

The isolation and purification of cytochrome b₅ with subsequent physiochemical characterization studies.

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Cytochrome b₅ is an electron-transfer hemoprotein that catalyses a variety of important reactions. These reactions include lipid biosynthesis, electron transport and adrenal steroidogenesis. The enzyme can be found in the outer mitochondrial membrane and the endoplasmic reticulum of lung, adrenal and hepatic tissue. Several studies have shown that the influence of CYT b₅ on steroid hydroxylases differs within species and their tissues. Adrenal tissues have been shown to produce either androgens or glucocorticoids depending on the influence of CYT b₅. CYT b₅ was isolated and purified from ovine liver microsomes according to previous methods. SDS-PAGE of the purified protein indicated that, in spite of stringent denaturing conditions, the enzyme was present in a monomeric and tetrameric form. MALDI-QTOF analysis has showed the protein has a molecular mass of 15 248. After isolation, antibodies were raised against the protein that recognised both its monomeric and tetrameric conformations (Anti-Agg) as well as only a monomeric conformation (Anti-Mono). Using affinity separation we aim to separate these two antibodies for immunohistochemical investigations of the tissue distribution of both conformations. It is possible that the monomeric and aggregate conformations of CYT b₅ may influence adrenal steroid production. By investigating the tissue distribution and amino acid sequence of the enzyme a better understanding will be obtained into mechanism of CYT b₅ interaction with steroidogenic cytochromes P450.