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Mass Spectrometry & Purification Techniques

Commentary

The Technique Involved in the Purification of Oxygen-Dependent Enzyme

Agnes Moricz*

Department of Chemistry, University of Hawassa, Great Rift Valley, Hawassa, Ethiopia

DESCRIPTION

The last stage in the heme production pathway is catalyzed by the oxygen-dependent enzyme known as Human Proto Porphyrinogen Oxidase IX (hPPO). Variegate porphyria is an autosomal dominant metabolic condition that has been related to mutations in the enzyme. Several researchers examined at eukaryotic cells as alternative platforms for the heterologous production of hPPO since some therapeutically significant hPPO variants could not be produced using a conventional bacterial-based method. They initially investigated the impact of N-terminal tags and different detergents on the yield and specific activity of hPPO generated by bacteria. They investigated the impact of the size and location of the fusion partners on the expression levels, specific activity, and intracellular targeting of hPPO fusions in mammalian cells by attaching several fusion partners to the N- and C-termini of hPPO. Ultimately, these findings imply that although enzymatically active hPPO can be produced heterologously in eukaryotic systems, the limited availability of the intracellular FAD co-factor probably has an adverse effect on yields of an accurately folded protein, creating the *E. coli* system the mechanism of selection for transgene hPPO excess production situations where the impact of post-translational modifications (absent in bacteria) on target protein activities are investigated, PPO overexpression in eukaryotic cells may be preferred.

Heme functions as a prosthetic group of several proteins involved in important biological activities such as oxygen transport, detoxification, photosynthesis, and respiration. Eight enzyme processes are involved in the multi-stage process of heme biosynthesis. The oxygen-dependent enzyme Proto Porphyrinogen Oxidase IX (PPO) catalyses the last stage in the heme biosynthesis by converting prototoporphyrinogen IX to prototoporphyrin IX, a precursor to both the chlorophylls found in plants and haemoglobin in mammalsHeme has a crucial role in the physiology of living things, thus PPO is the subject of extensive scientific and practical study. For instance, several pesticides target plant PPOs to suppress weeds in order to maintain and enhance agricultural productivity. On the other hand, human PPO (hPPO) is a protein that has undergone extensive research in relation to human health because variations in this enzyme can result in variegate porphyria, an autosomal dominant disease with a variety of neurological and cutaneous symptoms. There are currently known to be about 180 hPPO alterations, involving deletions, splice variations, and missense mutations. Around 50% of these hPPO variations are caused by missense mutations, which often result in reduced specific hPPO activity and variegate porphyria. Human PPO is made in the cytosol and carried to the inner mitochondrial membrane, where it joins forces with ferrochelatase, the final enzyme in the heme biosynthesis process, to create a complex. hPPO may be solubilized in the active form by using mild detergents since it lacks a membrane-spanning region and is only tangentially linked to the mitochondrial membrane. During the purification of heterologously generated hPPO, detergents are also utilised to cover hydrophobic areas of the protein surface. The membrane-binding domain, the FAD-binding domain, and the substrate-binding domain are the three structural domains that make up hPPO. It should be emphasised that flavin adenine dinucleotide (FAD), a noncovalently linked cofactor that employs molecular oxygen as the terminal acceptor of electrons, is present in all eukaryotic PPOs.

CONCLUSION

Researchers have successfully cloned hPPO from human placenta, heterologously produced it in *E. coli*, and homogenized it for the first time. Since then, the production of hPPO and its variants for *in vitro* biochemical, biophysical, and structural studies has typically been accomplished using this purification protocol (and slight modifications), which entails *E. coli* expression, Ni-NTA affinity purification, and a size-exclusion chromatography step. Since wild-type hPPO's prokaryotic expression is highly robust, the goal of this study was to investigate methods for hPPO's widespread heterologous expression in eukaryotic cells and contrast the properties of hPPO produced in each system.

Correspondence to: Agnes Moricz, Department of Chemistry, University of Hawassa, Great Rift Valley, Hawassa, Ethiopia, E-mail: Moricz158@gmail.com

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