Short Communications

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Cyanate inhibition of nitrate reductase

We have shown that nitrate assimilation by the green alga, *Chlorella vulgaris*, is inhibited during ammonium assimilation and suggested that an inhibitor of nitrate reductase ($NADH_2$:nitrate oxidoreductase, EC 1.6.6.1) is readily formed from ammonium[1]. In an attempt to identify such a compound, Chlorella was grown as described previously[1] with KNO_3 as nitrogen source, and cell-free extracts containing nitrate reductase activity were prepared. Nitrate reductase activity was measured as before[2] and the effect of a number of nitrogenous compounds on its activity investigated. Each compound was used separately, at a final concentration in the assay system of 10 mM. The compounds tested were urea, L-glutamic acid, L-aspartic acid, L-glutamine, L-asparagine, DL-arginine hydrochloride, L-ornithine hydrobromide, L-citrulline, L-alanine, L-serine, glycine, L-proline, L-β-phenylalanine, L-leucine, L-isoleucine, L-lysine hydrochloride, L-histidine, DL-methionine, L-cysteine hydrochloride and carbamyl phosphate. Of these compounds, only the last two had any significant effect on nitrate reductase activity; both were inhibitory, carbamyl phosphate markedly so. The strong inhibition by carbamyl phosphate was of interest because this compound could be a primary product of ammonium assimilation. Further study, however, showed that freshly prepared solutions of carbamyl phosphate were much less inhibitory than solutions which had stood at

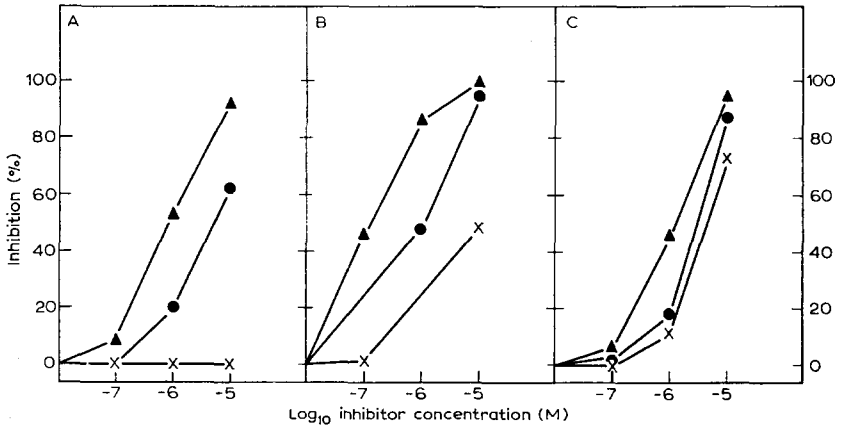

Fig. 1. The inhibition of nitrate reductase by cyanate (A), azide (B), and cyanide (C) in the presence of 0.1 (×—×), 1.0 (●—●) or 10.0 (▲—▲) mM nitrate. The reaction mixture contained in 1.0 ml, 0.5 ml cell-free extract (containing 1.1 mg protein per ml), 20 μmoles Tris buffer (pH 7.4), 0.1–10 mμmoles of inhibitor (KOCN, NaN_3 or NaCN), 0.1–10 μmoles of KNO_3 and 0.2 mg DPNH (Na salt). The mixture was incubated for 10 min at 29° and the nitrite formed measured.

room temperature for 2–3 h; this suggested that it was an hydrolysis product of carbamyl phosphate which was inhibitory. In alkaline solution, carbamyl phosphate is converted rapidly into cyanate while in acid solution it hydrolyses to CO_2 and NH_3 (ref. 3). We found that a carbamyl phosphate solution incubated at 37° at pH 13.0 for 15 min, or at pH 7.4 for 2 h, was highly inhibitory to nitrate reductase, at $5 \cdot 10^{-4}$ M concentration, whereas a solution incubated at pH 1.0 was without effect. This result suggested that it is cyanate which is a powerful inhibitor of nitrate reductase activity and this was confirmed. The inhibitory effect of cyanate can be demonstrated when either nitrite formation or DPNH oxidation is used as a measure of nitrate reductase activity. The amount of inhibition depends on the concentration of nitrate present; thus 10^{-5} M cyanate is ineffective in the presence of 10 mM nitrate but gives 90% inhibition when the nitrate concentration is 0.1 mM (Fig. 1). For comparison, Fig. 1 shows the effect of azide and cyanide on nitrate reductase activity; both are effective inhibitors but the degree of inhibition is somewhat less dependent upon nitrate concentration.

The inhibition of nitrate reductase activity by cyanate appears to be fairly specific. In 20 mM concentration we found cyanate had little effect on the activities of glutamate dehydrogenase (EC 1.4.1.4), malate dehydrogenase (EC 1.1.1.37) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49) from Chlorella, β-galactosidase (EC 3.2.1.23) and alkaline phosphatase (EC 3.1.3.1) of *Escherichia coli* and the thiosulphate oxidizing enzyme[4] from a Thiobacillus. 5 mM cyanate did, however, inhibit yeast alcohol dehydrogenase (EC 1.1.1.1) by about 40%. Cyanate, in 10 mM concentration, is reported to inhibit O_2 uptake by Azotobacter[5] and 1 mM cyanate inhibits nitrite oxidation by Nitrobacter by 60% (ref. 5). BUTT AND LEES[6] suggest that this latter inhibition results from the similarity between the cyanate and nitrite ions and such similarity might account for the inhibition of nitrate reductase since nitrite is the product of nitrate reduction. However, the similarity between the ions is not very marked since the cyanate ion is linear whereas that of nitrite is triangular in shape[7].

The inhibition of nitrate reductase by low concentrations of cyanate together with the readiness with which cyanate is formed from carbamyl phosphate in alkaline solution, suggest that, in Chlorella cells assimilating ammonium, small amounts of cyanate might possibly occur naturally and inhibit nitrate reduction. We have no direct evidence on this point.

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[1] P. J. SYRETT AND I. MORRIS, *Biochim. Biophys. Acta*, 67 (1963) 566.
[2] I. MORRIS AND P. J. SYRETT, *Arch. Mikrobiol.*, in the press.
[3] L. SPECTOR, M. E. JONES AND F. LIPMANN, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 3, Academic Press, New York, 1957, p. 653.
[4] P. A. TRUDINGER, *Biochem. J.*, 78 (1961) 680.
[5] J. A. RAKESTRAW AND E. R. ROBERTS, *Biochim. Biophys. Acta*, 24 (1957) 388.
[6] W. D. BUTT AND H. LEES, *Biochem. J.*, 76 (1960) 425.
[7] N. V. SIDGWICK, *The Chemical Elements and their Compounds*, Vol. 1, Oxford University Press, 1950, p. 673, 694.

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