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Differential roles of HypC and HupF proteins for hydrogenase synthesis in *Rhizobium leguminosarum*

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Summary

Some diazotrophic bacteria induce [NiFe] hydrogenases to recycle the hydrogen evolved by nitrogenase during the nitrogen fixation process. Biosynthesis of *Rhizobium leguminosarum* [FeNi] hydrogenase requires a number of accessory proteins (products of *hup* and *hyp* genes) that mediate the incorporation of Ni and Fe into the active site. Among them, HypC-paralog HupF and HupK are present in bacteria that synthesize hydrogenase in the presence of oxygen. Hydrogenase activity in mutant strains lacking either *hupF* or *hypC* genes was severely reduced, indicating that both proteins are essentials for biosynthesis of hydrogenase. Co-purification of StrepTag labelled variants of HupF and HypC by an affinity chromatography-based approach demonstrated interactions between HupL-HupF and HypC-HupK. Experiments carried out with strains induced for hydrogenase under 3% oxygen tensions indicated that HupF might provide additional stability to HupL under these conditions.

Introduction

The generation of hydrogen (H_2) as a consequence of the nitrogenase activity is a source of inefficiency for the nitrogen fixation process. Some rhizobia synthesize a hydrogen uptake (Hup) system with a [NiFe] hydrogenase that catalyses the oxidation of H_2 evolved during N_2 fixation.

[NiFe] hydrogenase is a membrane-bound enzyme that contains a large subunit (HupL) and a small subunit (HupS) in a αβ configuration. HupL contains the catalytic site ($NiFe(CN)_2CO$ cofactor). HupS contains three Fe-S clusters (two 4Fe-4S and one 3Fe-4S) through which electrons from H_2 are conducted to their primary acceptor. In *R. leguminosarum* bv. *viciae* 18 genes (*hupSLCDEFGHIJKhypABFCDEX)* clustered in the symbiotic plasmid are required for hydrogenase synthesis (Ruiz-Argüeso *et al.*, 2001). Analysis of *Escherichia coli* hydrogenase-3 has shown that a HypC-HypD complex carrying the $Fe(CN^-)_2CO$ cofactor intermediate is formed through the concerted action of these two proteins with HypF and HypE. Then HypC, after HypD dissociation, transfers the precursor cofactor to HupL (Blockesh & Böck, 2006).

Two of *R. leguminosarum* genes cited above, *hupF* and *hupK*, are absent in bacteria that synthesize hydrogenase under anaerobic conditions, such as in *E. coli*. Since these two genes are present in other hydrogenase systems expressed in the presence of oxygen, our working hypothesis is that both proteins might participate in a modified pathway adapted to the presence of oxygen. In this work, we have studied the functional roles of both paralog metallochaperones (HupF and HypC) and their interaction with other components of the biosynthetic system.

Materials and Methods

In-frame delections in each of the *hupL*, *hupD*, *hupF*, *hupK* and *hypC* genes were generated in plasmid pALPF1 as described by Manyani *et al*. (2005). This plasmid contains the whole *hup* cluster under the control of the promoter of *fixN* gene allowing microaerobic expression of hydrogenase activity in free-living cells. The resulting plasmids were transferred by conjugation to Hup^{-} *R. leguminosarum* UPM1155 strain. Hydrogenase activity was induced in cultures grown under continuous bubbling with a gas mixture containing O_2 concentrations of 1 or 3%. Hydrogenase activity was measured using an amperometric method with oxygen as electron acceptor as previously described (Ruiz-Argüeso *et al.,* 1978).

Results and Discussion

Hydrogenase activity of *hypC* and *hupF*-deleted mutant strains was drastically reduced (<10%) compared to the wild type, indicating that both proteins are essential for biosynthesis of hydrogenase. When expressed from a pBBR1MCS-based plasmid, HypC-StrepTag and HupF-StrepTag proteins complemented the Δ*hypC* and Δ*hupF* mutations, respectively, exhibiting levels of hydrogenase activity similar to those associated with wild type pALPF1. A cross complementation was not observed between HypC and HupF proteins. These data suggest that both proteins have differential effect on hydrogenase synthesis.

Co-purification experiments using StrepTag-labelled variants of HypC and HupF and antisera specific for HupL and HupK revealed the existence of direct interactions between HupL-HupF and HypC-HupK. This data are consistent with evidence reported for the hydrogenase system of *Ralstonia eutropha* (Ludwig *et al*., 2009). Furthermore, in our case the latter complex associates to the former one in a HupK-dependent manner, suggesting that the four proteins form a single complex that mediates the transfer of cofactor precursor into HupL.

Since HupF participates in hydrogenase systems induced in the presence of oxygen, we hypothesized that this protein might be involved in the adaptation of the system to higher oxygen tensions. To test this hypothesis, bacterial cultures from wild type strain (pALPF1) and *hypC* and *hupF*-deleted mutants were induced for hydrogenase activity in atmospheres containing either 1 or 3% O_2. Immunoblot analysis with antibodies against *R. leguminosarum* HupL showed that crude extracts from bacterial cultures of both mutant strains grown at 1% O_2 accumulated the unprocessed form of HupL. Analysis of crude extracts of Δ*hupF* mutant strains grown at 3% O_2 showed that unprocessed form of HupL was absent whereas it was present in the Δ*hypC* mutant, that contained normal levels of HupF. These data suggest that HupF might have a protective role of hydrogenase structural subunit HupL against higher oxygen levels.

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References

Blokesch M, Böck A (2006) *FEBS Lett* 580: 4065-4068.

Ludwig M, Schubert T, Zebger I, Wisitruangsakul N, Saggu M, Strack A, Lenz O, Hildebrant P, Friedrich B (2009) *J Biol Chem* 284: 2159-2168.

Manyani H, Rey L, Palacios JM, Imperial J, Ruiz-Argüeso T (2005) *J Bacteriol* 187: 7018-7026.

Ruiz-Argüeso T., Hanus FJ, Evans HJ (1978) *Arch Microbiol* 116: 113-118.

Ruiz-Argüeso T, Palacios JM, Imperial J (2001) *Plant Soil* 230: 49-57.