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Manganese as Substitute for Magnesium During Magnesium-limited Growth of the Cyanobacterium *Anacystis nidulans*

By HANS CHRISTIAN UTKILEN

Botanical Institute, University of Oslo, P.O. Box 1045, Blindern, Oslo 3, Norway

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An apparent inhibition of cell division in the cyanobacterium *Anacystis nidulans*, caused by low Mg^{2+} concentrations, was abolished by increasing the medium Mn^{2+} concentration. Thus the mean cell volume of this organism growing in a Mg^{2+}-limited chemostat culture decreased from an average of 1·3 to 0·4 μm^3 following an increase in the reservoir Mn^{2+} concentration from 9·5 to 15 μM. This increase in Mn^{2+} had no effect on the steady-state biomass concentration, while a further elevation of the Mn^{2+} concentration lowered the biomass concentration, seemingly by making Mg^{2+} less available to the organism. The cellular Mn^{2+} concentration increased, while cellular Mg^{2+} was unaltered, following an increase in the medium Mn^{2+} concentration.

INTRODUCTION

Although Mn^{2+} cannot replace Mg^{2+} for growth (Tempest, 1969; Utkilen, 1982), there is evidence indicating that Mg^{2+} can be replaced by Mn^{2+} in some cellular processes (Kennell & Kotoulas, 1967; Webb, 1968). Cell division could be one of the events where Mn^{2+} might substitute for Mg^{2+}. Deprivation of either Mn^{2+} (Alberts-Dietert, 1941) or Mg^{2+} (Finkle & Appleman, 1953) resulted in cell enlargement of *Chlorella*, and cell enlargement was also found when a diatom was deprived of Mn^{2+} (Von Stosch, 1942).

In the cyanobacterium *Anacystis nidulans*, cell division is influenced by Mg^{2+} (Utkilen, 1982) and cell division was dissociated from biomass production when the organism was grown in media containing 5 μM-Mg^{2+}. In order to examine whether Mn^{2+} could replace Mg^{2+} in cell division of *A. nidulans*, the concentration of Mn^{2+} in the growth medium of a chemostat limited by Mg^{2+} was progressively increased.

METHODS

Organism. Anacystis nidulans strain UTEX 625 of the Culture Collection of Algae, Department of Botany, University of Texas, was used.

Growth conditions. The organism was grown in a Mg^{2+}-limited chemostat as described earlier (Utkilen, 1982). The feed medium was designed to give a final concentration of 5 μM-$MgCl_2$, but when the Mg^{2+} concentration in feed medium was measured it turned out to be about 6 μM (Table 1). The difference between added and measured Mg^{2+} could be due to impurities from other chemicals, but the assay errors were large (25 and 15%), which also might account for some of the difference. The desired Mn^{2+} concentration was obtained by adding $MnCl_2$, which was autoclaved separately unless otherwise stated.

Cell number and volume. These were determined by a Coulter electronic particle counter (Model Z_B, industrial, Coulter Electronics Ltd, U.K.), as described earlier (Utkilen, 1982).

Analytical methods. Macromolecule and dry weight estimations were performed as previously described (Utkilen, 1982). Mn^{2+} and Mg^{2+} were determined by atomic absorption spectrophotometry (Perkin Elmer 306, Connecticut, U.S.A.), using air/acetylene.

RESULTS AND DISCUSSION

Marler & Van Baalen (1965) showed that about 60 μg H_2O_2 l^{-1} was formed in medium C (Kratz & Meyers, 1955) during autoclaving. This was due to a reaction between citrate and

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Table 1. *Mn^{2+} and Mg^{2+} concentrations in feed medium and culture medium, when 9·5 or 20 μM-Mn^{2+} was added to the reservoir of a chemostat limited by 6 μM-Mg^{2+} (D = 0·01 h^{-1})*

The steady-state cell number at the two Mn^{2+} concentrations is also shown. The concentrations of the cations are given ±S.D. (six determinations), while cell numbers are average values obtained from two samples.

Concn (μM) in feed medium		Concn (μM) in culture medium		10^{-7} × No. of cells ml^{-1}
Mg^{2+}	Mn^{2+}	Mg^{2+}	Mn^{2+}	
6·0 ± 1·5	4·7 ± 0·1	0	4·2 ± 0·8	4·0
6·0 ± 0·9	13·2 ± 0·9	1·9 ± 1·5	11·8 ± 0·4	10·1

Mn^{2+}. The same authors demonstrated that the growth of *A. nidulans* was extremely sensitive to H_2O_2. In order to examine whether increasing the Mn^{2+} concentration would have any inhibitory effect as a consequence of such a reaction, the concentration of this cation was increased to 20 μM (9·5 μM in medium C) in the reservoir before or after autoclaving. The results revealed that the steady-state biomass was about 80 μg ml^{-1} and the chlorophyll content 0·8% of dry weight in both cases. The different ways of handling Mn^{2+} therefore had no effect on the growth of *A. nidulans*. As a result of these preliminary experiments the additional amount of Mn^{2+} was autoclaved separately, since there was a heavy precipitation during autoclaving media that contained 20 μM-Mn^{2+}.

