Effect of Molybdenum Deficiency on Nitrate Reductase in Cell-free Extracts of *Neurospora* and *Aspergillus*

Although numerous investigators have shown that molybdenum is important for the utilization of nitrate nitrogen by fungi and higher plants[1,2], the role of the micronutrient element in the enzyme systems involved has not been determined. We have recently been able to show that the concentrations of nitrate reductase in cell-free extracts of molybdenum-deficient *Neurospora* and *Aspergillus* are markedly decreased to the extent of one-fifth to one-thirtieth of that of normal tissue. However, the nitrite activity, designated as nitrite reductase[3], of these extracts was significantly increased in *Neurospora* (5297*a*) and *Aspergillus* compared to that of the controls.

Neurospora crassa wild types 146 (microconidial type) and 5297*a* (macroconidial type) were grown on a modified Fries medium containing nitrate as the sole source of nitrogen, and on another in which ammonium nitrate and ammonium tartrate were supplied[3]. *Aspergillus niger* (*M* strain) was grown on a nitrate medium[4]. The removal of molybdenum was achieved by the copper and lead coprecipitation methods as previously described[2,4]; molybdenum was then added back to some of the media which served as the controls. Cell-free extracts and dry-weight measurements were obtained by a method described elsewhere[5]. Protein content of the enzyme extracts was determined by the biuret procedure[5]. The activities of the nitrate and nitrite reductases were measured by the appearance and disappearance, respectively, of nitrite and are expressed in the same units as defined earlier[3]. At least 80 per cent of these enzymes were present in the cell-free extracts as compared to the whole homogenates.

The accompanying table shows that the specific activity of nitrate reductase in cell-free extracts of molybdenum-deficient *Neurospora* is reduced to 10–50 per cent of that of the controls whether grown on a medium containing only nitrate or both nitrate and ammonia as the source of nitrogen. *Aspergillus* showed the same general response (see table) as *Neurospora*; the molybdenum deficiency obtained with *Aspergillus* was more severe as indicated by growth, and accordingly the decrease in nitrate reductase was greater, resulting in values 4–6 per cent of that of the controls. On the other hand, the specific activity of nitrite reductase in *Neurospora* (5297*a*) and *Aspergillus* was considerably increased in cell-free extracts of molybdenum-deficient tissues; in some instances a 15-fold increase was recorded. Preliminary experiments have indicated that the nitrate reductase in *Aspergillus* has the same requirements in terms of its specificity for reduced triphosphopyridine nucleotide and its activation by flavin adenine dinucleotide or flavin mononucleotide as in *Neurospora*. The nitrite reductase appears to be similar to that found in *Neurospora*.

The addition of molybdenum (10^{-3} microgram per 0·5 ml. reaction mixture) to the molybdenum-deficient extracts failed to increase nitrate reductase activity. The mixing of extracts of molybdenum-deficient and normal mycelia of *Neurospora* demonstrated the absence of an inhibitor or activator in the tissues in regard to both nitrate and nitrite reductase activities.

It has already been shown that individual deficiencies of iron, zinc and manganese in *Neurospora crassa* (5297*a*) resulted in a marked increase in nitrate reductase, while a nitrogen deficiency caused a striking decrease[3]. We have recently produced a copper deficiency in *Neurospora* (5297*a* and 146) by a double sulphide precipitation technique at *p*H 3·7 [2,4], which resulted in a four-fold increase in specific activity of nitrate reductase as compared with normal tissues. Thus only a nitrogen deficiency (which presumably affects protein metabolism) and molybdenum deficiency lower the nitrate reductase. Whether molybdenum is a component of the nitrate reductase system or whether it exerts its effect indirectly by influencing protein metabolism is not known. Experiments now in progress may elucidate this point.

Effect of Molybdenum Deficiency on Nitrate Reductase of *Neurospora* and *Aspergillus* Extracts
Units of nitrate reductase expressed as $\mu M \times 10^3$ of nitrate formed per mgm. protein

Fungus	Nitrogen source	Treatment	Per cent growth	Nitrate* reductase
Neurospora crassa No. 146	Nitrate only	+ Mo	100	20·5
		− Mo	29	4·8
5297*a*	Nitrate only	+ Mo	100	10·7
		− Mo	32	1·2
146	Nitrate and ammonia	+ Mo	100	48
		− Mo	50	20
5297*a*	Nitrate and ammonia	+ Mo	100	14·2
		− Mo	55	2·2
Aspergillus niger *M* strain	Nitrate only	+ Mo	100	45·3
		− Mo	5	1·6
M strain†	Nitrate only	+ Mo	100	38·8
		− Mo	31	2·4

* Reaction mixture contained the following: 0·1 ml. 0·1 *M* potassium nitrate, 0·02 ml. 10^{-3} *M* potassium cyanide, 0·04 ml. flavin mononucleotide (500 μgm./ml.), 0·04 ml. reduced triphosphopyridine nucleotide (2 μ*M*/ml.). The reaction mixtures were made up to 0·5 ml. with 0·1 *M* pyrophosphate buffer, *p*H 7·0.

'+ Mo' cultures contain 2 μgm. Mo per 125 ml. (*Neurospora*) and 2 μgm. Mo per 50 ml. (*Aspergillus*).

† In this experiment, 10^{-3} μgm. Mo only was supplied to the "− Mo" flasks.

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Note added in proof. Since this communication was submitted we have demonstrated that both the molybdenum content and specific activity of nitrate reductase increase with purification of the enzyme. Moreover, inactivation of the enzyme by dialysis against cyanide is accompanied by a decrease in molybdenum content of nitrate reductase. The inactivated enzyme is almost fully restored by the addition of molybdenum trioxide or sodium molybdate as compared to the other micronutrients.

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