

**Short Communication**

# Posttranscriptional Control of Nitrate Reductase of Cultured Tobacco Cells by Amino Acids

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## ABSTRACT

Using the inhibitor of RNA synthesis, 4,5',8-trimethylpsoralen plus near ultraviolet light, the half-life of the rate-limiting RNA species required for the induction of nitrate reductase in XD cells of tobacco was estimated to be 4 hours. Amino acids, the end product of nitrate assimilation, reduced the half-life to 1 hour. It is suggested that amino acids exert a posttranscriptional control on the enzyme level.

## RESULTS AND DISCUSSION

The NR activity in cells induced in the presence of tungstate was 10 to 20% that of the control cells (4). After exposure of the cells to TMP plus NUV light and addition of excess  $\text{MoO}_4^{-2}$ , NR activity increased during the next hour (Fig. 1). The increase of enzyme activity was almost completely inhibited by cycloheximide (10  $\mu\text{g}/\text{ml}$ ) (data not shown), indicating a *de novo* synthesis of the enzyme and not activation of preexisting nonfunctional enzyme protein. The conditions of the experiment were such (logarithmic phase cells instead of stationary phase cells previously reported [5]) that almost no activation by  $\text{MoO}_4^{-2}$  of nonfunctional enzyme took place. The amount of enzyme which developed after the addition of  $\text{MoO}_4^{-2}$  decreased as the time period between the inhibition of RNA synthesis and  $\text{MoO}_4^{-2}$  addition increased (Fig. 1). The plot of the net increase of enzyme activity calculated as the total increase minus the background activity of cells not given  $\text{MoO}_4^{-2}$ , as a function of the time of  $\text{MoO}_4^{-2}$  addition, is shown in Figure 1 (inset). The decreasing capacity of the cells to synthesize active enzyme cannot be attributed to decreasing rate of protein synthesis. It was shown previously that the incorporation of L-leucine into protein was not affected by TMP and NUV light for at least 5 h after the treatment (6). In cells which were incubated in the presence of casamino acids following inhibition of RNA synthesis, the amount of enzyme which developed after addition of  $\text{MoO}_4^{-2}$  was much smaller. Casamino acids do not have any effect on the *in situ* activity. This indicates that the capacity to synthesize active enzyme in cells incubated in the presence of casamino acids decreased in time more rapidly in the absence of casamino acids. The semilogarithmic plots of the results shown in Figure 1 (inset) are given in Figure 2. From the two straight lines, the rate of decay of the capacity to synthesize active enzyme can be calculated. The  $t_{1/2}$  values are 1 and 4 h for cells incubated with and without casamino acids, respectively.

The results indicate the presence of a short lived factor being required for synthesis of NR. It remains to be seen whether this is the mRNA coding for the enzyme. Amino acids reduce the availability or translatability of that factor by a mechanism not yet understood, thereby exerting a negative feedback control on the level of the enzyme (2). The reduced amount of the enzyme synthesized in the presence of amino acids reflects the reduced availability or translatability of the short lived factor in the cells.

The possibility that amino acids enhance the rate of decay of the NR itself seems unlikely. The enzyme which is formed after addition of  $\text{MoO}_4^{-2}$  to casamino acid-treated cells does not show any enhanced decay rate (data not shown). The latter would be expected if amino acids increase the rate of decay of the enzyme. The existence of an activation-inactivation mechanism as a means of controlling the NR has been already shown (7). It is suggested that the synthesis of NR can also be regulated at the translation

NR<sup>1</sup> of XD cells of tobacco is induced by nitrate or nitrite (2, 8), and is repressed by amino acids, the end product of nitrate assimilation (2). The mechanism of repression by amino acids is still obscure. The development of enzyme activity is due to *de novo* synthesis and the  $t_{1/2}$  of the enzyme molecule is no more than 4 h (9). Turnover of the enzyme molecule also occurs during diminishing activity, indicating continuous synthesis and breakdown of the enzyme and availability of the respective mRNA (9). This communication describes an attempt to estimate the  $t_{1/2}$  of the rate-limiting species of RNA required for the synthesis of NR in XD cells and suggests that amino acids have a posttranscriptional control of the enzyme.

