Structural characterization of nitrite reductases

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Introduction: Nitrite reductase (NIRS) are enzymes Responsible to convert nitrite in nitric oxyde (NO) in a process called denitrification. There are two different types of nitrite reductase described until now. The first class have heme based electron transport and the second class are copper based enzyme that have at least one type 1 copper center in the protein and the type 2 copper center Responsible for binding and reducing nitrite. Those enzymes have a huge importance on agriculture and health science.

Goals: In this field we are trying to characterize, structural and functionally, an enzyme from a bacteria called Bradyrhizobium japonicum that has a recently discovered N-terminal extended cytochrome C and cupredoxin-domain Cu-nitrite reductase.

Material and Methods: In order to produce mature cytochrome C the recombinant clone pET26b-BsNiR has to be transformed into Escherichia coli strain BL21(DE3) containing the plasmid pEC86 that encodes the cytochrome c maturation genes ccmABCDEFGH in E. coli (Arslan, E., (1998) Overproduction of the Bradyrhizobium japonicum c-type cytochrome subunits of the cbb3 oxidase in Escherichia coli. Biochem. Biophys. Res. Commun. 251, 744-747) (Cong Han Biochem. J. (2012) 444 (219–226)). The purification of the native version of the protein will be performed using chromatographic techniques**.**

Discusion and Results:. The BL21 (DE3) strain containing both plasmids was growth in LB media, supplemented with CuSO4 and haemin, and the over expression was induced by 0.5 mM IPTG for 16 hours at 18 ºC. After sonication treatment, the supernatant, was loaded on to a column with SP sepharose resin (GE Healthcare). The fraction containing BsNiR eluted, concentrated and then was applied on to a HiLoad 16/60 Superdex 200 gel-filtration column (GE Healthcare) twice.

Conclusion: Until this moment was possible produce a large amount of the protein, and partially purify the native version of BsNiR using a weak cationic chromatography and two steps of the gel filtration chromatography. The protein presents a reddish color and the gel filtration profile shows that the protein is not a monomer.

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