A CO-FACTOR DISTURBED NITRATE REDUCTASE DEFICIENT MUTANT

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Nitrate reductase (NaR) of fungi consists of an apo-enzyme and a Mo-bearing co-factor (see 3). Work on tobacco cell lines has shown that in a higher plant the same structure can be present (4). In order to obtain various mutant forms of NaR we are currently selecting NaR deficient mutants of pea.

An NaR deficient pea mutant has already been isolated and partly described (1,2). In that mutant, in vitro NaR activity is 5% of that of the parent cv 'Rondo'. Sufficient plant growth and seed production is possible only on an aerated liquid medium containing standard mineral solution (SMS, 1,2) with NH_4NO_3 as N-source. Under these conditions NaR is sufficiently induced.

The apo-protein of NaR from Aspergillus nidulans shows cytochrome c reductase (CR) activity sedimenting in sucrose gradient centrifugation with a sedimentation value of 8 s whereas the co-factor is also used by the enzyme xanthine dehydrogenase (XDH) (3). Therefore, the lesion in the enzyme NaR can be determined by studying CR and XDH activities.

For the XDH activity determination a procedure adapted to pea has been developed and will be published elsewhere. The XDH activity in mutant **E1** was about 25% of the parent variety 'Rondo'. This result indicates that mutant E1 is a co-factor mutant. For higher plants a fractionated CR activity assay has been described (5) which appeared to be suitable for pea. In cv Rondo grown on a medium with $(NH_4)_2SO_4$ as N-source no NaR activity was found. After fractionation a peak of CR activity with a sedimentation value of 4 s was found. The same peak was found in mutant **Ei** grown under these conditions. In an extract of cv Rondo grown on NH_4NO_3 as N-source NaR activity was found, together with CR activity, the latter sedimenting with peaks at 4 s and 8 s, the former with a peak of 8 s. Mutant E1 also showed two peaks of CR activity. The 8 s CR activity was much higher than in cv Rondo. This shows that in the mutant the apo-enzyme is still induced by nitrate. The increased activity possibly is due to absence or delayed functioning of a feedback system.

These results corroborate the conclusion that mutant E1 is a co-factor mutant. The designation cnx is based on the description of co-factor mutants of A. nidulans (3).

The 8 s CR activity peak in the mutant was shifted to a slightly lower s value. This lower value can directly be related to the co-factor deficiency by the assumption that the NaR molecule is missing the co-factor. This would mean that in mutant E1 the NaR molecule is defective in such a way that despite absence of the co-factor the molecule is still able to reduce cytochrome c. This assumption is in accordance with the scheme for the interactions of the apo-enzyme and co-factor given by MacDonald et al. (3). The availability of reliable tests both for CR activity and for XDH activity now enables us to test NaR deficient mutants directly on apoprotein- and/or co-factor deficiency. Using chlorate resistance (1) a number of NaR deficient pea mutants have already been isolated. When sufficient seeds are available they will be investigated.

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