## MATERIALS AND METHODS

The experimental procedure takes advantage of three previous observations: NR synthesized in the presence of tungstate ( $\text{WO}_4^{-2}$ ) is nonfunctional (5, 6); the translation of mRNA coding for NR which accumulates during induction with  $\text{NO}_3^-$  in the presence of  $\text{WO}_4^{-2}$  is not inhibited by TMP plus NUV light (3); excess  $\text{MoO}_4^{-2}$  counteracts the effect of  $\text{WO}_4^{-2}$  (5, 6). TMP plus NUV light were shown to inhibit RNA synthesis with no effect on the translation of preexisting translatable mRNA (3, 4). Thus, the net increase of NR activity following addition of excess  $\text{MoO}_4^{-2}$  at various times after inhibition of RNA synthesis can be taken as an estimate of the amount of the translatable or rate-limiting RNA species present in the cells at that time.

XD cells were grown and handled as previously described (2-4). Logarithmic phase cells, grown with casamino acids as nitrogen source, were harvested and resuspended in fresh medium containing 10 mM  $\text{KNO}_3$  and 0.2 mM sodium tungstate. The cells were incubated in this medium for 2 h and were then treated as described in figure legends. NR activity was determined *in situ* (1). RNA synthesis was inhibited by TMP and NUV light (3, 4).

<sup>1</sup> Abbreviations: NR: nitrate reductase; TMP: 4,5',8-trimethylpsoralen; NUV: near ultraviolet.