The steady-state dry weight for the Mg^{2+}-limited (6 μM) chemostat at D = 0·1 h^{-1} was 106 μg ml^{-1} when the reservoir contained 9·5 or 15 μM Mn^{2+}. Increasing the Mn^{2+} concentration to 20 or 100 μM reduced the steady-state dry weight to 85 μg ml^{-1}. The reduction in steady-state biomass was caused by an inhibition of Mg^{2+} uptake, since Mg^{2+} was detected in the culture medium at 20 μM-Mn^{2+} (Table 1). It was also found that the Mn^{2+} concentration in the feed medium was about 5 or 13 μM, when 9·5 or 20 μM-Mn^{2+}, respectively, was added to the reservoir (Table 1). This difference, which was not found for Mg^{2+}, could be due to precipitation in the reservoir. These results indicate that Mn^{2+} had a constant inhibitory effect on biomass production of *A. nidulans* over a wide range of concentrations above 13 μM in a chemostat limited by 6 μM-Mg^{2+}.

The most pronounced effect of increasing the Mn^{2+} concentration was on mean cell volume, since increasing the reservoir Mn^{2+} concentration from 9·5 to 15 μM resulted in a decrease of cell volume from about 1·4 to 0·4 μm^3. These minute cells were also obtained with 20 or 100 μM-Mn^{2+} in the reservoir. These cell sizes and the results in Table 1 were used to calculate intracellular concentrations of Mn^{2+} and Mg^{2+}. The cellular Mg^{2+} concentration was found to be about 100 mM at both 5 and 13 μM-Mn^{2+}, while the cellular Mn^{2+} concentration increased from about 9 to 35 mM over the same range of extracellular Mn^{2+} concentrations. Thus, although the cellular Mn^{2+} concentration increased almost fourfold, the organism was able to maintain its Mg^{2+} concentration. But *A. nidulans* could no longer deplete the medium of Mg^{2+} at 13 μM-Mn^{2+} or higher. A competitive inhibition of Mg^{2+} uptake by Mn^{2+} was unlikely, since the lowering of biomass concentration was the same with either 20 or 100 μM-Mn^{2+} in the reservoir.

The cell size of *A. nidulans* growing in a Mg^{2+}-limited chemostat culture decreased with increasing growth rate, while it increased with growth rate when SO_4^{2-} was the limiting nutrient (Utkilen, 1982). The mean cell volume, as a function of growth rate in a Mg^{2+}-limited chemostat culture with additional Mn^{2+}, followed the same pattern as for a non-Mg^{2+}-limited culture (Fig. 1). A Mg^{2+} shift-up from 5 μM to 1 mM, during balanced growth, resulted in a synchronized cell division of *A. nidulans* after 90 min and was accompanied by a marked decrease in cell volume (Utkilen, 1982). In order to investigate whether Mn^{2+} would have the same effect on cell division, the organism was grown in batch cultures as described earlier (Utkilen, 1982) and Mn^{2+} shift-ups to 15, 20, 50 and 100 μM were made by adding $MnCl_2$. These shifts revealed that the cell volume began to decline about 60 min after the Mn^{2+} shift-up, but the decrease in cell volume was not as marked as with a Mg^{2+} shift-up (Utkilen, 1982) and there was no synchronized cell division accompanying the decrease in cell volume.

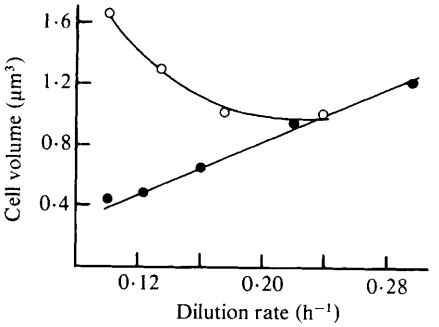

Fig. 1. Variation in mean cell volume with growth rate for a Mg^{2+}-limited (6 μM) chemostat of *A. nidulans* where the Mn^{2+} concentration in the reservoir was 9·5 μM (○) or 15 μM (●).

The results presented here indicate that Mn^{2+} could functionally replace Mg^{2+} in the cell division process during Mg^{2+}-limited growth. In doing so, Mn^{2+} was apparently more efficient than Mg^{2+}, since very small cells were obtained although most of the added Mn^{2+} was not taken up by the organism (Table 1). Increasing the concentration of Mg^{2+}, which was depleted from the medium, did not result in the same decrease of cell volume (Utkilen, 1982) though it resulted in a corresponding increase in biomass concentration (Utkilen, 1982). Therefore only a fraction of the additional Mg^{2+} would be available for cell division, in contrast to Mn^{2+} where no increase in biomass was observed. Mn^{2+} might in fact be less effective than Mg^{2+} in cell division, since no synchronized cell division was observed during a Mn^{2+} shift-up in contrast to that of a Mg^{2+} shift-up (Utkilen, 1982).

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