PreSens
PRECISION SENSING

Scientific Paper:

Applied and Environmental Microbiology (2015), Vol. 81, No. 11, pp. 3793 - 3799

Latex Clearing Protein (Lcp) of Streptomyces sp. Strain K30 Is a b-Type Cytochrome and Differs from Rubber Oxygenase A (RoxA) in Its Biophysical Properties

Jakob Birke[1], Wolf Röther[1], Dieter Jendrossek[1]
[1]Institut für Mikrobiologie, Universtät Stuttgart, Germany

Abstract:

Specific polyisoprene-cleaving activities of 1.5 U/mg and 4.6 U/mg were determined for purified Strep-tagged latex clearing protein (Lcp) of *Streptomyces* sp. strain K30 at 23 °C and 37 °C, respectively. Metal analysis revealed the presence of approximately one atom of iron per Lcp molecule. Copper, which had been identified in $Lcp1_{VH2}$ of *Gordonia polyisoprenivorans* previously, was below the detection limit in Lcp_{K30}. Heme was identified as a cofactor in purified Lcp_{K30} by (i) detection of characteristic α-, β-, and γ (Soret)-bands at 562 nm, 532 nm, and 430 nm in the visible spectrum after chemical reduction, (ii) detection of an acetone extractable porphyrin molecule, (iii) determination of a heme *b*-type-specific absorption maximum (556 nm) after chemical conversion of the heme group to a bipyridyl-heme complex, and (iv) detection of a b-heme-specific *m/z* value of 616.2 via mass spectrometry. Spectroscopic analysis showed that purified Lcp as isolated contains an oxidized heme-Fe^{3+} that is free of bound dioxygen. This is in contrast to the rubber oxygenase RoxA, a *c*-type heme-containing polyisoprene-cleaving enzyme present in Gram-negative rubber degraders, in which the covalently bound heme firmly binds a dioxygen molecule. Lcp_{K30} also differed from RoxA in the length of the rubber degradation cleavage products and in having a higher melting point of 61.5 °C (RoxA, 54.3 °C). In summary, RoxA and Lcp both are equipped with a heme cofactor and catalyse an oxidative C-C cleavage reaction but differ in the heme subgroup type and in several biochemical and biophysical properties. These findings suggest differences in the catalytic reaction mechanisms.

Keywords: Actinobacteria, *Streptomyces*, heme, oxygenase, cytochromes