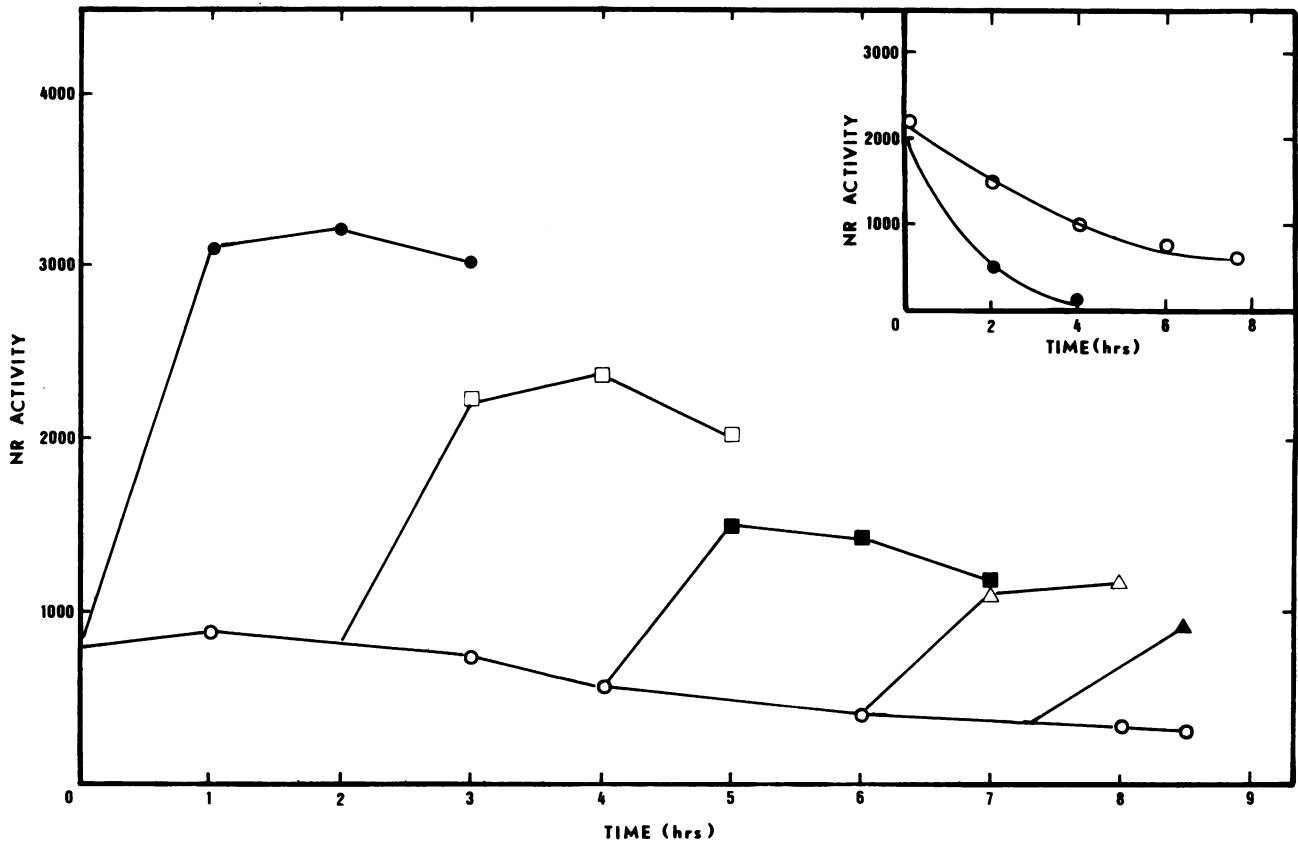
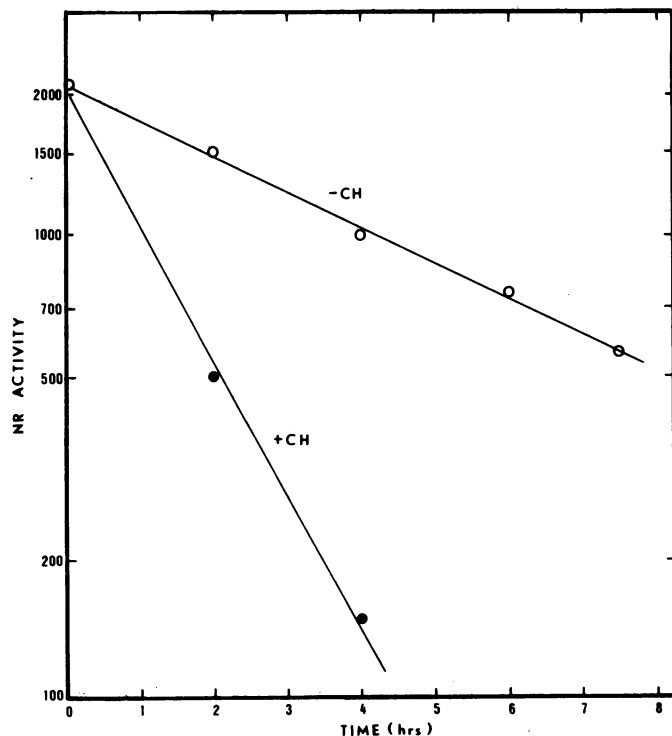


FIG. 1. Kinetics of development of NR activity after exposure to TMP and NUV light. At time intervals after exposure to TMP and NUV light,  $\text{MoO}_4^{2-}$  was added to different portions of the cell suspension, to a final concentration of 0.2 mM. At 1-h intervals after the addition of  $\text{MoO}_4^{2-}$ , NR activity *in situ* was determined.  $\text{MoO}_4^{2-}$  was added at: zero time (immediately after exposure to TMP plus NUV light) (bc), 2 h (□), 4 h (■), 6 h (Δ) and 7.5 h (▲). No  $\text{MoO}_4^{2-}$  (○). Inset: net increase of NR obtained 1 h after addition of  $\text{MoO}_4^{2-}$ , as a function of the time at which  $\text{MoO}_4^{2-}$  was added. Cells incubated with (●) and without (○) 1.5 g/l casamino acids.



level by controlling the availability of one or more of necessary factors, or by controlling the translatability of one or more of the necessary RNA species.

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FIG. 2. Half-life ( $t_{1/2}$ ) estimation of the translatable rate-limiting RNA species required for NR synthesis. A semilogarithmic plot of the results shown in Figure 1. Inset: cells incubated with (●) and without (○) casamino acids.