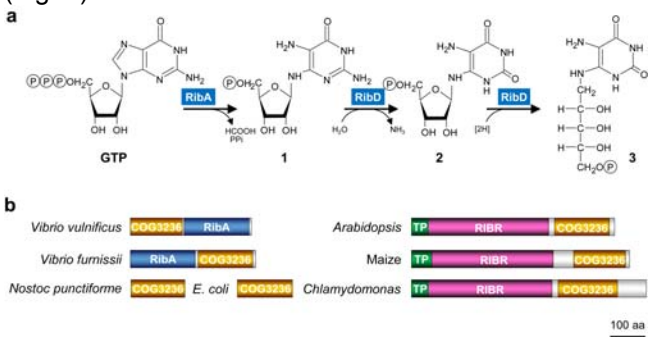

Figure 1: Evidence connecting COG3236 with the riboflavin synthesis pathway. (a) The first three steps of riboflavin synthesis and their enzymes in bacteria (blue) and plants (green). The first step is catalyzed by RibA (GTP cyclohydrolase II); steps two and three by RibD, a bifunctional deaminase-reductase. Intermediates 1, 2, and 3 are 2,5-diamino-6-ribosylamino-4(3*H*)-pyrimidinone 5′-P, 5-amino-6-ribosylamino-2,4(1*H*,3*H*)-pyrimidinedione 5′-P, and 5-amino-6-ribityl-amino-2,4(1*H*,3*H*)-pyrimidinedione 5′-P, respectively. P, phosphate. (**b**) Domain structures of representative fusions between COG3236 and RibA in γ-Proteobacteria, and between COG3236 and reductase in plants. Two stand-alone bacterial COG3236 proteins are also shown. TP, plastid targeting peptide.

Here we found by 13C NMR that COG3236 proteins are *N*-glycosidases that cleave the ribose-pyrimidine bond of the first two riboflavin synthesis intermediates.

Experimental

Intermediate 1 and 2 were synthesized according to [2] using 5 mM ^{13}C-^{15}N-GTP (Cambridge Isotope Laboratories) in 10 % deuterated-Tris buffer. To about 4mM intermediate 1 and 2, 100 ug of COG3236 protein were added. ^{13}C spectra were acquired during the enzymatic reaction at 22°C using the 600 MHz Agilent instrumentation and the NHMFL 1.5-mm ^{13}C-optimized HTS probe [3].

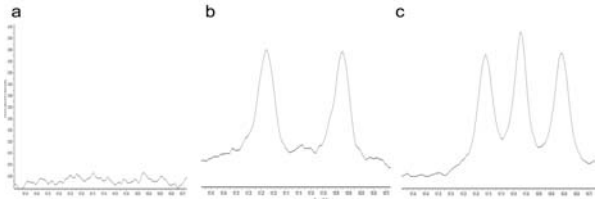

Figure 2: ^{13}C NMR spectrum from a, intermediate 1; b, COG3236 reaction on intermediate 1, and c; as b but spiked with 50mM ribose 5-phosphate standard. The region corresponds to the anomeric C1 carbon of ribose 5P.

Results and Discussion

We combined chromatographic (HPLC, data not shown) and spectroscopic (^{13}C-NMR) experiments to determine the function of COG3236 on intermediate 1 and 2 of the riboflavin pathway. Contrary to intermediate 1 spectrum (Fig.1a), a doublet appeared at 100.86 and 101.15 ppm after the COG3236 enzymatic reaction (Fig 2b). The signal was attributed to the anomeric C1 carbon of ribose 5-phosphate as shown in Fig. 2c.The same results were obtained with intermediate 2.

Conclusions

We described for the first time the function of COG3236 protein family, which cleaves the *N*-glycosidic bond of the early intermediates of the riboflavin pathway. By combining different approaches, we showed that COG3236 proteins illustrate two emerging principles in chemical biology: directed overflow metabolism, in which excess flux is diverted out of a pathway, and the pre-emption of damage from reactive metabolites.

Acknowlegments

This work was funded by NSF grant MCB-1153413 and NMR studies were supported by the NSF's National High Magnetic Field Laboratory User Program in the Advanced Magnetic Resonance Imaging and Spectroscopy (AMRIS) Facility in the McKnight Brain Institute of the University of Florida.

References

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[2] Bacher, A., *et al.*, Methods in Enzymology, **280**, 382-389 (1997).
[3] Ramaswamy, V., *et al.*, Journal of Magnetic Resonance, **235**, 58-65 (2013).