The Wisconsin Alumni Research Foundation (WARF) is seeking commercial partners interested in developing an improved method of producing functional membrane polypeptides, such as cytochrome b5.

OVERVIEW

Cytochrome b5 is an electron transport protein found in bacteria, protozoans, yeasts and mammals. This membrane protein is required for the activity of stearoyl-CoA desaturase (SCD), an integral membrane desaturase that plays a role in obesity, diabetes, hypertension, cardiovascular disease, immune disorders, degenerative neurological diseases and skin diseases.

Cytochrome b5 is a useful tool for researchers studying SCD activity. However, current methods of making large quantities of functional cytochrome b5 to use in assays are labor intensive and time consuming. Improved techniques for synthesizing full length cytochrome b5 are needed.

THE INVENTION

UW-Madison researchers have developed a simpler method of producing and purifying functional membrane polypeptides, such as cytochrome b5. This method utilizes a recombinant expression vector that expresses a fusion of cytochrome b5 and a solubilizing agent, such as maltose binding protein (MBP).

The vector can be used to express the fusion protein in solution. The protein optionally can be purified, and then a simple chemical or enzymatic cleavage reaction can release the MBP moiety when the functional, full length cytochrome b5 is needed.

In addition, the inventors discovered that the carboxy terminus of the cytochrome b5 peptide functions as a membrane anchor. To use this method to synthesize other functional membrane proteins that do not have a C-terminal membrane anchor, this anchor section can be fused to the protein, allowing it to spontaneously attach to a membrane when the solubilizing agent is released.
APPLICATIONS

- *In vitro* assays that utilize cytochrome b5 or other membrane proteins
- Studying SCD activity

KEY BENEFITS

- Enables synthesis of large quantities of pure, functional and full length cytochrome b5
- Fusion protein is soluble in aqueous solution.
- Does not require reconstitution, refolding or reassociation with the co-factor heme
- Fusion protein is highly stable and can be recognized by cytochrome b5 reductase
- MBP moiety or other solubilizing agent easily can be released when needed.
- Allows cytochrome b5 to be stored as a soluble fusion protein without the need for contaminating detergents
- Fusion protein may include an affinity purification tag.
- Can be used to produce other functional membrane polypeptides, including full length cytochrome b5 reductase, peptidoglycan glycosyltransferases, prostaglandin H2 synthase-1, monoamine oxidase B, cyclooxygenase-2 and carnitine palmitoyl transferase

ADDITIONAL INFORMATION

Tech Fields
Research Tools - Synthesis & purification

CONTACT INFORMATION

For current licensing status, please contact Jennifer Gottwald at jennifer@warf.org or 608-960-9854.