Poles,

Abstract

Characterization and Kinetics of the Biosynthesis of Some Nitrogen Fixation (*nif*) Gene Products in *Klebsiella pneumoniae*.

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Analysis of ^{14}C pulse-labelled proteins, synthesized by a Nif^+ *Klebsiella pneumoniae* strain and by a number of genetically mapped *nif*::Mu and *nif* deletion mutants, was performed by two-dimensional gel electrophoresis. In addition to the previously characterized *nifK*, *nifD*, *nifH* and *nifJ* products, the product of *nifF* was identified as a polypeptide of 10,000 daltons and pI about 4.5 and the product of *nifU* as a polypeptide of 22,000 daltons and pI 5. Moreover, the biosynthesis of *nifF* and *nifU* polypeptides was shown to be prevented in mutants affecting the regulatory gene *nifA*, which is known to control the biosynthesis of the other *nif* genes products so far identified.

Kinetic studies of both nitrogenase activity and of the biosynthesis of the six *nif*-specific polypeptides were performed with the Nif^+ strain, incubated either under conditions of derepression or under conditions of repression by NH_4^+ ions. Upon derepression, the biosynthesis of the six *nif* polypeptides, which belong to four different transcriptional units, was coordinated. Upon addition of NH_4^+ ions, the biosynthesis of the six *nif* polypeptides was rapidly abolished. However, the kinetics of residual biosynthesis, probably due to the transcription of pre-existing mRNAs, was not similar for the six *nif* products. The *nifU* product was no longer detectable after 5 minutes, the *nifF*, *K*, *D* and *J* products were not detectable after 30 minutes, whereas some *nifH* product was still slightly detectable after 60 minutes.

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