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Ancestral sequence reconstruction enlightens the structural basis of vitamin C biosynthesis

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PURPOSE OF THE ABSTRACT

Ascorbic acid (Vitamin C) is a key element in eukaryotes involved in several roles in the cell1. Apart from being a successful redox system counteracting the deleterious action of Reacting Oxygen Species (ROS)2, Ascorbic acid is also used as a cofactor in several metal-dependent monooxygenases responsible, for example, of hydroxylation reactions during collagen biosynthesis3. In nature, the final step of vitamin C biosynthesis is catalyzed by a class of flavin dependent enzymes called aldonolactone oxidoreductase4. In animals, the enzyme is responsible for the oxidation of L-gulono 1,4 lactone to ascorbic acid. The enzyme uses molecular oxygen as electron acceptor with the flavin cofactor that is covalently bound to the enzyme with a histidyl linkage. On the other hand, in plants, the enzyme (L-galactono 1,4 lactone dehydrogenase) poorly reacts with oxygen using cytochrome c as electron acceptor, without having the flavin covalently bound. Despite an extensive biochemical characterization5,6, no structural data have been reported so far for aldonolactone oxidoreductases leaving an open question about their mechanism of action.

By applying Ancestral Sequence Reconstruction (ASR) we were able to infer the evolutionary history of L-galactono 1,4 lactone dehydrogenase. The last common ancestors of embryophyta and viridiplantae were resurrected and characterized. The enzymes were recombinantly expressed in E. coli with a high yield. By deleting the mitochondrial target sequence at the N-terminus of the amino acid sequences the enzymes were purified as soluble cytoplasmatic proteins. Their substrate scope was investigated by testing a series of carbohydrates. Finally, the combination of site directed mutagenesis and x-ray crystallography allowed to highlight key features regarding the mechanism of action of this class of enzyme.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

structural biology | Ancestral sequence reconstruction | oxidoreductases

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