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 Birke¹, Wolf Röther¹, Dieter Jendrossek²
¹Institut für Mikrobiologie, Universität Stuttgart, Germany

Abstract:

Specific polyisoprene-cleaving activities of 1.5 U/mg and 4.6 U/mg were determined for purified Streptagged 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 Lcp1VH2 of Gordonia polyisoprenivorans previously, was below the detection limit in LcpK30. Heme was identified as a cofactor in purified LcpK30 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-Fe³⁺ 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. LcpK30 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