SHORT COMMUNICATION

Nickel Requirement of a Urease-deficient Mutant in *Aspergillus nidulans*

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The addition of nickel ions restored urease activity *in vivo* and ability to grow on urea in a mutant strain of *Aspergillus nidulans* otherwise unable to utilize urea. This strain carries a mutation in the *ureD* locus, one of four loci involved in urea utilization. No other urease-deficient strains tested responded to the presence of nickel ions.

The analogous characteristics of the *ureD* mutant and the nitrate reductase and xanthine dehydrogenase associated *cnxE* mutants in *Aspergillus nidulans* are discussed. It is postulated that the *ureD* locus is in some way involved in the production or incorporation of a nickel cofactor essential for urease activity.

INTRODUCTION

It has been reported that jack bean urease, despite its earlier contribution to the principle that enzymes need not contain metal ions (Sumner, 1964), does in fact contain stoichiometric amounts of nickel (Dixon *et al.*, 1975). Ureases, apparently similar to that in the jack bean, are widely distributed, being found in many bacteria (Larson & Kallio, 1954) and in fungi (Srb & Horowitz, 1944). This report presents evidence that nickel ions can restore both urease activity *in vivo* and growth on urea in a mutant of the ascomycete *Aspergillus nidulans* which is otherwise unable to utilize urea.

Aspergillus nidulans can utilize urea as a sole source of nitrogen but not of carbon (Pateman & Kinghorn, 1976). Mutants at four loci which are all deficient to some degree in the utilization of urea have been known for some time (Dunn & Pateman, 1972; Kinghorn & Pateman, 1977). They are all derived from *biA1*, a biotin-requiring strain. One locus, *ureA*, is known to be concerned with urea uptake and *ureA* alleles lack normal urea uptake activity (Dunn & Pateman, 1972). All the mutant alleles known at the *ureB*, *ureC* and *ureD* loci lack normal urease activity and grow poorly or not at all on urea as a sole nitrogen source. The *ureB* locus is closely linked to *ureA* and it may be that *ureB* is the structural gene for urease (Pateman & Kinghorn, 1977). The functions of *ureC* and *ureD* have hitherto been unknown. The findings reported here suggest that *ureD* may in some way be involved in the production of a nickel cofactor or in the insertion of nickel into the urease enzyme.

METHODS

Strains and media for growth tests. Pure cultures of strains containing each of the available mutant alleles at each locus described above were tested and the original *biA1* strain was used throughout as the wild-type. The strains were inoculated on to nitrogen-less minimal medium (basically that of Pontecorvo, 1953)

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Table 1. *Effect of nickel on the urease activity of the ureD9 mutant grown on various nitrogen sources and comparison with the wild-type strain*

The *ureD9* mutant was grown in nitrogen-less medium containing biotin, 10 mM nitrogen source as indicated and with or without 0.1 mM-nickel acetate. Urease activities are expressed as nmol urea hydrolysed min⁻¹ (mg protein)⁻¹.

Nitrogen source	Urease activity		
	<i>biA1</i>	<i>ureD9</i> (without Ni ²⁺)	<i>ureD9</i> (with Ni ²⁺)
L-Alanine	348	< 8	18
Sodium nitrate	239	< 8	20
L-Glutamine	242	< 8	17
L-Proline	258	< 8	13

containing biotin, 3 mM-urea unless otherwise stated, with or without nickel salts in the concentration range 0.01 to 1 mM, the latter concentration being lethal, and incubated at 37 °C for 48 h.

Growth of mycelium for enzyme assays. Mycelium was grown from a constant-sized inoculum of conidia, 130×10^6 per 200 ml of liquid nitrogen-less minimal medium containing biotin, a 10 mM nitrogen source as indicated, with or without 0.1 mM-nickel acetate, in shaken culture at 30 °C for 18 h. Cell-free extracts were prepared (Cove, 1966) and kept at 0 °C until assayed.

Urease assays. Urease activity was measured by a direct recording conductivity method (Lawrence & Moores, 1972). The hydrolysis of 20 mM-urea by 10 µl extract was followed in 1 ml 10 mM-tricine buffer, pH 7.5, at 37 °C. Protein concentration was determined by the method of Lowry.

RESULTS

Growth response to nickel

From plate tests it was found that only one strain responded to nickel ions; colonies of mutant *ureD9* grew to a diameter of approximately half that of the wild-type in the presence of 0.1 mM-NiSO₄. Higher concentrations did not further increase the growth of *ureD9* but rather had a toxic effect on all the strains. The response of *ureD9* was specific to nickel. It did not occur when CuSO₄ or MnSO₄ was added in the concentration range 0.01 to 10 mM, the latter concentration being lethal, and did occur when nickel acetate was added, the optimum concentration again being 0.1 mM.

The mutant strains were also tested for improved growth in the presence of nickel with 5 mM-L-arginine as the nitrogen source. Urease-deficient mutants grow poorly on this nitrogen source which is converted by arginase to L-ornithine and urea (Pateman & Kinghorn, 1976). Addition of 0.1 mM-NiSO₄ again improved the growth of *ureD9*.

Enzyme assays

Attempts were made to stimulate urease activity in *ureD9* and *ureD4*, *in vitro*. Nickel acetate at final concentrations of 0.5 to 2 mM was added to cell-free extracts in the glass cells of the conductivity apparatus and allowed to incubate for 2 min at 37 °C prior to the addition of urea to a final concentration of 20 mM. This treatment did not result in detectable urease activity in either strain.

An *in vivo* response was, however, demonstrable. Nickel acetate was added during growth to cells of both the wild-type and *ureD9*. After various trials, 0.1 mM-nickel acetate in the growth medium proved to be the maximum concentration which permitted reasonable growth of the cells. This concentration decreased the weight of cells harvested by more than half compared with medium without nickel acetate but did not alter the wild-type urease activity per mg protein. Strains containing each of the available alleles at each locus were assayed after growth on 10 mM-L-alanine in the presence or absence of 0.1 mM-nickel acetate. The urease activity of *ureD9* was significantly increased by the addition of nickel acetate.

The results of further assays of *ureD9* and the wild-type grown on various nitrogen sources are shown in Table 1. On all four nitrogen sources the presence of nickel acetate increased the urease activity of *ureD9* from an undetectable level ($< 3\%$) to 5 to 8% of that of the wild-type.

DISCUSSION

The characteristics of the *ureD9* mutant with respect to nickel and urease activity are analogous to those of the *cnxE* mutants in *A. nidulans* with respect to molybdenum and the activities of nitrate reductase and xanthine dehydrogenase. Mutations at the *cnxE* locus result in the loss of these two enzyme activities. The addition of molybdenum to the growth medium results in a significant increase in the two enzyme activities and in growth on nitrate and purines of the *cnxE* mutants. Although only this one class of *cnx* mutant actually responds to the addition of molybdenum, it seems that all the *cnxA*, *B*, *C*, *D*, *E*, *F* and *H* loci are in various ways involved in the production and incorporation of a low molecular weight protein-molybdenum cofactor which, together with the structural gene proteins, results in nitrate reductase and xanthine dehydrogenase activities (Kinghorn & Pateman, 1977).

Pursuing the analogy between these two systems, the most likely interpretation of our results is that with the *ureB* locus as the structural gene for a urease polypeptide the *ureD* locus is in some way responsible for the production or incorporation of a nickel cofactor essential for urease activity. Despite the failure of the *ureC* mutants to respond to the addition of nickel, the possibility of the *ureC* locus having some role concerning nickel cannot be excluded.

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